The Bulletin of BISMiS

Published by Bergey's International Society for Microbial Systematics

Volume 3, part 1 – July 2012

The Bulletin of BISMiS

Published by Bergey's International Society for Microbial Systematics

ISSN **2159-287X**

Editorial Board

Director of the Editorial Office: William B. Whitman, *Athens, GA, USA*

Editor: James T. Staley, *Seattle, WA, USA*

Associate Editor: Paul A. Lawson, *Norman, OK, USA*

Editorial Board Members: Hans-Jürgen Busse, Jongsik Chun, Paul De Vos, Michael Goodfellow, Brian P. Hedlund, Peter Kämpfer, Wen-Jun Li, Wolfgang Ludwig, Bruce J. Paster, Fred A. Rainey, Ken-ichiro Suzuki, Martha E. Tujillo, William G. Wade, Naomi L. Ward and William B. Whitman

Managing Editor: Aidan C. Parte, *Sudbury, MA, USA*

Publisher and Editorial Office

Bergey's International Society for Microbial Systematics
Department of Microbiology
527 Biological Sciences Building
University of Georgia
Athens, GA 30602-2605
USA

email: bergeys@uga.edu

Copyright

The copyright in this publications belongs to Bergey's International Society for Microbial Systematics (BISMiS). All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (BISMiS), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden. The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

© 2012 Bergey's International Society for Microbial Systematics

On the cover

Cluttered Desk of an Old-time Microbiologist, a watercolor by Noel Krieg.

The Bulletin of BISMiS

Contents of Volume 3, part 1

Review

Evolution and diversification of oxygen metabolisms of aerotolerant anaerobes in the order *Bacillales* and other bacterial taxonomic groups **1**
Daichi Mochizuki, Naoto Tanaka, Morio Ishikawa, Akihito Endo, Yuh Shiwa, Nobuyuki Fujita, Junichi Sato and Youichi Niimura

The topsy-turvy world of a microbial systematist **19**
Zhiheng Liu

Sailing through the scientific ocean - my research on the systematics of actinomycetes **29**
Ji-Sheng Ruan

The Bulletin of BISMiS (2012), Volume 3, part 1, pp. 1–17

Evolution and diversification of oxygen metabolisms of aerotolerant anaerobes in the order *Bacillales* and other bacterial taxonomic groups

Daichi Mochizuki,[1] Naoto Tanaka,[2] Morio Ishikawa,[3] Akihito Endo,[4] Yuh Shiwa,[5] Nobuyuki Fujita,[6] Junichi Sato[1] and Youichi Niimura[1]

The relationship between the distribution of bacterial oxygen-protective/oxygen-utilizing enzymes, their phylogeny and implications on taxonomy are described in this review. The NADH oxidase (Nox)- or AhpF–AhpC (Prx) systems, which function in both hydrogen peroxide-scavenging or oxygen metabolism are widely distributed in bacterial obligate aerobes, facultative aerobes, aerotolerant anaerobes and obligate anaerobes. Although biologists regard the advent of oxygen production as a major transformational period of Earth's early biosphere about 2.5 Ga bp, if and how this change has influenced the evolution of bacteria is not well understood. However, the high correlation we report between the bacterial phylogenetic tree based on 16S rRNA gene sequences and the evolutionary trees derived from the amino acid sequences of the Nox and AhpF provide support for the view that bacteria evolved and diversified to produce disparate taxa from the *Bacillales* to various Gram-stain-negative bacteria that include a variety of species with enzyme systems to cope with oxygen toxicity and utilization. Among the Gram-stain-negative bacteria included in this study were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*. Additional studies of the evolution of these systems may aid in furthering our understanding of bacterial evolution in response to the production of oxygen and its increase in concentration in the biosphere.

Introduction

A wide variety of studies have revealed that diverse metabolic systems such as those involved with reactive oxygen species (Imlay, 2008), thiol proteins (Ritz and Beckwith, 2001) and biometals (Archibald, 1986; Yamamoto et al., 2000) are found among bacteria. Investigations of these enzymes together with bacterial phylogeny will aid in elucidating how prokaryotes have adaptated to the changes in the early environment on Earth due to the availability and increasing concentration of oxygen in the biosphere.

