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Opening ceremony of the Inaugural Meeting of BISMiS in Beijing, May 2011.

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Recommendations for a new bacterial species description based on analyses of the unrelated genera *Aeromonas* and *Arcobacter*

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Assigning a strain or a group of strains to a new bacterial species and describing it formally requires at least its phenotypic characterization in order to establish its distinctiveness from existing species and 16S rRNA gene sequences and DNA-DNA reassociation results with closely related species (classically those with 16S rRNA similarities >97%). More recently Multilocus Sequence Analysis (MLSA) has been proposed as a potential alternative for DNA hybridization. These are common requirements for species in all genera, irrespective of how different they are. In the present study, the advantages and disadvantages of each of these requirements is reviewed and analyzed using two unrelated bacterial genera *Aeromonas* (*Gammaproteobacteria*) and *Arcobacter* (*Epsilonproteobacteria*) as models with some additional examples from their close neighbors *Vibrio* and *Campylobacter*. The genus *Aeromonas*, defined in 1943, is considered to have a complex taxonomy because it includes a tight group of species (and several subspecies) with 16S rRNA gene similarities ranging from 96.9 to 100%, some of which have required reclassification. In contrast, the genus *Arcobacter*, was defined 20 years ago (1991) from members of the genus *Campylobacter*, and the presently known species show 16S rRNA gene similarities ranging from 92.1 to 98.9%, and none of the species have required reclassification. Therefore, while the similarity of the 16S rRNA gene cannot be used to delineate closely related species of *Aeromonas*, this is still an excellent tool for *Arcobacter*, something that may change in the near future with the addition of new species. Several specific topics are discussed, such as the number of isolates needed to define a new bacterial species, the molecular approaches used for recognizing new species and the procedure for validly publishing a new species name. In addition, the need for developing genus-specific guidelines for describing new bacterial species, recommendations for avoiding common errors in the laboratory, and a species definition for prokaryotes in the 21st century are presented. Emphasis is placed on the two factors that govern the proper description of new bacterial species, the precision of the performance of experimental work and the correct interpretation of results.

Introduction

The reliable identification of bacterial strains as belonging to a known or a new species requires a robust taxonomy. The "ad hoc committee for the re-evaluation of the species definition in bacteriology" (CSDB) has settled the criteria and methods to be applied for defining and describing new bacterial species (Stackebrandt et al., 2002) generating a homogeneous approach of universal application (Rosselló-Móra and Amann, 2001; Schleifer, 2009; Tindall et al., 2010). A new species description should include as many strains as possible, phenotypic diagnostic properties, analysis of 16S rRNA gene sequences and if there is a similarity with closely related species above 97% (Stackebrandt and Goebel, 1994) or 98.7–99.0% (Stackebrandt and Ebers, 2006) DNA–DNA hybridization (DDH) results. Additional recommendations include the analysis of the sequences of at least five housekeeping genes (Multilocus Sequence Analysis, MLSA) and DNA typing methods for establishing inter- and intra-specific genomic relatedness. All these criteria for defining species have been extensively reviewed in other studies (Rosselló-Móra and Amann, 2001; Schleifer, 2009; Tindall et al., 2010).

More recently, information derived from whole genome

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sequences or indexes derived from them, such as the Average Nucleotide Identity (ANI; Konstantinidis et al., 2006) and the Maximal Unique Matches (MUM; Deloger et al., 2009) have been envisaged as alternatives to replace DDH in the near future (Ritcher and Rosselló-Móra, 2009; Tindall et al., 2010). In addition, faster sequencing techniques such as pyrosequencing, which relies on the detection of pyrophosphate release on nucleotide incorporation rather than chain termination with dideoxynucleotides (Petrosino et al., 2009; Ritcher and Rosselló-Móra, 2009) are also available now.

This paper presents current criteria and boundaries for defining new bacterial species emphasizing their advantages and disadvantages (Table 1) and describing how they have been applied to two unrelated bacterial genera *Aeromonas* and *Arcobacter* (with examples from their close neighbors *Vibrio* and *Campylobacter*). The use and importance of MLSA today as an alternative technique for replacing DDH will be underlined with specific examples.

The genus *Aeromonas* described in 1943 (Martin-Carnahan and Joseph, 2005), which now includes 26 species and 8 subspecies (Table 2), was originally proposed on the basis of only phenotypic characters from bacteria recognized as early as 1891 and has evolved through advances in taxonomic techniques. In contrast, the genus *Arcobacter*, was created in 1991 when molecular techniques were already in practice. Some *Arcobacter* spp. originally considered "aerotolerant campylobacters" were described on the basis of only phenotypic characteristics and DNA mol% G+C content (Vandamme et al., 1991, 1992, 2005). Currently, 12 species have been described (Table 2). These two genera provide good contrasting examples that illustrate some of the difficulties with current taxonomic practices. The genus *Aeromonas* which was described almost 70 years ago has a complex taxonomy dotted with reclassifications, controversy and problems of terminology. In comparison, the relatively new genus *Arcobacter* is almost free of these problems. Issues with terminology are rampant in *Aeromonas*, and are also found in many other older genera. They are linked to old species' and subspecies' names that are pending for Judicial Commission Opinions or have been considered synonyms of those of other species (Martin-Carnahan and Joseph, 2005; Janda and Abbott, 2010). Unfortunately many non-specialists are still using these names because these are the labels associated with 16S rRNA gene sequences at GenBank. This problem will be discussed along with other topics, such as the number of isolates that are needed to define a new bacterial species, the molecular approaches that enable new species to be recognized and the procedure for validly publishing a new species name. In addition, this paper discusses the need for developing genus-specific guidelines for describing new bacterial species, makes recommendations for avoiding common laboratory errors and presents a proposal for a species definition for prokaryotes in the 21st century.

Advantages and disadvantages of the requirements for new bacterial species' descriptions - analysis of the unrelated genera *Aeromonas* and *Arcobacter*

Phenotypic methods

Phenotypic characterization includes morphological and physiological properties and chemical composition (using standardized techniques) and leads to a set of diagnostic tests for species' identification and discrimination (Rosselló-Móra and Amann, 2001; Stackebrandt et al., 2002; Schleifer, 2009; Tindall et al., 2010). A new species cannot be described unless it can be differentiated from known species by at least one phenotypic property (Wayne et al., 1987; Rosselló-Móra and Amann, 2001). Disadvantages (Table 1) include the lack of reproducibility (due to poor precision, different methodologies, different strain response etc.) the large number of tests and the specialized skills needed (Rosselló-Móra and Amann, 2001). Some of these disadvantages cannot be attributed to the phenotypic methods *per se* but to the performance or behavior of the bacteria. A major disadvantage of phenotypic tests is that they generate many inconsistent results that confuse the current taxonomy.

An important aspect, often neglected, is that type strains from closely related species should be tested in parallel (Rosselló-Móra and Amann, 2001; Tindall et al., 2010). Sometimes, authors copy previously published data (Tindall et al., 2010) without any re-evaluation. This is important because contradictory results can be found that must be verified and corrected. For instance, when we re-evaluated nitrate reduction in *Arcobacter mytili* it was found to be positive for the three strains included in the description (Collado et al., 2011) contradicting our previously published data (Collado et al., 2009a). Likewise, growth in 4% NaCl, described as positive in the definition of the new species *Arcobacter trophiarum* (De Smet et al., 2011), was later found by us to be negative for the type strain and two other additional strains (Figueras et al., 2011c). However, after contacting the authors of this new species to alert them of these discrepancies they repeated the tests and found similar results to ours, so this was amended (40% response instead of the 100%) on the proofs of the paper describing this new species (Dr Kurt Houf, personal communication).

Table 1. Advantages and disadvantages of the recommended standard requirements for defining new bacterial species

nd, Not determined. Other advantages and disadvantages for the use of the 16S rRNA gene, DNA–DNA reassociation and MLPA are reviewed in the study of Schleifer (2009). Rosselló-Móra and Amann (2001) provided the guidelines for the recognition of prokaryotic species.

Requirement	Designated limit	Advantages	**Disadvantages**[a]
Number of strains	As many as possible[b,c]; 5[d], 10[c,d], 25[b]	More precise phenotypic diversity and genetic characterization; type strain can be better selected[d]	Isolation is a matter of luck so the number of strains cannot be controlled; waiting for a specific number of strains is a loss to science[e].
The type strain must be deposited in two culture collections		The type strain is the reference material for comparison	Normally only the type strain is deposited
Phenotypic characterization[f]	As many characteristics as possible, at least one differential with the closest species[b,g].	Evidence of intra-species diversity; provides important physiological properties and ecological information[b]; enables development of diagnostic identification systems.	No absolute guarantee exists that other strains will have the same phenotypic response; subjective interpretations of results even if standardized procedures are employed[b]; lack of reproducibility of results for certain tests in/and among laboratories[b]; high intra-species variability with no clear-cut (+ and −) characters limits the delineation of the species; methods are not always detailed in the descriptions of new species; time-consuming and specialized skills[b].
DNA–DNA reassociation	70% ($\leq$ 5°C ΔT_m)[g]; 80%[b]	Reference technique for the delineation of all bacterial species[g,h,i]; reflects the overall genome similarity among the compared strains.	Does not measure direct sequence identity but efficiency of the hybridization of the DNA strands[b]; possible lack of reproducibility with a high degree of experimental error that has lead to some erroneous species descriptions; not readily available at many laboratories; complex, time consuming and expensive[h]; the method is outdated in the genomic era[i].
16S rRNA gene sequence similarity derived from >1300 nt with 0.5% ambiguities	>97%[j]; 98.7–99%[i]	Universal marker and primers; gene with unlikely lateral gene transfer[h]; sequences available for many taxa; genus specific signature regions for comparison with other taxa; it can guide the selection of strains for DDH.	Different species may have an identical sequence and may not be useful for separating closely related species[h]; it is a multicopy gene with possible variability among the copies (microheterogeneities)[h], which has led to some erroneous species descriptions; sequences of poor quality or erroneously labeled in databases[i].
Mutilocus phylogenetic analysis (MLPA) of a minimum of five hoousekeeping genes	nd	Genes are present in all bacterial species; Higher discriminatory power than 16S rRNA gene[h]; correlates well with DDH results; provides cumulative databases for comparisons; intra- and inter-species delineation limits can be established[k]; recognizes redundant strains derived from the same clone.	Not enough sequences available in databases for comparisons; Lack of universal primers[h]; they cover only a small proportion of the whole genome[h]; a different set of genes may be required for different taxa[h], however, this has not been extensively demonstrated; the criteria for the selection of the genes are not always clear[h].
DNA fingerprinting	nd	Recognizes redundant strains derived from the same clone; reveals genetic diversity among the strains; targets the whole genome; fast and easy to perform.	Possible lack of reproducibility; not suitable for the separation of species in the absence of a broad database; in-house databases are difficult to share.

[a]Disadvantages include some that are not due to the method per se but to the lack of reproducibility of results or poor performance at different laboratories that have had consequences in the taxonomy and that is why are considered a limitation.
[b]Roselló-Mora and Amman (2001).
[c]Ursing et al. (1994).
[d]Christensen et al. (2001).
[e]Oren (2004).
[f]Numerical taxonomy in phenotypic analysis has been reviewed by Roselló-Mora and Amann (2001) and complementary data such as chemotaxonomic properties (cell-wall composition, lipids, fatty acids, etc.) despite being recommended are not included in the table (for more information see Roselló-Mora and Amann, 2001; Tindall et al., 2010).
[g]Wayne et al. (1987).
[h]Schleifer (2009).
[i]Stackebrandt and Ebers (2006).
[j]Stackebrandt and Goebel (1994).
[k]Martínez-Murcia et al. (2011).

The phenotypic diagnostic characteristics for a given new species are used later by other researchers for discriminating this species from their isolates. However, the stable differential characteristics (+ and – responses) given in the original description can vary when tested with other strains. This may be due to poor standardization of the methods used, but may also result from the intrinsic variable nature of phenotypic characters of the bacteria involved (that may or may not be expressed equally) due to the natural adaption of bacteria to different circumstances or ecosystems. Therefore, because phenotypic characterization may produce spurious results, one cannot guarantee that a strain is correctly identified at the species level (especially for complex species in complex genera like *Aeromonas*, *Arcobacter*, *Vibrio* and *Campylobacter* among others) if it has not been further verified with reliable molecular methods. Misidentifications occur not only at the species level but also at the genus level; *Aeromonas* are commonly misidentified as *Vibrio* on the basis of phenotypic characters (Chacón et al., 2002; Park et al., 2003; Figueras, 2005; Janda and Abbott, 2010). Furthermore, after performing many studies with *Aeromonas* strains recovered from clinical and environmental samples using molecular identification methods, we have demonstrated that both the routinely used biochemical tests and the commercial identification systems (i.e. API20E, Vitek, BBL Crystal, MicroScan W/A), fail to correctly identify *Aeromonas* strains at the species level (Kozińska et al., 2002; Castro-Escarpulli et al., 2003; Park et al., 2003; Soler et al., 2003; Figueras, 2005; Ørmen et al., 2005; Beaz-Hidalgo et al., 2010a). This is especially relevant for *Aeromonas hydrophila* because these systems typically identify erroneously up to 70–80% of the strains as belonging to this species (Soler et al., 2003; Figueras, 2005; Beaz-Hidalgo et al., 2010a). This has, in turn, led to an overestimation of the clinical and environmental relevance of this species at the detriment of more relevant species. Molecular identification showed *Aeromonas hydrophila* to be the 3rd or 4th most abundant species after *Aeromonas caviae*, *Aeromonas veronii* and *Aeromonas aquariorum* when investigating clinical strains (Figueras, 2005; Alperi et al., 2008; Figueras et al., 2009). Strains of *Aeromonas sobria*, *Aeromonas hydrophila* and *Aeromonas caviae*, identified phenotypically, masked a higher diversity of other species, including new species like *Aeromonas piscicola* or *Aeromonas aquariorum* (Beaz-Hidalgo et al., 2009; Figueras et al., 2009; Martínez-Murcia et al., 2008; Beaz-Hidalgo et al., 2010a; Figueras et al., 2011b). Furthermore, the variable phenotypic characteristics of the *Vibrio splendidus*-like and the *Vibrio harveyi*-like groups make it virtually impossible to discriminate species of these groups, which were only recognized using molecular methods (Beaz-Hidalgo et al., 2008, Pascual et al., 2009; Beaz-Hidalgo et al., 2010b).

In *Arcobacter*, phenotypic differentiation of species is also difficult because these bacteria neither ferment nor oxidize carbohydrates (Vandamme et al., 2005) and misidentifications as *Campylobacter* have been reported (Diergaardt et al., 2004) as well as erroneous identifications when using the API Campy system (Atabay et al., 1998). Recognizing *Arcobacter* spp. in different types of samples (water, food, animal or human feces) relies on molecular methods like m-PCR or 16S rDNA-RFLP (for a review, see Collado and Figueras, 2011). These methods, despite their faults, have produced relative accurate information on this genus (Collado and Figueras, 2011). This differs from the genus *Aeromonas*, where many studies have drawn conclusions from strains that were identified only from phenotypic tests, the usefulness of which is questionable. Nowadays, these studies should be totally discouraged, and a re-evaluation of phenotypic characteristics is recommended with many genetically well-characterized strains and well-described standardized tests for each species.

DNA G+C content (mol%)

The DNA guanine cytosine content (mol% G+C) was the first genetic character used in the description of new species and is one of the requirements of the CSDB for the description of a new genus (it was not included in Table 1, which has species requirements). It has been established that strains that differ by more than 10% may belong to the same genus, while a value of 5% is the common range within a species, although a similar G+C content does not necessarily imply that strains are closely related (Rosselló-Móra and Amann, 2001; Schleifer, 2009). The DNA G+C content of *Aeromonas* is 57–63 mol% (Martin-Carnahan and Joseph, 2005) while for *Arcobacter* it is 26.8–35 mol% (Vandamme et al., 2005).

DNA–DNA relatedness

The determination of the DNA–DNA relatedness, which establishes the similarity between two genomes, is the mandatory reference method for determining whether two micro-organisms belong to the same species (Wayne et al., 1987; Stackebrandt et al., 2002; Rosselló-Móra, 2006; Stackebrandt and Ebers, 2006; Schleifer, 2009; Tindall et al., 2010). The different DDH methods have been reviewed by Rosselló-Móra (2006). DNA–DNA results can be expressed as percentage reassociation similarity and increment in melting temperature (ΔT_m) of reassociated DNA strands, normally being the first parameter generally used in the descriptions of new species (Stackebrandt and Ebers, 2006). As shown in Table 1, a group of strains (or a single strain) that show less than approximately 70% DNA–DNA similarity with the closest related species (type strains) are con-

Table 2. Currently accepted *Aeromonas* and *Arcobacter* species and techniques used for their classification at the original descriptions[a]

Two candidate new species "*Aeromonas lusitana*" and "*Aeromonas cavernicola*" have recently been recognized (unpublished; Martínez-Murcia and co-workers).

Taxa	Year	DNA G+C content (mol%)	DNA–DNA	16S rRNA gene	DNA typing	Housekeeping genes/ MLPA[b]
Genus *Aeromonas*						
Aeromonas hydrophila[c]	1943	+	–[d]	–[e]	–	–
Aeromonas salmonicida[f]	1953	–	–[d]	–[e]	–	–
Aeromonas sobria	1976	+	–[d]	–[e]	–	–
Aeromonas media	1983	+	–	–[e]	–	–/–
Aeromonas veronii	1987	+	+	–[e]	–	–/–
Aeromonas shubertii	1988	–	+	–[e]	–	–/–
Aeromonas eucrenophila	1988	+	+	–[e]	–	–/–
Aeromonas caviae	1984	+	+	–[e]	–	–/–
Aeromonas trota	1991	–	+	–[e]	–	–/–
Aeromonas jandaei	1991	–	+	–[e]	–	–/–
Aeromonas allosaccharophila	1992	–	+	+	–	–/–
Aeromonas encheleia	1995	–	+	–	–	–/–
Aeromonas bestiarum	1996	–	+	–	+	–/–
Aeromonas popoffii	1997	+	+	–	+	–/–
Aeromonas simiae	2004	–	+	+	+	–/–
Aeromonas molluscorum	2004	+	+	+	+	–/–
Aeromonas bivalvium	2007	+	+	+	–	–/–
Aeromonas haianensis[g]	2007	–	+	+	–	–/–
Aeromonas tecta	2008	–	+	+	–	+/–
Aeromonas aquariorum	2008	–	+	+	+	+/–
Aeromonas piscicola	2009	–	+	+	–	+/–
Aeromonas fluvialis	2010	–	+	+	–	+/+
Aeromonas sanarellii	2010	–	+	+	–	+/+
Aeromonas taiwanensis	2010	–	+	+	–	+/+
Aeromonas diversa	2010	+	+	+	+	+/–
Aeromonas rivuli	2010	–	+	+	–	+/+
Genus *Arcobacter*						
Arcobacter nitrofigilis[h]	1983	+	–[i]	–[j]	–	–/–
Arcobacter cryaerophilus[k]	1985	+	–[i]	–[j]	–	–/–
Arcobacter butzleri[l]	1991	+	+	–[m]	–	–/–
Arcobacter skirrowii	1992	+	+	–[m]	–	–/–
Arcobacter cibarius	2005	+	+	+	+	–/–
Arcobacter halophilus	2005	+	+	+	–	–/–
Arcobacter mytili	2009	+	+	+	+	+/–
Arcobacter thereius	2009	+	+	+	+	–/–
Arcobacter marinus	2010	+	+	+	–	+/–
Arcobacter trophiarum	2010	+	+	+	+	+/–
Arcobacter defluvii	2011	–	+	+	+	+/–
Arcobacter molluscorum	2011	–	+	+	+	+/–

[a]Phenotypic tests were evaluated on all species and are therefore not included. For the specific authorship for *Aeromonas* species described up to 2004 see Martin-Carnahan and Joseph (2005), and for the rest see Figueras et al. (2011a). For the specific authorship for *Arcobacter* species, see Vandamme et al. (2005) and Collado and Figueras (2011).

[b]Sequencing was carried out for 1–4 housekeeping genes in the original description (+). The term MLPA refers to the analysis of minimum five housekeeping genes, which has been carried out for all *Aeromonas* species and included in the recent descriptions of new species (Alperi et al., 2010a, 2010b; Figueras et al., 2011d). A complete MLPA with seven genes was recently finished (Martínez-Murcia et al., 2011). For *Arcobacter* species no MLPA with 5 housekeeping genes is yet available.

[c]*Aeromonas hydrophila* includes three subspecies: *Aeromonas hydrophila* subsp. *hydrophila*, *Aeromonas hydrophila* subsp. *dhakensis* and *Aeromonas hydrophila* subsp. *ranae*. An amended description for *Aeromonas hydrophila* was provided by Huys et al. (2002).

[d]Popoff et al. (1981) carried out the DDH for all the marked species and defined the hybridization groups (HG).

[e]Martínez-Murcia et al. (1992) established the phylogeny of the genus derived from the 16S rRNA gene for all the marked species.

[f]*Aeromonas salmonicida* includes five subspecies: *A. salmonicida* subsp. *salmonicida*, *A. salmonicida* subsp. *smithia*, *A. salmonicida* subsp. *achromogenes*, *A. salmonicida* subsp. *smithia*, and *A. salmonicida* subsp. *pectinolytica* (Martin-Carnahan and Joseph, 2005).

[g]The species name of *Aeromonas haianensis* (Wang et al., 2007) has so far not been validly published.

[h]Originally described as *Campylobacter nitrofigilis* and reclassified as *Arcobacter nitrofigilis* comb. nov. by Vandamme et al. (1991).

[i]Vandamme et al. (1991)

[j]16S rRNA gene sequences were performed by Thompson et al. (1988) who suggested that they may belong to a new genus before a reclassification by Vandamme et al. (1991).

[k]Originally described as *Campylobacter cryaerophila* and reclassified as *Arcobacter cryaerophilus* comb. nov. by Vandamme et al. (1991).

[l]Originally described as *Campylobacter butzleri* and reclassified as *Arcobacter butzleri* comb. nov. by Vandamme et al. (1992).

[m]Wesley et al. (1995).

sidered a new species (Wayne et al., 1987). However, these values should not be used as a strict species boundary (Martínez-Murcia et al., 1992; Rosselló-Móra and Amann, 2001; Rosselló-Móra, 2006; Richter and Rosselló-Móra, 2009; Tindall et al., 2010). DDH results obtained for some clearly different species have led the 70% limit to be considered too broad so a more restricted limit of 80% (Table 1) has been proposed (Rosselló-Móra and Amann, 2001; Rosselló-Móra, 2006 and references therein).

There are many limitations attributed to the DNA–DNA results (Table 1) but probably the most important are the possible experimental variations, and the fact that the quality of the results (as occurs with the phenotypic characterization) cannot be verified by reviewers, contrary to what can be done with sequences. The CSDB has promoted the search for alternative methods to DDH that do not show these limitations. Popoff carried out the first DDH studies on the genus *Aeromonas* in 1981 and established the different hybridization groups (HG). Although it is still used in the latest edition of *Bergey's Manual* (Martin-Carnahan and Joseph, 2005), this terminology is outdated and should be abandoned (Janda and Abbott, 2010).

In the genus *Aeromonas* large discrepancies between DDH results for the same strains have led to species descriptions that have later required reclassification. For instance, *Aeromonas culicicola* was proposed as a new species because the obtained DDH result with the closest species *Aeromonas veronii* was only 42% (Pidiyar et al., 2002), but the 79–88% DDH results found between the latter two species together with the lack of phenotypic differentiation led Huys et al. (2005) to consider *Aeromonas culicicola* a synonym of *Aeromonas veronii*. Some extreme cases are the DDH results for *Aeromonas encheleia* and *Aeromonas* sp. hybridization group 11 (HG11), for which Esteve et al. (1995) obtained 12% compared to the 84% obtained by Huys et al. (1997) when they recognized them as synonyms; and *Aeromonas veronii* and *Aeromonas allosaccharophila*, where Esteve et al. (1995) found a value of 0% compared to the 80% found by Huys et al. (2001). The latter two species are now accepted as clearly different species (Saavedra et al., 2006; Martínez-Murcia et al., 2011). In other instances the reference strains chosen for DDH experiments were not the type strains as they should be (Rosselló-Móra and Amann 2001; Tindall et al., 2010), leading to incorrect conclusions being drawn. This is the case with *Aeromonas ichthiosmia*, where the 60% DDH result obtained with one *Aeromonas veronii* strain (not the type) led Schubert et al. (1990a) to propose this new species, although it was later recognized to be a synonym of *Aeromonas veronii* on the basis of a 100% 16S rRNA gene similarity (Collins et al., 1993) and new DDH results (84–96%) obtained with the type strain (Huys et al., 2001). Similar examples probably exist in other bacterial genera. It must be remembered that results of DDH experiments are only a measure of the efficiency of the hybridization of the DNA strands (Rosselló-Móra, 2006) and differences such as those mentioned may occur. There are a number of reasons for those differences: physico-chemical parameters, genome size, the presence of large plasmids, DNA purity and other factors that influence the hybridization results, as indicated by Stackebrandt and Ebers (2006). Although these may not be considered a disadvantage of the method *per se* we consider it so because of the confusion it may generate.

The selection of species for DDH experiments has been guided classically by the 16S rRNA gene sequence similarity of >97% between a new strain and that of the type strains of known species (Stackebrandt and Goebel, 1994). However, based on new analysis more restricted values (98.7–99.0%) have been proposed for this selection (Stackebrandt and Ebers, 2006). In *Aeromonas*, there is no clear correlation between DDH results and the 16S rRNA gene similarity. For instance, a species with 99.5% 16S similarity showed a DDH value of 66% (*Aeromonas fluvialis–Aeromonas veronii*) (Alperi et al., 2010b), while others with identical 16S rRNA gene sequences showed a reassociation value of 65% (*Aeromonas piscicola–Aeromonas bestiarum*) (Beaz-Hidalgo et al., 2009). This strongly suggests that the similarity of these genes is not a useful tool for selecting the strains for the DDH experiments in this genus. This also occurs in other genera like *Vibrio*, *Campylobacter*, etc. Despite the fact that DDH has in general been very useful in ordering taxonomy, it will soon become an outdated "emeritus taxonomic tool" (Stackebrandt and Ebers, 2006; Richter and Rosselló-Móra, 2009), an opinion that we totally support.

The 16S rRNA gene

The use of 16S rRNA gene sequences has revolutionized prokaryotic taxonomy as a tool for establishing phylogenetic relationships (Rosselló-Móra and Amann, 2001; Schleifer, 2009; and references therein). According to the CSDB, the description of a new species should include an almost complete, high quality, 16S rRNA gene sequence (>1300 nt, <0.5% ambiguity). However, sequence variations (<1%) in the operon copies of the 16S rRNA gene (microheterogeneities) occur in many bacteria and may be as high as 11.6% in some thermophilic bacteria (Alperi et al., 2008; Schleifer, 2009). These are informative variations that should be carefully interpreted and annotated (Alperi et al., 2008 and references therein). A major advantage (Table 1) is that sequences are available for many taxa in databases (GenBank, etc.) allowing both similarity comparisons and phylogenetic

analysis. However, not all sequences of GenBank are correct and/or correctly labeled, which may result in a lot of confusion (Stackebrandt and Ebers, 2006; Janda and Abbott, 2007; Tindall et al., 2010). Another important drawback is that as more sequences from more species became available it was apparent that within several genera (i.e. *Aeromonas*, *Campylobacter*, *Vibri*o, etc.) some species that were accepted as different had almost identical sequences. This gene, therefore, lost its usefulness for the delineation of those species in those genera (Rosselló-Móra and Amann, 2001; Schleifer, 2009; Richter and Rosselló-Móra, 2009; and references therein; Beaz-Hidalgo et al., 2009, 2010a; Alperi et al., 2010a, 2010b; Figueras et al., 2011a). Nevertheless, the 16S rRNA gene shows signature sequences that are very specific at the genus level and give high resolution for splitting closely related genera (Martínez-Murcia, 1999; Rosselló-Móra and Amann, 2001; Martínez-Murcia et al., 2007; Tindall et al., 2010). Consequently, despite the aforementioned limitations, it is important that those sequences remain a mandatory requirement for species descriptions in order to support that they belong to the genus. However, errors when assigning a species to a genus may occur. For example, the species *Aeromonas sharmana* shared only ≤95.3% similarity of its 16S rRNA gene with various species of the genus (Saha and Chakrabarti, 2006). The lack of the genus signature regions in the 16S rRNA gene and of the typical genus-specific phenotypic traits revealed that this species does not belong to the genus *Aeromonas* (Martínez-Murcia et al., 2007). This was confirmed later using other housekeeping genes (Miñana-Galbis et al., 2009).

In the genus *Aeromonas* the 16S rRNA gene is highly conserved, ranging from 96.8 to 100% (Martínez-Murcia et al., 2007). Strains from *Aeromonas salmonicida*, *Aeromonas bestiarum* and *Aeromonas piscicola* may share identical sequences or at most have two nucleotide differences (Martínez-Murcia et al., 2005; Beaz-Hidalgo et al., 2009; 2010a). Without including the latter species, the similarity ranges from 96.8 to 99.8% (Alperi et al., 2010a). Consequently, as commented, neither the 97% 16S rRNA gene similarity nor the newly proposed 98.7–99% limits (Stackebrandt et al., 2002; Stackebrandt and Ebers, 2006) can be applied to this genus (Figueras et al., 2011a). Microheterogeneities in the operon copies of the 16S rRNA gene have been found to occur in 8% of the more than 900 *Aeromonas* strains investigated and these have an impact on their taxonomy (Alperi et al., 2008). For example Pidiyard et al. (2002) proposed the new species *Aeromonas culicicola* because the strains showed an ambiguous position in the 16S rRNA tree and had low DDH values with *Aeromonas veronii* as has already been mentioned. This species was later considered a synonym of *Aeromonas veronii* (Huys et al., 2005). The ambiguous phylogenetic position was generated by the microheterogeneities, present in seven positions of the 16S rRNA gene of *Aeromonas culicicola* (Figueras et al., 2005), and was one of the reasons that led to this error (Alperi et al., 2008).

In contrast to *Aeromonas*, the inter-species similarity of the 16S rRNA gene among *Arcobacter* species ranges from 92.1 to 98.9% (Collado and Figueras, 2011) and can therefore still be considered a good tool for separating the species of this genus. Notice that the lower similarity (92.1%) between *Arcobacter thereius* and *Arcobacter halophilus*, is clearly below the classical <95% threshold suggested for the separation of genera (Rosselló-Móra and Amann, 2001, and references therein). Despite this, the newly proposed 98.7–99% values (Stackebrandt and Ebers, 2006) were considered more appropriate for the new species *Arcobacter molluscorum* (Figueras et al., 2011c). It is probable that as more species of *Arcobacter* are described, the 16S rRNA gene will lose its discriminatory capacity, as is the case with *Aeromonas* or observed also for *Campylobacter* where some strains of the species *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* have identical 16S rRNA gene sequences (Gorkiewicz et al., 2003). The same occurs with *Vibrio harveyi* and *Vibrio campbellii*, which share nearly 100% 16S rRNA gene sequence similarity (Thompson et al., 2007).

The 16S rRNA gene can help to identify uncultured bacteria. The phylogenetic analysis of the 16S rRNA gene sequences of *Arcobacter* deposited in databases shows that there are still many new species waiting to be described, some of which come from novel environments, e.g. cyanobacterial mats, contaminated oilfields, coral, plankton, tubeworms, abalone, cod larviculture, and snails (Collado and Figueras, 2011, and references therein). This may also be true for other genera.

DNA typing methods

DNA typing methods, or fingerprinting, generally refer to techniques which differentiate isolates that belong to the same strain (i.e. derived from the same clonal group), and may be useful for determining the inter- and intra-species relatedness (Rosselló-Móra and Amann, 2001; Stackebrandt et al., 2002; Tindall et al., 2010). The main advantage is that some of them are easy and quick to carry out, but, on the other hand, may have poor reproducibility (Table 1). These techniques have been recommended as mandatory for new species' descriptions in order to prevent the use of redundant isolates, as occurred with *Aeromonas culicicola* and *Aeromonas simiae* (Figueras et al., 2006). All *Arcobacter* species described recently with more than one strain have

included molecular genotyping in order to differentiate the isolates (Table 2).

Multilocus Phylogenetic Analysis (MLPA)

As the CSDB indicate, the sequences of a minimum of five housekeeping genes (i.e. those that encode vital functions for the bacteria) can be useful for delineating species and serve as an alternative method to DDH if a sufficient degree of concordance between the methods can be demonstrated. This approach was first termed Multilocus Sequence Typing (MLST) and was used as a strain-typing method (intra-species level) that assessed differences of gene sequences by allele numbering (Maiden et al., 1998). The MLST name has been used inappropriately in studies that calculated the total sequence similarity. However, for the phylogenetic analysis derived from the nucleotide sequences, Gevers et al. (2005) proposed the more general term Multilocus Sequence Analysis (MLSA). In our view, if information is used to ascertain inter-species phylogenies shown by phylogenetic trees, it seems even more appropriate to use the term Multilocus Phylogenetic Analysis (MLPA) (Alperi et al., 2010a,b; Figueras et al., 2011a; Martínez-Murcia et al., 2011) as we will refer to it here.

Housekeeping genes have many advantages (Table 1): (i) they evolve faster than the 16S rRNA gene and have higher resolution for differentiating closely related species, (ii) results (i.e. sequences) are reproducible (so their accuracy can be verified) and (iii) databases can be constructed and can be shared via the Internet, allowing strains to be identified quickly (Christen, 2008; Bishop et al., 2009; Schleifer, 2009; Tindall et al., 2010). Concordance has been demonstrated between MLPA and DDH results in several bacteria, including *Aeromonas* and *Vibrio* (Richter et al., 2006; Martens et al., 2008; Pascual et al., 2009; Serrano et al., 2010; Martínez-Murcia et al., 2011). Furthermore, new species of *Vibrio* (Beaz-Hidalgo et al., 2010b, and references therein) and *Aeromonas* have already been described using this approach (Alperi et al., 2010a, 2010b; Figueras et al., 2011a). In the latter new descriptions, DDH studies were carried out with the closest neighbor species selected on the basis of the higher similarities of both, MLPA and 16S rRNA genes, which were not always coincident. Recently an MLPA of *Aeromonas*, including seven concatenated genes (*gyrB*, *rpoD*, *recA*, *dnaJ*, gyr*A*, *dnaX* and *atpD*) for which inter- and intra-species boundaries have been established, has been completed (Martínez-Murcia et al., 2011). The MLPA increases resolution, buffers away chronometric distortion effects (derived from horizontal gene transfer, etc.) and makes more robust (bootstrap values about 100% for species clusters) the overall phylogenetic relatedness (Zeigler, 2003; Gevers et al., 2005; Martens et al., 2008; Bishop et al., 2009; Schleifer, 2009; Serrano et al., 2010; Martínez-Murcia et al., 2011). However, it is essential that sufficient representative strains of each species are included in the analysis. In *Aeromonas* one single gene (*gyrB* and/or *rpoD*) is an excellent tool for species delineation and for preliminary recognition of potentially new species (Yañez et al., 2003; Soler et al., 2004; Martínez-Murcia et al., 2008; Alperi et al., 2010a, 2010b; Beaz-Hidalgo et al., 2010a; Figueras et al., 2011a) and to detect wrongly identified or described taxa (Figueras et al., 2009; Martínez-Murcia et al., 2007; 2009; Beaz-Hidalgo et al., 2009; Figueras et al., 2011a, 2011b). Based on *gyrB* and *rpoD* housekeeping genes *Aeromonas hydrophila* subsp. *dhakensis* (Huys et al., 2002) has been proposed as a synonym of *Aeromonas aquariorum* (Martínez-Murcia et al., 2009), but a formal validation is still pending.

Good agreement with the accepted taxonomy of the genus *Arcobacter* has been shown using the genes *rpoB–rpoC*, *gyrA*, *gyrB*, *rpoB* and *hsp60*, separately, but an MLPA (with five concatenated genes) that includes all accepted species is not yet available. Recent descriptions of new species have introduced the analysis of one or more of the mentioned genes (Collado et al., 2009a, 2011; Figueras et al., 2011c). In addition, when Miller et al. (2009) concatenated the seven genes (*aspA*, *atpA*, *glnA*, *gltA*, *glyA*, *pgm*, and *tkt*) of an MLST for arcobacters and carried out a phylogenetic analysis, they also found that this method was useful for discriminating among the species studied.

To our knowledge so far, specific genes useful for species descriptions have not yet been proposed within the guidelines for defining species available for certain bacteria orders, families or genera (a list of published minimal standards is provided by J.P. Euzéby at http://www.bacterio.cict.fr/minimalstandards.html). This has led to many studies investigating different genes. Although this may be interesting for guiding the selection of the best ones, it hampers a broader validation of the already described genes with larger databases.

In conclusion, MLPA is today considered an excellent alternative to DDH for the delineation of new species (Stackebrandt et al., 2002; Gevers et al., 2005; Ritcher et al., 2006; Martens et al., 2008; Pascual et al., 2009; Serrano et al., 2010; Martínez-Murcia et al., 2011), an opinion that we share. Furthermore, the MLPA approach has the advantage that isolates are simultaneously genotyped by sequencing because if five genes show identical sequences, it is likely these isolates belong to the same strain. DNA typing methods would still be needed when dealing with a large number of isolates in order to select different strains (Martínez-Murcia et al., 2000; 2008; Figueras et al., 2011b).

Number of isolates required for describing new bacterial species

The CSDB encouraged description of a new species using more than one strain (Table 1). This was based on the arguments published by Christensen et al. (2001), who believed, as did others (Ursing et al., 1994; Rosselló-Móra and Amann, 2001), that as many strains as possible (minimum five) are needed because a single one may not allow diversity to be described, hampering later identifications. Christensen et al. (2001) also considered it necessary because 40% of the new taxa described between 1999 and 2000 were based on a single isolate, of which 1.5% were reclassified later. According to those authors the most obvious reason for these reclassifications was the strong weight given to the 16S rRNA gene sequences. However, in our view these authors did not consider the possible impact of DNA–DNA results, as has been shown to affect *Aeromonas*. Since the publication of Christensen et al. (2001), the number of descriptions with 1 or <5 strains has risen exponentially (Felis and Dellagio, 2007) because, in practice, often only one or only a few strains are found. Recently we demonstrated that a single strain was as representative of the new species *Arcobacter trophiarum* as the ten isolates of the description (Figueras, 2010, and unpublished results). In the genus *Aeromonas*, three species (11.5%) have been defined from one strain and seven species (26.9%) from ≤5 strains. None of these species have required a reclassification apart from *Aeromonas culicicola* because of the reasons mentioned before and is not linked at all with the number of strains. In *Arcobacter*, two species (16.7%) were defined from only one strain and six species (50%) from <10 strains. According to the minimal standards for *Arcobacter* and other genera of the family *Campylobacteraceae*, preferably not fewer than 10 strains should be included in the description of a new species (Ursing et al., 1994). However, species like *Arcobacter thereius* and *Arcobacter mytili* that have been described with fewer than 10 strains were well recognized later on in our laboratory (Collado et al., 2009, and other unpublished results).

Another issue to be considered for the description of a new species is that the type strain (Table 1) must be deposited in two different culture collections (Tindall et al., 2010). This means that only the type strain is publicly available, unless the authors deposit the rest of the isolates. This limits future comparative studies of the species at the intra-species level. We consider that for the description of a new species, authors should be encouraged to deposit in culture collections several strains if available, as has also been recommended recently (Stackebrandt, 2011). When deciding which collection to deposit the strains of a new species, it should be borne in mind that some collections are more easily accessible and less expensive than others yet offer the same degree of quality. Furthermore, reference or type strains are given free of charge on interchange (one by one) at most public collections.

What are the factors that influence the recognition and discovery of new species?

Discovering strains of a new species depends mainly on luck, which we cannot control, and on the techniques that are used to identify the isolates (Figueras, 2010). We believe it is very important to describe new species as soon as possible, irrespective of the number of strains. This allows other researchers to recognize them, as long as methods used are accurate enough (i.e. molecular techniques including gene sequencing). The 16S rDNA-RFLP identification methods described for *Aeromonas* and *Arcobacter* (Borrell et al., 1997; Figueras et al., 2000; 2008) has proven to be very powerful. Their design included first a computer simulation of the digestions of the 16S rRNA gene sequences of all the type strains of the accepted species to search for enzymes able to produce differential RFLP patterns for all of them, and second an experimental verification with several strains including the types (Borrell et al., 1997; Figueras et al., 2000, 2008). After using these techniques routinely, for more than 3000 strains of *Aeromonas* and 800 of *Arcobacter*, different patterns from those previously described have been recognized and have corresponded either to new species (Collado et al., 2009a, 2009b, 2011; Figueras et al., 2011a, 2011c) or to microheterogeneities of the 16S rRNA gene of known *Aeromonas* species (Alperi et al., 2008). Once the species *Aeromonas popoffii* was described (Huys et al., 1997), we enlarged the 16S rDNA-RFLP identification method by adding this species (Figueras et al., 2000) and very soon 11 new strains had been identified (Soler et al., 2002). Further, its implication in a clinical case was recognized for the first time when sequencing the 16S rRNA gene (Hua et al., 2004). Also the implication of *Aeromonas aquariorum* (Martínez-Murcia et al., 2008) in clinical cases was discovered on the basis of the *rpoD* sequences within a year of its description (Figueras et al., 2009). This species has also been found recently in an unknown environment – chironomid egg masses from Israel (Figueras et al., 2011b). However, the 16S rDNA-RFLP method has limitations because common patterns are obtained for *Aeromonas aquariorum* and *Aeromonas caviae* (Figueras et al., 2009) or *Aeromonas salmonicida*, *Aeromonas bestiarum* and *Aeromonas piscicola* (Beaz-Hidalgo et al., 2009; 2010a). Nevertheless, in combination with the *rpoD* gene all strains of the latter species can be identified (Beaz-Hidalgo et al., 2010a; Figueras et al., 2009, 2011b). The direct sequencing of the *gyrB* gene is routinely used [by one of the authors

at the Molecular Diagnostics Centre (MDC) laboratory] and enabled the recognition of rare species like *Aeromonas simiae* and *Aeromonas allosaccharophila* (Saavedra et al., 2007; Fontes et al., 2010). In some studies, once the genetic identity of the strains was confirmed, biochemical tests were re-evaluated and key differential tests were discerned (Soler et al., 2002; Martínez-Murcia et al., 2005; Figueras et al., 2009; Beaz-Hidalgo et al., 2010a).

In *Arcobacter*, the identification strategy included in parallel both the RFLP and an m-PCR method developed by Houf et al. (2000) for the simultaneous detection and identification of *Arcobacter butzleri*, *Arcobacter cryaerophilus* and *Arcobacter skirrowii*. This approach revealed that the m-PCR produces misidentifications (Figueras et al., 2008; Collado and Figueras, 2011). Incongruent results found between the methods have led us to identify strains of *Arcobacter thereius*, *Arcobacter nitrofigilis* (the type species of the genus) and *Arcobacter trophiarum* for the first time since their description and to recognize three new species, *Arcobacter mytili*, *Arcobacter defluvii* and *Arcobacter molluscorum* (Collado et al., 2009a, 2009b, 2011; Collado and Figueras, 2011; Figueras et al., 2011c). All these studies have in common the use of more accurate molecular techniques. Furthermore, by applying them, knowledge has increased about the diversity and geographic distribution of rare and/or recently described species.

What is a validly published new species name?

We consider it important to introduce the concept of a "validly published new species name" as this is not always fully known by non-taxonomists.

According to Rules 25a and 27 of the *Bacteriological Code* (1990 Revision) the name of a new taxon is not valid until it has been validly published, either by publication in *International Journal of Systematic Bacteriology* (IJSB), since renamed *International Journal of Systematic and Evolutionary Microbiology* (IJSEM), or by appearing in a Validation List in IJSB/IJSEM, if the "effective" publication was in a publication other than IJSB/IJSEM. Effective publications must be in the form of printed material made generally available to the scientific community. For validation to occur, the published paper is sent to the IJSEM's List Editor who checks (and corrects if necessary) the name and the compliance with the Bacteriological Code; the new name is then published in a Validation List ("List of new names and new combinations previously effectively, but not validly, published"), the date of publication of that issue of IJSEM being the official date of valid publication (Euzéby, 2005).

Currently, the major shortcoming of this process is the lengthy period between a new species being accepted and "in press"and being published in the IJSEM, which delays the availability of the new names for science. Rule 25b, adopted in 1992, also states that no other kind of publication is accepted as effective (effective publication). However, there are good reasons why, in the modern Internet era of electronic publication these rules should be changed, and indeed at the Instanbul International Committee on Systematics of Prokaryotes (ICSP) meeting in 2008 the rules were amended to enable electronic effective publication, but this amendment will not come into effect until the minutes from the meeting are published in IJSEM. In addtion, if a new species is "in press" online at IJSEM, we believe this should comprise an effective published description of the taxon. In this way, the new name could simultaneously be extracted and appear on an online "notification list of new names".

Once a name has been validly published, even if it is not correct, it remains validly published in perpetuity. This enables traceability, but many old, currently invalid names still remain on 16S rRNA gene sequences at public databases (GenBank, etc.). This is one of the main reasons why non-specialists still use old names in the current literature. This practice should be avoided by verifying status in the current taxonomy each time a name is used (http://www.bacterio.cict.fr/).

In the case of *Aeromonas*, old names still being used associated with 16S rRNA gene sequences at GenBank are *Aeromonas enteropelogenes* (Schubert et al., 1990b) instead of *Aeromonas trota* (Collins et al., 1993; Huys et al., 2002), *Aeromonas punctata* (Zimmermann, 1890) and *Aeromonas hydrophila* subsp. *anaerogenes* (Schubert, 1964) instead of *Aeromonas caviae* (Schubert and Hegazi, 1988; Martínez-Murcia et al., 1992; Huys et al., 2002) and *Aeromonas culicicola* (Pidiyar et al., 2002) or *Aeromonas ichthiosmia* (Schubert et al., 1990a) instead of *Aeromonas veronii* (Collins et al., 1993; Huys et al., 2001). These names correspond to species that have been synonymized or are pending Judicial Commission Opinions for priority of the name and are never used by specialists of this genus (Martin-Carnahan and Joseph, 2005; Janda and Abbott, 2010).

Interpretation of results and expertise

The importance of double-checking the data and correctly interpreting the results

As indicated, the MLPA is the best tool for defining new species, while the use of single genes is useful for identification, as the 16S rRNA gene sequences may be for certain genera like *Arcobacter*, etc. However, wrong interpretations may still occur. Sequences are objective in nature (but are subject

to bias by sequence errors), but the sequence-derived interpretation for establishing a species designation is not, and scientists should correctly interpret the information derived from the similarity and phylogeny. They must sequence appropriate genes of the right size, review the quality of their sequences thoroughly using sequences without errors and ambiguities, evaluate carefully the chromatograms and alignments, and translate the nucleotide sequences into the amino acids to ascertain possible sequencing mistakes (Tindall et al., 2010). Nowadays, clinical laboratories often use sequences of the 16S rRNA gene (MicroSeq500 system among others) for the identification of groups of medically important bacteria (Janda and Abbott, 2007). Therefore, they should be aware of the limitations of this gene and move toward the use of housekeeping genes, as highlighted by Janda and Abbott (2007, 2010). The sequencing capability has been so popularized that everyone can obtain a sequence without the need to question its quality. In fact, Stackebrandt and Ebers (2006) criticized careless handling of 16S rRNA gene sequences deposited in public databases that appear to be direct downloads from computer printouts, and lack rigorous inspection of quality and secondary-structure. Many researchers lack an understanding of the different algorithms from which phylogenetic trees are derived and take this information as truth, while in reality they are only theoretical hypotheses pending confirmation.

Researchers often place too much importance on the location of a new species in relation to their closest neighbors by the 16S rRNA gene and are surprised when another gene places the strain in another position. This is perfectly normal owing to their different modes of evolution (Richter et al., 2006; Martens et al., 2008; Christen, 2008; Pascual et al., 2009; Serrano et al., 2010). Presently, only the MLPA trees with the concatenated sequences can be taken as the best theoretical approximation if enough representative strains of each species are tested. Bootstrap values "inform" about the robustness or weakness (values above or below 70%) of strains clustering for each species. This is particularly important as they "inform" about the stability of clustering when new strains are added to the tree. Is the chosen outgroup correct? (Tindall et al., 2010). Does the tree change with and without an outgroup or using other sequences as the outgroup? Does it make sense to include the 16S rRNA gene in the MLPA? Many papers concatenate the 16S rRNA gene with the other housekeeping genes (Beaz-Hidalgo et al., 2009; Pascual et al., 2009; Serrano et al., 2010). This conflicts with the fact that housekeeping genes used in such analysis should in principle be single-copy genes (Zeigler, 2003) and not a multigene family, as the 16S rRNA gene is. Furthermore, the 16S rRNA gene has a completely different mode of evolution from protein-encoding genes (Rosselló-Móra and Amann et al., 2001; Gevers et al., 2005; Tindall et al., 2010). While rRNA is constrained by secondary functional structures, the protein-enoding genes are subjected to a degenerate code. Since the 16S rRNA gene is so highly conserved compared to protein-encoding genes, it contributes very few informative positions in the concatenated tree.

When defining new species, attention has to be paid to the quality of the phenotypic results. How many times has the specific test been repeated? Only once? With or without a positive and a negative control? Using the type strains of other species? Faced with discordant results, how many times were the results re-tested? Were the complex phenotypic tables double-checked for typographical errors? In our experience these are common mistakes that should be double-checked, not using the first typed table, but rather going back to the laboratory notebook. Were the DDH experiments performed in duplicate or triplicate for the direct and reciprocal reactions? Were there any discordant data? Were the discordant results disregarded or repeated? Were certain data eliminated in the calculation of the mean to avoid a high standard deviation? A pair of strains with known DDH values could be included each time experiments are performed as a quality control.

Which is more important, the established boundaries or the scientific expertise?

Expertise in a specific bacterial genus provides more powerful and reliable information for defining the typical species boundaries, than the fixed theoretical boundaries established for delimiting all prokaryotic species. Expert groups have been set up by the ICSP in the form of subcommittees (http://www.the-icsp.org/), mostly grouped by families, which will update knowledge (the minutes of the meetings of the subcommittees can be consulted in the IJSEM) and propose guidelines for defining species. However, specific guidelines for genera seem more appropriate than for families as they could fix more precisely the criteria that need to be applied. For instance, should a species be delineated at the 70% or at the 80% DNA–DNA similarity level? What is the usefulness of the 16S rRNA gene? If MLPA has already proven to be a superior alternative technique to DDH, why should DDH be used?

Specific guidelines on minimal standards should be reviewed and, if necessary, updated regularly (every 5–10 years). These criteria should be made available to reviewers by the editors of the journals in order to guarantee a uniform application in future publications. Some groups of bacteria, such as *Aeromonas*, lack specific guidelines for species descriptions as discussed elsewhere (Janda and Abbott, 2010) and those that exist for *Arcobacter* embrace all the family

Campylobacteraceae and have not been updated since their publication (Ursing et al., 1994). The latter may, in addition, be too general, considering the peculiarities of each genus within a family.

The species definition for prokaryotes in the 21st century

As indicated by Rosselló-Móra and Amann (2001), there is no approved definition for prokaryotes species. That is why we took inspiration and modified some of the existing definitions given by those authors to propose a new one based on our experience "*a species is a category that circumscribes genomically a single strain* or *a group of strains (not clonally identical*), *sharing a high degree of similarity (considering the specific boundaries recognized within the specific genus both for the 16S rRNA gene* and *for five*, or *more if necessary*, *housekeeping genes in an MLPA that have proven useful*) and *showing distinctive phenotypic characteristics (comparatively tested under highly standardized conditions*) *to differentiate it from other validly named species of the same genus*". In our view, this definition or a similar one, articulates what we and many other publications consider reflects a new bacterial species for the 21st century.

Perspectives and conclusions

The description of any new species should in our view match our stated definition above. However, the MLPA should include (if available) at least 4–5 *bona fide* strains of each species together with the type strain. The number of strains needed to define a new species cannot be pre-established. Therefore, a single isolate is as valid as a group of isolates because identification by sequencing will later provide more isolates and increase the intra-species diversity. The sequence of the 16S rRNA gene (no less than 1300 nt. and with a clear annotation of microheterogeneities, if present, and no ambiguities "N") should still be mandatory for genus confirmation, although its value at the species level may be limited. We envisage that in the next 20 or 30 years pyrosequencing will enable information to be obtained from a higher proportion of the genome than MLPA, using techniques like the ANI, MUM, etc., among others (Richter and Rosselló-Móra, 2009), or even from complete genomes. The latter cannot be used at present because genomes are not available from all species within a given genus, although several projects are ongoing. Furthermore, key criteria for comparison will have to be defined.

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What is a bacterial species? I will know it when I see it

Micah I. Krichevsky

An objective definition of bacterial species does not exist. Starting from operational descriptions of the concepts of isolate, strain, and species, the process of building a species description depends on three subjective decisions: (1) the spectrum of individual isolates to be used to construct the species description, (2) the methods used to characterize the isolates, and (3) the computational (or subjective) method(s) used to build the species description. The discussion that follows describes the subjective aspects of the three decision components. The resulting species description has a component of uncertainty. By analogy with quantum mechanics, the methods used to describe and define a species affect the perceived position of the species in the biological universe. Biologists have evolved *ad hoc* strategies to cope with this uncertainty. Foremost among the strategies is the allowance of competing taxonomies in the rules of nomenclature (except in virology). No judicial mechanisms exist for deciding on the scientific merits of the use of a taxon name other than the opinion of the user. The user must choose a taxonomic framework to organize thought and communicate. As a practical matter, communication does not require the use of species designations. Communication of specific attributes of isolates or strains are necessary and sufficient. In other cases, the communication is at a higher taxonomic level.

Introduction

The following scheme illustrates the iterative nature of establishing a collection of biological objects from nature (or from other collections) and classifying the organisms thus obtained. The scheme is largely method independent and applies to all organisms.

Establishing a strain collection for accession and study

- Sample econiche(s) to classify predominant phenetic and/or genetic biotypes
- Accumulate representatives of each major biotype
- Assign biotypes to appropriate putative taxa
- Establish minimum phenetic and/or genetic span for inclusion into each biotype, e.g. Gram-, obligate aerobe or Gram-, facultative aerobe

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Data gathering on isolates in each category

- Collect data on physiology, biochemistry, morphology, serology, DNA homology and/or macromolecular sequences, etc. of each isolate in each major biotype
- Data management and quality control
- Store and search data (simple bookkeeping)
- Evaluate tests
- Compare results of repeats as controls
- Feed back results to investigator
- Generate reports

Analysis

- Compare by direct matching with “authentic” strains
- Evaluate tests for applicability and discriminatory power
- Do statistical analysis
- Determine feature frequencies
- Choose taxonomy (by informational content for intended use)

Classify isolates

- Establishment of identification methods
- Construct keys
- Construct probabilistic matrix

As the collection grows, the above process is repeated and the taxonomy is updated. With each update cycle, the boundaries will shift with the inclusion of new isolate descriptions. Why not use a "standard" taxonomy? As stated above, there is no "official" scientific definition of the boundaries of each taxon. Subjective decisions abound throughout the planning and execution of the process.

The common process of delineating a new species of bacteria is to start with an isolate in pure culture; then, if possible, find additional identical isolates to constitute a strain; and find a number of similar isolates to form a species from the descriptions of each of the isolates. The working concepts of these categories, for the purposes of this discussion, are described as:

Isolate

- Observations of a clone
- A single assemblage of data (a record)
  - ◊ Phenotype
  - ◊ Serotype
  - ◊ Genotype
  - ◊ Virulence
  - ◊ Host range
- One "true" value/observation
- Incidence = isolate can only occur once in time and place
- Source of isolation usually quite important
  - ◊ Utility increases with specificity of source description

Strain

- Series of isolates or clonal subcultures that are "identical"
- Isolate records compared to establish identity
- Any single isolate the definitive record
- Incidence of strain - two step process

1. Identity = exact match to the definition (limited by methods)
2. Time and place of isolation

- Only the incidence data are further analyzable

Species

1. Record computed from multiple strains

- Strains different, but not too different
- Operational species definition - 2 components
  - ◊ Idealized strain description
  - ◊ Hypothetical median organism (changes with added strains)

2. Decision boundary of allowable variation

- Codified (computed statistic)
- Personally operational
  - ◊ Experience
  - ◊ Key

The description of an isolate is the result of specific objective observations, including those of time, place and conditions of isolation. Since the above definition of a strain requires identity of the recorded results of the isolates, it too is objective. However, this definition implies that the original source clone is known and that all measurable characteristics remained constant. The assemblage of strains included in the putative species depends on three subjective decisions. These are: (1) the spectrum of individual isolates to be used to construct the species description, (2) the methods used to characterize the isolates, and (3) the computational (or subjective) method(s) used to build the species description.

The English philosopher John Locke (1632–1704) argued that nature does not make species. People do, as a mechanism to facilitate communication of a collection of similar ideas under one general term. In addition, Locke stated that the boundaries of species are opinions rather than natural borders. This lack of natural borders precludes an objective definition of species.

Consider the definition of species by Cowan (1978):

"**Species** [abbreviated to sp. or spp. (plural)].

"1. A category, definable only in terms of position (below genus) in a hierarchical system.

"2. A taxonomic group, definable in terms of the characters of the constituent members.

"3. A concept; that it is useful cannot be denied, but the user must realize that the species does not exist and is not an entity.

"To summarize: a species is a group of organisms defined more or less subjectively by the criteria chosen by the taxonomist to show to best advantage and as far as possible put into practice his individual concept of what a species is."

Jacques Monod in *Chance and Necessity* (1971) (as translated from the French by Austryn Wainhouse, 1971) stated:

"…values and knowledge are always and necessarily associated in action…

"…the principle of objectivity as the condition of true knowledge constitutes an ethical choice and not a judgement arrived at from knowledge…

"…it is from the ethical choice of a primary value that knowledge starts."

What follows is a discussion of how choices affect the outcome of the construction of a species description such that a universal objective definition of the species concept eludes us.

The spectrum of individual isolates to be used to construct the species description

The February 2011 issue of the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM) contained descriptions of the approximately 23 new species. Most new species descriptions listed the properties of a single isolate. Eight articles listed more than one. Thus, there is no objective way to determine the variation of properties within the several species. Under the terms of the various Biological Codes of Nomenclature these are new species and must be so considered.

The accumulation of knowledge of variation within a species largely rests with subsequent finding of similar isolates. The finder of such isolates chooses if the new isolates are similar enough to be classified as belonging to the subject species. Until the variation within the species is assessed, there is no certainty that the type strain is "typical" of the species.

The finding of new isolates of a species results from either the microbiologist choosing where and when to look for new isolates (e.g. common in epidemiology) or by happenstance of finding them among the isolates encountered in the course of the multiple isolation work. In both cases, the choices of where and how to look result from subjective considerations. This is the usual circumstance wherein new isolates are discovered. One imperfect exception to this practice is the use of "selective" media.

The methods used to characterize the isolates

Two categories of methods define the species descriptions. The first is those methods that classify the isolates. The classic use of serotyping found great favor in classifying *Salmonella* strains. DNA–DNA hybridization is another example that found great favor. In the current issues of the IJSEM, classifying by the 16S rRNA gene sequence is by far the favored method to demonstrate the uniqueness of putative species.

The recent application of whole genome sequencing to describing an isolate results in the building of taxonomies which are isolate specific. As the data accumulate, yet another picture of microbial taxonomy will emerge.

The second category of defining method is the listing of phenetic expression of properties of the isolates and their accumulation into the overall description of the species. Here the choices exerted by the describers vary markedly overall. For example, which of the multitude of carbohydrates (if any) are chosen for utilization tests? In practice, there is great variation in the list of carbohydrates tested. In the published species descriptions, the phenotypic information is given. However, the justification for specific uniqueness commonly rests on the sequence analysis.

The computational (or subjective) method(s) used to build the species description

A species designation results from interpretation of a set of factual observations. The designation is a linguistic fact. The species is not an objective biological fact, *per se*. Yet, it is often treated as a factual entity. Diversity studies are often couched in terms of species diversity. *E. coli* is a convenient and useful metaphor for a group of organisms ranging from benign to deadly. Under the Code of Nomenclature, the same biological entity may have multiple names associated with it as long as those names are contained in the Approved List (if prokaryotes) or are validly published under the applicable Code.

Until computers became readily available to taxonomists, the method of choice in building species descriptions and the relationships among them was to build logical hierarchi-

cal keys. Much discussion (not always friendly) ensued at meetings as to whose key was best, i.e. "truth". Even now, the biological community recognizes and codifies the idea that multiple interpretations of a set of biological facts are possible and are common. The Codes of Nomenclature of all non-viral life forms allow for competing taxonomies. In his search for a coherent model for species, Charles Darwin in his letters to Joseph Hooker, as edited by F. Darwin, complained: "After describing a set of forms as distinct species, tearing up my M.S., and making them separate, and then making them one again. I have gnashed my teeth, cursed species, and asked what sin I had committed to be so punished."

DeSantis et al. (2006) analyzed the level of correspondence of taxonomies of archaea and bacteria at the phylum level. They report incongruent taxonomic nomenclature among the five curators' databases even at the phylum level (DeSantis et al.). The article contains an elegant five-way Venn diagram of phylum-level nomenclature shared by the independent curators. The diagram symbolizes the number of individual names recognized by the multiple curators. The counts for phylum or candidate division names recognized by the individual candidates are Pace 88, Hugenholz 88 (not all the same ones as Pace), Ludwig 50, NCBI 68, and RDP 31.

The issue of competing taxonomies pervades biology since the analytic methods (whether computational or judgmental) allow different interpretations of the underlying data. The decision process of choosing among competing taxonomies is inherently complex. Studies of bumble bee taxonomy serve to illustrate the issue. Cameron et al. (2007) described a phylogeny of bumble bees. Bell (2007) describes the use of the University of Illinois supercomputer facility by the group of investigators in developing a preferred tree for bumble bee evolution. "In Cameron's studies, millions of possible trees with different branching patterns are constructed during the computer analyses of species relationships, representing different possible ways all the species in a study could be related. Out of these millions of possible trees, the one thought to represent the most accurate tree based on a variety of criteria is chosen." Thus, their choice of the final tree is a subjective interpretation of the data by their opinion as to the appropriate criteria. No matter how well thought out, the *a priori* criteria have a necessarily subjective component. Williams et al. (2008) followed this with a new subgeneric classification. They describe the dilemma of choosing among alternative treatments as follows. "The precise choice of level within a phylogenetic tree for labeling a supraspecific taxon with a particular nomenclatural rank is essentially arbitrary, and there is a large literature discussing this problem…."

In the aforementioned issue of IJSEM, a number of the articles used the same computational methods to demonstrate the validity of their taxonomic calculations of the uniqueness of the isolate(s). The resulting dendrograms demonstrate the required separation to attain species designation. The most common method of calculation was the neighbor-joining tree. To give credence to the calculations some used two methods of calculation of the cladograms. The second was by a maximum-parsimony algorithm. When the joining points of the two agreed, the authors used the agreements as indicating the validity of their diagrams. This is entirely rational and provides a useful gestalt of the group of organisms under study. However, there are a number of linkages that are not denoted as agreed by the two methods. Thus, the disagreements imply evidence for two competing taxonomies (differing at least in detail) from the same data.

The recent development of practical whole genome work has marvelous potential for increasing knowledge of strains but does little to objectively define a species. In all methodological cases, the issue boils down to the insoluble problem of objectively defining a universal boundary between taxa no matter the methodology. As more and more data are collected, the boundaries will inevitably blur.

In constructing these diagrammatic relationships, the cohort of strains included in the study includes the new strain(s) as well as individual strains representative of other taxa deemed by the investigator to be in the same general biological space as the "unique" strain(s). Thus, the other strains in the cohort usually are single representatives (often the type strains) of the various other species. The position of each representative chosen commonly has an uncertain position within the species. No attempt is made to ascertain the boundaries among the species. In fact, to attempt to do so likely would exceed the practical resources of even the most dedicated of investigators.

Basing the definition of uniqueness of sequences as definition of species presents a judgmental issue of boundaries. How many base pairs difference are enough to define a new strain? A new species? In the above working definition of "strain", a difference of even one attribute defines a new strain. However, this stringency is impractical when considering the error rate in determining the sequence. This conceptual conflict applies to all sequence work. Thus, the question arises as to how many base pair differences should designate that two isolates are members of different strains? Is the differential number 1, 2, 5, 20? Is a universal number attainable? Even more difficult is the same question with

regards to species.

The two books by Isaacson (2007) and Kumar (2010) describe in detail the disagreements between Einstein on the one hand and the Copenhagen group, i.e. Bohr, Heisenberg et al., on the other, on the possibility of making exact determinations of the location in space of an electron as it orbits the nucleus (grossly oversimplifying the concepts here.) There is a parallel between physics and taxonomy.

Einstein was a determinist believing that the positions could be determined absolutely. Heisenberg is famous for the uncertainty principle wherein the exact position can only be described statistically. The analogy with taxonomy is that the search for a determinist solution to the definition of a taxon is not achievable. The taxon must be described in statistical terms wherein the boundaries and position in the biological universe can only be described by arbitrarily chosen methods of interpreting selected observational data. Further, as with quantum mechanics, the act of observing affects the answer.

Sneath and Sokal (1973) attributed to Sokal and Rolf the following postulate of an uncertainty principal for taxonomy: "It is impossible to know the natural (phenetic) classifications of a group of organisms above a certain level of precision, since conventional or numerical classifications by independent taxonomists would always disagree to a certain extent."

The dividing line between data and their interpretation is critical to the process of building taxonomies and the converse of identification. All taxonomies and identifications are subjective interpretations of observations. Thus, there cannot be an objectively correct taxonomy or identification. Hans Yu of Health Canada (personal communication) states: "There can be a consensus which lends credence to the identification." This statement applies to taxonomies as well.

Coping with uncertainty

Microbiologists have evolved *ad hoc* strategies to cope with such issues as: (a) the type strain has an uncertain position within the cluster or clade defined as comprising a species. (b) The boundaries of any cluster or clade are uncertain. (c) The closer we get to species, the less certain the result.

Wallowing in the mud of uncertainty prevents placing the strains under consideration in any organized framework. Some classification scheme must be chosen as an indexing system to allow coherent thought. Species names are useful pointers to information. They help the user place a biological entity into the context of the overall biota just as the Dewey Decimal System helps place a book in a library. However, biologists, for good reasons, allow competing taxonomies and have not agreed on even an operational definition of a taxon (especially at the species level) let alone a deterministic or absolute definition. All biologists, and especially microbiologists, must continue to develop and use operational criteria as working definitions. The emphasis for microbiologists stems from the variety of mechanisms for genetic change and exchange.

The first consideration for "prokaryotologists" is to consult the "List of Prokaryotic Names with Standing in Nomenclature" (http://www.bacterio.cict.fr/). The names on the list are approved as to the validity of the publication of the list. Any name on the list may be used. The last published name has no more standing than a previously published name. Either can be used in publication. The IJSEM gives (or points to) the description of the organism as originally described. Thus, the selection of a name from the list is subjective. This is a problem which awaits future solution in the rules of nomenclature.