Depending on the bacterial species, oxygen exerts either positive or negative effects on their growth. Enzymes that protect bacteria from oxygen toxicity or utilize oxygen must have an important effect on their growth. We first introduce the distribution of hydrogen peroxide-scavenging and oxygen-metabolizing enzymes of aerotolerant anaerobes in the order *Bacillales* and other organisms that have these enzymes using *Amphibacillus xylanus* as a reference, based on their published bacterial taxonomy and available biochemical data. In addition, genomic analyses were conducted by using information available from public databases. Based on the data analyzed, we finally discuss the distributions of the related enzymes with the phylogenetic taxonomy of bacteria.

In general, bacteria are divided into five groups: obligate aerobes, facultative aerobes, microaerobes, aerotolerant anaerobes and obligate anaerobes, and their behaviors to oxygen are different in each group (Leadbetter, 2002).

Contact details

[1]Department of Bioscience, Tokyo University of agriculture, Tokyo Japan.

[2]NODAI Culture Collection Center, Tokyo University of Agriculture, Tokyo, Japan.

[3]Department of Fermentation Science, Tokyo University of Agriculture, Tokyo, Japan.

[4]Functional Foods Forum, University of Turku, Turku, Finland.

[5]Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture, Tokyo, Japan.

[6]Biological Resource Center, National Institute of Technology and Evaluation, Tokyo, Japan.

Corresponding author: Youichi Niimura - niimura@nodai.ac.jp

© BISMiS 2012

Table 1. Main oxygen-metabolizing enzyme systems discussed in this review

Enzyme or enzyme system	Reaction	Comment
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$	Uses H_2O_2 as substrate, heme- or Mn-catalase
NADH peroxidase	$H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$	Uses H_2O_2 as substrate
H_2O-producing NADH oxidase	$O_2 + 2NADH + 2H^+ \rightarrow 2H_2O + 2NAD^+$	H_2O-producing NADH oxidase
NADH oxidase (Nox)	$O_2 + NADH + H^+ \rightarrow NAD^+ + H_2O_2$	H_2O_2-producing NADH oxidase
NADH oxidase (Nox)–AhpC (Prx)	$O_2 + NADH + H^+ \rightarrow NAD^+ + H_2O_2$	Uses O_2, and H_2O_2 and ROOH* as substrate
	$H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$	
Alkyl hydroperoxide reductase (AhpF)–AhpC (Prx)	$H_2O_2 + NADH + H^+ \rightarrow 2H_2O + NAD^+$	Uses H_2O_2 and ROOH* as substrate

*Alkyl hydroperoxide.

Table 2. Phenotypic properties of the family *Bacillaceae**†

Characteristic	*Bacillus*	*Alkalibacillus*	*Amphibacillus*	*Anoxybacillus*	*Cerasibacillus*	*Filobacillus*	*Geobacillus*	*Gracilibacillus*	*Halobacillus*	*Halolactibacillus*	*Lentibacillus*	*Marinococcus*	*Oceanobacillus*	*Paraliobacillus*	*Pontibacillus*	*Saccharococcus*	*Tenuibacillus*	*Thalassobacillus*	*Virgibacillus*
Spore formation	+/−	+	+	+	+	+	+	+	+	−	+	−	+	+	+	−	+	+	+
Growth																			
Aerobic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic	+	−	+	+	−	nd	+	+	−	+	−	−	+	+	−	−	nd	−	+
Heme-catalase	+/−	+	−	+/−	+	+	+/−	+	+	−	+	+	+	+	+	+	+	+	+

*Symbols: nd, no data; +, positive; −, negative; +/-, some species are positive, some species are negative.
†Based on *Bergey's Manual of Systematic Bacteriology*, 2nd edition.

Obligate aerobes can not grow in the absence of oxygen, on the other hand, obligate anaerobes can not grow in the presence of oxygen. Microaerobes require oxygen, but can tolerate it only at low concentrations. While typical facultative aerobes such as *Escherichia coli* enhance their growth by the utililzation of oxygen, the growth of aerotolerant anaerobes such as most lactic acid bacteria is suppressed by oxygen.

In this way, the growth properties of most bacteria are affected by the presence or absence of oxygen. However, the aerotolerant anaerobe *Amphibacillus xylanus* grows well with the same cell yield and growth rate regardless of the presence or absence of oxygen (Niimura et al., 1990; Niimura and Suzuki, 2009). A recent study reported that *Amphibacillus xylanus* shows favorable growth even at 80% oxygen (four times higher than atmospheric concentration). However *Amphibacillus xylanus* is readily distinguished from aerobes because *Amphibacillus xylanus* lacks both a respiratory chain and catalase and uses an NADH oxidase–AhpC (Prx) system for both its oxygen metabolism (Niimura et al., 1993; Nishiyama et al., 2001) and hydroperoxide-scavenging reaction (Niimura et al., 1995, 1996). In addition to this enzyme system, several oxygen-metabolizing enzymes not related to a respiratory chain and hydrogen peroxide-scavenging enzymes other than catalase have been identified in obligate aerobes (Antelmann et al., 1996; Mongkolsuk et al., 1997), facultative aerobes (Murphy et al., 1984; Hansson and Haggstrom, 1984) and obligate anaerobes (Kawasaki et al., 2004, 2007, 2009; Diaz et al., 2004) (see detailed data in Table 5). These enzymes therefore have been of interest