Choosing a taxonomic framework will place valid names of interest in a biological context. For example, such compendia of taxonomy as the various editions of *Bergey's Manual* (http://www.bergeys.org/) and/or *The Prokaryotes* (published by Springer in both print and online versions) describe the attributes of the various species. The publication intervals among these various sources necessarily lag behind the publication of the ever expanding list of newly named species. In spite of this lag, both works provide useful taxonomic frameworks. Both rely on expert knowledge and ability of the contributing experts to synthesize the relationships among the varied groups of prokaryotes. Many textbooks and manuals describe specific groups of microorganisms such as those related to human and/or animal disease, habitat (soil,water, etc.) and commerce (e.g. dairying, antibiotics, enzymes).

Commercial identification systems update their taxonomic databases more frequently. They may emphasize specific kinds of organisms over others such as having a clinical bias. Generally, these systems actually do not decide if the submitted organism is identified. They rely on providing a statistically derived score denoting the similarity with the closest candidates in their database. The algorithms used by the vendors vary. Therefore, the numerical values are not comparable among different systems. For example, DNA hybridization thresholds and ranking often are not congruent with those of 16S rRNA gene sequence data.

Macromolecular sequence databases are useful if used with

caution. The strain of interest is matched against the library of sequences in the bank. Most of the banks return a list of sequences ranked by a measure of similarity. The note of caution stems from the annotation of the sequences. The annotation is not curated by taxonomic experts. Throughout the databases, scientific names are lacking or are erroneous. An exception to the lack of curation is the Ribosomal Data Project (http://rdp.cme.msu.edu/) which provides both a taxonomic structure to the sequences as well as tools for analyzing sequences. The sequence banks are not identification tools *per se*, but serve as useful pointer systems when the information is combined with confirming information. Taxonomies can be, and are, built by application of sophisticated algorithms. As previously mentioned, differing algorithms will differ in the outcomes to varying degrees.

Dongying Wu et al. (2009) published "A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*". The article presents an elegant maximum-likelihood phylogenetic tree of the bacterial domain based on a concatenated alignment of 31 broadly conserved protein-coding genes. The tree is useful as a taxonomic framework. One wonders what differences would be found by using a different algorithm for computing the tree such as neighbor-joining? What would result if a somewhat different set of protein-coding genes were selected? These questions should not be construed as criticism of the authors' selections. They only indicate that their opinions matter to the final outcome.

Opinionated epilogue

This message was sent by this author to some practicing microbiologists with varying professional perspectives on use of species names. "I had occasion to look at numerous hospital lab records. Often, they contain notations such as "atypical *E. coli*" for the identification but had no information as to what was "atypical" of the isolate. The information as to what made the isolate atypical was discarded. What was kept in the record was the antibiogram. In both cases, the level of identification is driven by the end use of the information. Can any of the group suggest other areas of isolate characterization that are similarly driven and stop before a "complete" identification?" The following answers came back as personal communications.

Sue Whitehead, Interior Health Authority, Canada, (personal communication) stated "…that "identification" in a clinical Microbiology laboratory may be different - for a human pathogen versus an unlikely human pathogen. In the latter case an identification of P. fluorescens group is just as good as a more exact ID in the majority of patient samples - not a pathogen, not important to have a further ID unless sterile site, immunocompromised patient etc. We spend our energy/time/resources on identifying pathogens and performing susceptibility testing on those so for non-human pathogens we just rule out pathogenic status and stop."

Jorge Lalucat, Universitat de les Illes Balears, Spain, pointed out: "In many publications on the physiology or enzymatic activities of specialized bacteria, only the genus is indicated and no further taxonomic investigations are mentioned. Also in many papers on microbial ecology."

Many disciplines involve assessing risk and hazard of the use of micro-organisms, e.g. product developers, safety committees, regulators, clinical laboratories, etc. The assessors generally aim for a species-level name. Obtaining "identification" down to a group of related species also allows development of more detailed analyses through consideration of surrogate relatives. This is the functional equivalent of the above clinical scenario, except that they may be looking at ecological characteristics rather than, or in addition to, clinical ones to judge the likely presence or absence of a hazard.

The practical aspect common to all of these examples is the use of taxonomies and other tools to locate the isolate in their biologic neighborhood. Then, the need is for strain specific attributes.

The microbiologist has many framework tools available. It is the microbiologist's responsibility to select among them for adaption and adoption to the problem at hand. Probability, with its attendant uncertainty, requires professional judgment to select working boundaries for species. Only usage by the community of microbiologists defines species.

As new organisms are found, evolve, and exchange genetic information, the group classifications change. Hence, the concomitant uncertainty will prevent the development of an overarching, static, objective definition of species.

"To summarize: a species is a group of organisms defined more or less subjectively by the criteria chosen by the taxonomist to show to best advantage and as far as possible put into practice his individual concept of what a species is." (Cowan. *ibid.*)

Stated less elegantly, "I will know it when I see it".

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The Bulletin of BISMiS (2011), Volume 2, part 1, pp. 25–32

Report of the Inaugural Meeting of Bergey's International Society for Microbial Systematics in Beijing, China, 19—23 May 2011

The meeting was held at the Friendship Hotel in Beijing. The Opening Ceremony began in Ju Ying Hall of the Friendship Palace of the hotel in the afternoon and evening of May 19. Several of the brief welcoming talks and the closing talk are provided verbatim to provide a written record of this historical meeting.

Opening Ceremony

Lixin Zhang

Chair, Organizing Committee and Secretary-in-General, BISMiS 2011

Dear Distinguished guests, Ladies and Gentlemen,

Here we are, and welcome to Beijing! After 2 years of preparation, on behalf of the local organization committee, I would like to extend the warmest welcome to all distinguished guests and participants of the Inaugural conference of Bergey's International Society for Microbial Systematics (BISMiS 2011). Louis Pasteur once stated the importance of microbes: "the role of the infinitely small is infinitely large". Indeed, the invisible microbes brought together a huge diversity of over 370 participants from 27 counties who have gathered to exchange ideas, establish or further develop research networks, promote global friendships and to explore the importance of microbial systematics.

The conference is entitled "Microbial Systematics: Concepts, Practices and Recent Advances" and I believe it will revolutionize microbial systematics across its various divisions. In the next 3 days, 53 speakers and 150 poster presenters will showcase their best scientific work in four symposia and the Young Scientist Forum.

Once again, I wish to express our sincere gratitude to all the participants for their time, effort, and preparation for this meeting, the supporters, sponsors and co-organizers including Bergey's Manual Trust, the Chinese Society for Microbiology and the Chinese Academy of Sciences, Institute of Microbiology for all their efforts in ensuring this meeting is a success.

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Michael Goodfellow

Chair, Bergey's Manual Trust

Member, Organizing Committee, BISMiS 2011

Distinguished guests, colleagues, ladies and gentlemen I would like to start by thanking Lixin Zhang, Liz Ashforth and other members of the Local Organizing Committee for all of the time consuming and selfless work they have undertaken on behalf of Bergey's Manual Trust in organizing this the inaugural meeting of Bergey's International Society for Microbial Systematics (BISMiS). The Trust is also grateful to the organizations who have sponsored the meeting. BISMiS has been launched by members of the Trust but is, in fact, the brainchild of Professor Jim Staley, who preceded me as Chairman of the Trust, a non-profit organization with the mission to promote prokaryotic systematics.

Microbial systematics is a fundamental scientific discipline which is being sidelined in many parts of the world, not least in Europe and the USA. This worrying situation also reflects the decline in Microbiology as a distinct scientific discipline in many institutes of higher education. It is difficult to ac-

count for this state of play at a time when Microbiology is pivotal to meeting some of the major challenges facing humankind, as exemplified by the need for fine chemicals, new catalyzts and novel natural products, notably antibiotics.

It is, however, most encouraging that microbial systematics in particular and microbiology in general are thriving here in China, a country which has a long and distinguished record in the development and practice of scientific concepts. Indeed, the flowering of microbial systematics in this part of the world was one of the main reasons why the members of Bergey's Manual Trust were enthusiastic about having the inaugural meeting of BISMiS in China. We were particularly happy to have the meeting here in Beijing partly given its central location but also because this great city is the home of many fine institutes where microbial systematics features on the agenda. These scientific establishments include the Institute of Microbiology of the Chinese Academy of Sciences, one of the renowned centers for microbial systematics in the world.

It is clear from the scientific programme that there is a lot of interest in microbial systematics both as a pure and applied discipline. Indeed, the future of the subject looks bright essentially due to the remarkable advances being made in biosystematics and genomics. This will become increasingly clear given the microbiological feast that awaits us over the next few days. We can also look forward to existing research collaborations being deepened and extended and new scientific networks being set up by the young people who will be driving microbial systematics forward in years to come. Finally, we need to remember that we are not driven by a wish to underpin our scientific beliefs but by a wish to find out, which is the exact opposite.

James T. Staley

President, Bergey's International Society for Microbial Systematics

Welcome, all of you, to the first BISMiS meeting!

Thank you, Lixin Zhang, Michael Goodfellow and all others of the Local Organizing Committee who have put so much time and effort into organizing this inaugural meeting of BISMiS. I also wish to thank each of you who are participating in the meeting.

I want to briefly tell you about how BISMiS originated and what its mission and goals are.

Since the discovery of the Tree of Life about 20 years ago, microbiologists have begun to learn about the unanticipated

James Staley.

and vast diversity of microbial life on Earth. This enormous diversity is one of the greatest unsolved mysteries of Science. Its study will lead not only to the discovery of novel life forms, but to the advancement of other fields such as biotechnology and medicine.

In 2008, Bergey's Manual Trust voted unanimously to form a new international society to address the scientific needs in this area. BISMiS officially began in 2010. In addition to this overarching goal, BISMiS has other aims:

1. To promote excellence in research and education in microbial systematics

2. To provide a global forum for microbial systematists, such as this meeting here in Beijing

3. To publish scientific literarure such as *The Bulletin of BISMiS*, the first issue of which was published in 2010

I invite each of you to attend the BISMiS Members' meeting which will be held on Sunday, 22 May. You do not have to be a member to attend. At the meeting we will present and discuss some of the most popular ideas that BISMiS members submitted as possible areas to pursue by the Society.

In addition, we will announce the results of the election for new officers. BISMiS began by appointing officers from Bergey's Manual Trust, but they will be replaced by members of BISMiS through election.

We encourage those of you who have not yet joined to become members. Application forms are available in the lobby at the registration desk.

I like to think of BISMiS as a research vessel or ship whose purpose is to explore the uncharted diversity of microbial life in the biosphere, even at the very limits at which life itself exists.

As we launch the good ship BISMiS on its voyage of discovery, it will be up to the members and officers to set their course of exploration, so that it will lead to a fuller and better understanding of life as well as to the enlightenment of Mankind.

Thank you Beijing for launching the good ship BISMiS!

And, *Bon Voyage*, BISMiS.

Keynote Speech and Welcome Reception

Nobel Laureate Barry Sharpless delivered the keynote speech entitled "Click chemistry evolving – destinations unknown".

Barry Sharpless.

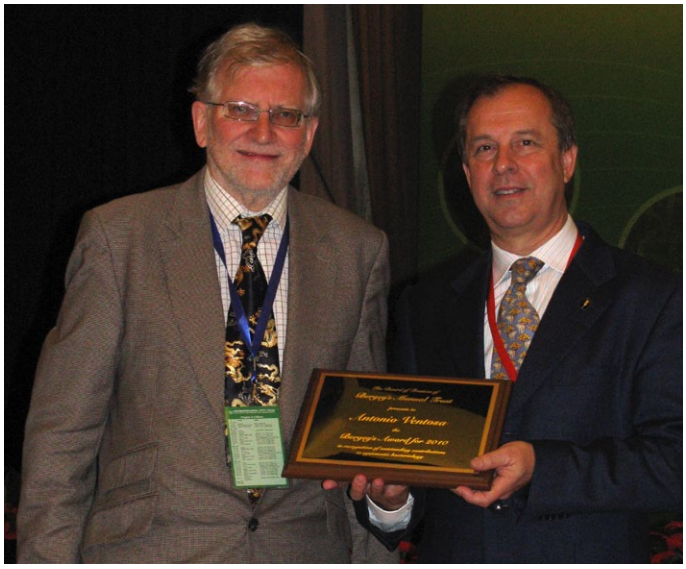
The Welcome Reception included a marvelous banquet with Cultural Evening entertainment. The entertainment began with Folk Music, a Peking Opera (*The Drunken Concubine*), Rolling Light acrobatics, the Peking Opera, *Monkey King*, Chinese yo-yo, and concluded with the Sichuan Opera, *Face Changing*.

Scientific Sessions

All of the talks and discussions were held in Ju Ying Hall, 20–22 May. The opening session began with Antonio Ventosa delivering the Bergey Award lecture for his lifelong work on halophilic *Bacteria* and *Archaea*. Following his presentation he received the Bergey Award from Michael Goodfellow. The next issue of the *The Bulletin of BISMiS* will have a special section that includes some review articles by those who presented talks at the meeting.

BISMiS Members Meeting

The BISMiS members meeting was held at 4:50 pm on 22 May 2011 in the main auditorium, Ju Ying Hall, at the Friendship Hotel in Beijing. The meeting was an open meeting to all who attended the conference as well as BISMiS members. The attendance at the members meeting was estimated at 300, including approximately 40 who were BISMiS members. The meeting was called to order with the President's gavel at 16:55 by James T. Staley.



Antonio Ventosa (right) receiving his Bergey Award from Michael Goodfellow.

The President's gavel.

Agenda and Minutes

1.0 Introductions and origin of BISMiS

The President thanked Lixin Zhang, Michael Goodfellow and other organizers of the inaugural meeting of BISMiS for planning the exciting program and making all the other arrangements that have made this meeting so successful. The President introduced the officers of BISMiS and provided some background to the audience on the origin and goals of BISMiS. BISMiS originated in 2010 based on an idea presented to Bergey's Manual Trust (BMT) in 2007 at the Trust meeting in Athens, Georgia. The idea was discussed at the International Union of Microbiological Sciences in Istanbul in 2008 where a survey was distributed to elicit the interest of other microbiologists. The survey was also conducted online at the Trust's website, http://www.bergeys.org/ in 2008. Based on the results of the survey, the Trust decided to move

forward with organizing BISMiS in 2009 with a start date of 2010. To expedite the formation of BISMiS, the initial officers were appointed from the officers of BMT with the aim of having elections as soon as possible.

2.0 Initial year's activities of BISMiS

The President reported on the initial year's activities of BISMiS.

2.1 Membership drive. The initial year's activities of BISMiS began with a membership drive. Ads were published about the society in various microbiology-related publications, such as ASM's *Microbe*. Also, the BMT website advertised the formation of the society and provided on-line application forms for members.

2.2 Adopting a Constitution. A draft of the Constitution was prepared by the President, President-Elect, Secretary and Treasurer. It was circulated to BMT for approval. After the Trust approved it, it was sent to all current members of BISMiS for input. A number of members made suggestions to improve the draft. Virtually all of the recommendations were adopted and included in a revised version that was sent out for voting in early 2011. 58 out of 75 members voted to approve the final draft of the constitution. No members voted against it. Therefore, the percentage of members who voted yes was 77% which is greater than the 2/3rds needed to amend the Constitution.

The approved Constitution of BISMiS is provided in the Appendix of this issue of *The Bulletin of BISMiS*.

2.3 *The Bulletin of BISMiS*. The official publication of BISMiS is *The Bulletin of BISMiS*, the first issue of which was published in December 2010. *The Bulletin* is published twice yearly, December and July. It publishes opinion articles, mini-reviews, meeting reports, biographies and autobiographies (by invitation only). No original research articles are published. Articles are exclusively available to members for the first 6 months, then they are made available to the public. *The Bulletin* is free to members. The Editor of *The Bulletin* is James T. Staley, the Associate Editor is Paul Lawson, and the Editorial Board members are currently Hans Jürgen Busse, Jongsik Chun, Michael Goodfellow, Brian Hedlund, Peter Kämpfer, Wolfgang Ludwig, Fred A. Rainey, Ken-ichiro Suzuki, Martha E. Trujillo, Paul De Vos, William B. Whitman, William Wade and Naomi L.Ward. The Managing Editor is Aidan C. Parte. The Editorial Board met on 21 May with members who attended the BISMiS meeting.

2.4 Member's ideas. A recent on-line request was made to members to determine if they had special ideas they would like BISMiS to pursue to better serve their own needs and that of the field of microbial systematics. Three members indicated an interest in setting up collaborations with other BISMiS members. Ten other members provided suggestions for ideas for all BISMiS members. These suggestions were then distributed to all members for comment and ranking.

Because there was insufficient time to discuss all of these ideas at the members meeting, two were mentioned and one, whose advocate was in attendance at the Beijing meeting, presented his for discussion. The three ideas that were briefly discussed are:

1. Should BISMiS actively recruit those who are interested in eukaryotic micro-organisms and provide a forum for them as well?

The President noted that there were quite a few presentations at this meeting on fungi and yeasts. These members and others interested in eukaryotic micro-organisms, i.e. protists and algae are welcome in BISMiS. Furthermore, if there were enough of these members, they could form their own subdivision within BISMiS and have a special publication section in the *The Bulletin of BISMiS*.

2. Should BISMiS initiate a global initiative for a decade-long program (2012 to 2021) to advance the study of microbial biodiversity?

Since almost all microbiologists would benefit from such a research and educational program, it might be an excellent way to bring members together with a common goal. This could begin by organizing international colloquia funded by those countries that have international research programs.

3. Iain Sutcliffe was the only BISMiS member who submitted an idea and was also in attendance at the Beijing meeting. Therefore, he was asked to present his ideas about the adoption of a simplified method to describe and name species of *Bacteria* and *Archaea*. He received important feedback from the audience who in general shared his concern about the difficulties in naming new species but no conclusion was reached in how this might be best pursued. This is clearly an important area for future discussion.

3.0 Treasurer's and Membership Reports – William B. Whitman

3.1. William B. Whitman discussed the ongoing membership drive. Applications for Full and Student Members were provided at the meeting and he encouraged all who were in attendance to apply. William Whitman then provided a report on

William B. Whitman.

the membership, which was updated following the meeting:

Type of membership	Number of members	
	31 Dec. 2010	1 June 2011
Lifetime	8	11
Full	53	81
Student	0	2
Total	61	94

3.2 BISMIS Treasurer's report 2010

REVENUE 2010:	$	
Dues income:		
Lifetime, 8	4000	
Full, 53	2650	
Student, 0	0	
Total dues	**6650**	
Charter certificate income, 41	410	
Contributions	691	
Total revenue:	**7751**	
EXPENSES 2010:	$	
Bank fees*	848	
Legal fees	216	
Total expenses	**1064**	
INCREASE (DECREASE) IN NET ASSETS		$6687
NET ASSETS 1 JANUARY 2010		$0
NET ASSETS 31 DECEMBER 2010		$6687

*Includes Merchant E-Solutions and USEPay fees to allow credit card payments of initial set up, $175; monthly fee, $25 per month for 10 months; transaction and other fees, $423.

Fred Rainey with Chinese students Li Ma (left) and Quanxin Yang (right).

4.0 Election results – Fred Rainey

Fred Rainey, as Secretary of BISMiS, conducted the election of officers, which was held online prior to the Beijing meeting. The two offices that were up for election and voted upon were the President-Elect (Vice-President) and Secretary. Fred reported the results of the vote were: Brian Austin, new President-Elect, and Martha E. Trujillo, Secretary.

Brian Austin.

Martha E. Trujillo.

Prior to closing the meeting, the President's gavel was transferred by Jim Staley to President-Elect Michael Goodfellow. Michael accepted the gavel. He then proceeded to explain that, since he has recently retired from the Trust and wished to pursue other interests in his retirement, he had regrettably decided not to become President of BISMiS. The gavel was then transferred to Fred Rainey who accepted the role of President of BISMiS.

5.0 Adjournment

Fred Rainey, the new President of BISMiS, declared adjournment of the first BISMiS members' meeting.

Closing Ceremony

Lixin Zhang thanked all who had attended the meeting and gave special thanks to those who presented talks and posters and helped with the arrangements for the meeting, including co-chairs Guoping Zhao, Li Huang, and Zixin Deng. Lixin then asked Zixin Deng and Michael Goodfellow to provide closing comments for the meeting.

Zixin Deng.

Zixin Deng

Co-Chair, BISMiS 2011

Dr Deng thanked the approximately 400 attendees. He indicated that, in addition to the 350 registrants, there were about 40 student assistants – those who wore green shirts – who also attended the meeting and aided with the arrangements, registration and other activities. He also noted that participants came from over 20 countries or regions. He concluded that the meeting had been a great success.

Michael Goodfellow

Chair, Bergey's Manual Trust

I would like to start where I began at the Opening Ceremony by thanking the Local Organizing Committee, headed by

Guoping Zhao and Li Huang.

Lixin Zhang, for putting on what has clearly been a highly successful conference that has got *Bergey's International Society for Microbial Systematics* off to a flying start. I would also like to thank the sponsors for their invaluable contributions and the many young scientists who have looked after all of our needs. We all hope that your careers flourish in years to come.

Lixin asked me to give an overview of what has been achieved over the last four days, but I do not think this is necessary. What is really important is that we have had a great time, existing collaborations have been strengthened and new ones established. This means that we all leave here motivated to promote our interests in prokaryotic systematics from both conceptual and practical points of view. I'm pleased to be able to say that some of the more conceptual contributions will be the subject of articles to be published in a Special Issue of *Antonie van Leeuwenhoek* while others will be published in the *The Bulletin of BISMiS*.

Several colleagues have expressed surprise that I did not contribute to the Discussion which Peter Kämpfer and Iain Sutcliffe introduced on "Criteria for the characterization of prokaryotes for taxonomic purposes". I did not do so for several reasons. First, I think it is very important that card-carrying prokaryotic systematists, like myself, listen to what members of the microbiological community we serve have to say. Indeed, several serious issues were raised during the discussion by the likes of Ying Huang, Paul Jensen, Paul Lawson and Jűrgen Wiegel. It was particularly worrying to hear that important elements of the microbiological community do not understand the importance of the nomenclatural type concept which was introduced by Bergey's Manual Trust many years ago. It is vitally important that at a time of rapid change that systematists build upon what has been achieved to date as they lay the platform for future work.

I have to admit to being quietly amused that much of the discussion was focused on the role of chemical characters

in prokaryotic systematics. I'm a firm advocate of the importance of chemotaxonomy though I'm very surprised that several of the chemical procedures which Dave Minnikin and I introduced or popularised almost 50 years ago are still in use despite spectacular advances in analytical chemistry. The time honored procedures include those designed to detect what we used to refer to as "minniquinones" in homage to Dave Minnikin. Perhaps the time has come to apply modern techniques to detect key chemical markers.

Another reason for my silence was that I've been around long enough to remember similar charged debates in the past. Indeed, last time round I organized the ad hoc committee which squared the simultaneous use of genotypic and phenotypic criteria in the delineation of prokaryotic taxa (see Wayne et al., 1987, *International Journal of Systematic Bacteriology* 37: 463–464), a paper that has underpinned the direction of travel in the description of new taxa for nearly 25 years. Given the dramatic advances currently being made in acquiring taxonomic data, notably from the genomics revolution, it would seem appropriate for another ad hoc committee to be set up to chart the way ahead. Such a committee would need to be representative of the whole microbiological community and could be spearheaded by Bergey's Manual Trust, possibly in league with other relevant organizations.

In the very first plenary lecture I was intrigued when Barry Sharpless drew from a novel by Tolstoy to support his thesis that "revolutionary thinking" is an important factor in driving scientific research forward. Like Barry, I admire the works of Tolstoy and would like to remind you all of the very beginning of my favorite novel, Anna Karenina: "Happy families are all alike, every unhappy family is unhappy in its own way" (the Oblanovskys were an unhappy family). It is quite clear from this wonderful conference that we are all part of a very happy family. Clearly, we need to remain so by building upon our collegiate spirit to tackle the problems we currently face as microbial systematists not least in the description of new taxa.

I now have a very pleasant duty to perform as my last act as Chairman of *Bergey's Manual Trust*. At the Annual Meeting of the Trust held prior to this conference it was agreed unanimously that two highly distinguished Chinese microbial systematists, Jisheng Ruan and Zhiheng Liu, be recipients of this year's Bergey Medals. I would now like Jisheng and Zhiheng to join me on the stage while I say a few words about their many contributions to prokaryotic systematics that make them worthy recipients of the award that has been bestowed on them.

Bergey Medal recipients Jisheng Ruan (left) and Zhiheng Liu (right) with Michael Goodfellow, Chair of Bergey's Manual Trust.

Presentation of Awards

On behalf of the organizing committee, Michael Goodfellow and Elizabeth Ashforth presented awards to several young scientists who made outstanding contributions to the meeting. In addition, several awards were made to those who had submitted outstanding posters to the meeting.

Young Scientist award winners (left to right): Xiao-Ying Rong, Beile Gao, Hong Wei Zhou, and Lorena Carro. Jiun-Yan Ding was not present.

Poster award winners (left to right): Kamlesh Jangid (collected by William Whitman), Ying Zhou and Qi Wang (Qi Wang not in picture), Daisuke Yamamuro, Senthil K. Murugapiran (collected by Brian Hedlund), C.R. Marks (collected by Paul Lawson), Cheng Zhang Fu, Zhe Xue Quan, Chang Fu Tian, Diana Andrea Gil Rivera and Mariel Segura del Pilar. Eun Ju Choi was not present.

The Bulletin of BISMiS (2011), Volume 2, part 1, pp. 33–60

Microbial systematics, “weaving threads into cloth”

Kazuo Komagata

I was born in 1928 into a country doctor’s family and grew up in the small town, Urasa in Niigata Prefecture, 200 km north of Tokyo. Urasa is still known for its heavy snowfalls. My father, Kinji Komagata, was a physician and loved science, and my mother, Fumi Komagata, was a nurse. He had a Leitz microscope and investigated specimens from the patient with it, and he used a Royal Company typewriter for the preparation of prescriptions. The microscope and typewriter influenced my personality, and I became interested in living things. As long as I remember, I spent cheerful days in elementary school, riding a bicycle and catching dragonflies. When I was in the first year class in junior high school, World War II broke out, and the school days entered a bleak period.

During the war, in 1945 I entered the Department of Agricultural Chemistry, Morioka College of Agriculture and Forestry, where I encountered applied microbiology. However, education in the college was still affected by the war. Fortunately, after graduation in 1948, I became a research student in the Laboratory of Fermentation, Faculty of Agriculture, the University of Tokyo. The laboratory was under the directorship of Professor Kin-ichiro Sakaguchi and Professor Kei Arima. At the time I graduated from college, the Japanese educational system differed from the American system, and I took a three-year college curriculum. Therefore, I have neither a Bachelor’s nor Master’s degree. I will describe my life and research over the years below (Figure 1).

Figure 1. Professor Kazuo Komagata.

Laboratory of Fermentation, the University of Tokyo

Professor K. Sakaguchi was highly cultured, receptive, generous, and outstanding in every way. He educated and trained many students, researchers, and fellows who have flourished and contributed to academia and industry not only in Japan but also in other countries. He was a leading Japanese microbiologist who had great foresight.

In the early days of applied microbiology in Japan, research interests were focused on traditional fermentations such as sake, soy source, miso (soy-paste), vinegar, etc., and the micro-organisms involved were the target of study. Professor Teizo Takahashi, who was the mentor of Professor K. Sakaguchi, shifted his interests away from the traditional fermentations to focus on microbial biochemistry and physiology. Therefore, he paved the way to modern microbiology in Japan. Under his direction, Professor K. Sakaguchi studied the production of organic acids by molds, and another student, Professor Toshinobu Asai, worked on the systematics of acetic acid bacteria. Professor K. Arima, a student of Professor K. Sakaguchi, worked on the improvement of penicillin production, and obtained a pigmentless mutant of *Penicillium chrysogenum* Q 176, which is a well-known penicillin-producing mold. The mutant made it easier to purify penicillin, and contributed significantly to the development of penicillin production in Japan.

I worked for Professor K. Arima as a research student for several years. He was generous and fair, and like Professor K. Sakaguchi, he fostered many students and fellows. Professor K. Arima was an internationally recognized microbiologist, and served as President of the International Union of Microbiological Societies (IUMS). I owe in large part my career as a microbiologist to my great mentors Professor K.

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Figure 2. Visit of Dr S. A. Waksman to Sakaguchi Laboratory in 1952. Front row from left: Professor K. Sakaguchi, Dr S. A Waksman and Professor Y. Sumiki.

Sakaguchi and Professor K. Arima (Figure 2).

Splitting of aromatic compounds and pseudomonads.

In the early 1950s, Professor K. Arima told us “We, applied microbiologists, have been interested in glucose and other sugars as carbon sources. In the near future, non-carbohydrate materials will be employed as carbon sources”. In retrospect, this seems a likely prediction for the investigation of petroleum and methanol as raw materials in succeeding generations. Thus, we began the study of bacterial splitting of aromatic compounds, imaging decomposition of lignin and focusing on monohydroxybenzoic acid because this acid has three isomers and was regarded as the model aromatic acid compound. Consequently, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid assimilating-bacteria were isolated. Importantly, many of these bacteria belonged to the genus *Pseudomonas*. Further, metabolic pathways of the isomers revealed a new metabolic pathway in the splitting of *m*-hydroxybenzoic acid (Arima et al., 1954a, 1954b, 1954c, 1955a, 1955b; Sugiyama et al., 1958, 1960a, 1960b). Interestingly, strains of fluorescent pigment producing pseudomonads decomposed this acid through gentisic acid, which are now identified as *Pseudomonas putida*. In contrast, strains of fluorescent pigment non-producing pseudomonads split it through protocatechuic acid. These strains are now identified as *Comamonas testosteroni*. However, *p*-hydroxybenzoic acid was metabolized through protocatechuic acid both by strains of fluorescent pigment producing and pigment non-producing pseudomonads. These findings were reported in 1954. Consequently, I became interested in bacterial systematics because a good correlation was found between the metabolic pathway of monohydroxybenzoic acids and the two groups of the genus *Pseudomonas*. This brought about the realization that bacterial systematics needs to be studied from different angles or different facets, and convinced me to become a bacterial systematist.

Institute of Applied Microbiology, the University of Tokyo

In 1954, I moved to a newly established institute, the Institute of Applied Microbiology, the University of Tokyo, where I worked under the guidance of Professor Hiroshi Iizuka.

Bacteria in rice

At the beginning of the 1950s, Japan had a problem with imported rice because the rice was contaminated with mycotoxin-producing molds such as *Penicillium citrinum*, *Penicillium islandicum*, and others. Professor H. Iizuka had been studying the microflora of the moldy rice, and I engaged in the isolation of micro-organisms from the rice. During the course of the study, a large number of aerobic bacteria were found, accounting for 10^6–10^7 per gram of rice seed. Surprisingly, they existed between the hulls and the grains of the rice seed because few micro-organisms were found in polished rice. In addition, the number of the bacteria decreased by several orders of magnitude on old rice seed stored for several years, and the rate of germination of the rice seeds concomitantly decreased (Iizuka, 1960). Further, most of the bacteria were pseudomonads, fermentative bacteria, and a small number of coryneform bacteria. Yellow

colony-forming strains were among the strains isolated, and later they were identified as *Pseudomonas oryzihabitans* and *Erwinia herbicola* (Iizuka and Komagata, 1963a, 1963b, 1963c; Iizuka et al., 1963; Kodama et al., 1985; Komagata and Iizuka, 1964; Komagata et al., 1964). These findings gave hinted to me that the genus *Pseudomonas* consisted not only of fluorescent pigment producing strains, but also of yellow insoluble pigment producing strains, and those bacteria had a moderate symbiotic relation with the seed rice, which indicates a role of pseudomonas in ecological niche. In the light of the pigmentation, the genus *Pseudomonas* was classified into three groups; a fluorescent group that produces water-soluble fluorescent pigments, an achromogenic group that does not produce pigments, and a chromogenic group that produces water insoluble yellow pigments (Iizuka and Komagata, 1962).

Micro-organisms in petroleum and natural gas fields in Japan

Concomitantly, the microbiological study on petroleum and natural gas was being pursued in Iizuka's laboratory. Professor K. Sakaguchi suggested this project because he had been interested in the microbiology of petroleum and natural gas, and a certain Japanese oil company became interested in his idea and plans, and facilitated the study. I was also engaged in this subject, and tried to study the distribution of the micro-organisms found in an oilfield and a natural gas field in Japan, from which not only hydrocarbon-assimilating bacteria were isolated but also microbes found commonly in soil. Micro-organisms were isolated mainly by the agar plate method using a variety of different media. Samplings of oil brine were made at outlets of pipes from which crude oil was introduced into oil storage tanks, and direct samplings from oil layers were obtained with the bottom-hole sampler that was used in petroleum mining. Further, injection water for the waterflood, crude oil, sewage, soil, etc. were obtained wherever they could be collected. The waterflood is an injection of water into the oil layer to maintain the inner pressure, and employed as a secondary recovery method in the shallow layer in petroleum mining. Oil brine and river water were used as the injection water after treatment with aluminium sulfate and filtration through a sand-filter. Strains of Gram-stain-positive hydrocarbon-assimilating bacteria were then identified as "*Corynebacterium hydrocarboclastus*" that was widely distributed in soil samples in the fields, which is presently identified as *Rhodococcus erythropolis*. Interestingly, Gram-stain-positive hydrocarbon-assimilating bacteria such as *Rhodococcus erythropolis* retained the activity of assimilation of hydrocarbons, but Gram-stain-negative hydrocarbon-assimilating bacteria like pseudomonads easily lost the activity during the successive culture (Iizuka and Komagata, 1964a). A large number of bacteria were isolated from oil brine, natural gas brine, and related materials. Most were Gram-stain-negative oxidative and non-oxidative bacteria (Iizuka and Komagata, 1964b), and a small number of Gram-stain-positive coryneform bacteria (Iizuka and Komagata, 1965a).