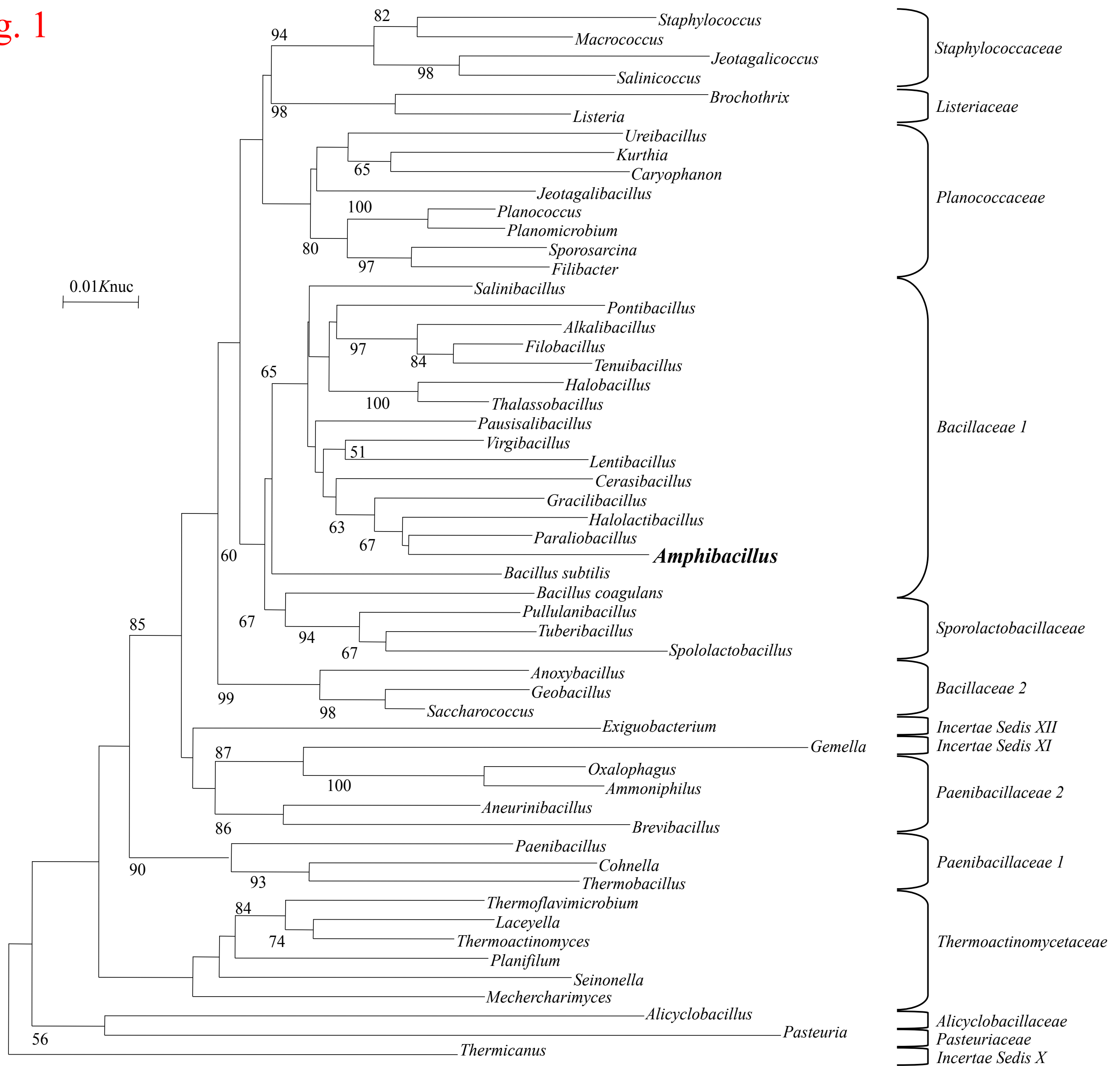


Figure 1. Phylogenetic tree of the order *Bacillales* based on 16S rRNA gene sequences. The genus *Thermicanus* was used as an outgroup. Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 50% are shown. The bar represents the unit length of the number of nucleotide substitution.

in studies of the comparative physiology of bacteria.

In Section 1 we discuss the distribution of the oxygen-metabolizing and hydrogen peroxide-scavenging enzymes found in aerotolerant anaerobes, based on biochemical data. The biochemical data were confirmed by using genomic information available in public databases in Section 2. Finally, the relationship between the divergence among these enzyme systems and their implications for bacterial evolution are discussed in Section 3.

The list of the primary targeted enzyme systems discussed in this article are found in Table 1. These include:

Hydrogen peroxide-scavenging enzymes: catalase, NADH peroxidase, and NADH oxidase (Nox)- or alkylhydroperoxide reductase (AhpF)–AhpC (Prx). AhpC now belongs to the peroxiredoxin (Prx) family, and the NADH oxidases

Table 3. Phenotypic properties of *Amphibacillus xylanus* and related species[a,b]

Characteristic	*Amphibacillus xylanus*	*Amphibacillus fermentum*	*Amphibacillus tropicus*	*Amphibacillus sediminis*	*Amphibacillus jilinensis*	*Halolactibacillus halophilus*	*Halolactibacillus miurensis*	*Halolactibacillus alkaliphilus*	*Streptohalobacillus salinus*	*Natronobacillus azotifigens*	*Paraliobacillus ryukyuensis*	*Paraliobacillus quinghaensis*	*Gracilibacillus dipsosauri*	*Gracilibacillus halotolerans*
Spore formation	+	+	+	+	+	−	−	−	−	+	+	+	+	+
Aerobic growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	+	+	+	w	+	+[1]	+	−	−	ANR
Cytochromes	−	−	−	−	nt	−	−	nt	nt	nt	+	nt	+	+
Quinones	−	−	−	−	−	−	−	−	+	nt	+	+	+	+
Catalase	−	−	−	−	−	−	−	−	−	+	+	+	+	+
Products in anaerobic growth:														
Lactate	−	+	+	nt	nt	+[2]	+[2]	+[2]	+	−	+	−	−	−
Acetate	+	+	+	nt	nt	+	+	nt	+	+	+	−	−	−
Formate	+	+	+	nt	nt	+	+	nt	−	+	+	−	−	−
Ethanol	+	+	+	nt	nt	+	+	nt	+	+	+	−	−	−
Pyruvate	+	−	−	nt	nt	−	−	nt	−	−	−	−	−	−

[a]Symbols: nt, not tested; w, weak; ANR, anaerobic respiration; +, positive; −, negative.