Microflora of the Higashiyama oil mine

The microflora of the Higashiyama oil mine was interesting because this oil mine, unlike drilling oil wells, was similar to the mining of coal. The oil mine is located in Niigata Prefecture, which is in the north of Japan. It is recorded that oil outcrops appeared on the surface ground, and an oil well was dug by hand in 1873. During Word War II, the Japanese Government attempted to make an oil gallery due to extreme shortage of oil, and galleries were built through 300 m of the slope and 300 m of the level in 1944. The galleries consisted of two main levels connected to each other through passages (Figure 3).

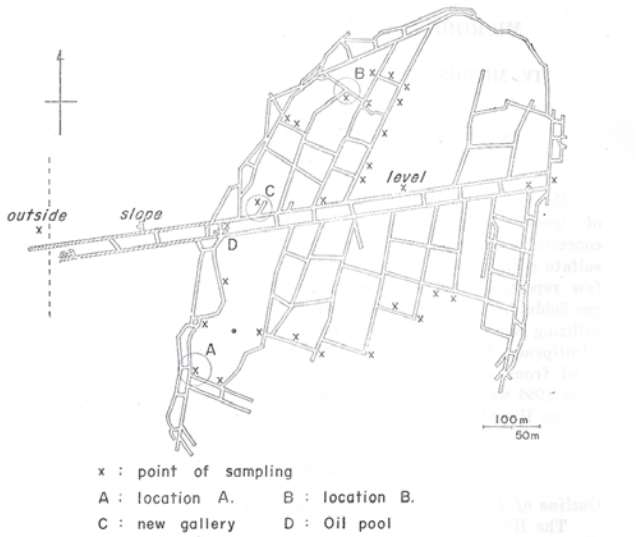

Figure 3. Map of Higashiyama oil mine.

The galleries were tunneled toward oil layers, and extended about 12,000 m in length. The galleries were dug under the cap rock, and oil seeped sand could be seen by the naked eye. Crude oil seeped from the sand of the gallery wall, or from leaks that were bored toward the inner parts of the oil layers. Some parts of gallery walls were dry because oil seeps were depleted, and others seemed wet because the oil was still seeping. The seeped crude oil was conducted to open trenches, collected in a pool under the ground, and then pumped up to an oil tank outside. The inside of the oil galleries was maintained at 14–18°C and about 90% humidity throughout the year. Fresh air was provided by artificial ventilation. The Higashiyama oil gallery was closed for eco-

Figure 4. Sampling by Professor K. Sakaguchi and Professor H. Iizuka at the Higashiyama oil mine in 1957. From left: the second, Professor Sakaguchi, the fourth, Professor H. Iizuka. Photo taken by K. Komagata.

nomic reasons in 1962.

Since there were the tunnels, researchers could go underground, investigate the oil layer with the naked eye, and take samples directly from the layers that were composed of oil spilled sand, oil brine, cutting pieces of boring, and others. Considerable numbers of micro-organisms were found in the samples from the oil gallery, and the number of the micro-organisms varied with the sample. Generally, about 10^4 to 10^6 aerobic bacteria were isolated from 1 g of oil seeped sand and water sand, but a few micro-organisms were found in oil depleted sand and sandy clay that were dry and pulverous and did not show an oily appearance (Iizuka and Komagata, 1965b). The aerobic bacteria mainly belonged to the fluorescent group of pseudomonads. From a sample that was considered to be rain water leaked from the surface ground, about 10^5 aerobic bacteria were isolated from 1 ml of the sample on nutrient agar plates, but only a small number of bacteria grew on 3% NaCl nutrient agar plates. Adaptation to the environment of the oil gallery was apparent from the fact that nearly the same number of colonies appeared on both nutrient and 3% NaCl nutrient agar plates from oil brine in which the salt concentration was about 3%.

Distribution of molds was relatively limited. In oil rich sand and mud of the trenches, black molds, probably belonging to the family Dematiaceae, were found, and *Aspergillus versicolor* and *Aspergillus sydowi* were abundantly isolated from oil poor sand. However, such molds were not necessarily isolated from all samples. No actinomycetes were found in the samples from the oil gallery, although they were abundantly isolated from the soil of the surface ground. It is noteworthy that the Higashiyama oil gallery was composed of taxonomically restricted micro-organisms, whereas many kinds of micro-organisms such as bacilli, *Penicillium* and other molds, and streptomycetes were abundantly isolated from the soil of the surface ground (Figure 4).

During the course of a two-year investigation (three times at the Higashiyama oil gallery), a very slow succession was recognized in the microflora. In oil-rich sites, the oil sands demonstrated 10^6 aerobic bacteria per 1 g at a depth of 20 cm in 1958, but the number decreased to 10^3 aerobic bacteria per 1 g at a depth of 10 cm in 1959. The black molds were observed as before, but a few aspergilli appeared. The color of oil sands changed from black to brownish, and became slightly dry. From the oil-poor site, characteristic aspergilli were observed in 1957, but the number of the micro-organisms decreased to 10^3 per 1 g of oil sand at the surface. Moderate numbers of *Aspergillus sydowi* were found, but few aerobic bacteria were isolated from oil sand in 1959. The kinds and numbers in the soil outside the gallery had remained almost unchanged during the years. Thus, it seemed likely that the microflora of the oil gallery had gradually changed to resemble that of oil poor sands.

At the beginning of the study, the microflora of the Higashiyama oil gallery was supposed to be complicated because air was artificially ventilated and work was performed in the gallery by humans who might introduce different kinds of micro-organisms. However, experimental results were contrary to the expectations. The microflora of the Higashiyama oil gallery was relatively simple and stable. It is obscure whether the micro-organisms existed before the opening of

the gallery or they invaded after the opening. Probably, the constants of temperature, humidity, and the presence of petroleum controlled the microflora of the gallery. However, it seemed likely that the microflora of the Higashiyama oil gallery has been slowly changing to the oil poor sand type. Such a transformation may be interesting in petroleum microbiology and microbial ecology.

Microflora of the Yabase oilfield

Japanese oilfields are small on the scale, and the depths of wells are shallow. The Yabase oilfield is the largest one that is located in Akita Prefecture, the northeastern area of Japan. In the Akita area, oil outcrops have been known since olden times, and in fact, there is a stream called "Kusozu Gawa" that means "petroleum stream" in Japanese. This oilfield was discovered in 1933, existing over a range of 10 km long and 500 m wide. Petroleum was produced only from shallow layers of approximately 200 m in depth. After development of the shallow layer, deeper layers of over 1000 m in depth were discovered in 1948. Petroleum was pumped up from the shallow layers, and produced from the deeper layers by oil gushers. The waterflood was employed for the shallow layers, and approximately 800 kl a day was injected in the early 1950s.

Considerable numbers of micro-organisms were found in the samples obtained in the Yabase oilfield, but the number varied among samples. Approximately 10^4–10^6 aerobic bacteria, which were mainly pseudomonads, including hydrocarbon-assimilating bacteria, and 10^2–10^3 anaerobic bacteria, including sulfate reducers, were found in 1 ml of oil brine coming up from the shallow layers (Iizuka and Komagata, 1965c). Generally, the numbers of micro-organisms found in the oil brine obtained with the bottom-hole sampler were less than those at the outlets of pipes, but the reason was not apparent. The bacterial numbers were almost the same in oil brine obtained at different depths of a well with the bottom-hole sampler. The numbers in oil brine varied with different samplings, even from the same well. Changes of pipes or casings, hydrofracturing, and other treatments of the well in petroleum mining may have exerted effects on the microflora. Hydrocarbon-assimilating bacteria were distributed in the bottom water of oil tanks, injection water of the waterflood, oil brine, etc. Large numbers of terrestrial inhabitants such as bacilli, molds, and actinomycetes were isolated from soil and sewage. The microflora of the oil brines clearly differed from those of the surface ground.

Microflora of the Niigata natural gas field

In Japan, approximately 1700 million m^3 of natural gas were produced in the early 1960s, and supplied as city gas and raw materials for the chemical industry. The Niigata gas field is the largest in this country. The field is 30 km long and located northwest to southwest along the coastline of the Japan Sea in Niigata Prefecture. The natural gas of the field is of the "dissolved in water" type, and is produced with almost equal volumes of gas brine. The gas lift procedure is employed in a large number of wells, and the natural gas dissolved in gas brine is separated from it with a gas separator (Figure 5).

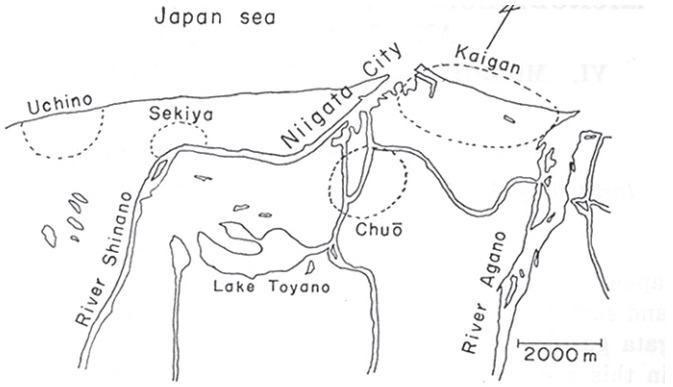

Figure 5. Map of Niigata gas field.

Gas outcrops have been recorded in the Niigata area since olden times, and small amounts of the gas have long been used as a fuel for homes. However, production of natural gas has been developed in the Niigata gas field only since the discovery in 1940 of a well that produced 1750 m^3 of the gas per day, and gas layers extended from 200 m to 2000 m deep. Samplings were carried out at the gas separator, and gas brine was directly obtained with a bottom-hole sampler. Micro-organisms were isolated from gas brine, sewage, river water, soil, etc., as described in the above section, *Microflora of the Yabase oilfield*.

Considerable numbers of micro-organisms were found in the gas brine from the Niigata gas field, but the number varied with the sample (Iizuka and Komagata, 1965d). Approximately 10^5–10^7 aerobic bacteria were isolated from 1 ml of gas brine from the shallow layer of a depth of 300–600 m, and they mainly belonged to the fluorescent pseudomonads. However, a small number of bacteria were found in the gas brine from the deep layer of over 1000 m. Methane-oxidizing bacteria were found in several samples of oil brine, but no hydrocarbon-assimilating bacteria were isolated. Anaerobic sulfate reducing bacteria were not found. Terrestrial micro-organisms such as bacilli, molds, actinomycetes were not found in the gas brine, but they were abundantly isolated from the soil near the well and at other places. The micro-

flora of the gas brine was different from that of the surface ground. The environment of the shallow layer may permit the growth of micro-organisms, since the temperatures of the gas brine are about 25–30°C, and pH is usually 6.0–7.2. Internal pressure of the gas well is lower for the depth than expected. Unusual effects caused by hydrostatic pressure are not expected on the micro-organisms in gas brine, since a number of bacteria grow under a hydrostatic pressure over 200 atmospheres. The pseudomonads isolated from gas brine were similar to those found in oil brine.

In the course of the three-year investigation on the Niigata gas field, the number of bacteria in gas brine did not show any differences among the wells. However, some differences were revealed by taxonomic studies on the bacteria isolated. Wells were divided into five groups by the bacteria found in the gas brine. Newer gas wells, which were located in the southeast district, belonged to Type A, and older gas wells, which were mainly in the northeast, belonged to Types D and E. Type A consisted of few bacteria, Type B of a few flavobaceria, micrococci, etc., Type C of *Pseudomonas stutzeri*, *Pseudomonas putrefaciens* (later reidentified as *Shewanella putrefaciens*), etc., and Type D of *Pseudomonas desmolytica* and *Pseudomonas dacuhnae* (later reidentified as *Comamonas* and *Delfia*) that belonged to the achromogenic group of the genus *Pseudomonas*, and Type E of *Pseudomonas schuylkilliensis* (later reidentified as *Pseudomonas putida*), *Pseudomonas azotoformas* that belong to the fluorescent group of the genus *Pseudomonas*. A succession of the microflora from Type A to Type E was recognized during the three years. The change was faster than expected. The tendency of the succession from Type A to Type E paralleled the age of the gas wells and the decrease of chlorine in gas brine. As the pseudomonads of the fluorescent group were aquatic and terrestrial inhabitants, it was believed that fresh water seeped in, but no marine water because no marine bacteria were isolated. It was remarkable that in contrast with the oil brine, no anaerobic sulfate reducers and hydrocarbon-assimilating bacteria were found in gas brine.

In the course of the study of rice, and the petroleum field and the natural gas field, a large number of bacteria were isolated, but their taxonomic positions were unknown. Consequently, my systematic studies and ecological considerations of bacteria can be traced to the study of these bacteria.

Central Research Laboratories, Ajinomoto Company

In 1960, I moved to the Central Research Laboratories, Ajinomoto Company, which is the biggest producer of sodium glutamate. This amino acid has long been used as a seasoning in Japan. Soon after joining the Central Research Laboratories, I was asked to establish a laboratory of microbial systematics because the company recognized the importance of micro-organisms for future development and patent strategy. Thus, I decided to raise the training of the staff to become high-level researchers of microbial systematics covering a variety of micro-organisms, and to set up a culture collection that was essential for microbial systematics as well as to solve microbial problems arising from consumer complaints, which is valuable for the company and would provide good sources of unknown micro-organisms. The company employed good staff, had excellent research facilities, and gave us the freedom to pursue this approach. Therefore, a large number of papers were produced in cooperation with the staff and under highly advanced conditions. During work in the Ajinomoto Company, I received my Doctor of Agriculture (PhD) in 1960 from the University of Tokyo for the taxonomic study of the genus *Pseudomonas*. I was promoted to chief microbiologist in 1965.

The following major areas of study were pursued in the laboratory.

Microbiological study of frozen food

In the early 1960s, frozen food came into the market in Japan, but few papers relating to the microbiological study of the frozen food had been published. Therefore, micro-organisms capable of growing at low temperatures and the microflora of frozen food attracted attention as areas of study. Samples included frozen raw shrimp, squid, oyster, and trout, and frozen fish battered with wheat flour and breadcrumbs, etc., and they were obtained directly from the retail market. The micro-organisms were isolated at 0°C and 30°C with Tryptone agar plates for bacteria and actinomycetes and yeast extract-malt extract agar plates for yeasts and molds. The kinds and numbers varied with samples. Aerobic bacteria were the main member of the microflora of the frozen food, followed by yeasts. Few actinomycetes and molds were found. On a mean, 10^3 to 10^6 of micro-organisms per gram were found in the frozen food, and they consisted of the following groups: 44.4% of Gram-stain-negative rods, 15.3% of Gram-stain-positive cocci, 14.0% of Gram-stain-positive straight rods, 16.7% of Gram-stain-positive pleomorphic rods, and 8.8% of yeasts. Further, the micro-organisms isolated from the frozen food were divided into three groups on the basis of the relation to temperatures for growth: Group 1 (psychrophiles) consisted of those growing at 0°C but not at 30°C, Group 2 (mesophiles) of those growing at 30°C but not at 0°C, and Group 3 (psychrotorelant mesophiles) of those growing at both temperatures. They were composed of 20% of Group 1, 16% of those of Group 2, and 64% of those

of Group 3. Gram-stain-negative rods were most abundantly isolated from the frozen food (Komagata et al., 1964).

Almost all of the bacteria isolated from the frozen food were regarded as psychrotorelant mesophiles. Gram-stain-negative rods were identified as *Pseudomonas taetrolens*, *Pseudomonas schuylkilliensis* (now *Pseudomonas putida*), *Pseudomonas maltophilia* (*Stenotrophomonas maltophilia*), *Pseudomonas* sp., *Flavobacterium* spp., *Aerobacter cloacae* (*Enterobacter cloacae*), *Alcaligenes faecalis*, etc. Gram-stain-positive rods were identified as *Brevibacterium helvolum*, *Brevibacterium ammoniagenes* (now identified as *Corynebacterium ammoniagens*), *Brevibacterium* spp., *Arthrobacter citreus*, *Arthrobacter tumefaciens*, *Arthrobacter* sp., *Sarcina lutea* (*Micrococcus lutea*), *Bacillus pumilus*, etc. (Komagata and Ogawa, 1966a, 1966b, 1966c).

The yeast flora of frozen food comprised mainly asporogenous species. (Taxa follow the then-known nomenclature.) They were represented by the genera *Candida* (42%), *Torulopsis* (16%), *Trichosporon* (13%), *Cryptococcus* (11%), *Rhodotorula* (7%), *Debaryomyces* (7%), *Sporobolomyces* (2%), and *Aureobasidium* (2%). Further, yeast isolates were identified as *Sporobolomyces roseus*, *Debaryomyces hansenii*, *Cryptococcus laurentii*, *Cryptococcus albidus*, *Cryptococcus luteolus*, *Torulopsis candida*, *Candida lipolytica*, *Candida cruvata*, *Candida zeylanoides*, *Candida guillermondii*, *Trichosporon cutaneum*, *Trichosporon pullulans*, *Rhodotorula infirmo-miniata*, *Rhodotorula glutinis*, and *Aureobacidium pullulans* (Komagata and Nakase, 1967a, 1967b). In addition, two obligately psychlophilic yeast species, *Candida curiosa* and *Candida punicea*, and two psychlotorelant species, *Candida salmonicola* and *Candida glaebosa*, were described (Komagata and Nakase, 1965).

The above results showed that the microflora of frozen food consisted of different kinds of micro-organisms, including the micro-organisms in the raw and auxiliary source materials, and those that were due to microbial contamination during the distribution processes. Therefore, micro-organisms of food should be investigated at the species level, and further, the total number of micro-organisms makes little sense to investigate.

Taxonomic study of coryneform bacteria

In the course of the microbiological studies of rice, petroleum and natural gas, and frozen food, identification of coryneform bacteria isolated from the above materials were reported in each case. Further, this group of bacteria attracted attention to applied microbiologists in the late 1950s because some coryneform bacteria were reported to produce a large amount of glutamic acid in culture media. This made it possible to produce glutamic acid on an industrial scale. Consequently, I became involved in identification of glutamic acid producing bacteria from the viewpoint of bacterial systematics as well as the profit of the company. Since the systematics of coryneform bacteria has been studied completely on the basis of chemosystematics, and many students and fellows in my laboratory were involved with this study, the details will be discussed in a separate section below.

Taxonomic significance of base composition of yeast DNA

DNA base composition was first introduced into bacterial systematics in the early 1960s, but little attention was paid to yeast systematics. However, DNA base composition was examined in a large number of yeast strains covering the genera *Hansenula* (Nakase and Komagata, 1968, 1969, 1971b), *Hanseniaspora* and *Kloeckera* (Nakase and Komagata, 1970a), *Pichia* (Nakase and Komagata, 1970b), *Debaryomyces* (Nakase and Komagata, 1971a), *Cryptococcus* and *Rhodotorula* (Nakase and Komagata, 1971c), *Torulopsis* (Nakase and Komagata, 1971d), *Saccharomyces* (Nakase and Komagata, 1971e), *Candida* (Nakase and Komagata 1971f), and yeast-like fungi (Nakase and Komagata, 1971g). (Taxa follow the then-known nomenclature.) Particular interests were given to one group with high G+C contents and the other group with low G+C contents. Generally speaking, most species in Saccharomycetaceae belonged to the low G+C group and basidiomycetous yeasts in Heterobasidiomycetes were included in the high G+C group (Nakase and Komagata, 1971f). Later, this led to differentiation of ascomycetous yeasts and basidiomycetous yeasts. Dr Takashi Nakase and his group extended the study to chemosystematic approach of yeast systematics.

Moniliella isolated from synthetic vinegar

In 1966, a turbid vinegar sample was sent to the laboratory, and we were asked to determine the cause of the turbidity. At that time, a cheap vinegar was made from acetic acid produced by a chemical process and other ingredients, and was called “synthetic vinegar” (this type of vinegar is rarely produced in Japan today). Microscopic investigation revealed some moldy materials in it, a mold strain was isolated, and identified as *Moniliella acetoabutans*, which grew at pH 3.0 adjusted with acetic acid (Awao et al., 1971). This is the first case of isolation of this mold in Japan, and tells us that materials arising from consumer complaints can be good sources for the isolation of new micro-organisms.

Histochemical study of the development of mushrooms

Almost all of the mushrooms belong to the Basidiomyce-

tes, and form fairly developed fruiting bodies (carpophores). This group of micro-organisms was interesting for understanding morphogenesis and cell differentiation in multicellular organisms. Further, comparison of mycelia cultured on solid media with those in submerged cultures provided a clue about the industrial application of the mushrooms. A culture of *Coprinus kimurae* was isolated from a carpophore on rice straw in 1967. The primordia and carpophores formed at 24°C-culture under white light (about 140 lux) and dark conditions at 12-h intervals, and they were subjected to histochemical study at various intervals. The developmental process was divided into seven stages with morphogenesis (Figure 6).

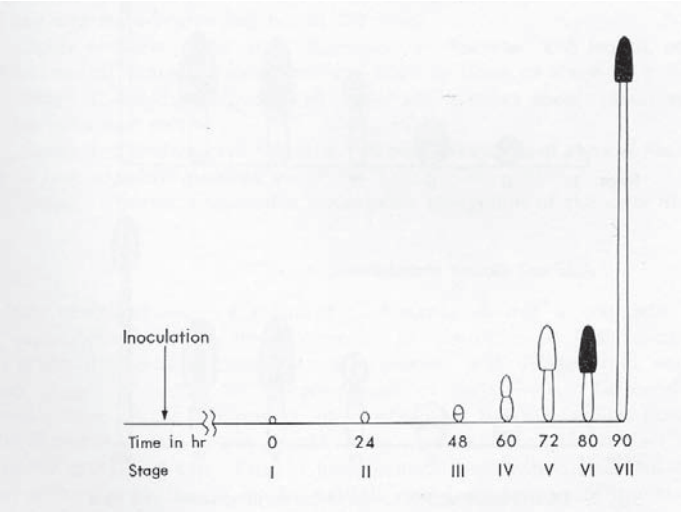

Figure 6. Schematic illustration of morphogenesis of *Coprinus kimurae*.

Activities of cytochrome oxidase, succinic dehydrogenase, and alkaline and acid phosphatases were mainly found at active growth zones of *Coprinus kimurae* such as the top part of the stalk and the margin of the cap. Both kinds of phosphatases were not detected in gills at early stages, but were detected in gills at later stages when the cap matured and spores formed (Figure 7) (Komagata and Okunishi, 1969).

Also, the developmental process of the carpophore of *Polyporellus brumali* was studied histochemically. This fungus formed primordia that developed into tubular stipes, and the pileus developed out of the apex of the stipe at a later stage of development. Acid phosphatase was stained intensively throughout the development of the carpophore but alkaline phosphatase was not detected. Acid phosphatase, esterase, peroxidase, cytochrome oxidase, and succinic dehydrogenase were detected in growth zones of this fungal carpophore such as at the apex of stipes (Okunishi and Komagata, 1975). In confirmation of these enzymic studies, further histochemical work would clarify the locality in which the enzymes play their roles and the significance of these enzymes in the morphogenesis of the Basidiomycetes.

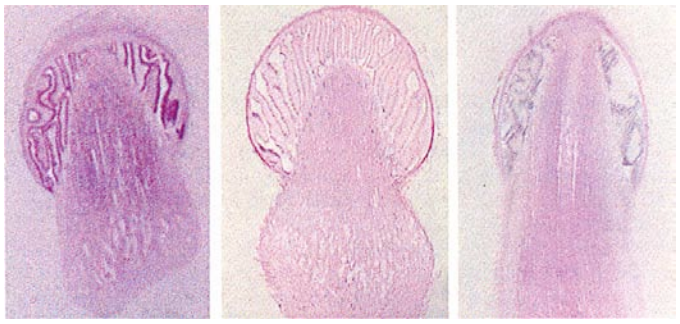

Figure 7. Distribution of RNA, and acid and alkaline phosphatases in *Coprinus kimurae*. From left: RNA at stage 4, acid phosphatase at stage 4, and alkaline phosphatase at stage 4.

Reduction of lag time in bacterial growth.

Bacterial growth under various cultural conditions is an interesting topic in bacterial physiology and biochemistry, and the reduction of lag time is important in the light of applied microbiology. Therefore, bacterial growth was investigated with a Biophotometer (Jouan, Paris), which is equipped with six photocells and monitors turbidity of bacterial cultures at 2-min. intervals. First, the effects of inoculum size and ingredients of media were examined in 24 strains belonging to 15 species and 11 genera. Inoculum size greatly affected the length of lag time, but not the growth rate. Two types of reduction of lag time were found: One occurred when the lag time was reduced regularly in parallel with an increase in inoculum size, and the other occurred when the lag time was reduced in response to other factors. For example, the composition of the media and supplementation of ingredients influenced the length of the lag time, but the effect of compounds used varied with each strain. No ingredient was found among the compounds employed that would change an irregular reduction of lag time to a regular reduction of lag time with an increase of inoculum size. Reduction of lag time when inoculum size was increased ten times was termed L_{10}, and a simple calculation of doubling time was discussed on the basis of cell numbers in the inoculum size and L_{10} (Shida et al., 1975a).

The effects of glucose and sodium chloride supplemented with culture media, initial pH of the media, and cultural temperature were examined in connection with inoculum size. In higher concentrations, glucose and sodium chloride greatly influenced the lag time and growth rate of bacteria, but the concentrations affecting the growth rate varied with strains. An effect similar to those of glucose and sodium

chloride was observed at lower initial pHs. Even when inoculum size changed, the growth rate of bacteria was not affected by these environmental factors. Temperature also influenced both lag time and growth rate. In the range of temperatures tested, lag time became longer and the growth rate was reduced at lower temperatures than at higher temperatures. Moreover, the growth rate with a large inoculum size was greater than that with a small inoculum size (Shida et al., 1975b).

In addition, the effect of inoculum size and the growth phase of the seed culture on main cultures was examined. Lag time and L_{10} were influenced by growth phases of seed cultures. Two types were found in the change of lag time: One was when the length of the lag time varied with the growth phase of the seed culture, and the other was when the length of lag time did not change by the growth phase of the seed culture. Two types were also found in the change of L_{10}: One is the type influenced by the growth phase of the seed culture, and the other is the type not affected by the growth phase of the seed culture. From these results, the influence of growth phase of the seed culture on the lag time and L_{10} of the main culture did not reduce to a common principle. It was presumed that variable L_{10} would be caused by the inoculum composed of cells from different growth phases. It was concluded that bacterial growth was not regulated by a single characteristic, but was attributable to the characteristics of each bacterial strain (Shida et al., 1977).

The study of microbial systematics carried out at the Central Laboratories, Ajinomoto Company became seeds for my subsequent studies. I very much appreciate the support and understanding of the company.

Return to the Institute of Applied Microbiology, the University of Tokyo

In 1968, I returned to my old laboratory at the Institute of Applied Microbiology, the University of Tokyo, and was appointed to Associate Professor. At the time, the university had suffered from the strong student movement, and sometimes encountered violence. The institute had been placed under severe circumstances as well. I was deeply concerned about the desolation of the spirit of the students and staff, and the decline in research activity. However, circumstances retuned to normal with time, and my concern proved unfounded. Under such conditions, I began to educate and train students and fellows, and initiated the study of microbial systematics. In fact, only one gas chromatography instrument was provided, and studies of the cellular fatty acids of *Pseudomonas* strains and methanol-utilizing bacteria were immediately commenced. I focused the study on chemosystematics because reliable data could be obtained by chemical analyses.

I was promoted to Professor in 1978, retired in 1989 from the University of Tokyo at the official retirement age, and became Emeritus Professor in 1989. Further, I became Honorary Member of the Society for Bioscience and Bioengineering, Japan, in 1988; Honorary Member of the World Federation of Culture Collections in 1990; Life Member of the International Committee on Systematic Bacteriology, Division of Bacteriology and Applied Microbiology, IUMS, in 1994; and Life Member of the Japan Society for Bioscience, Biotechnology, and Agrochemistry in 1998.

I received an Award of Food Hygienic Society in Japan for microbial studies on frozen food in 1968, the Senior Scientist Award from the Japan Society for Bioscience, Biotechnology, and Agrochemistry in 1988, the Van Niel International Prize from the Division of Bacteriology and Applied Microbiology, IUMS in 1999, and the Bergey Medal from Bergey's Manual Trust in 2005.

Figure 8. IUMS Congress at Munich in 1978. From left: Dr M. Kocur (Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; at present, Czech Collection of Microorganisms, Czech Republic), unknown person, Professor Eiko Yabuuchi (Gifu University, Gifu, Japan), and K. Komagata

I served as member for the Subcommittee on *Corynebacterium* and coryneform bacteria, the International Committee on Systematic Bacteriology (1974–1994), as chairman for the Committee on Data Coding, the World Federation for Culture Collections (1976–1981), as board member for the Agricultural Chemical Society of Japan (at present the Japan Society for Bioscience, Biotechnology and Agrochemistry) (1979–1983), as commissioner for the Judicial Commission of International Committee of Systematic Bacteriology (1982–1994), as vice-president for the Society of Fermenta-

tion Technology (at present the Society for Biotechnology, Japan) (1983–1987), as member for the Subcommittee on *Flavobacterium* and *Cytophaga*-like bacteria, (1986–1990), and as vice-chairman for the International Committee on Systematic Bacteriology, (1986–1994).

I became acquainted with many distinguished microbiologists through international activities in microbiology, and I feel happy and honored to maintain deep friendship and establish trust with them (Figure 8).

Microbial chemosystematics

Modern bacterial systematics requires precise methodology and practice, and a culture collection with a number of strains with unique characteristics also plays a crucial role. Consequently, I focused on chemosystematics in our investigations of modern bacterial systematics. In a broad sense, microbial chemosystematics is the study of the microbial classification, identification, and phylogeny based on the information derived from semantides and cellular constituents that are deeply related to the maintenance of life. Chemosystematics has employed such characteristics as DNA base composition, DNA–DNA relatedness, cellular fatty acid composition, quinone systems, etc.

Chemosystematics is based on the following principles: (1) The progress of molecular biology and related fields has made it possible to study the interrelation and phylogeny of micro-organisms on the basis of the information derived from semantides and cellular constituents, (2) the development of instrumental analysis has made it possible to determine small quantities of cellular constituents from a large number of specimens in a short time, (3) the development of computers has made it possible to treat various sources of data mathematically, (4) the development of preservation techniques for micro-organisms has made it possible to preserve fastidious micro-organisms and distribute them to researchers all over the world, and (5) the growth of culture collections has made it possible to provide a large number of authentic cultures for systematic comparison.

A variety of bacterial strains have been isolated from rice, petroleum and natural gas fields, frozen food, etc. for the ecological study of micro-organisms. The isolates have been maintained in the laboratory and deposited in the culture collections, and are still useful for the systematic study and their application. In view of the perspective of bacterial systematics, a horizontal line indicates bacterial groups to be studied and a vertical line represents isolation sources. Therefore, the combination of the chemosystematic data and the use of unique isolates provided the basis for my further study.

Cellular fatty acid composition and quinone systems of the genus *Pseudomonas*

Cellular fatty acid composition was introduced in bacterial systematics in the mid-1960s, and its usefulness was previously acknowledged. The composition of 50 strains of the genus *Pseudomonas* was determined by gas chromatography, and straight chain saturated acid of $C_{16:0}$, and straight-chain unsaturated acids of $C_{16:1}$ and $C_{18:1}$ were commonly found in a large number of the strains tested (Ikemoto et al., 1978b). In contrast, the composition of *Pseudomonas maltophilia* was remarkably different from those of other species, and iso-branched $C_{15:0}$ accounted for approximately 50% and $C_{16:0}$ did for 15% of total acids. Similarity values calculated on the basis of fatty acids exhibited a good correlation with taxonomic groups of this genus. Quinone systems were then determined for 63 strains of 35 species in the genus *Pseudomonas*, and three quinone systems, Q-8, Q-9, and Q-10, were recognized (Yamada et al., 1982). Further, the genus *Pseudomonas* was classified into nine groups with the composition of 3-OH fatty acid and quinone systems (Oyaizu and Komagata, 1983). Group 1 consisted of the species with 3-OH $C_{10:0}$ and 3-OH $C_{12:0}$, and Q-9, in which typical *Pseudomonas* species, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, etc. were included; Group 2 was composed of species with 3-OH $C_{14:0}$ and 3-OH $C_{16:0}$, and Q-8, in which *Pseudomonas cepacia* and *Pseudomonas solanacearum* were contained; Group 3 was characterized by the presence of 3-OH $C_{10:0}$ and Q-8, to which *Pseudomonas acidovorans*, *Pseudomonas testosteroni*, and *Pseudomonas iners* were belonged; Group 4 comprised species with 3-OH $C_{12:0}$ and 3-OH $C_{14:0}$, and Q10, in which *Pseudomonas diminuta* and *Pseudomonas vesicularis* were included; Group 5 consisted of species with 3-OH $C_{12:0}$, 3-OH $C_{11:0}$ iso., and 3-OH $C_{13:0}$ iso, and Q-8, in which *Pseudomonas maltophilia* was included; Group 6 did not contain any 3-hydroxy acid, in which *Pseudomonas paucimobilis* was included; Group 7 was composed of species with 3-OH $C_{14:0}$ and Q-10, to which a methanol-utilizing *Pseudomonas extorquens* was belonged; Group 8 consisted of species with 3-OH $C_{8:0}$ and Q-8, in which *Pseudomonas palleronii* was contained; and Group 9 was composed of species with 3-OH $C_{10:0}$, 3-OH $C_{12:0}$, 3-OH $C_{14:0}$, and 3-OH $C_{14:1}$, and Q-8, to which *Pseudomonas avenae* was included. According to the classification with r-RNA–DNA homology by Palleroni et al. (Palleroni et al., 1973), Group 1 corresponded to their Group I, but now is regarded as the genus *Pseudomonas sensu stricto*, Group 2 was comparable to their Group II, now represents *Brukholderia cepacia* and *Ralstonia solanacearum*, Group 3 was comparable to their Group III, but is now regarded as *Delfia acidovorans*, *Comamonas testosteroni*, and *Marinobacterium georgiense*, Group 4 corresponded

to the genus *Brevundimonas*, *Brevundimonas diminuta* and *Brevundimonas vesicularis*, and Group 5 was equivalent to the genus *Stenotrophomonas* including *Stenotrophomonas maltophilia*. Further, presently Group 6 is regarded as the genus *Sphingomonas* containing *Sphingomonas paucimobilis*, Group 7 as the genus *Methylobacterium extorquens*, Group 8 as *Hydrogenphaga* with *Hydrogenphaga palleronii*, and Group 9 as *Acidovorax* including *Acidovorax avenae*. This indicates a good correlation between chemosystematic data and nucleic acid hybridization. However, more recently, a correlative relationship was found between numerical analysis based on phenotypic characteristics and values of DNA–DNA relatedness, but a poor correlation was recognized between values of DNA–DNA relatedness and phylogenetic data based on 16S rRNA gene sequences (unpublished). Therefore, systematics of the genus *Pseudomonas* and related genera is on the road to completion.

Systematics of methanol-utilizing bacteria and yeasts

In the mid-1960s, being deeply concerned by the serious food shortages in the world, researchers became interested in the production of single cell protein (SCP) from normal paraffins (*n*-paraffins) for the feed, and industrial plants were established worldwide for its production. However, the plans ceased eventually because of the social movement against the use of SCP for feed. Consequently, methanol drew attention as a substitute of *n*-paraffins because it was cheap and mixed with water, and a large number of methanol-utilizing micro-organisms were isolated. In response, the First International Symposium on C_1-compounds was held in Tokyo in 1974. Rational and clear systematics of micro-organisms involved was essential to avoid patent problems.

Thus, the cellular fatty acid composition and quinone systems were determined for 145 strains of Gram-stain-negative methanol-utilizing bacteria, and the bacteria were divided into four groups with the combination of morphological characteristics, assimilation of carbon compounds, cellular fatty acid composition, and quinone systems (Ikemoto et al., 1978a; Urakami and Komagata, 1979). Group 1 included polarly flagellated rods, and showed the cellular fatty acid composition consisting of a large amount of $C_{16:0}$ acid and $C_{16:1}$ acid (Type A) and the presence of Q-8. So-called "obligate methanol-utilizing bacteria" were included in this group; group 2 contained polarly flagellated rods, and the cellular fatty acid composition consisted of a large amount of $C_{18:1}$ acid (Type B) and the presence of Q-10; group 3 comprised *Microcyclus* (now *Ancylobacter*) strains with the Type B cellular fatty acid composition and the presence of Q-10; and group 4 consisted of *Hyphomicrobium* strains with the Type B cellular fatty acid composition and the presence of Q-9.

Further, an electrophoretic comparison of enzymes was made on 155 strains of the methanol-utilizing bacteria (Urakami and Komagata, 1981). Phenazine methosulfate (PMS)-linked methanol dehydrogenase, phosphoglucomutase, glucose-6-phosphate dehydrogenae (NADP-dependent), isocitrate dehydrogenase (NADP-dependent), and catalase were detected in almost all of the strains tested. The strains were divided into two types with the electrophoretic pattern of PMS-linked methanol dehydrogenase. The first type of the enzyme was characterized by not migrating into gel at pH 8.9, and was found in so-called "obligate methanol-utilizing bacteria" (the above-mentioned group 1). The second type of the enzyme moved into the gel during electrophoresis, and found in *Pseudomonas extorquens* (now *Methylobacterium extorquens*) and related bacteria (group 2), *Ancylobacter* strains (group 3), and *Hyphomicrobium* strains (group 4). The presence of hexose phosphate synthetase and the absence of β-hydroxypyruvate reductase were characteristic of the ribulose monophosphate pathway in the methanol-utilization of the strains of the group 1, and the absence of hexose phosphate synthetase and the presence of β-hydroxypyruvate reductase would indicate a functioning serine pathway in the methanol-utilization in the groups 2 and 4. Esterases were detected in the strains of groups 2, 3, and 4, but not in those of group 1. A considerable number of strains showed the same patterns in esterases, and such strains exhibited identical electrophoretic patterns in all other enzymes tested. Dendrograms were drawn on the basis of kinds of enzymes and relative mobilities, and the clustering in each of the groups showed a good correlation with grouping based on phenotypic characteristics, cellular fatty acid composition, and quinone systems.

In addition, the methanol-utilizing bacteria were classified into nine groups with DNA base composition, DNA–DNA relatedness, and other chemosystematic data (Urakami et al., 1985). Group 1 consisted of "obligate methanol-utilizing bacteria", group 2 of *Protomonas* (*Methylobacterium*) strains, group 3 of *Ancylobacter* strains, group 4 of *Hyphomicrobium* strains, group 5 of *Xanthobacter* strains, group 6 of isolates, group 7 of "*Acetobacter sadu*" strains (would be identical with *Acidomonas* strains), group 8 of *Paracoccus denitrificans* strains, and group 9 of a *Thiobacillus novellus* strain.

The combination of chemosystematic data showed a rational background for the creation of a framework for systematics of methanol-utilizing bacteria, and this strategy and tactics extended to systematics of methane- and methylamine-utilizing bacteria (Urakami et al., 1987a).

Further, research brought about the proposal for new genera *Protomonas* (now *Methylobacterium*) (Urakami and Komagata, 1984), *Acidomonas* (Urakami et al., 1989), and *Aminobacter* (Urakami et al., 1992), the emendation of the genus *Methylobacillus* (Urakami and Komagata, 1986), and characterization of the genera *Methylophaga* (Urakami and Komagata, 1987b), *Hyphomicrobium* (Urakami and Komagata, 1987c), *Mycoplana* (Urakami et al., 1990), and *Xanthobacter* (Urakami et al., 1995). Methanol-utilizing bacteria were also employed for commercial production of co-enzyme Q that is now used as a nutritional supplement.

Yeasts were promising micro-organisms for the production of SCP from methanol as well. However, systematics of methanol-utilizing yeasts was not studied in detail in the mid-1970s, and several yeasts with very similar characteristics were assigned to different yeast species. Thus, known methanol-utilizing yeast strains and new isolates were studied in the light of their chemosystematic characteristics including DNA base composition, DNA–DNA relatedness, quinone systems, cell wall mannans, electrophoretic comparison of enzymes (Lee and Komagata, 1980a, 1990b; Lee and Komagata, 1983), and immunodiffusion with methanol oxidases against antisera to methanol oxidases from reference strains (Lee, 1982). As a result, the yeast strains were classified into four major groups (Komagata, 1991; Lee and Komagata, 1983). Group 1 consisted only of *Candida boidinii*, which is the first methanol-utilizing yeast species described by Ogata et al. (Ogata et al., 1969), and most abundantly isolated from a variety of natural sources. Yeast strains of this group showed a lower DNA base composition compared with other methanol-utilizing yeasts and the presence of Q-7; Group 2 contained strains of a large number of yeast species such as *Pichia angusta*, *Pichia glucozyma*, *Pichia minuta*, *Pichia philodendra*, *Pichia pini*, *Candida cariosilignicola* (Later, this species was reidentified as *Pichia methylivora* by Kurtzman, 1998.), *Candida methanosorobosa*, *Candida succiphila*, etc., and they showed the presence of Q-7; Group 3 comprised strains only of *Pichia methanolica*, and indicated the presence of Q-7 and Q-8 with a ratio of approximately 1:1; and Group 4 consisted of strains of *Pichia capsulata* and *Pichia pastoris*, and showed the presence of Q-8. Almost all of the known methanol-utilizing yeasts examined so far assimilated pectin from which methanol may be formed by hydrolysis of pectin. From this point of view, methanol-utilizing micro-organisms may play an important role in the decomposition of plant materials and the carbon cycle in nature.

Systematics of coryneform bacteria

Coryneform bacteria were isolated from rice and the materials taken at the Japanese petroleum and natural gas fields in the mid-1960s. At that time, the systematics of coryneform bacteria was ambiguous, and the isolates had to be determined only on the basis of phenotypic characteristics. The systematics of coryneform bacteria relied upon particular characteristics that were then emphasized to establish the genera. For example, the genus *Corynebacterium* was established on the basis of pathogenicity, the genus *Microbacterium* on the production of lactic acid, the genus *Cellulomonas* on the decomposition of cellulose, and the genus *Arthrobacter* on its soil habitat. However, little attention was paid to coryneform bacteria in applied microbiology.

Microbial production of glutamic acid

In 1957, Japanese microbiologists, Dr Shukuo Kinoshita and his co-workers, reported the isolation of a bacterium that produced glutamic acid directly from glucose and accumulated a large amount of this amino acid in culture medium, and named this bacterium *Micrococcus glutamicus* (Kinoshita et al., 1957; Kinoshita et al., 1958). Because glutamic acid production became very important commercially in Japan, some background will be provided about the development of this industry. The Japanese have widely used a kind of sea tangle, *Laminaria*, "kombu" in Japanese, as an umami seasoning for a long period of time. In 1908, Professor Kikunae Ikeda at the University of Tokyo crystallized a substance with the flavor of "kombu", and identified it as monosodium glutamate. After this, sodium glutamate was commercially produced by the hydrolysis of wheat protein, followed by the hydrolysis of soybean protein. This flavoring material was sold in a chemically pure state. However, the raw materials were not easily obtained just after World War II because they had been imported. Therefore, Japanese workers attempted to produce glutamic acid from other raw materials, and they succeeded in producing glutamic acid from glucose by employing glutamic acid producing bacteria in 1957. This success was a global breakthrough toward the commercial production of amino acids by using microorganisms. In addition, the L-isomer of monosodium glutamate tastes good, but the D-isomer does not. Fortunately, glutamic acid producing bacteria produce only L-glutamic acid. Currently, glutamic acid is produced by glutamic acid producing bacteria in modern industrial lines over the world. Indeed, the annual production worldwide is estimated at more than 2,000,000 tons.

Studies of the production of glutamic acid have been undertaken from the viewpoint of biochemistry, genetics, regulation of metabolism, and other aspects. The microbial production of lysine and other amino acids was achieved by employing mutants in which part of the metabolic pathway was blocked. The extensive application of glutamic acid producing bacteria is a beautiful success of application of

coryneform bacteria. Microbial production of amino acids and nucleotides is a significant achievement of modern Japanese biotechnology.

Soon after Kinoshita's reports, a considerable number of glutamic-acid-producing bacteria were isolated and new species were described according to minor differences of phenotypic characteristics. Dr Shigeo Abe and his coworkers reported the characteristics common to glutamic acid producing bacteria (Abe et al., 1967). At that time, the workers emphasized such characteristics as Gram-stain-positive, pleomorphic rods, aerobic, requirement of biotin for the growth, *meso*-diaminopimelic acid (*meso*-DAP) in the cell wall, and high production of glutamic acid from glucose. The presence of *meso*-DAP in the cell wall was only a characteristic from the viewpoint of chemosystematics. Further, glutamic acid producing bacteria were members of the coryneform bacteria. When the microbial production of glutamic acid was being enthusiastically studied in the past 60 years, more than 15 species, and even 5 genera, of glutamic acid producing bacteria were described, but all the species shared most of the same characteristics. This confusion might come from the indefinite systematics of coryneform bacteria. In addition, a problem rose whether glutamic acid producing bacteria were related to *Corynebacterium diphtheriae* pathogenic to humans. Therefore, the systematic study of coryneform bacteria including glutamic acid producing bacteria was a critical and crucial problem not only from the viewpoint of bacteriology, but also from that of the Japanese microbial industry. Thus, I will date back to our early study of the systematics of coryneform bacteria, particularly focusing on the systematic position of glutamic acid producing bacteria.

Grouping of coryneform bacteria reported by Yamada and Komagata in 1972

Approximately 50 years ago, the study of the systematics of coryneform bacteria including glutamic acid producing bacteria was attempted, as many as possible non-pathogenic coryneform bacteria were collected from culture collections and other sources, but only 120 strains were obtained. A number of phenotypic characteristics were investigated, and DNA base composition and the principal amino acid in the cell wall were determined. As a result, it was concluded that a large number of phenotypic characteristics were of little value for the systematics of coryneform bacteria, and named strains did not always exhibit the characteristics in the original descriptions. Therefore, the names had to be ignored, and the actual characteristics of each strain were compared with one another. Chemosystematic data were also recognized as useful criteria for the systematics of this group of bacteria.

The cell division of coryneform bacteria was observed by time-lapse microscopy and microcinematography. Broadly speaking, bacterial cell division was classified into three types. The simple type of cell division was found in the cells of *Alcaligenes faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Bacillus megaterium*, *Bacillus subtilis*, etc.; the snapping type in *Brevibacterium ammoniagenes* (*Corynebacterium ammoniagenes*), *Brevibacterium lactofermentum* (*Corynebacterium glutamicum*), *Corynebacterium diphtheriae*, *Corynebacterium xerosis*, *Microbacterium flavum* (*Corynebacterium glutamicum*), *Micrococcus glutamicus* (*Corynebacterium glutamicum*), etc.; and the bending type in *Arthrobacter atrocyaneus*, *Arthrobacter citreus*, *Arthrobacter globiformis*, *Arthrobacter simplex*, *Arthrobacter tumefaciens*, *Arthrobacter ureafaciens*, *Brevibacterium lines*, *Cellulomonas biazotea*, *Cellumomonas fimi*, "*Corynebacterium hydrocarboclastus*" (*Rhodococcus erythropolis*), *Microbacterium lacticum*, etc. The cell division of coryneform bacteria belonged to either the snapping type (Figure 9) or the bending type, not to the simple type (Komagata et al., 1969).

Further, principal diamino acids in the cell wall were determined, and *meso*-diaminopimelic acid (*meso*-DAP), LL-diaminopimelic acid (LL-DAP) and lysine were found as

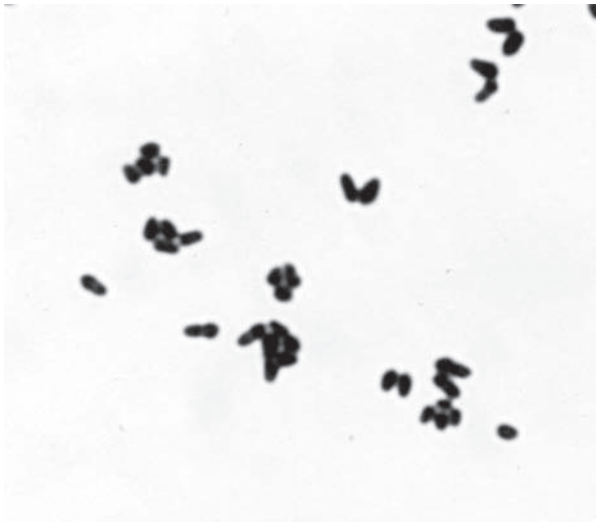

Figure 9. Cells and snapping division of glutamic acid producing bacterium.

the principal amino acids (Yamada and Komagata, 1970a). Further, DNA base composition of coryneform bacteria was found to be widely distributed from 46% to 72%, and the specific range was not recognized for the genera, with the exception of the genus *Cellulomonas* (Yamada and Komagata, 1970b). In addition, phenotypic characteristics of coryneform bacteria were investigated (Yamada and Komagata, 1972a). Finally, coryneform bacteria were divided into

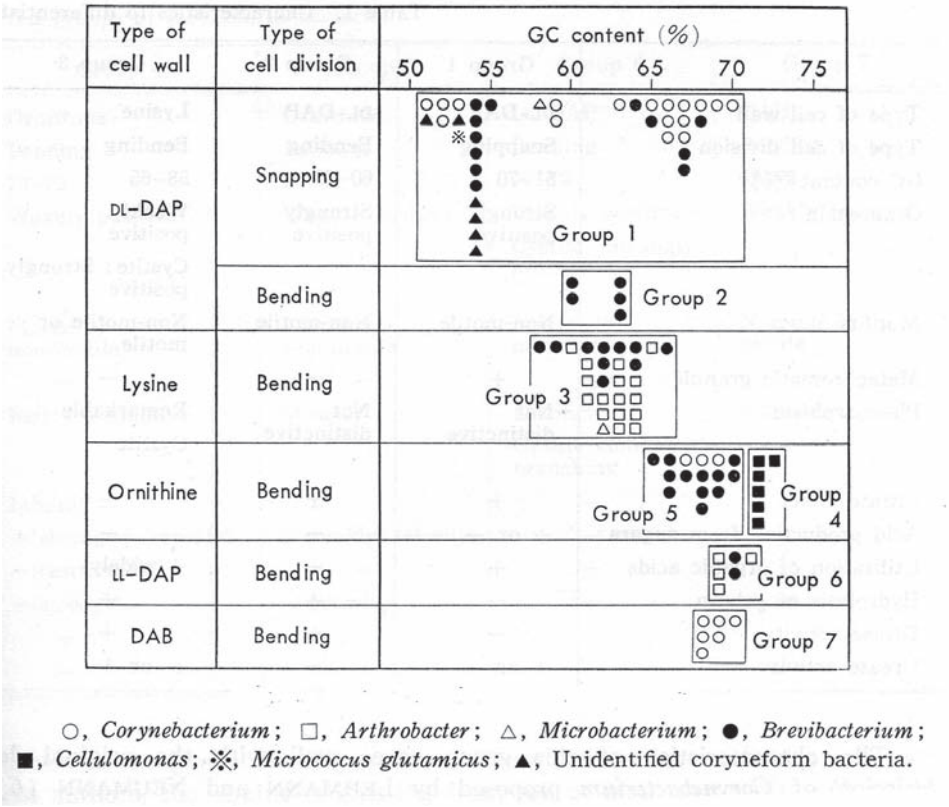

Figure 10. Framework of coryneform bacteria by Yamada and Komagata (1972).

seven groups on the basis of the combination of the mode of cell division, DNA base composition, and the principal amino acid in the cell wall as shown in Figure 10 (Yamada and Komagata, 1972b). Further study made it clear that these groups were comparable with the genera of coryneform bacteria. In addition, named strains were scattered over different sections in this figure. For example, Group 3 was identical with the genus *Arthrobacter*, but included named strains of *Brevibacterium*, *Arthrobacter*, and *Microbacterium*. This told us that chemosystematic data were useful to make clear the relationship of coryneform bacteria. Further, this figure indicates that the framework of the generic concept of coryneform bacteria was drawn with the chemosystematic data. In the light of the data, the genus *Curtobacterium* was established for Group 5. This idea seems likely to be the original framework of the systematics of coryneform bacteria. In fact, new data have been supporting this framework, and the framework has since been improving for a better understanding of coryneform bacteria.

When we were working on the systematics of coryneform bacteria, Professor Karl-Heinz Schleifer and Professor Otto Kandler in Germany reported the murein type of the bacterial cell wall (Schleifer and Kandler, 1972). Their data were very useful and supported strongly the construction of the generic concepts of coryneform bacteria, and some of the principal diamino acids were corrected. Glutamic acid producing bacteria and *Corynebacterium diphtheriae* were reported to have the same murein type. Fortunately, Professor K.-H. Schleifer and Professor O. Kandler employed the same strains as those we investigated. A correlation was found between the groups reported by the murein types reported by them and by us. This finding strongly encouraged because both groups received the same strains with the same origins from culture collections. Therefore, it was possible to directly compare the data obtained by both groups. This emphasizes the importance of culture collections to basic microbiology.

Chemosystematic characteristics of coryneform bacteria

Following DNA base composition, DNA–DNA relatedness was determined for coryneform bacteria, and the bacteria were divided into four groups; group 1 consisted of coryneform bacteria with a glycolyl type of cell wall, group 2 comprised glutamic acid producing bacteria, group 3 composed of *Brevibacterium linens* strains, and group 4 consisted of coryneform bacteria with LL-DAP in the cell wall. Consequently, glutamic acid-producing bacteria differed clearly from other coryneform bacteria. This indicates the heterogeneity of coryneform bacteria (Suzuki et al., 1981). Before

the report of Suzuki et al. (1981), coryneform bacteria and nocardioform bacteria were classified into two groups based on the acyl type, glycolyl type and acetyl type, of the glycan moiety of the cell-wall peptidoglycan (Uchida and Aida, 1977, 1979).

As mentioned above, the cellular fatty acid composition was found to be useful for the systematics of the genus *Pseudomonas* and methanol-utilizing bacteria. Consequently, the composition was determined for coryneform bacteria, and the bacteria were divided into two major groups according to the cellular fatty acid composition. Type I consisted of straight-chain fatty acids, and Type II consisted of branched-chain fatty acids. Coryneform bacteria with *meso*-DAP showed the composition of Type I, and glutamic acid producing bacteria and *Corynebacterium diphtheriae* showed the composition of Type I (Suzuki and Komagata, 1983).

Brevibacterium linens strains had *meso*-DAP in the cell wall and the cellular fatty acid composition was of Type II. The composition differed clearly from those of glutamic acid producing bacteria with the composition of Type I. The cellular fatty acid composition was useful for separating the strains in both *Corynebacterium* and *Brevibacterium*. Approximately 30 years ago, *Corynebacterium* strains were not easily separated from *Brevibacterium* strains by other characteristics. *Arthrobacter*, *Cellulomonas*, and *Curtobacterium* strains have the cellular fatty acid composition of Type II. *Curtobacterium pusillum*, which was isolated from oil brine, produced an interesting fatty acid, ω-cyclohexylundecanoic acid (Suzuki et al., 1981). Phospholipid composition was a good marker for the classification of coryneform bacteria as well as the cellular fatty acid composition (Komura et al., 1975). Particularly, the presence of phosphatidyl ethanolamine (PE) is useful for separating several genera. Glutamic acid producing bacteria contained a trace amount of PE.

A systematic interest of the isomers of 2,4-diaminobutyric acid (DAB) in the cell wall was reported for the genera *Agromyces*, *Clavibacter* and *Rathayibacter*, and the type strains of all the subspecies of *Clavibacter michiganensis* were confirmed to have D- and L-DAB in almost equal proportions in their cell-wall peptidoglycan (Sasaki et al., 1998). This resulted from the employment of an HPLC procedure that separates L-isomer and D-isomer of DAB in the bacterial cell wall. As mentioned above, the development of instrumental analysis would make it possible to identify coryneform bacteria more precisely.

Coryneform bacteria had menaquinone as a major quinone, and approximately eight isoprenologs, MK-8, MK-8(H_2), MK-8(H_4), MK-9, MK-9(H_2), MK-9(H_4), MK-11, and MK-12, were known to this group of bacteria (Collins and Jones, 1981; Yokota, 2001). Glutamic-acid-producing bacteria had MK-9(H_2), and differed from *Corynebacterium diphtheriae* with MK-8(H_2) (Yamada et al., 1976).

Recently, phylogenetic relationships of bacteria are studied on the basis of rRNA gene sequences, particularly 16S rRNA and 5S rRNA. First, 5S rRNA gene sequences of some coryneform bacteria was determined (Park et al., 1987), and they constructed a phylogenetic tree. Subsequently, a more detailed phylogenetic tree of actinobacteria was depicted with 16S rRNA gene sequences, and in this tree, the genera in coryneform bacteria were separated from one another (Suzuki, 2001; Yokota, 2001).

Some arthrobacters have long been known to form cystites, but details have not been clarified. This was due to the lack of experimental reproducibility and growth on a defined medium. Subsequently, a defined medium was developed for the study of cystite formation of *Arthrobacter ureafaciens* NRIC 0157^T (Tanaka et al., 2000). Cystites differed clearly from vegetative cells in cell size, structure of the cell wall, and other features (Figure 11).

A balance of the concentration of magnesium ion and potassium ion induced the formation of cystites. Further, some antibiotics (for example, tetracycline) induced the formation of cystites (Tanaka et al., 2001). Interestingly, many arthrobacters with the A3α-type of cell wall produced cystites, and they were located in the same cluster in the phylogenetic tree based on the 16S rRNA gene sequence. Consequently, a potential morphogenesis would be induced by cultural conditions, and in this case, the induction may reflect their phylogeny (Tanaka et al., 2002).

Improvement of Yamada and Komagata's framework

The generic concepts of coryneform bacteria are well constructed by chemosystematic data. Extensive improvement of Yamada and Komagata's framework has been reported in recent years. The classification of coryneform bacteria has been studied mainly in the light of chemosystematics and phylogenetic information. Glutamic acid producing bacteria are known to be neighbors of *Corynebacterium diphtheriae*, but they differ clearly from one another on the basis of 16S rRNA gene sequences.

Therefore, glutamic acid producing bacteria were included in a single taxon that belonged to the genus *Corynebacterium* according to their chemosystematic data and phylogenetic relationship. Because *Micrococcus glutamicus* had priority over other names of glutamic acid producing bacteria, all the glutamic acid producing strains were identified

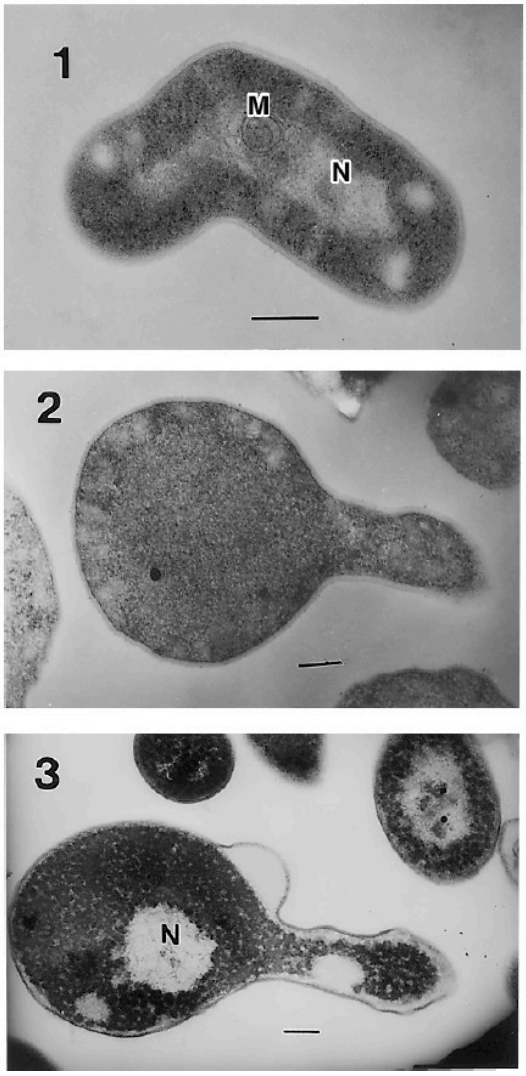

Figure 11. Ultrathin sections of a vegetative cell and cystites of *Arthrobacter ureafaciens* NRIC 0157^T. (1) A vegetative cell grown in YPM liquid medium; (2) a cystite induced in YPM-Tc liquid medium (induced with tetracycline); and (3) a cystite produced in CT medium (induced by nutritional imbalance). Bar markers represent 200 nm. M, mesosome; N, nuclear region.

as *Corynebacterium glutamicum* (Abe et al., 1967; Yamada and Komagata, 1972). This was since confirmed by rRNA gene restriction patterns, DNA–DNA relatedness, and chemotaxonomic data (Liebl et al., 1991).

Value of chemosystematic data for predicting anamorph—teleomorph relationships between the genera *Rhodotorula* and *Rhodosporidium*

The genus *Rhodotorula* is an asporogenic and non-fermentative yeast genus characterized by the production of pink to red pigments. However, strains of this genus have not shown any promise in applied microbiology. Therefore, this genus may be said to be “a genus left behind” or “a genus of little interest” because its merits and demerits have not yet been recognized for humans. Nonetheless, several Japanese researchers became interested in this genus, and taxonomic, physiological, and ecological studies have been reported. Dr Isao Banno observed the basidiomycetous life cycle of *Rhodotorula* yeasts and established a new genus *Rhodosporidium* (Figure 12) (Banno, 1963, 1967), which is noteworthy in yeast systematics.

A considerable number of *Rhodosporidium* species have been since described, but the species were found by chance. As mentioned above, DNA base composition was determined for *Rhodotorula* and *Rhodosporidium* strains, and anamorph–teleomorph relationships were suggested based on DNA base composition. As a result, not only was DNA base composition introduced into yeast systematics, but also quinone systems, carbohydrate composition of cell wall, DNA–DNA relatedness, and electrophoretic comparison of enzymes were also introduced. Therefore, the anamorph-teleomorph relationships of the genera *Rhodotorula* and *Rhodosporidium* were inferred from the combination of chemosystematic data, and decisively confirmed by cell conjugation.

Anamorph—teleomorph relationship between Rhodotorula glutinis var. rufsa and Rhodosporidium toruloides

Rhodotorula strains were classified into four groups based on DNA base composition, and they were Group 1 with G+C content of 66.8 to 68.5 mol%, Group 2 with G+C content of 60.0 to 61.2 mol%, Group 3 with G+C content of 55.4 to 58.8 mol%, and Group 4 with G+C content of 50.0 to 50.7 mol% (Nakase and Komagata, 1972).

The heterogeneity of *Rhodotorula glutinis* gave rise to interests in yeast systematics because named strains of this species and varieties were distributed in three of the groups. Quinone systems of *Rhodotorula* and *Rodosporidium* strains were determined, and the presence of Q-8, Q-9, Q-10, and Q-10(H_2) was also reported in these yeasts. In the light of a common ubiquinone system of Q-9 and similar DNA base composition (approx. 60%) shared between *Rhodosporidium toruloides* strains and *Rhodotorula glutinis* var. *rufusa* RJ 5, Professor Yuzo Yamada and Professor Keiji Kondo pursued and successfully demonstrated the formation of teliospores after cell conjugation of a strain with mating type A of *Rhodosporidium toruloides* (Yamada and Kondo, 1973). As a result, *Rhodotorula glutinis* var. *rufusa* RJ 5 was identified as a strain with the mating type a of *Rhodosporidium toruloides*. This is the first elucidation of an anamorph-teleomorph relationship between *Rhodotorula* and *Rhodosporidium* obtained from chemosystematic data

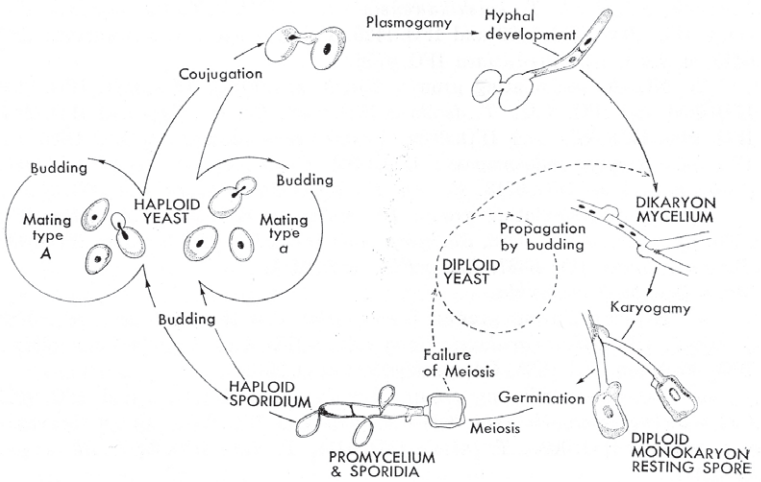

Figure 12. The life cycle of *Rhodosporidium toruloides* (I. Banno, 1967).

and confirmed clearly by cell conjugation.

After this successful study, a relationship of another strain of *Rhodotorula glutinis* var. *rufusa* to *Rhodosporidium* strains was deduced from an electrophoretic comparison of enzymes (Yamazaki and Komagata, 1981). Strains with the mating type of *Rhodosporidium* species indicated mostly similar patterns in the electrophoresis of enzymes such as fructose-1,6-biphosphate aldolase (FA), 6-phsophogluconate dehydrogenase, NAD-dependent malate dehydrogenase, hexokinase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, and glutamate dehydrogenase. *Rhodotorula glutinis* var. *rufusa* YK 117 produced enzyme patterns mostly identical with those of the mating type strains of *Rhodosporidium toruloides*. Considering the electrophoretic comparison of enzymes, DNA base composition, and quinone systems, a cell conjugation experiment was carried out between *Rhodotorula glutinis* var. *rufusa* YK 117 and each of the mating type strains of *Rhodosporidium toruloides*. Confirming the cell conjugation results, *Rhodotorula glutinis* var. *rufusa* YK 117 was identified as a strain with mating type a of *Rhodosporidium toruloides*.

Anamorph—teleomorph relationship between Rhodotorula glutinis var. salinaria—Rhodosporidium sphaerocarpum

A relationship between some *Rhodotorula* strains and *Rhodosporidium sphaerocarpum* was suggested on the basis of DNA base composition, but no strains with the mating type were found in the *Rhodotorula* strains obtained from culture collections and other sources. In contrast, enzyme patterns of 108 *Rhodotorula* and *Rhodosporidium* strains were investigated, and *Rhodotorula glutinis* var. *salinaria* YK 118 and *Rhodosporidium sphaerocarpum* strains were found to produce identical zymograms with all enzymes tested. After a cell conjugation test, *Rhodotorula glutinis* var. *salinaria* YK 118 was identified as a strain with the mating type a of *Rhodosporidium sphaerocarpum* (Yamazaki and Komagata, 1981).

Anamorph—teleomorph relationship between Rhodotorula glutinis var. glutinis—Rhodosporidium diobovatum

A relationship between some *Rhodotorula glutinis* strains in Group 1 of Nakase and Komagata and *Rhodosporidium diobovatum* was suggested on the basis of DNA base composition (approx. 67mol%). The same inference was made from the quinone system (Q-10) and the G+C content, but no haploid strains of *Rhodosporidium diobovatum* were found in *Rhodotorula* strains studied. On the basis of the electrophoretic comparison of enzymes, *Rhodotorula glutinis* var. *glutinis* YK 104 was recognized to produce identical zymograms with those of *Rhodosporidium diobovatum* strains. After the cell conjugation test, *Rhodotorula glutinis* var. *glutinis* YK 104 was identified as a strain with the mating type α of *Rhodosporidium diobovatum* (Yamazaki and Komagata, 1981).

Furthermore, *Rhodotorula glutinis* strains that were similar to *Rhodosporidium diobovatum* with respect to an identical ubiquinone system (Q-10) and similar G+C contents (approx. 67mol%) attracted attention, but these strains produced slightly different enzyme patterns from those of *Rhodosporidium diobovatum*, although the strains produced two enzymes, aldolase and 6-phosphogluconate dehydrogenase, with the same relative mobility (Rm). Possible haploid

mating partners were studied by the cell conjugation test, and *Rhodotorula glutinis* YK 108 was finally identified as a strain with the mating type α of *Rhodosporidium diobovatum* (Hamamoto et al., 1984). *Rhodotorula glutinis* YK 108 showed rather high DNA–DNA relatedness with the type strain of *Rhodosporidium diobovatum*. Thus, *Rhodotorula glutinis* strains in Group 1 of Nakase and Komagata were probably the haploid mating strains of *Rhodosporidium diobovatum*.

According to the chemosystematic studies of the *Rhodotorula* and *Rhodosporidium* strains, a considerable number of strains with the mating type of the *Rhodosporidium* species were presumed in the *Rhodotorula* strains, and their mating types were decisively determined by cell conjugation. In modern yeast systematics, one of the most interesting targets of study is to find the teleomorph for anamorphic strains. The combination of chemosystematic data would be useful for searching for haploid teleomorphic strains among the putative anamorphic yeast strains. The discovery of new teleomorphs is very likely based on the above-mentioned findings, and yeast systematics will continue to flourish based upon a consideration of information from various sources including chemosystematics, cytology, phylogeny study, and other related studies of modern biology.

Other studies

In addition to the above-mentioned studies, bacterial systematics was carried out on the genera *Planococcus* and *Marinococcus*, *Pseudomonas* species, the genus *Rubrobacter*, and the genus *Sporolactobacillus*.

During the study of frozen food, several motile cocci were isolated from frozen boiled shrimp and prawn that were identified as a new species, *Planococcus kocuri*, based on chemosystematic data (Hao and Komagata, 1985). Also, halophilic motile cocci with *meso*-diaminopimelic acid in the cell walls were identified as a new genus *Marinococcus*, and *Marinococcus albus* and *Marinococcus halophilus* were described (Hao et al., 1984). Chromogenic pseudomonads isolated from rice were reidentified as *Pseudomonas oryzihabitans*, and isolates from clinical specimens as *Pseudomonas luteola* (Kodama et al., 1985). These species were included in the chromogenic group of the genus *Pseudomonas*. Aromatic compound-decomposing *Pseudomonas desmolytica* and *Pseudomonas dacuhnae* strains were reclassified into *Comamonas acidovorans* and *Comamonas testosteroni*, and they were included in the achromogenic group of the genus *Pseudomonas* (Tamaoka et al., 1987). *Arthrobacter radiotolerans* was reidentified as a new genus *Rubrobacter*, and new species *Rubrobacter radiotolerans* was described based on radiotolerance and chemosystematic data (Suzuki et al., 1988). Since a few strains of the genus *Sporolactobacillus* were known, a number of new strains of this genus were isolated from soil and other materials, and they were classified into four new species (Yanagida et al., 1997). Further, a new method for determination of DNA base composition was developed, making it possible to determine the composition accurately and quickly (Tamaoka and Komagata, 1984).

Figure 13. K. Komagata (left) and Dr T. Hasegawa.

As mentioned above, no single chemosystematic approach is applicable to microbial systematics, however, a combination of different approaches is rational and useful.

Japan Collection of Microorganisms, Institute of Physical and Chemical Research

In 1981, I was appointed Director of the Japan Collection of Microorganisms (JCM) at the Institute of Physical and Chemical Research, and served concurrently as professor for the Institute of Applied Microbiology, the University of Tokyo.

In 1971, the Japanese Government decided to promote research in the life sciences and to establish a culture collection of micro-organisms. In the light of the official request, JCM was established in 1981 at the Institute of Physical and Chemical Research. My task was to build up the foundation and development of the culture collection, advocating “research and culture collection”. Thanks to staff and officials, JCM has grown into one of the leading culture collections in the world. For my entire career, I learned much of systematics, and philosophy and practices for the management of culture collections from Dr Takeji Hasegawa who was a former director of the Institute for Fermentation, Osaka. I am grateful for his many kindnesses (Figure 13).

Figure 14. Visit of Professor K. Arima to the Japan Collection of Microorganisms. From left: K. Komagata and Professor K. Arima.

The International Code of Nomenclature of Bacteria requires that the type strain of a new species be deposited in at least two established culture collections in different countries. Bacterial systematics would not be achieved without reliable and permanent culture collections. Further, micro-organisms are widely used for biological science, and new advances in biochemistry, genetics, and molecular biology are essentially due to the study of micro-organisms as model forms of life. Countless numbers of microbial strains are isolated during the study of general and applied microbiology, and the attributes of a large number of strains are improved. Therefore, reliable culture collections are needed as the depository and for the further study and application of the cultures. In addition, the culture collection should play a role in off-site (*ex situ*) conservation of microbial diversity (Figure 14).

Chemosystematic data and sequence data of nucleic acids of micro-organisms appear to be "finger prints", and are reliable for the characterization of individual microbial cultures. In addition, the data are useful for the management of the culture collection. I retired in 1989 from JCM at the official retirement age.

The World Federation for Culture Collections (WFCC) and the World Data Centre for Microorganisms of the WFCC (WDCM) have functioned as the international organizations relevant to the culture collection. WDCM was relocated from Queensland University, Australia, to the Institute of Physical and Chemical Research in 1986 (Komagata, 1987), and I served as director for WDCM (1989–1989). Further, I served as board member for WFCC (1981–1988). The Japan Federation for Culture Collections (at present Japan Society for Culture Collections, JSCC) has played a role in development and promotion of the culture collection in Japan. I served as president (1981–1988) and board member for JSCC, and presented the plenary lecture entitled "Milestone in Japanese Culture Collections" at the 10th International Congress for Culture Collections in 2004 (Figure 15). I frequently visited culture collections in Southeastern Asian countries, and encouraged students and personnel at the culture collections for developing the collections in the countries and building up networks among the collections.

Figure 15. The plaque of the WFCC. Professor M. Watanabe, the President of ICCC-10 (left), and K. Komagata (right). Professor H. Iizuka, the president of ICCC-1, prepared the plaque, designed with a ginko leaf that was made of a piece of 800-year-old ebony. He suggested passing the plaque to the presidents of the future ICCCs, and the plaque is in the office of a new president.

Micro-organisms are not only of value for the production of useful substances but they also play unique and important roles in elemental cycles with plants and animals. Broadly speaking, humans depend on individual micro-organisms in biotechnology and diverse ecosystems on earth. Micro-organisms are also a source of significant gene pools, and these gene pools must not be lost in this or later generations. From this point of view, micro-organisms can be regarded as a cultural heritage and a cultural property common to all humans. Therefore, micro-organisms must be transferred to future generations in normal and healthy conditions. From this point of view, culture collections play decisive roles in science and the conservation of biodiversity on earth.

Laboratory of Microbiology, Tokyo University of Agriculture

After retirement from the University of Tokyo and the Institute of Physical and Chemical Research, I moved to Tokyo University of Agriculture in 1992, which is a private university relevant to agricultural science, and has a long history of more than 100 years. I engaged in education and research of microbiology at the laboratory of microbiology, and worked in a lively and delightful atmosphere thanks to Professor Michio Kozaki and Professor Tai Uchimura. I retired from Tokyo University of Agriculture in 1999 at the retirement age.

Study of acetic acid bacteria

One of my research interests was the study of acetic acid bacteria because systematics of acetic acid bacteria in a modern sense was initiated by Professor T. Asai in the mid-1930s in Japan (Asai, 1935), and I worked for him in the mid-1960s (Asai et al., 1964). He was interested in isolation sources of acetic acid bacteria, and used a variety of fruits including kaki (persimmon), date, peach, Japanese pear, citrus, strawberry, cherry, apple, apricot, grapes, fig, and pineapple. After detailed study, he classified the acetic acid bacteria into two genera, *Gluconobacter* and *Acetobacter*. The genus *Gluconobacter* consisted of acetic acid bacteria isolated from fruits and oxidized glucose more vigorously than ethanol, and the genus *Acetobacter* comprised the bacteria isolated from vinegar materials and oxidized ethanol more actively than glucose. The two genera also differed clearly from each other in oxidation of acetate and growth temperature range as well. This study shows two important things. One is the establishment of the genus *Gluconobacter* based on biochemical characteristics in the mid-1930s, and the other is the focus on the isolation sources of the bacteria other than vinegar and related materials. Following Asai’s studies, Japanese microbiologists, Professor Keiji Kondo and Professor Minoru Ameyama, extended the source habitat from fruits to flowers, and named the isolates from lily *Acetobacter aurantium* (Kondo and Ameyama, 1957). Later this species became a basis of the creation of the genus *Fateuria* (Swings et al., 1980). Further, Professor Yuzo Yamada in Japan introduced quinone systems into systematics of acetic acid bacteria in the late 1960s (Yamada et al., 1969), and Professor Jozef De Ley and his group in Belgium reported numerical analysis of acetic acid bacteria (Gosselé et al., 1983a; 1983b). Recently, acetic acid bacteria are phylogenetically included in the family *Acetobacteraceae* in the class *Alphaproteobacteria* and the genus *Frateuria* in the class *Gammaproteobacteria*, and 13 genera (including the genus *Frateuria*) have been described.

Since 1964, my laboratory has been studying the bacterial flora of acetic acid bacteria in Southeastern Asia and Japan, and described so far two new genera *Asaia* (Yamada et al., 2000) and *Kozakia* (Lisdiyanti et al., 2002), and, in addition, five new species and three new combinations in the genus *Acetobacter* (Lisdiyanti et al., 2000, 2001), one new species in the genus *Kozakia*, and five new species in the genus *Asaia* (Yamada et al., 2000; Katsura et al., 2001; Suzuki et al., 2010). Further, the genus *Acidomonas* was emended (Yamashita et al., 2004), and synonymy of *Gluconobacter asaii* as *Gluconobacter cerinus* (Katsura et al., 2002), identification of *Frateuria* strains isolated from Indonesian sources (Lisdiyanti et al., 2003), diversity of acetic acid bacteria in Indonesia, Thailand, and the Philippines (Lisdiyanti et al., 2003), reclassification of *Gluconobacter hansenii* strains (Lisdiyanti et al., 2006), and other related studies have been reported.

Establishment of the genus Asaia

During the isolation of acetic acid bacteria from Indonesian sources, eight interesting bacterial strains were isolated from flowers and glutinous rice. The strains produced acid from glucose but not from ethanol, and did not grow in the presence of 0.35% of acetic acid. Furthermore, they were phylogetically included in the family *Acetobacteraceae*. Therefore, I regarded these strains as acetic acid bacteria, even though they did not produce acid from ethanol. This is very unusual in acetic acid bacteria. Subsequent studies showed that the strains were sufficiently unique to justify the naming of a new genus. The genus was named the genus *Asaia* in honor of Professor T. Asai, and the type species was named *Asaia bogorensis* of which specific epithet was derived from Bogor, Indonesia where the type strain of the species was isolated (Yamada et al., 2000).

Strains of the *Asaia* species were since isolated from flowers and fruits in tropical countries, and the genus *Asaia* had been assumed to be a genus of the acetic acid bacteria with specific niches in the tropics. Therefore, my laboratory extended the sites of isolation of *Asaia* strains from Southeastern countries to Japan, which extends approximately 3000 km from the north to south. Isolation sites covered a range from the northern area, Hokkaido, to the southern area, Okinawa, and 345 strains were isolated from 776 samples consisting of 684 from flowers, 56 from fruits, 30 from fermented foods, and 40 from other sources. Two media were used, one of which was designed for isolation of *Acetobacter* strains and the other was suitable for isolation of *Asaia* strains. The isolates were classified into genera, and *Asaia* strains were most abundantly isolated, followed by *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* strains, and a few strains were of *Frateuria* and *Saccharibacter*. No *Acidomonas* and

Figure 16. Professor K. Sakaguchi and his fellow pupils. Front from left: Professor Hiroshi Iizuka, Professor Kin-ichiro Sakaguchi, Professor Yonosuke Ikeda, and Professor Kei Arima. Back from left: Professor Teruhiko Beppu, Professor Kazuo Komagata, Professor Toshimasa Yano, Professor Gakuzo Tamura, Professor Ko Aida, Professor Keiji Yano, Dr Kenji Sakaguchi, Professor Hiuga Saito, and Professor Yasuji Minoda.

Kozakia strains were obtained. Also, the *Asaia* strains were isolated from Akita, Niigata, Tokyo, and Yamanashi located in the temperate zone in Japan. In addition, three new species were described based on the characterization of isolates from flowers collected in the northern area (Suzuki et al., 2010). Consequently, the genus *Asaia* is not an acetic acid bacterium whose distribution is limited to tropical countries, but can be regarded as a cosmopolitan bacterium. This indicates that a variety of isolation sources and enrichment media are essential for the study of distribution of certain micro-organisms.

More recently, *Asaia* strains were reported to be isolated from the peritoneal dialysis fluid of a patient with a medical history of end-stage renal disease and secondary diabetes (Synder et al., 2004), and from blood of a man with a history of intravenous drug abuse (Tuuminen et al., 2006). Further, *Asaia* strains were isolated from an Asian malarial mosquito vector, *Anopheles stephensi* (Favia et al., 2007). According to our research, the finding of *Asaia* cells in adult mosquitoes is logical because *Asaia* strains are widely distributed in nectar-bearing flowers. When adult mosquitoes visit flowers to collect nectar, lay eggs, hatch larvae, and then new offspring come into being, young mosquitoes will suck up the nectar. As a result, *Asaia* cells will move to the organs of the mosquitoes during their life spans. Therefore, members of the genus *Asaia* are important to microbiologists for their taxonomic interest and ecological behaviors. In addition, besides *Asaia* strains, the isolation of *Acetobacter* and *Gluconobacter* strains from clinical specimens was newly reported (Alauzet et al., 2010).

Other studies

A facultative anaerobic sporeforming xylan-digesting bacterium, which lacks cytochrome, quinone, and catalase, was isolated and identified as a new genus and new species *Amphibacillus xylanus* (Niimura et al., 1990). Also, the characterization of *Bacillus brevis* strains, which were known protein producers, resulted in the establishment of the genera *Brevibacillus* and *Aneurinibacillus*, and several new species on the basis of numerical analysis of phenotypes, DNA base composition, DNA–DNA relatedness, cellular fatty acid composition, quinone systems, and immunological analysis (Nakamura et al., 2002; Shida et al., 1994a, 1994b, 1995, 1996a, 1996b, 1997a, 1997b; Takagi et al., 1993). Furthermore, a method for the determination of isomers of lactic acid with HPLC was developed, and has been used for systematics of lactic acid bacteria (Manome et al., 1998). In additions, papers relevant to lactic acid bacteria were reported (Iino et al., 2001, 2002, 2003a, 2003b; Sakamoto et al., 1996a, 1996b, 1998; Tanasupawat et al., 1988, 1991, 1992a, 1992b, 1992c, 1992d).

“Weaving threads into cloth”

As mentioned above, microbial production of amino acids originated in Japan, and its success is in great part due to the study of traditional foods and fermentations. In other words, such practical studies led to the stimulation of basic research, while new findings in basic research brought about ideas for new fermentations and new biotechnologies. Further, harmonization between basic research and application should be emphasized. This can be said: There are no walls between basic microbiology and the application of micro-organisms. This thought stems from the philosophy of Professor K. Sakaguchi. I express my cordial appreciation to my mentors, professors, and seniors for their mentorship, encouragement, and kindness, and I am also grateful to colleagues, students, fellows, and others for their cooperation and friendship. Further, I am profoundly grateful to my wife, Tadako Komagata, and my family for their understanding and cooperation with my work. Also, I thank my brother, Yasuo Komagata, and his wife, Kiyoko Komagata who helped me spiritually and financially for a long time because my father was sickly at later stages in life.

The combination of chemosystematic data, phylogenetic information, and the use of unique isolates has led to great progress in modern microbial systematics. With the accumulation of more detailed data and new isolates, the bacterial species and generic concepts will be better established, and bacterial systematics will become much more precise and much more conclusive. This is why microbial systematics is analogous to “weaving threads into cloth”.

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My career as a curator of microbial cultures

Tian-shen Tao

I was born on Hanzheng Street, Hankow, China, on 20 February 1934. Hankow is now combined with the cities Wuchang and Hanyang and is named Wuhan, which is located on both the Yangtze and Han river banks. I spent my childhood on Hanzheng Street until the age of four. Then, because of the war with Japan, my family decided to move to Chongqin, the largest city in southwestern China, which was the Provisional Capital of China, during the anti-Japanese war. This was before the Japanese occupation of Wuhan during October 1938.

My father was a lawyer. Besides his general legal activity, he was the legal adviser for the Italian Consulate in Hankow. Unfortunately, he suffered septicemia after a surgery before antibiotics such as penicillin were discovered. He passed away in 1936 and left behind his wife, two daughters and four sons. I am the youngest in my family (Figure 1). So, I have three older brothers and two older sisters. The eldest child is the daughter of my father's first wife. She married at the time the Japanese war began.

My mother was a housewife although she was educated in Nanking and Shanghai, respectively. My father's death left my mother deeply broken-hearted. For that reason, my mother hoped that one of her children would become a doctor or pharmaceutical maker. This is the one of reasons I chose to study bacteriology for my life's profession.

My grandfather on my father's side was a general manager of banks and also a personal investor. He owned property and houses but he did not pay much attention to the education of his children. But, my father studied and worked hard which had a great deal to do with his successful career.

My grandfather on my mother's side was a district official during the latter part of the Qing Dynasty. He also was a Chinese painter of pleasant landscapes. I saw one of his paintings hanging on the wall of the Yekaitai drugstore (now, the Jianming Pharmaceutical Factory). He also knew traditional Chinese medicine, but it was a hobby not a profession for him. He was familiar with the educator Chen Shi

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Figure 1. A recent photo of Tian-shen Tao.

(1891–1953), who established the first private university, Zhonghua University in Wuchang, which is now combined with Huazhong University and named Huazhong Normal University. So, my aunts and uncles studied at the Zhonghua University in Wuchang and in Chongqin in the 1930s and 1940s.

As the Japanese invading army drew near Wuhan, in October of 1938, my mother, who had learned about the shocking Nanking massacre, decided to move our entire family to Sichuan, where my maternal grandmother's family had moved to in advance. Shortly after my mother and uncles made funeral arrangements for my grandfather, we left for Sichuan in a cautious manner. We went to Shashi and Yichang by steamship, and stopped over 1 or 2 weeks to wait for tickets. At that time, the war situation was very dire. We could not buy steamship tickets from Yichang to Wanxian, and therefore had to take a large wooden boat, which my mother's relative used for the transport of press packing cotton. Luckily, the boat could accommodate some passengers and the boat's boss with his wife and boatmen were kind to us. However, the stretch from Yichang to Wanxian, was treacherous all the way, passing through the three gorges of the Yangtze River and battling against the water current with a wooden boat was very difficult and dangerous. The boat had a sail. If there was no wind, the boatmen had to rock the scull hard to move it forward. Moreover, when encountering a dangerous shoal, the passengers had to leave the boat to decrease its weight and walk along with the boat.

Most of the boatmen also disembarked to tow the boat along. The voyage reminds me of the well known oil painting, *The Volga Boatmen* by the Russian painter Rebin. The boss's wife rode the stalk of the boat's helm with her legs standing on the stern and regulated the direction of forward motion by moving her legs slightly. Moreover, she also played a drum with one hand and waved a small flag with the other to direct the boat crew how to go left or right. Sometimes, when facing a very dangerous shoal, the passengers had to leave the boat to decrease its weight and walk along with the boat. My oldest brother who was 17 often carried me on his back when we were following the boat. Sometimes, the boss allowed the old and weak to remain on the boat when passing less dangerous shoals. Everything was done to ensure the safety of passengers and the crew.

Late in autumn of 1938 we arrived in Wanxian of Sichuan and remained there for several days. Finally, we went by steamship to Chongqing where my aunts and maternal uncle were waiting for us. We rented a residence located in Juelingshi on the south bank of the Yangtze River, Chongqing. This was near the campus of Zhonghua University. The notable sinologist, Professor Lu Run-ju, lived next door and the youngest son of the president of the University lived upstairs. The Japanese air force often bombed Chongqing, particularly on the north bank of the Yangtze River, the location of the principal urban district of Chongqing. Sometimes, the fire bombs led to a day-long conflagration. Although we lived in the south bank urban district, in order to avoid the bombing, we often hid in a bomb shelter of the University or went to an American agency to seek asylum. I do not remember its exact name, but on the Yangtze River there was an American warship that had an American flag stretched on its deck to deter Japanese aviators from targeting it.

The frequent bombing raids appalled and worried the residents of Chongqing. Therefore, the city designed and hung big balloons on the roof of the Bank of Meifeng which was the tallest building close to the Yangtze River. A yellow balloon provided a preliminary warning to the residents to prepare protective measures, a red one announced the imminent arrival of hostile aircraft, and a green balloon informed residents they could safely return home.

One day, a bomb hit the building near our housing. Fortunately, we had gone to the bomb shelter of the University, however, when we returned, our apartment had collapsed by the shock wave from the bombing. My mother quickly asked my aunt to bring me to the school where she taught and my mother and my second eldest brother rescued the vestigial materials from the ruins. Prior to the attacks, my oldest brother had to discontinue his studies for a job at Tianfu Company. After learning our housing was destroyed by a bomb, my oldest brother immediately returned, and then our whole family moved to Tianfu away from the city district of Chongqing. In Tianfu, we resided in the living quarters for staff. From then on, I spent my childhood and studied in primary school there.

The Tianfu Company is a coal mine located in Beipei, a special district of Chongqing. The mining region is located in a long canyon. Originally, it was an ordinary mine, until the outbreak of the war with Japan. Then, the mine equipment lines moved from the east and middle mines located in Tianfu. Also, many scientific and technological personnel also arrived in Tianfu to make it become a primary coalmine using mechanized coal mining. At that time, the famous national capitalist, Lu Zuo-fu (1893–1952) was the director, and the notable coal-mining engineer, Sun Yue-qi (1891–1995) was the general manager, who had been the former vice-chairman of CPPCC (Chinese People's Political Consultative Committee). The famous geologist, Dr Weng Wen-hao (1889–1971), the former director of the National Resources Commission often consulted at Tianfu. Moreover, many dignitaries such as Dr H.H. Kun visited the Tianfu Company. Once when there was a performance in Tianfu Square for the celebration of Chinese Lunar New Year, Ju Zheng (1876–1951), the former president of Judicial Yuan was asked to make a speech. He said: "We are fighting the Japanese invader, we fight with iron, we fight with coal." I have always remembered it, although I was very young. It means that science and technology are becoming great powers for a country. At the same time, many engineers and technicians, such as coalfield, electric, coal transportation engineers and coalfield geologists worked in Tianfu, which became an academic and technological center, which was an especially appropriate place for me to grow up.

Early education

I began primary school in 1940. I studied for 4 years in Wenxinchang primary school near my family housing. Then I studied in the Tianfu Company's primary school for 2 years. This was during the time when World War II was raging. At that time, goods and materials were in shortage. For example, the paper used for newspapers was very rough and crisp. Sometimes, a grass blade fell from the page and removed several Chinese characters. Luckily, basic food staples were sufficient to support daily life. Many farm produce and related products came from Sichuan, a land of abundance, which profited well during the war. Moreover, the Tianfu Company paid good salaries and benefits for their staff. At that time, the livelihood of our family was supported entirely by my oldest brother's income. My second eldest

brother supported himself elsewhere in Sichuan with a part-time job that also allowed him to study.

One day, a lot of photos were displayed on the walls of the Wenxinchang Country Government building. They showed the miserable sight of London after it was bombed. This shocked me very much. Merely, the protracted length of the war made us anxious. How we looked forward to the end of the war so we could return to our former residence in Hankow.

In April 1945, the president of the United States of America, Franklin Roosevelt, passed away. The Tianfu junior school held a memorial meeting. Every Boy Scout held the crabstick (the Boy Scout's "sign") with his right hand and marched around the stadium to express our mourning. This was my first participation in a grand memorial.

During summer vacation of my fifth grade in primary school, my third eldest brother ran home to tell me that Japan announced its unconditional surrender. The war was over! This followed the atomic bomb explosions on Hiroshima and Nagasaki in Japan, and the entry of the Soviet Union Army to the Northeast of China to fight against the Japanese invading army which caused major problems for the invaders. These events indicated that the war would be over soon. However, events happened so quickly that people were at a loss as to what to do. After we celebrated the end of the Japanese war, people from the east and middle regions of China urgently wanted to return home from Chongqing. But the communication media were not providing essential information. Also, bus and ship tickets were rare. People had to wait for a long time to buy a ticket. As a result, our family came back to Hankow at different times. First, my second eldest brother thumbed a lift with my uncle and he arrived to Hankow in the winter of 1945 and he got a job at once. After that, he and my uncle and my cousins began to discuss how to divide my grandfather's inheritance, which caused a lot of problems. My mother, eldest brother and I remained in Chongqing a while longer.

The consequences of the return to Hankow affected the daily lives and education expenses, especially for the youngest members of our family, including my third eldest brother and my second eldest sister. When our entire family returned to Hankow, my oldest brother lost his job in Tianfu Company that supported our entire household for many years with its good salary and benefits. In contrast, in Hankow, all of expenses for daily life and education were different from Tianfu.

After I finished primary school, in the autumn of 1946 I attended Daxiong High School, which was established by a famous Buddhist figure, Tai Xu Buddhist monk (1889–1947). The school was in a distant suburb of Chongqing and was surrounded by mountains and trees with a bluish sky. There were many tall, large gingko trees and the teachers had excellent educational ideas for studying science and English.

More than 1 month later, my oldest brother got two ship tickets to Shanghai for my mother and me to return to Hankow. We embarked on a ship named Meijiang, which was an iron barge that had been refitted from loading goods to carrying passengers because of the need to accommodate all the displaced war refugees. We waited for the barge to be rebuilt for days before we could board. The rebuilt barge without propulsion was parallel trussed with a landing craft that was loaded full of weapons. In fact, it was evidence of an ominous omen, namely, that the civil war would begin soon, however, my mother and I, as a child, were unaware. It was not surprising that we could not buy ship tickets to return to our native place. Later we learned that most transportation companies were required to transport troops and weapons to the east and middle regions by the Kwamintang Government.

When we arrived in Hankow, it was 11 November 1946. I was sent to a school named Enan High School in Wuchang to continue my junior study. My mother met many of her relatives when we returned to Hankow. She played Mahjong almost every day with them, while I spent my holidays watching movies, Chinese and American films. One semester later, I was transferred into the First Male Hankow High School for study. The schoolmaster Du Ze-yao was one of Xiong Room's disciples. A Xiong Room's disciple refers to someone who had studied under the philosopher, Xiong Shi-li (1885–1968), who had three students, Tang Jun-yi, Mou Zong-san, and Xu Fu-guan. They are the representatives of Neo-Confucianism who are well known in Western countries.

The civil war from 1946 to 1949 became more and more intense and the American General Marshall's mediations between Kuomintang and the Communist Party ended in failure. The civil war occurred during my study in junior high school at the same time from 1946 to 1949. Also, the armaments buildup in the military of the People's Liberation and Kuomintang armies occurred at that same time. These events were shocking to the city residents and the leaders of my school. Therefore, the school arranged for early graduation of the students ahead of the holidays. As a result, I received my diploma for finishing junior high school about April 1949. The Kuomintang army retreated from Wuhan to the South of China. Finally, the People's Liberation Army (PLA) entered Wuhan without any obstruction. The resi-

dents in Hankow warmly welcomed PLA. My third older brother and I stood on the front gate of the Salt Industrial Bank of Hankow and watched the PLA march down one of most magnificent streets in Hankow.

Thus, I was quickly transformed from a capitalistic world to a socialistic world on 16 May 1949. Meanwhile I spend a very long summer vacation in 1949. In the fall I passed an examination and entered the Wuhan First Male High School which had been previously named the Hankow school.

The new schoolmaster, Mr Jiang Jia-rui originally taught mathematics and his son, Professor Jiang Din-xian, is a notable composer who made incidental music for the famous chef-d'oeuvre, "Kangding Love Song". Then Mr Jiang Jia-rui accepted another position as head of Wuhan's Research Institute of Culture and History. Mr Zhang Wei-zhi served as the new schoolmaster for our high school. He was an outstanding speaker. When he spoke, the audience was always large. He was regarded as a gentleman. For example, when he was near others who were talking in a group he always kept his distance. Later, he became chief of the Wuhan city educational bureau.

Before liberation, the school provided foreign language lessons in English with six lessons a week. Then, after liberation, the students could select English or Russian as their foreign language course. My oldest brother advised me to select English. He recommended that I continue my English study which began in junior high school through senior high school. Now, I realize he was right. Some of my classmates selected Russian. After that, we went to study in different universities, but all of us were required to study Russian as a foreign language, starting with the Russian alphabet. So, from then on, I neglected English until I graduated from college, I resumed my study of English again while I was a teaching assistant. I am recounting my experience of my foreign language education because I believe it was crucial in aiding my understanding of the full meaning, such as when I read reviews on microbiology.

I had many excellent, bright, and healthy classmates in senior high school. When we took the university entrance exam, most of my classmates selected engineering as their major, whereas only two boys including me selecting biology. I graduated from senior high school in 1952.

University education

In 1952, when the implementation of the First Five Plan began in new China, and the country decided to speed up and increase the level of personal training. In order to promote the development of higher education, universities and colleges were reorganized using a list of priorities. First, many universities and colleges were combined with others or some sections of a university isolated as independent universities or colleges, for example, the Agricultural College in Wuhan University was combined with Hubei Agricultural College as a new college named Huazhong Agricultural College (now, Huazhong Agricultural University). Second, in order to quickly train personnel, the education system was modeled on that of the Soviet Union. And in the same year, higher education initiated a system of uniform examination and selection of their major and university in a uniform manner. All university students enjoyed public support including free tuition, boarding costs, and accommodation. However, the candidates could not choose discretionally the university they attended or their major. The candidates could be assigned to a university and a specialty. Each candidate could select three universities and three specialties in order of priority. Then, after examination, based on test performance plus the candidate's behavior in senior high school, as well as the above selection, the candidate was assigned to a specialty and university. I requested and was selected for Huazhong Agricultural College in the specialty of soil science and fertilizer, which was my first preference, and I was glad to begin my university life.

Ms Chen Wen-xin, who was a student of Professor H.K. Chen, was assigned as our tutor when I was a freshman. She was very kind and encouraged self-development for all her students. This deeply influenced me. Later she went to the Soviet Union for further study. She became a notable taxonomist on systematics. Later, she was always concerned about me and my wife, and encouraged us in our careers. My wife and I regarded her as a teacher and a friend.

Therefore, the new education system with specialty majors replaced the old system. As a result all of the courses were arranged by the school so the students could not take elective courses. For our specialty of soil science and fertilizer, freshmen were taught Chinese modern history as a political course, and general chemistry, general physics, and botany as basic courses. In addition, Russian was taught as the foreign language and physical training was a common course.

Although I was a quiet student, occasionally, I played table tennis or badminton with my classmates, but they usually won. The first year, we studied on the campus of Wuhan University with its beautiful surroundings including Luojiashan, a low mountain forested with different trees and a large Eastern Lake with ripples and dark bluish water. I was glad to spend my freshman year in the campus. It is worth mentioning that in May 1953, when the head of the Soviet Union, Joseph Stalin (1879–1953) passed away, the schools

in Wuhan city suspended classes for 3 days to grieve over his death. Also, the university students held a general assembly in the central square in Wuchang. This was the second time I participated in a memorial meeting for a national leader, following Franklin Roosevelt in Sichuan as I described previously.

During summer vacation, 1953, the subsection of Huazhong Agricultural College (HAC) was moved from the campus of Wuhan University to the principal part of HAG on the campus of the original Hubei Agricultural College. Thus I left the beautiful campus of Wuhan University. Then I began the second year of my university life. We had other courses such as plant physiology, microbiology, and soil science as basic disciplines. Professor H.K. Chen gave lectures for microbiology and his postgraduate helped him to direct the experiments. In Chen's Lab, every student had a nice microscope made by Carl Zeiss Co. (Jena) to do microscopy. When we went to the Lab, everything was in order. The students enjoyed the curriculum. Professor Chen self-designed teaching materials and used a textbook translated from the Soviet Union as the reading reference after class. I expressly remember that he emphasized the importance of type strains for bacteriology in a lecture and introduced some culture collections such as ATCC and NCTC. From that time on, I took notice of the significance of type cultures in microbiology.

In April to July 1954, an unbroken spell of rainy and wet weather struck the Yangtze valley regions. The campus of HAC is near Sha Lake connected to a river. During heavy rains the level of the river and lake rises. Because of the heavy spring and summer rains, the school faced the arduous task of flood control. During the flood control, students and some of young teachers helped with the flood control by building embankments. At one point, the level of the lake was so high that parts of the campus were flooded. Thus, the heads of HAC had to evacuate the teachers and some of students to safe places. Some of my classmates and I were transferred to free housing near the campus of Wuhan University with higher topography. This record-breaking flood occurred in July or early August 1954.

During the flood, all of the students assisted in flood control and their classes met off and on at the college. As a result of this flood, the buildings of HAC suffered substantial damages which were evident when I returned to the campus for my third year of study in autumn 1954. My specialty name, the Specialty of Soil and Fertilizer, was changed to the Department of Soil and Agro-chemistry. Then, we began to study practical courses, such as soil surveying and soil cartography, crop cultivation, plant protection, etc. Unfortunately, at the end of 1954 and the early 1955, heavy snow and unusually cold weather struck the middle regions of China. In Wuhan, the lowest temperature dropped to –18°C. Because the record-breaking flood and freeze affected vegetable production and supply, many students suffered an unusual disease, Raynaud's disease. I was one of the victims. This disease causes peripheral vascular damage and is due to vitamin deficiencies from lack of vegetables and fruit in the diet. In this disease, when I went to sleep, toe aches were unbearable, and moreover, some patients had difficulty walking. Sometimes my classmate Yang Xing-bang, a strong boy, carried me on his back to class or the lab. Later, with the improvement of weather, the disease slowly subsided but all victims thought they were facing disaster.

Following graduation and based on my performance in chemistry I was assigned to the chemical section of the Changjiang Academe's Changjiang Water Resources Committee (Changjiang refers to the Yangtze River) along with three other students for graduate practises. The Committee and its Academe were established to prepare for the construction of the Three Gorges Water Conservancy Project on the Yangtze River. There were a large number of professionals in different fields, including experts from the Soviet Union that served as advisors for the Committee and Academe. So, I had a rare opportunity to learn from them that scientific and humanistic literacy is important for scientists in the Academe. A soil chemist from the Soviet Union came to the lab to discuss soil chemical analyses. He was very knowledgeable and modest. At the same time, I actively prepared to take part in the entrance exams for postgraduate schools. I passed my qualifications, but the deputy head of the Department in HAC, Professor Zhou Zheng-hao asked me to postpone applying for the exams because the Department needed a teaching assistant.

Early professional career

Thus, I postponed my postgraduate study program. After graduation, based on the arrangement by Professor Zhou on behalf of the Department, I was assigned as a teaching assistant to the soil science teaching and research section. I was the youngest in the section. The lecturer, Zhuang Zheng-de, spoke excellent English because of his experience as a translator for the U.S. Army in India during World War II. He often taught me how to sort out scientific references, and loaned me an English book *Soil Conditions and Plant Growth* written by E.W. Russell. Another lecturer, Yang, spoke English very well and was kind to me. He gained my respect as one of my teachers. He enjoyed a long life of more than 90 years.

I used both English and Russian to study soil science and

microbiology. Moreover, I continued to prepare to become a postgraduate in microbiology. However, it required the approval of the Department and depended on the work needs and the uniform training program for younger teachers. Three years later, I was assigned to the chemical teaching and research section as a teaching assistant under the direction of Professor Huang Rong-han. He was good at analytical chemistry and his lectures drew a large audience. I studied chemical analyses of soil. Therefore, my postgraduate studies in microbiology were postponed for more than 5 years. At times, I even considered applying for entrance exams for soil chemistry or geochemistry.

As a postgraduate

When Professor Chen learned of my thoughts, he told me "the Department will allow you to apply for the exams this year and I will be your supervisor for your postgraduate study". This occurred one day in April 1962. Of course, I was glad to agree to the arrangement. A few days later, the Department informed me to stop my preparation for the exams. I smoothly passed the exams and entered into the graduate school of HAC along with other admitted postgraduates including Chen Yao-bang, who studied mycology under the direction of Professor Yang Xin-mei, and Fu Ting-dong, who studied crop breeding under the direction of Professor Liu Hou-li. The three of us had been teaching assistants, and others who were admitted postgraduates were graduating university graduates. All of us shared the same graduate hall, and the only female postgraduate resided in a dormitory. Although we came from different Departments, we quickly became a friendly team that helped each other. It was the first of September 1962, and I was 28.

Because I had been a teaching assistant, I entered the lab to begin my research project at once. All of us postgraduates took a philosophy course, the dialectics of nature and respective basic courses. Professor Chen taught me microbial physiology and advanced microbiology. Prior to winter vacation of the first year, Professor Chen selected many reviews and important papers on microbiology and typed a list of them for my study. One morning each week, he quizzed me and discussed the papers with me. This teaching method improved my understanding of basic microbiology. The graduate school required that each student must study two foreign languages. Professor Chen asked me to take English as my first foreign language and German as my second foreign language. Later, when we realized the heavy study burden it placed on me, Professor Chen asked me to stop taking German. Later, Professor H.K. Chen was always concerned with my development (Figure 2).

Figure 2. Professor H.K. Chen, a former supervisor, visiting Tian-shen Tao at his home.

My research project was "studies of the mechanisms of releasing phosphate from silicate minerals by bacteria and other micro-organisms isolated from soil". Originally, the Soviet Union agronomist Alexanderov reported that *Bacillus mucilaginosus* was able to release potassium from silicate minerals for plant utilization. Subsequent researchers even claimed that the organism could release phosphate from apatite. Because silicate minerals such as mica, feldspar, etc., are very hard insoluble materials, the mechanism used by organisms to release this plant nutrient from silicate mineral comprised an interesting research topic.

Lecturer, Zhou Qi (now professor) suggested the project would expand the scope of research on dissolved phosphate released by bacteria from soils. This initiative also led me to study the biodiversity and taxonomy of soil bacteria. Therefore, in addition to the mechanism research, I isolated many strains from different soils. They were *Bacillus* spp. and *Streptomyces* spp. Lecturer Li Fu-di (now professor) who studied the taxonomy of *Actinomycetes* in Professor Yan Sun-chu's lab, returned from Beijing. I learned from him how to identify this group of organisms. The mechanism research remains a difficult problem, as even now, soil microbiologists in China still study it. My research on this resulted in only partly providing evidence for understanding the interaction between the mineral particles and bacteria. Therefore, I also focused on the isolated organisms. The strains of *Bacillus mucilaginosus* had different dissolving potentials for apatite. The organism produces a large capsule around the cell under nitrogen limitation conditions. A large number of strains needed preliminary identification and characterization. So, I earnestly read new editions of *Bergey's Manual of Determinative Bacteriology*. It helped me later on to become a taxonomist and enabled me to obtain positions in culture collections in different organizations in China.

Professor Chen was familiar with bacterial taxonomy. Dr

H.G. Thornton was his supervisor when he studied at the Rothamsted Experimental Station in the U.K. in 1936–1940. Dr P.H.H. Gray and Dr Thornton established the genus *Mycoplana* and the two species *Mycoplana dimopha* and *Mycoplana bullata* in 1928. The genus included four species until 1990.

Professor Chen knew my aspiration to become a bacterial taxonomist. He indicated that the genus *Pseudomonas* was an important group of bacteria in soil and other environments. As a professor who worked in the agricultural field, he and his students were required to focus their research on practical projects in agriculture. This was the requirement from superiors in those years. The superior repeatedly emphasized that the theory must be combined with practice. Therefore, Professor Chen had to follow this principle in both his teaching and research. Insofar as my research project was concerned, the Department head also considered that it placed too much emphasis on theory and not enough on practice.

Professor Chen also arranged for me to join a rhizobia production project in the teaching factory of HAC during several months of my postgraduate study, although there was no connection with my research. Of course, I obtained some practical knowledge, but my wish to study my collection of soil strains was greatly diminished.

Career after graduation of postgraduate

When I graduated in November 1965, three of us graduates waited for employment. At that time, there was not an established degree system in China. So, we did not have a title. Meanwhile, on 8 December 1965, I married Yue Ying-yu, a teaching assistant in the same College. We do not have any children. In March 1966, I was placed at the Institute of Soil and Fertilizers (now, the Institute of Agricultural Resources and Regional Planning), Chinese Academy of Agricultural Sciences (CAAS) in Beijing. In the Institute, the main research fields were the reclamation of saline alkali soils, soil surveys on a large scale, soil fertility and cropping systems, chemical and organic fertilizers and their application, etc. In addition, there was a microbiological section. Some senior experts supervised different research projects such as microbial fertilizers, gibberellin production and its application, and stress resistance of plants caused by related micro-organisms. When I arrived at the Institute, the head of personnel asked me to work in a soil science analysis lab to determine the cation exchange capacity (CEC) of a number of soil samples from Albania. It was a temporary job for me and I worked with my new colleague, Mr Huang, and learned the operation of the microbiological lab from him. He told me that most of key personnel in the lab had been in basic research linked to practical agriculture. This was the principal objective of the whole Institute. Soon after, I was assigned to the basic research point, Gaobeidian farm, which focused on a production brigade in a village. Seven to eight of us lodged in different peasants' cottages. Our host and his wife and children were kind to us. I found that I must adapt to a new life in a strange place and learned from my coworkers and from farmers to become familiar with agricultural production. But soon after, the Institute recalled all staff to return to Beijing to participate in the Culture Revolution Movement. When I returned to the Institute, the daily research work still carried on, intermittently. A research group, directed by senior expert Yin Xin-yun, studied agricultural antibiotics and microbial fertilizers. Strain 5406 was used as microbial fertilizer, which gives plants stress resistance and strain G4 had a strong effect on plant growth stimulation. The group also isolated many strains from different soils that were awaiting study. The expert Yin decided that the group needed someone to study bacterial taxonomy and considered me as a candidate. Yin had good relationships with many farmers. His product, microbial fertilizer strain 5406, was quite profitable. Moreover, the idea of using microbial strains to inoculate compost was readily grasped by farmers as a way to make fertilizer. Only a few simple microbiological techniques were involved. His research direction was timely; therefore, the research work was supported by the Movement.

Also, he studied antibiotics and their application to plant protection. Among the agricultural antibiotics, neiliaomycin is a broad spectrum fungicide and was used to prevent apple tree canker (*Valsa mali*). It also had a preventative effect for plant disease. During the cultural revolution, Yin's research projects went smoothly. Later, the expert Yin asked me to join his group and it was approved by the leader of the Institute. When I joined the group, Yin asked me to identify some strains. So, I was able to pursue microbial taxonomy. Yin asked the members of the group including me to go with him for a field investigation on the application of neiliaomycin and he asked Wu Da-lun and me to identify the strains. Thus, we collected books and other related reference material for our experiments. The library of CAAS had ample sources of Chinese and foreign language books and journals. The requirements in the Institute and the references in the library satisfied the needs for our characterization of the strains in *Streptomyces* spp. My co-worker Wu and I checked out related books, and read some related journals and noted the essential contents. At the same time, we prepared reagents, biological materials including reference strains from our lab and other culture collections in Beijing so that we would be able to undertake the work the Institute leaders assigned us. Wu and I went to the Shenyang Chemical Engineering In-

stitute for cooperative study, not only to identify the strains, but to select strains with increased neiliaomycin potency. In the winter, Wu and I went to the Institute in Shenyang and we and our copartners began to work at once. The work in the Institute in Shenyang was convenient for us. The first reason we enjoyed this was that the Institute in Beijing was far from where Wu and I worked, so we could spend an entire day without interference by the Movement. Second, there was a microbiological lab at the attached fermentation factory in the Institute. So, the two tasks for us were: strain identification and culture breeding for increased antibiotic production. We used ultraviolet radiation and chemicals as mutagenesis factors for culture breeding. And based on the four principal features proposed by Dr L. Ettlinger along with other characters, we were able to identify the strains of *Streptomyces* spp. The features included colony characteristics, i.e., spore mass color, pigmentation of the substrate mycelium, diffusible exopigments, spore chain morphology, ornamentation of spore surface, and melanin reaction. In addition, we also determined the carbon utilization of the strains.

I remembered that the related report written by Dr Gottlieb from ISP (International *Streptomyces* Project) had been published. The 8th edition of *Bergey's Manual of Determinative Bacteriology* had not yet been published. But we had the *Actinomycetes* Vol. I, Vol. II, and Vol. III, by S. Waksman and his co-workers for reference at that time. We used six different media for cultivation of the strains and observed the colonial characteristics and noted their color using the Color Standard and Color Nomenclature made by Ridgeway at the American Bureau of Standards in 1919. I found it most useful to write down the color with its color tag number while observing colonies of the *Streptomyces* strains.

Using light microscopy, it was easy to observe the strains and note the morphology of the spore chain, (a) straight, to flexuous (*Rectiflexible*), (b) hooks, loops or spirals with one to two spirals (*Retinaculiaperti*), and (c) spirals (*Spirales*). We repeatedly observed the morphology of our strains by microscopy. Wu was careful and skillful, and wrote everything he considered significant in a notebook. So, I enjoyed working with Wu and we became good friends. Since there was no electron microscope at the Institute in Sheyang, we could not observe the ornamentation of the spores. I had previously participated in a workshop for the study of clay minerals in soil in an Institute, in 1957. Therefore, in order to study electron microscopy, Wu and I visited above the Institute, the Institute of Metal Research, Chinese Academy of Sciences, in Shenyang located at South Lake in the city. We asked the famous metal physical scientist Professor Gou Ke-xin for permission to use their TEM. He lent his assistant Mr Liu to assist us. Mr Liu was interested in looking at the microbial samples. Wu and I prepared the samples using the duplication method, and Mr Liu operated the TEM. The TEM observations showed that the spores of our strain 5406 and strain G4 had smooth surfaces, whereas the spores of strain SF 106 had a spiny surface similar to cocklebur seeds. With other experimental results, we could identify the strains 5406 and G4 being similar to the *Streptomyces fradiae* Waksman and Henrici 1948, and strain SF 106 which produced leiliaomycin was similar to *Streptomyces noursei* Brown et al. 1953. At the same time, the lab in the Institute assigned chemists to purify antibiotics from the fermentation solution of strain SF 106 by chemical methods, and use the crystal materials for determination of primary chemical structure with infrared spectroscopy, etc. The fingerprint of one of our pure materials was similar to that of actidione (cycloheximide) which was produced by the species *Streptomyces noursei*. But, strain SF 106 still had some features to distinguish it from *Streptomyces noursei*.

I recall that during our work in Shenyang, I returned to Beijing for a short vacation. When I returned from vacation to find a bus from the Shenyang railway station to the Institute, I heard a babble of many voices, one of whose was Wu. When I found Wu and I asked him "Why are you here?" he told me that he had hepatitis A. He was going to Beijing to be treated. I regretted his loss. Fortunately, Li Xiu-yu, another co-worker had come to the Institute in Shenyang. Li also worked very well, and she was a pleasant person who was kind to everyone. Finally, we finished our work on the identification of the strains and some of the work on the antibiotics. Then we returned to Beijing to consult with the taxonomist Professor Yan Sun-chu in the Institute of Microbiology about the strain identifications. After seeing our results, Professor Yan indicated that strain SF 106 was similar to *Streptomyces ahygroscopicus*, which he had studied, but strain SF 106 was hygroscopic and had other different features. Thus, he recommended that we describe it as a new species in the genus *Streptomyces*. Professor Yan and we decided that it was necessary to undertake additional studies to classify strains 5406 and G4 before we published our results.

I asked for time off to see my wife in Hubei and invited senior microbiologist Guan Miao-ji to go to Shenyang to continue the strain breeding. She went to Shenyang at once where she and her co-workers made good progress. When I finished my leave and returned to Beijing, The Institute had received an order to move to Shandong Province except for Yin's group which stayed in Beijing where it was merged with the Institute of Atomic Energy in Agricultural Application. At the same time, Yin's strains of micro-organisms were separated into two duplicate collections, one remained

in Beijing, and the other one was transported to the Institute at Shandong.

On the way towards a culture collection

Originally, Yin's group members and I requested that I stay in Beijing for continuation of the research work. But the leader of the Institute politely refused our request. Then, I went to Shandong's new address for the Institute with others in early spring, 1971. After relocating at a large farm near railway station for a short time, the Institute moved to Dezhou located on Beijing–Shanghai railway line where it merged with the Dezhou Region Agricultural Institute.

The leader assigned me to continue the microbial fertilizer work with the main task of developing a culture collection. Just then we heard that Dr Huang Shu-wei from ATCC may visit China. At that time, our Government emphasized the importance of foreign affairs. When a foreign scientist came to visit, the department that had a common interest would arrange a formal reception. So, a Section of the Ministry of Agriculture identified the Institute of Atomic Energy in Agricultural Applications in Beijing to receive Dr Huang. Thus, the leaders of Institute in Beijing consulted with the leaders of Institute in Dezhou and asked me to loan personnel to Beijing for making preparations for Dr Huang's visit. I went to the Institute and met Yin and other members of the group. I checked out related materials from the Library including *Culture Collections of Microorganisms*, which is the Proceedings from the first Conference on Culture Collections (ICCC-I) in Tokyo, Japan. From it, I learned the basic information on the operation of the major culture collections in the world, such as ATCC, NCTC, CBS, NRRL, etc., which were reported by curators or directors during the conference. Further, some experts and scientists introduced the principal methods for preservation of micro-organisms, including lyophilization, liquid drying, mineral oil sealing and cryopreservation. Dr Huang reported on the ultralow temperature preservation for algae, and she also reported the method in detail at a special meeting following the ICCC-I in Hokkaido, Japan. The pioneers for suggesting the establishment of the international organization of culture collections, such as Dr S. Martin (Canada) and Professor V. Skerman (Australia) and their activities and great efforts deeply moved me so that I recognized the importance of culture collections and I was glad to undertake the work. It was a pity that Dr Huang did not visit the Institute. Thus I came back to the Institute in Dezhou to carry out my daily work. Meanwhile, the Institute in Dezhou set up an experimental factory for the production of agricultural antibiotics for the prevention of plant diseases. The leaders urged us to finish the identification of the strains, so we went to Beijing to ask Professor Yan for advice on the above issue, and under his direction we proposed strain SF106 as a new species, *Streptomyces hygrospinosus*, and published it in *Acta Microbiologia Sinica* in Chinese.

In 1976, when Chairman Mao Ze-dong (1893–1976) passed away, all Chinese people grieved and mourned. I joined the memorial meeting at the door at the Institute in Dezhou, because my stomach was upset. This was the third time I joined in the memorial services for a national leader.

During that time my wife and I lived in Dezhou. Because I was sick quite often, we applied to move to Wuhan, my home town, for employment. After this was approved, my wife and I went to Wuhan Microbial Pest Factory for a new job in the early spring 1977. My friend Yu Zi-liu welcomed us to join his lab, the Central Experimental Laboratory in the Factory, where the main products were *Bacillus thuringiensus* used as insecticide, and the antibiotic ginggangmycin used to treat rice disease, banded sclerotial blight (*Hypochnus sasakii*).

Also under the direction of Professor Yan, we proposed a new species of *Streptomyces qingyangensis* for strain 5406 and strain G4. We published it in *Acta Microbiologica Sinica*, and we gave the standard description of the strains such as the photos by TEM and SEM microscopy. Later, some popular books adopted our descriptions on strain 5406 which is commonly used as a microbial fertilizer in China. However, afterwards, with the development of taxonomy in bacteriology, I believed that the genus *Streptomyces* needed reclassification. Many years later, my friend, Li Li carried out more experiments on strains 5406 and determined its 16S rRNA gene sequence and compared it with the GenBank database. He found that strain 5406 was closely related to *Streptomyces fradiae*. He called to inform me about it. I told him this was okay with me. Moreover, in fact, as early as the 1950s, someone had preliminarily classified strain 5406 as *Streptomyces fradiae*. Therefore, I accepted this. Of course, we would have liked to publish new bacterial taxa at that time.

Two years later, in March 1979, The Institute of Soils and Fertilizer moved from Dezhou to Beijing and the leaders of the Institute asked my wife and I to come back to the Institute. Thus, I returned to the research field again to work on culture collections, which I wanted to do for many years. When I returned to Beijing, the administrators emphasized personnel training, and in particular, asked the staff to learn English. I joined in English training courses, and in addition to reading, the teachers taught English writing, speaking and comprehension. The latter two were the main areas of training. Later, some of the students including me went

to Guangzhou as TOEFL (Test Of English as a Foreign Language) training for preparation to study abroad.

In the TOEFL training course, the teachers who came from the USA, were experienced in teaching non-English speaking students. The students in the class studied hard, because they understood that going abroad to study was difficult. However, I think that the teaching was not so effective because of the older age of the students. But, we studied hard and even had sleepless nights. I was the one of them. After I passed the examinations, I came to Beijing to wait for a notice to go abroad. According to the plan, I was selected to go to Australia for study. Prior to leaving, I asked my former supervisor Professor H. K. Chen to take advantage of the occasion of his visit to Australia and a recommendation letter was written for me by Professor Zhang Nai-feng, which was sent to Professor Victor Skerman who was at the University of Queensland. Professor Skerman accepted my request and sent a letter to Professor Zhang to invite me to come to his lab. Our Government used a special loan from the World Bank to support a number of young and middle-aged scholars to go abroad for study and I was one of them. The Australian government agency ADAB (Australian Development and Assistance Bureau) handled our arrangements. In September 1981, my friends Rong, Chen, and I passed through Hong Kong and arrived in Sydney, Australia. The ADAB arranged for us to study English for up to 6 months in Sydney. In my English course there were six to ten students, who learned according to their own time-frame. At one time, the six students in our class came from Thailand, Indonesia, and Myanmar (Burma), and I from China. Except me, the other classmates spoke quite good English, such as a woman who had obtained her MD from New Zealand.

The teacher was tall woman who had a wealth of knowledge. She knew that I was the oldest student and in need of understanding and speaking English, even though I had TOEFL training. So, she spent as much time as possible with me and gave class work for the other students. Often she spoke English so quickly that I couldn't follow her. But I felt that I improved my English during the training courses. In addition, the Australian Government gave every student a good stipend so that we happily lived in Sydney, and made us used to life abroad. However, as an older student, I urgently wished to enter the lab for study, so at times, I was disheartened during my stay in Sydney.

After I completed my English examinations in Sydney, I flew to Brisbane in March 1982. I experienced otalgia (earache) in the small plane we flew to Brisbane so I was somewhat uneasy. The official of ADAB in Brisbane, Mr Smith, met me at the airport and drove me to the office of ADAB in Brisbane, where two or three officials took me in and explained the conditions of study to me. After lunch, Mr Smith drove me to my apartment, where a friend of mine greeted me, and then he took me to see Professor Skerman in his lab. Professor Skerman showed me his micromanipulator and taught me how to use it for the isolation of single microbial cells isolated on an agar plate. He mentioned that Mr Smith was his distant relative and with this and his kindness, my tense mood began to relax. Now, I began to experience the highly efficient mode of operation in a foreign country. From then on, I began my study of the taxonomy of bacteria under the direction of Professor Skerman and also to learn from my peers. I thank ADAB for supporting my studies.

During my studies in Skerman's lab, there was a PhD student, Peter Franzmann, Hylan and Wanchern Potacharoen from Thailand, Andang Sukara from Indonesia, a part time MD student, and other assistants. Peter was kind to me, and the others also were friendly. In order to study many fields on microbiology within the limited 2 years, I did not take a project, but I asked Professor Skerman to provide a broad range of study for me (Figure 3).

Figure 3. Tian-shen Tao at the Great Court at the University of Queensland, Brisbane, Australia, in 1983.

First, Skerman taught me about systematic bacteriology by his lecturing; because my understanding of English was limited, and his voice changed by his hearing difficulty, he often wrote down some important points on a scraps of paper to show me so I could understand better. Then I studied *Bergey's Manual* by myself to enhance his lectures. We met often after lunch, when he taught me face to face with his teaching microscope and the use of the micromanipulator. In addition, I also learned much about general microbiology in which he was very knowledgeable. Moreover, he explained the Bacteriological Code in detail to me, Wenchern and Andang, and he wrote down on the board all the impor-

tant points. He emphasized time and again that the study of the Code was difficult. After his explanation, I attentively read the Code, and asked him some of the difficult aspects which he explained to me again. Later, when I returned to China, Dr and Professor Chen Wen-xin and I translated the Code into Chinese and published it.

Professor Skerman gave me a copy of the *Approved Lists of Bacterial Names* and told me about the editing process and the background of World Data Center (WDC, now WDCM), which he established. As a member of the Executive Board of WFCC, Professor Skerman was responsible for accepting new members for WFCC. Therefore, I contacted the responsible person of Chinese Society of Microbiology (CSM), and the Society joined WFCC as a new affiliate organization member. It is interesting to note that the WDCM, which later moved to Japan, just recently relocated to China.

In Professor Skerman's Lab, I isolated many pure cultures from the campus lake using his micromanipulator and the cultures from single cells were preserved with lyophylization in Dr Lindsay Sly's Lab. When I came back to China, Professor Skerman also gave me many teaching organisms and the duplicates of the above-mentioned strains that I isolated while there. Dr Sly helped me to obtain the quarantine certificate for leaving from Australia.

Professor Skerman also asked me to work with Dr Lindsay Sly on the University of Queesnland's culture collection and experiments for DNA G+C mol% determination. These studies were later published by Dr Sly and others in the *Journal of Microbiological Methods*. While I was in Dr Sly's lab, I subcultured most of the cultures preserved in the Collection QUM. Each culture was subcultured on fresh agar slants and smears were stained and examination by microscopy. So, I had observed each micro-organism which I had subcultured. Of course, I concentrated my attention on bacteria, specifically, the organisms which I have never seen, such as *Planctomyces*, *Caulobacter*, etc. As a person who was mainly engaged in microbiological work in agricultural sections, I had not worked with pathogenic organisms, so, before I worked with them, Professor Skerman arranged for me to be immunized. And I also worked with Dr Sly to preserve bacteria using a lyophylizer which was made by the Department Workshop according to a French design. I learned from Dr Sly how to use a meter to program the control of the temperature for cooling and preservation of temperature-sensitive organisms before placing them in the liquid nitrogen tank. The information I gained at the University of Queensland was very important for my future work in China.

I also studied microbial physiology with Dr H.W. Doelle in the Department of the University of Queensland. He taught me an enzymological method for determination of generation time of bacteria using a Beckman automatic spectrophotometer. It was a pity I did not successfully finish these experiments because I had a gastralgia. A doctor from New Zealand at the university hospital treated me with a mixture containing aluminium and I slowly recovered over a few days.

During my studies at the University of Queensland, my alma mater, Huazhong Agricultural College (HAC), held a workshop on the ecology of rhizobia. The leaders of HAC invited Dr R. Date to present a lecture. Dr Date was at the CSIRO (Commonwealth Scientific and Industrial Research Organization) in Brisbane near the University of Queensland campus. Dr Date was very short of staff to help with the preparation of biological materials including the preparation of different immune sera from different rhizobial strains. Since he knew that I was from HAC and had studied in Professor Skerman's lab, Dr Date consulted with Professor Skerman to invite me to work in his lab for a period of time to help prepare for the workshop. Therefore, I worked with Dr Date for a time. Since his lab was close by, it was very convenient for me to prepare the antisera.

Dr Date asked one of his young students to help me to make immune sera using rabbits. So, his student and I went to the farm facility where the rabbits were kept. He drove and we happily talked all the way. Finally, we killed the rabbits to harvest the antisera. The young student was too kind to kill the rabbits, so I did it. We obtained a sufficient amount of high-titer antisera and preserved it for all rhizobia strains by lyophilization with a lyophilizer made by Edward Co. Dr Date took the sera dispensed in small bottles with him to China for demonstrations of how to trace strains of rhizobia in the field.

During my stay in Australia, the Government, Professor Skerman and others were very hospitable. The private organization, Australia–China Friendship Society in Brisbane, organized one Australian family to host a Chinese student. The president of the Society, Mr Wu Gui-lin, introduced me to Dr John Tong's family in Brisbane. Dr Tong, a retired pathologist, and the members of his family were very kind to me. He invited me to spend Christmas Holiday at his home with his other close friends, and also invited me to his son's dairy farm for summer holidays for several days. So, I had a wonderful time in Australia, despite my occasional poor health.

After I finished my study in the Department of Microbiology at the University of Queensland under the direction by Professor Skerman, I returned back to Beijing, China, in March 1984, when I was 50 years old. The leaders of the Institute of Soils and Fertilizer reassigned me to work in the Collection, China Agricultural Culture Collection (ACCC). Just then, our Government prepared to call the Second Conference of Culture Collections. As a member of Academic Group on the Chinese Committee of Culture Collections for Preservation and Administration, I joined the other members to prepare for the meeting. At the conference, there were several special reports. I gave an introductory presentation on the culture collections in the world. Wang Zhi-lun introduced activities of ATCC where he had studied for 2 years.

In the 1900s only a few Chinese went abroad to study culture collections and returned to China. Wang was at ATCC, Yuan Zeng-lin was at the Institute of Serology in Denmark, and I was at University of Queensland, Australia.

After a conference, I translated the *International Code of Nomenclature of Bacteria* (1976 Revision), and invited Professor ChenWen-xin to correct my first manuscript for translation of the *Bacteriological Code*. Later, one of her students, Luo Chuan-hao joined us to translate Appendices 4 and 5. Finally, we asked Professor Wang Da-shi to make a final correction. The Chinese version of the Code was published by the Scientific Press of China, in Beijing, in 1989. Before the publication, Luo went to Germany for study.

When Luo completed his MD study under the direction of Professor Chen Wen-xin, he came to our Collection, ACCC for work, and he wanted work with me. Consequently, we invited Hu Wen-lin to work together to set up a database for search of antibiotics and the micro-organisms that produced them. Hu had studied physics and he was the main administrator of the mini-computer in our Institute because of his familiarity with computers. Luo and I collected information from the Library of CAAS. So, we obtained hardware and software needed for the database. It was our aim to enable a user to put in keywords to access information from the database. With regards for this, we applied for and obtained funding from the National Natural Science Foundation of China (NSFC). Later, we completed the database and passed the job evaluation.

In the meantime, Professor Li Fu-di at HAC and I suggested that the Ministry of Agriculture of China formally invite Professor V. Skerman to come to China for a visit and to give lectures on culture collections and systematic bacteriology. He accepted the invitation gladly, and came to Beijing in October 1985. I accompanied him during his entire visit.

Figure 4. Tian-shen Tao interprets a lecture by Professor V.B.D. Skerman in Beijing, China, in 1985.

First, in Beijing, Professor Skerman explained the importance of culture collections for microbiology, and tersely lectured about systematic bacteriology by use of lantern slides (Figure 4). He also made a short film showing how to isolate a single cell using his micromanipulator. He showed those who were interested how to operate the micromanipulator to isolate a single bacteria cell on an agar plate. They were all amazed by his lecture and the micromanipulation.

Then, Professor Skerman went to HAC in Wuhan where his host, Professor Li Fu-di, organized a workshop along the same lines as that in Beijing, but the training was much more extensive. In addition, he lectured on the *Bacteriological Code* in detail. Most of the postgraduates in HAC, and some teachers and scientific researchers on taxonomy or culture collection attended the workshop in Wuhan. All participants at the workshop were delighted with Professor Skerman's lectures, his slideshow and his demonstration of micromanipulation as well as Professor Li's thoughtful arrangements. Meanwhile, Li invited Professor Skerman to visit some places of historical interest near Wuhan. But, he was not interested as he wished to do more to help Chinese youth understand microbial taxonomy. Therefore, Professor Li canceled some travel for him. He only visited the Gezhouba Dam which was under construction. After we returned to Beijing from Wuhan, we accompanied him to visit some large culture collections at different institutions in Beijing. Professor Skerman had an informal discussion with the directors or curators, and had some interviews. He was interested to visit the Great Wall (Figure 5). During Professor Skerman's visit to China, I had another chance to learn from him, which was very helpful for my teaching and research.

Figure 5. Professor V.B.D. Skerman and Tian-shen Tao at the Great Wall, Beijing, China, in 1985.

The officials of the Ministry of Agriculture gave a banquet for Professor Skerman and another distinguished guest, Professor Goodman from the U.S.A., to thank them for their successful visit and lectures in China. Finally, on a bright and sunny day in early November 1985, we saw Professor Skerman off at the Beijing Airport. A few days later, a sudden cold wave came to Beijing. Fortunately, he had already returned. Mr Han Kai aided me in hosting Professor Skerman during his visit to Beijing.

On 8 March 1985, our Government established a patent system, and began to put patent law into practice. The Chinese Patent Office designated two culture collections for acceptance and preservation of patent cultures involving patent cases coming from at home and abroad, and accepted responsibility according to international practices. They are the Culture Collection in the Institute of Microbiology in Beijing (now CGMCC) and the China Center for Type Culture Collection (CCTCC) at Wuhan University in Wuhan.

CCTCC accepted the task with foreign affairs for preservation of patent culture. It required that the Collection increase its staff. In view of my aspiration to work in Wuhan, my former supervisor, Professor H. K. Chen, recommended that my wife and I join the Collection. The leaders of Wuhan University readily agreed to the recommendation.

My wife and I quickly made arrangements to resign from CAAS, and we went to Wuhan University to report for duty in the summer of 1986. Originally there were four full-time staff members and two part-time staff at CCTCC. Since it was established in 1985, the Collection had accepted many patent cultures including bacteria, fungi, viruses, cell lines, and a few plant tissue cultures. A young man, Qu San-pu who studied aquaculture, joined us soon after we arrived. The work-load and requirements were considerably heavy

Figure 6. Tian-shen Tao working at the CCTCC in 1987.

for the Collection. Fortunately for us, Professor Peng Zhenrong often assisted us by directing our work. At that time, due to the limitation of development in Wuhan, it was difficult for us to contact foreign clients for patent cultures, especially cell lines that had to be delivered under refrigerated conditions by aeroplane. But, without an international airport in Wuhan we had to receive the cell line package before the dry ice evaporated in Beijing or Shanghai. But, only CCTCC undertook the task for receiving patent cell lines at that time. Moreover, we set up a self-governed building for use of our Collection. I began to enroll a graduate for research work, because our country urgently needed improved talent (Figure 6). Due to the limitation of lab facilities, the graduate student, Li Gao-xia, selected database research as her main project. Another student carried out experiments in a borrowed lab until our new building was completed.

With the development of our work on patent cultures, an expert in the Patent Office of our Government suggested that we visit large culture collections in America. Thus, four of us went to the U.S.A. for a visit in September 1987. We first visited NRRL in Peoria, Illinois State University, where Dr Cletus P. Kurtzman hosted us. He introduced us to the operation of the facilities and provided the history of the collection. We then toured the building where we visited most of the labs and listened to each lab host's presentation. A Chinese-American mycologist, Dr Wang Li-hua, aided us in understanding more about the collection. Dr Kurtzman also

Figure 7. Tian-shen Tao presents a seminar describing the CCTCC to NRRL, in Peoria, Illinois, USA, in 1987.

Figure 8. Chinese microbiologists visit the ATCC, in Rockville, Maryland, USA in 1987. From left: Zhou Zhong-gui, Tian-shen Tao, Dr S.C. Jong, Dr R. Gherna.

invited me to present a seminar to their staff. I talked about our collection and some of our research work and showed the slides for them. However, my English was not fluent enough to express my ideas very well (Figure 7).

Dr Wang Li-hua and her husband Dr Wang invited us to visit their house and we later ate dinner at a Chinese restaurant. The visit to America reminded us of the common saying: "seeing is believing". Through the visit, all of us had the feeling that we learned a lot from the foreign scientists. When I saw a lyophilization machine made by NRRL personnel themselves based on the design of a French scientist, it reminded me of the same type of machine that I first saw in Dr Sly's lab in Australia. I also remembered that a scientist had reported in ICCC-I about above-mentioned lyophilization machine at NRRL, and Professor Gao Shang-yin at Wuhan University had an offprint that mentioned the story about the machine, written by yeast taxonomist Dr L.J. Wickerham. I also saw a Pyrex glass beaker with many old markings on the experiment table for use. I think that America is a rich country, but most scientists are not ostentatious or extravagant. I admire their spirit of saving and care of public property. But, we also saw wasteful consumption in cities. I think that a comprehensive view is helpful. That was my impression for my first visit to the U.S.A.

Then, we went to the ATCC in Rockville, Maryland. There, Mrs B. Brandon, the associate director for administration of the ATCC, hosted us. A Chinese-American mycologist, Dr S.C. Jong, also helped arranged our activities (Figure 8). I had met Dr Jong in Beijing previously, so this helped make our visit more comfortable. Through Dr Jong's connection, we visited the Dental Institute of NIH, in Bethesda, Maryland, and met Dr Micah Krichevsky, whom I had also met previously in Australia. Dr Morrison Rogosa and Dr

Figure 9. Chinese scientists visit the Dental Institute, NIH, in Bethesda, Maryland, USA in 1987. Back row, from left: Li Guang-wu, Zhou Zhong-gui; front row, from left: Tian-shen Tao, Dr M. Rogosa, Dr M. Krichevsky.

Krichevsky gave us a copy of *A Coding of Microbiological Data for Computers* edited by Rogosa, Krichevsky, and Colwell (so-called RKC system) (Figure 9). It was very useful for our teaching and research in bacteriology. In ATCC, at Dr Jong's suggestion, we transacted the formalities and became an affiliate member of WFCC for our collection, CCTCC, and six staff members of our collection became individual members of WFCC. Also Dr Jong told us that the ICCC-VI would be held in America in 1988, and the conference would be held on the campus of the University of Maryland. He hoped to see us in America again. We were thankful for the news so we would make arrangements to participate in the conference. In ATCC, we had recommended one of our staff, Zheng Cong-yi, to further study there for cell line preservation and its quality control etc. and got

assent from director, R. Stevenson for Cong-yi to learn at ATCC. Later, Cong-yi studied at ATCC under the direction of Dr R. Hay and when Cong-yi came back from ATCC, it was very helpful for the relevant work of our collection.

After finishing visit in ATCC, we went to Washington, DC, by subway train. We visited some museums, the Washington Monument, and admired Lincoln Memorial.

Then we went to the Lilly Center of Eli Lilly and Company in Indianapolis, Indiana. Lilly was one of our important clients for the deposit of patent cultures (Figure 10). They showed us the company and the Center history and the present situation by a special film in the reception hall. A receptionist spoke Chinese to explain it to us, and under her guidance, we visited some of the labs that were separated by glass windows in a corridor. We understood that this is routine for protection of trade secrets. Especially, the receptionist told us euphemistically about in Chinese. We asked to meet Dr Yao in corridor, who studied *Actinomycetes* taxonomy.

The patent lawyers of the Center told us that they urgently hoped the Chinese government would quickly sign the Budapest Treaty in order to enhance the use of patents for culture deposition. We answered that this is a matter for the Chinese government, but we would explain their recommendation to our Patent Office.

Then we went to Boston and Chicago to visit Harvard University and the University of Chicago. At Harvard University, I saw the glass flowers exhibition and they said that the arts with scientific significance were made by both father and son (Leopold and Rudolph Blaschka), and no one could do them again. As they recognized, art products could not be reproduced, and science could not replace art.

We went to New York City by Greyhound bus from Boston. In New York, we visited our cooperative institution, the New York Section of Patent Agency which belongs to the China Council for Promotion of International Trade (CCPIT) to meet our friend, Mr Dong whose office was located in one of the sections on the 25th floor of the World Trade Center. Years later, I saw with my own eyes online that the twin towers were destroyed in the terrorist attack of 11 September 2001. It shocked me very much. As a person like me in my childhood who suffered the wounds of war, how I detested the violence and resolutely held my denouncement of this act of terror. Finally, we flew to Hong Kong and returned to China. This visit provided us with a lot of information about culture collections and patents.

In 1988, I was assigned by Wuhan University to take part in the Sixth International Conference on Culture Collections (ICCC-VI) in the U.S.A. I met my former supervisor, Professor Skerman, and Dr S.C. Jong, Professor Peter H.A. Sneath, Dr C.P. Kurtzman, Dr Lindsay Sly, et al., and I also met my friend Dr Peter Franzmann. My friends and I in CCTCC proposed posters for the conference to introduce our work on culture collections and patent culture deposition. Also, at Dr Jong's recommendation, I joined the Committee of Education of WFCC. The other members of the Committee had been Mrs. B. E. Kirsop, Dr Komagata etc. WFCC arranged the next conference, namely, ICCC-VII, which would be held in Beijing, China. As a representative, Dr Song Da-kang gladly received the information.

Figure 10. Chinese delegation visits Eli Lilly Center in Indianapolis, Indiana, USA, in 1987. From left: Li Guang-wu, Zhou Zhong-gui, a lawyer of the Eli Lilly and Company, Tian-shen Tao.

During the conference, Dr Song Da-kang and I discussed with Dr M. Krichevsky organizing a workshop on quality control of cell line preservation, during the ICCC-VI in Beijing.

As our new building was completed, we improved our work and research conditions of our laboratories, then we added additional facilities, and we subscribed to several periodicals including the *International Journal of Systematic Bacteriology*. We also purchased the 1st edition of *Bergey's Manual of Systematic Bacteriology*, volumes 1–4. They are not only useful for our jobs and research work but also useful for teaching. In addition, we produced related books in Chinese for Chinese readers using the above information, because it was not so easy for local institutions to buy foreign books.

At the same time, I applied for and received support from the National Natural Scientific Foundation for research. For this, we twice went to Shenlongjia Nature Reserve for isolation of micro-organisms to obtain the natural resources from a special region, in 1990 (Figure 11) and in 1992. We isolated nearby micro-organisms at a local hotel using simple materials which we brought with us from Wuhan University.

Figure 11. The investigation group from Beijing Agricultural University at the Shenlongjia Nature Reserve of China in 1990. Back row, from left: Li Ying, Qin Xiao-tin (who now works at the CDC in Atlanta, Georgia, USA), the local jeep driver with the group, Wu Hou-bo; front row, from left: Gao Ju-liang, Tian-Shen Tao.

In this way, we believe that our soil strains were representative of their original habitat.

So, my co-worker, from Beijing Agricultural University (now, China Agricultural University) and I led some graduates to the Shenlongjia Areas Nature Reserve for sampling different soils. The samples were used for studying the microflora, in particular the bacterial composition. We isolated micro-organisms and brought them back to our labs for further study. Originally, there was a lush virgin forest in Shenlongjia, which was associated with mystery and unfathomable legends, such as the presence of many albino animals and barbarians. Now, most of the virgin forests has been harvested and secondary forests have grown. While the primeval forest was cut in Shenlonjia, some of big trees were kept *in situ* for showing the original appearance of the forest. We saw very large Chinese hemlock (*Tsuga chinensis*) there (Figure 12).

We worked with a local forester who knew the area and the vegetation quite well. He was happy to help us, but on one occasion he mistakenly led us to a remote region with mountains and precipices. We were on isolated hilltops. Everyone became separated and could not help one another. We bare-handedly climbed forward, and sometimes stopped to have a rest. But we were still separated. We could only go forward and not retreat. However, finally, we found the right way to our destination. In thinking about our misadventure

Figure 12. A large Chinese hemlock from Shenlongjia illustrates the original appearance of the primeval forest.

afterwards, it makes my blood run cold. However, as a result of this field trip, we were able to isolate many microbial cultures and most of them are actinomycetes. We brought them back to our lab for further study (Figures 13 and 14)

In 1990, the 15th International Congress of Microbiology was held in Osaka, Japan. I was assigned by the leaders of the Department of Biology, Wuhan University, to present at the congress. I had just finished the field sampling and isolation of micro-organisms in Shenlongjia Nature Reserve back in Wuhan before I made arrangements to go to Japan. I again met Professor Skerman during the congress. Once during coffee break, he talked with me about my studies in Australia. He said that, if I wished to study abroad again, I should not go to Australia, and he suggested the DSM to me or another culture collection as a place to study to increase my knowledge of microbial taxonomy. I thanked him for his suggestions, but I told him that I could not study abroad again because I had to stay in China for my job for the long-term development of our Collection, CCTCC. However,

Figure 13. Field group collecting soil samples in Shenlongjia Nature Reserve of China in 1992. Center: graduate student, Wang Ying-qun.

we need as quickly as possible to send our personnel to go abroad for study. He suggested that I should meet with others who might have suggestions. Once, during a break between sessions during the congress, I happened to sit beside Dr Akio Seino so I talked with him. I recommended that my friend, Fang Cheng-xiang in our Collection, could go to his lab for study in Japan. He promised to try to help. After I returned to our Collection, I asked Fang to make contact at first hand with Dr Akio Seino for the related matters. After some time passed, Dr Seino invited Fang to the Institute of Fermentation, Osaka (IFO), to study under the direction of Dr T. Hasegawa and Dr A.Yokota. I think that as a developing country, China urgently improved personnel level on science and technology at that time. Thus, Fang was able to go to Japan for his further study. When Fang returned to our Collection, he recognized that he had learned a lot from IFO, and he continued his close contacts with his Japanese friends.

In the 1990s, our Collection had increased facilities and

Figure 14. Post-graduate student Yang Zhao-hui (now in the USA) and Tian-shen Tao shown isolating microorganisms from soils in Shenlongjia Nature Reserve of China in 1992.

software, and improved conditions in experimental housing. We also established our working system so teaching and research became a normal operation for our scientists. In addition to our daily job of patent culture depository, the main personnel in our Collection began to carry on research. Zheng studied cytology and virology, Fang studied systematic bacteriology as well as microbial genetics, and my wife and I studied systematic bacteriology on investigation of natural resources. I began to sort out the nomenclature of the genera of bacteria on the scientific name of Chinese translation. At the same time, Dr Yang Rui-fu and his co-workers also carried out similar work and expanded the coverage to all species of prokaryotes. In a meeting, Yang proposed to combine the two projects together and this proposal was supported by the all of the participants in the meeting. Later, Dr Yang rui-fu and I, and our co-workers, began to compile a dictionary that was published in 2000. It was entitled *The Scientific Names of Bacteria with English Explanation and Chinese Translation*. More than 5000 bacterial names with standing in nomenclature have been collected in it and the Latinized names with both English explanation and Chinese translation, reference of original publications for each names and other related information. Two indexes of specific epithet names and translated names in Chinese could save readers much trouble in finding correct names. I remembered that in a symposium on systematics of *Actinomycetes* in Kunming, China, Dr Michael Goodfellow commented how nice it was. So, I presented a copy of the dictionary to him.

The legal expert in the Chinese Patent Office (now, Chinese Intellectual Property Office), Hu Zuo-chao invited me to edit a book named *Patent and Biotechnology* to meet the needs of some of those who are engaged in the patent business. I called on several scientists who also understand the patent

Figure 15. Guests from ATCC visit the CCTCC in Wuhan, China, in 1992. From left: Dr R. Stevenson, Tian-shen Tao's wife and one of the curators of our Collection, Yue Ying-yu, Tian-shen Tao, Mrs B. Brandon.

profession to write the book. And I was pleased that my elder relative, the famous poet and calligrapher Zhao Pu-chu, wrote the title of the book in Chinese calligraphy, and it was later published by Chinese Scientific Press in Beijing.

In 1990, the ICCC-VII was held in Beijing, China. My friends and I participated in the conference. We proposed some posters and Qi San-pu in our Collection was invited to take part in the workshop for the quality control of cell line preservation during the conference. Professor Yuan Cenglin from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) in Beijing, China, directed the workshop. After the conference, we invited Dr R. Stephenson, Dr S.C. Jong, and Mrs B. Brandon of ATCC to visit our Collection (Figure 15). We accompanied them and informally discussed the profession and exchanged ideas on culture collections. The president of Wuhan University, mathematician Qi Min-you, held a banquet for them and our staff.

With the development of Chinese patent business, the Chinese Government decided to formally sign the Budapest Treaty whose complete name is the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Usually in order to meet the legal requirements of sufficiency of disclosure, patent applications and patents must disclose in their description the subject matter of invention in a matter sufficiently clear and complete to be carried out by a person skilled in the art. When an invention involving a micro-organism, completely describing said invention in a description to enable third parties to carry it out is usually impossible. This is why, in the particular case of inventions involving micro-organisms, a deposit of biological material must be made in a recognized institution. The Budapest Treaty ensures that an applicant, i.e. a person who applied for a patent, need not

Figure 16. Representatives from WIPO visit the CCTCC in Wuhan, China, in 1994. From left: Tian-shen Tao, Mrs B. Brandon, Mr Wang Zhong-fa.

deposit the biological material in all the countries where he/she wants to obtain a patent. The applicant needs only to deposit the biological material at one recognized institution in all countries party to the Budapest Treaty. After our Government signed the Budapest Treaty, any applicant, whether it is a Chinese or foreign person, could treat his/her biological material with his/her patent as he/she likes.

After our Government signed the Budapest Treaty, the Government required the two Collections, our Collection and the Collection in Beijing, for deposit of patent micro-organisms to have International Depository Authority (IDA) according to Article 7 of Budapest Treaty outlining the requirement for each facility to become an IDA. Thus, during a summer vacation, I wrote the document with the assistance of one of our staff, Qu San-pu, for our Collection to apply for an IDA from the World Intellectual Property Organization (WIPO). Then WIPO assigned two inspectors, Mrs B Brandon and Mr Wang Zheng-fa, to visit our Collection for inspection on our application for an IDA.

We received Mrs B. Brandon and Mr Wang Zheng-fa from WIPO and showed our applying document and accompanied them to inspect our facilities, the hardware and the software, and housing. On behalf of our Collection, CCTCC, I orally stated our request to join IDA. After that, they indicated they were satisfied with our document, the oral report as well as the facilities and recognized that CCTCC could meet the requirements by WIPO for an IDA (Figure 16). Finally, they told us that they would transmit our document and report what we had stated to the relevant section of WIPO. This was the second time Mrs B. Brandon visited CCTCC, but this time, she spoke on behalf of an inspector assigned from WIPO to visit. Soon thereafter, WIPO approved and confirmed that the two Chinese culture collections were members of IDA.

Afterwards, foreign applicants had no need to deposit patent cultures and other biological materials in China. Then, our Collection suddenly did less work for the patent profession,

and we could use more time to take part in other things, especially in research work.

After retirement

In 1997, according state system, the personnel division of Wuhan University informed me that I was of retirement age. But the new leaders of CCTCC hired me for one more year. Therefore, I left our Collection at the end of 1998. After I retired from Wuhan University, I came back to Beijing again and still live in the yard of CAAS. At this time, I have enough time to do things that I like to do.

Professor Chen Wen-xin in China Agricultural University and my other friends organized a group to edit a short edition on systematic bacteriology in Chinese for the needs of the Chinese microbiological community. I was retired and had time to organize the writing of this book. Then, I invited Dr Yang Rui-fu, Dr Dong Xiu-zhu as the editor-in-chiefs for the book, and I also invited Dr Chen Wen-fang to be vice editor-in-chief because he had taken a lot of time to edit the book. We developed a writing outline and named the book as Systematics of Prokaryotes (conciseness). According to the system of the Taxonomic Outline of the Prokaryotic Genera, in the new edition of *Bergey's Manual*, we edited our above book. However, the space of our book was limited. We did not deal with all of genera of the prokaryotes, but selected only some representative groups to discuss their taxonomy. And in this book, we wrote two chapters to discuss the Bacteriological Code and its significance in prokaryotic systematics and bioinformatics as well as its application to prokaryotic taxonomy, respectively. We also included some taxonomic techniques such as molecular biological methods for taxonomy in the book. In my personal opinion, the book is the introductory version in Chinese for the new edition of the *Bergey's Manual*, of course, which was edited and published during this period. Our book was published by the Chemical Engineering Press in Beijing, 2007.

In 2003, The Ministry of Science and Technology had organized some large nationwide projects. They were research bases and large scientific instrument, scientific data, natural scientific literature, nature and technological resources, online technological environment, and commercialization of research findings. I was invited as one of advisors for discussion of the preparation for the establishment of the above projects. Among them, the nature and technological resources included eight platforms, such as crop germ-plasmas, microbial resources, etc. Dr and Professor Liu Xu, the vice president of CAAS took charge of the project, nature and technology resources, and Professor Jiang Rui-bo, the director of ACCC led the subproject, microbial resources. Liu and Jiang often invited me as one of advisors for discussion on how to arrange and operate the above projects and evaluate the results.

For the project, microbial resources, there were nine large culture collections as the main participants, which also organized many regional microbial collections, universities, and other relevant institutions, and the main task for the project was the arrangement and integration of their existing practical microbial cultures and other biological materials in collections and their corresponding information. The practical cultures basically must be well preserved *in situ*, but all participant institutions would edit their directories of preserved microbial strains, respectively, and all of information about them would be submitted to the General Information Website, www. escience.gov.cn. The website is the portal for the Platform of Natural Science and Technology Infrastructure which is supported by the Ministry of Science and Technology and the Ministry of Finance of People's Republic of China. The website was established in September 2009 formally, and the website builds on information sharing so as to realize the material resource sharing of the data and working technology. During the implementation of the projects, the directors or curators of the culture collections organized and invited many experts to draft the standards and protocols for description of representative groups of micro-organisms, and laid down the operational specifications on microbiological experiments relating to the preservation of micro-organisms such as the operational rules etc. as well as the basic diagnosis of cultures, such as the standard Gram stain method, etc. All of the activities provided a good foundation for the development of biological resources centers in the future. I was honored to participate in the above activity as one of the advisors. And I also learned a lot during the activities and from my co-workers and peers, including many younger counterparts, such as Dr Gu Jin-gang, Dr Zhang Xiao-xia and others.

During that time, I recommended that Professor Jiang Rui-bo, the director of the ACCC, invite Dr Dagmar Fritze of the DSMZ to visit the ACCC. She gladly received Jiang's invitation to come to China. She gave lectures and discussed the taxonomy of the bacilii group of bacteria with us. The staff of ACCC exchanged their experience of culture collections with her. They believed that her visit was very successful, and they obtained useful knowledge from her. The leader of the Institute of Soils and Fertilizer and Professor Jiang recommended Zhang Xiao-xia to go DSMZ for a short period of training and if possible to work with Dr Fritze. Later, Zhang Xiao-xia went to DSMZ for study under the direction of Dr Fritze.

I welcomed Dr Fritze and also accompanied her visit to Dr Yang Rui-fu's Laboratory. Dr Fritze gave a seminar in the Institution. Her talk and visit were warmly welcomed. Moreover, I suggested to Professor Jiang to invite the Chairman of WFCC, Dr D. Smith of Commonwealth Agricultural Bureau International (CABI) in U.K., to come for a visit, and his visit brought many useful conferences, such as the Dictionary of Fungi, etc., to ACCC. Dr Smith was accompanied by us during his visit to the Institute of Microbiology. At the same time, the leader of the Institute recommended to him that ACCC would assign Gu Jin-gang to CABI for study. Later, Gu went to study under the direction of Dr Smith. In my personal opinion, sending younger staff abroad to study with the clear target of learning about culture collections, as well as other relevant knowledge, was very helpful for the further development of the ACCC. For this, I only played a role as mediator. As a matter of fact, I also learned from the youth. For example, Dr Gu and Dr Zhang quite often exchanged study information with me so I have learned some bioinformatics from them, because younger people acquire new knowledge more quickly than I as an older man.

Figure 17. Chinese microbiologists visit the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands in 2005. From left: Fang Cheng-xiang, Cheng Ci, Dr J.A. Stalpers, Tian-shen Tao, and a scientist from CBS.

During the activities of the projects, the leaders organized several international activities. In 2005, they organized a delegation to visit the CBS collection (Figure 17) in the Netherlands and CABI in U.K. (Figure 18) and I went with them as well because Gu Jin-gang from ACCC was studying there. The visits to the two culture collections were very rewarding.

In 2007, with a delegation of Chinese from the microbiological community, I attended ICCC-XI in Germany, spon-

Figure 18. A delegation of Chinese scientists visits the Commonwealth Agricultural Bureau International (CABI) in the UK in 2005, where Dr David Smith presented their work to us.

Figure 19. Tian-shen Tao and Brian J. Tindall at the DSMZ in Braunschweig, Germany, in 2007.

sor by the DSMZ. I met Professor Erko Stackebrandt, Dr D. Smith, Dr D. Fritze, et al., again, and we visited the laboratories and advanced experimental facilities which served as a model and an inspiration for our own culture collections (Figure 19).

After the conference, we went to the Institute of Pasteur in Paris, France. I accompanied Professor Fang Cheng-xiang to visit the Nobel Laureate Dr F. Jacob. Fang asked some questions of him and he patiently answered. I was very impressed when we looked around the Pasteur Museum. We marveled at how well Pasteur's relics were preserved. Finally, we revered Pasteur's mausoleum, which is delicate, artful and solemn, and stately. This is the best reminiscence

to pay tribute to Louis Pasteur.

I also appreciate Dr Cowan's concept of the "taxonomic trinity", namely classification, nomenclature and identification, for taxonomy of micro-organisms. I found that the genus of bacteria *Microsphaera* Yoshimi et al. 1996 was illegitimate in bacteriological nomenclature because the name *Microsphaera* had been used as a genus of fungi previously. For this reason, I invited Professor Chen Wen-xin and Dr Chen Wen-feng for discussion with my wife and me and we wrote a paper to propose changing the bacterial genus name *Microsphaera* Yoshimi et al. 1996 to the name *Nakamurella* and the family name *Microsphaeraceae* Rainey et al. 1997 to the name *Nakamurellaceae*. The paper was published in IJSEM in 2004.

In recent years, I was the second compiler-in-chief to produce a dictionary, the *Scientific Names of Bacteria with English Explanation and Chinese Translation*, Second Edition. It has been finalized and Dr and Professor Yang Rui-fu and I have read the manuscript proofs and it has been given to the Chemical Engineering Press for publication. We hope that it will be useful for microbiological community in China.

Originally, in 1985, the Chinese Government established a committee named the China National Committee for Terms in Sciences and Technologies (CNCTST) for the unification of terms in sciences and technologies. I was one of editors of *Chinese Terms in Microbiology*, the 2nd Edition. It is one of a series of *Chinese Terms in Sciences and Technologies*, including *Biochemistry and Molecular Biology*, *Immunology*, *Genetics*, etc. For these, every term has a standard Chinese name with a concise paraphrase (interpretation), and a corresponding English term. The editor-in-chief, Professor Cheng Guang-sheng, provides a unified draft that will be submitted to the Subcommittee for publication.

I am still devoted to contining my life's cause, but since I have retired, I only gather many references on culture collections and systematic bacteriology, including teaching materials, electronic books, PowerPoint presentations from friends or downloaded data from the Internet, and so on. As a senior microbiological worker, I also introduced some important references to my friends and peers. They are: (1) the *Guideline for the Establishment and Operation of Culture Collections of Microorganisms*, published by WFCC; (2) *The Biodiversity of Microorganisms and the Role of Microbial Resource Center*, published by WFCC and UNEP; (3) the books *Methods in Microbiology*, Volumes 3A and 3B; (4) *Culture Collection on Microorganisms*, as stated above. I also emphasized the use of websites, such as NCBI Taxonomy home page, List of Prokaryotic Names with Standing in Nomenclature (LPSN), MycoBank, StrainInfo, uBio, etc., for obtaining relevant information on biological resources. I think that every person who is engaged in work in culture collections or biological resource centers should understand the above-mentioned references and should be familiar with using the above websites for searching information.

I have enjoyed working in my field of culture collections and systematic bacteriology.

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Bergey's International Society for Microbial Systematics

Constitution and Bylaws

ARTICLE 1. NAME

The name of this organization shall be *Bergey's International Society for Microbial Systematics*, hereinafter referred to as BISMiS.

ARTICLE 2. OBJECTIVES

The objectives of BISMiS shall comprise the following scientific and educational activities:

Section 1. To promote the highest global standards of scientific research in bacterial and archaeal systematics.

Section 2. To support the scientific exploration of microbial diversity in order to comprehend the vast diversity of microbial life.

Section 3. To promote the global production and dissemination of scientific knowledge regarding bacterial and archaeal systematics through publications, meetings, workshops and reports.

Section 4. To support global efforts to enhance the education of all peoples regarding current knowledge about bacterial and archaeal systematics and diversity.

BISMiS was formed by Bergey's Manual Trust in 2010 following a survey of microbial systematists who expressed interest in an international organization to represent their scientific and educational needs in the field. BISMiS was formed exclusively as a non-profit branch of Bergey's Manual Trust for the above stated scientific and educational purposes. No part of its assets, income or profit shall be used for the personal benefit its members, directors or officers.

ARTICLE 3. MEMBERS

BISMiS shall consist of the following categories of members, who shall be nominated and elected as provided in the Bylaws.

Section 1. Full members. An individual who endorses the objectives of BISMiS and who holds a bachelor's degree in microbiology or a related field, or who has had an essentially equivalent background is eligible to apply for Full Membership. Full Members, including Honorary and Emeritus Members, are eligible to vote, hold elective office, serve on committees and boards and have access to *The Bulletin of BISMiS* and other materials of the society.

Section 2. Student members. A student who is matriculated in microbiology or a related field who does not hold a bachelor's or doctorate degree shall be eligible to become a Student Member. Student Members shall not have the right to vote or hold office.

Section 3. Honorary members. A person who has made outstanding contributions to microbial systematics shall be eligible for nomination and election as an Honorary Member. Honorary status is the highest recognition given to members and carries the same privileges as Full Member.

Section 4. Emeritus members. A person who has been a full member for at least 20 years preceding retirement from professional work and who is 70 years or older may apply for Emeritus status, which has the same benefits and rights of Full Members.

Section 5. Corporate, Foundation and Patron membership. Corporations, Foundations and Patrons, i.e., individuals who are not microbial systematists, that have an interest in supporting the objectives of BISMiS may join the society and have a vote but cannot become officers.

ARTICLE 4. OFFICERS

Section 1. Elective Officers. The Elective Officers shall be President, President-Elect and Secretary. Each officer shall serve a two-year term following the bi-annual meeting of BISMiS. Nomination and election of Elected Officers shall be conducted as provided in the Bylaws.

Section 2. Duties of the elective officers shall be:

a. The **President** shall preside over all the meetings of BISMiS and the Executive Board, and shall perform such duties as needed and parliamentary usage require, in accordance with this Constitution and Bylaws. The President shall also have the responsibility of organizing the

general meeting of BISMiS along with the Executive Board, including the time, location and program of the meeting.

b. The **President-Elect** shall preside in the absence of the President in addition to performing those duties that are delegated by the President. The President-Elect shall become President following the general meeting of BISMiS.

c. The **Secretary** shall maintain a register of the full, sustaining and honorary members of BISMiS; shall notify the membership of meetings and other pertinent matters; shall receive abstracts or manuscripts submitted for presentation at the general meeting as well as any reports that may ensue from the general meeting and other meetings.

Section 3. Treasurer. The Treasurer of Bergey's Manual Trust shall be the Treasurer of BISMiS. The BISMiS account including revenues from Memberships of all categories, meetings, publications and other activities of BISMiS will be kept separate from that of *Bergey's Manual*, a separate branch of Bergey's Manual Trust. The Treasurer's duties also include submitting tax information to the US government to ensure that BISMiS maintains its non-profit status.

Section 4. Executive Board. The executive board shall comprise the President, President-Elect, Secretary, Treasurer and Chair of Bergey's Manual Trust.

Section 5. Executive Officers. BISMiS may employ an Executive Director and Staff to assist in carrying out the policies of BISMiS if needed and can be supported by revenues of BISMiS.

ARTICLE 5. ORGANIZATION

In order to implement its objectives, BISMiS may need to institute Boards and Committees for specific purposes.

Section 1. Boards and Committees. Chairs and members of organizational groups shall be appointed by the Executive Board on an ad hoc basis. Permanent boards and committees may also be instituted if necessary.

Section 2. International Academy of Microbial Systematics. The officers shall appoint a Board to form an International Academy of Microbial Systematics (the Academy) to recognize noted microbiologists who have made significant contributions to microbial taxonomy. Those who have been elected to membership shall be called Fellows of International Academy of Microbial Systematics. Members of BISMiS may nominate other members or non-member affiliates of BISMiS who have made substantive contributions to the field by providing a curriculum vitae of the nominee along with a description of his or her major contributions. The nominees shall be evaluated by the Board, which will decide whether they shall become Fellows of the International Academy of Microbial Systematics.

The Academy shall have a Board of Governors, the members of which are elected by Fellows.

The Academy shall assess fees as needed for its operation.

ARTICLE 6. PUBLICATIONS

Publications such as *The Bulletin* of BISMiS and related communication services such as web sites will be administered through the Bergey's Editorial Office. Revenue received from publications that are above expenses of the Editorial Office will accrue to BISMiS in its separate BISMiS account.

Section 1. The Bulletin of BISMiS. *The Bulletin* shall be an official online publication of the society. *The Bulletin* does not publish original research articles. The content shall be restricted to biographies, autobiographies (by invitation), mini-review articles, opinion articles, reports of taxonomic committees, educational articles, obituaries and letters to the editor that pertain to the subject of microbial systematics and other items of interest to members of BISMiS.

The Editor of *The Bulletin* shall be appointed by the Executive Board for a term of three years, which may be renewed once. The Editor shall serve at the discretion of the Executive Board. The Editor shall be responsible receiving and deciding on the appropriateness of manuscripts, appointing an editorial board and staff, and all other aspects of the journal's publication.

ARTICLE 7. MEETINGS

BISMiS may hold meetings at specific international locations as necessary. Meetings shall occur on alternate years beginning with 2011. The President and Executive Board of BISMiS shall appoint a Chair of the Meetings Committee, who is located at the site for the forthcoming meeting at least one year prior to the meeting. The Chair shall establish a Local Organizing Committee that shall work with the President and Executive Board of BISMiS to organize and advertize the meeting.

Other *ad hoc* meetings may be organized by the Executive Board to address specific topics in microbial taxonomy and/or produce documents to support the educational and professional goals of BISMiS.

ARTICLE 8. TAX-EXEMPT STATUS

Section 1. BISMiS shall operate exclusively as a non-profit scientific and educational organization. None of the income generated from BISMiS shall be used for the benefit of members, officers or directors except that compensation for services provided by employees for services provided in Article 2 of the Constitution.

Section 2. BISMiS shall only partake in activities that are permitted for a corporation exempt from federal income tax under section 501(c)3 of the United States Internal Revenue Code of 1954.

ARTICLE 9. ARCHIVES

Archives shall be maintained for the collection, preservation and display of historical materials relating to BISMiS.

ARTICLE 10. AMENDMENTS

Section 1. An amendment to the Constitution may be proposed in writing by at least 30 members of BISMiS. Amendments must have a 2/3rds majority of Full Members in order to pass.

ARTICLE 11. DISSOLUTION

Section 1. If BISMiS is dissolved, the assets shall be distributed entirely to international scientific or educational organizations that qualify under the provisions of the United States Internal Revenue Service Code, Section 501(c)3.

Bylaws of BISMiS

(At this time there are no Bylaws for BISMiS. However, as specific needs arise that require the support of members, Bylaws may be adopted as an alternative to passing an amendment to the Constitution, in order to carry out the wishes of the majority of members.)

ARTICLE 1. AMENDMENTS TO BYLAWS

An amendment to the Bylaws may be proposed in writing by at least 15 members. Bylaw amendments will require a 3/5ths favorable vote of Full Members in order to pass.