[b]Based mainly on *Bergey's Manual of Systematic Bacteriology*, 2nd edition, Volume 4 (Logan and De Vos, 2009).

[1]Obligately fermentative but aerotolerant.

[2]Main product was lactate.

(Nox) and AhpF now belong to the same peroxiredoxin oxidoreductase family. The Nox– or AhpF–Prx system shows a high scavenging activity for both hydrogen peroxide and various kinds of hydroperoxides. The NADH oxidase (Nox) producing H_2O_2 is distinguished from H2O-producing NADH oxidase.

- Oxygen-metabolizing enzymes: respiratory chain, H_2O-producing NADH oxidase, and Nox–AhpC (Prx).
- Bifunctional oxygen-metabolizing and hydrogen peroxide-scavenging enzymes: Nox–AhpC (Prx) system.
- NADH-generating system: glycolytic pathway, TCA cycle, and pyruvate dehydrogenase complex (PDH).

Section 1. Distribution of oxygen-metabolizing and hydrogen peroxide-scavenging enzyme systems in bacteria

The aerotolerant anaerobe *Amphibacillis xylanus* is used as the reference organism in this section because the enzyme system of the organism has been well characterized (Niimura et al., 1989, 1990).

A. *Amphibacillus xylanus*

Horikoshi et al. (1982) investigated the xylan-assimilable alkaliphilic bacteria because xylan is a major component of hemicellulose. Most strains isolated were actually aerobic bacteria that required oxygen for their cultivation. Bacteria that exhibit good growth regardless of the presence or absence of oxygen are considered to be most suitable for biomass utilization. For the purpose of xylan biomass utilization, Niimura et al. (1990) attempted to isolate strains capable of assimilating xylan under alkaline conditions regardless of the presence or absence of oxygen. Although typical enrichment culturing was unsuccessful, strains were finally isolated from alkaline compost and were used for this screening (Niimura et al., 1990). The isolated organisms were Gram-stain-positive, spore forming, rod-shaped bacteria that lacked a respiratory chain

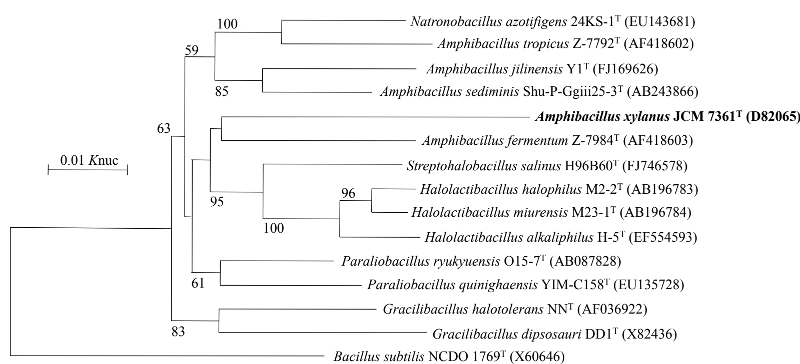


Figure 2. The phylogenetic relationship of *Amphibacillus* species and its relatives of the *Bacillus* HA group. Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 50% are shown. The bar represents the unit length of the number of nucleotide substitution.

and catalase, but showed the same cell yield and growth rate under both aerobic and anaerobic conditions. Since there was no appropriate genus and species to accommodate this bacterium, the name *Amphibacillus xylanus* was proposed (Niimura et al., 1990). The genus *Amphibacillus* currently contains five species and is placed in the family *Bacillaceae* (Figure 1 and Figure 2).

The explanation for the same good growth of this species under both aerobic and anaerobic conditions is that their aerobic and anaerobic pathways produce a similar amount of ATP (Figure 3A; Niimura et al., 1989, 2000). Importantly the amount of ATP produced ranges from one to two times more than that of the glycolytic pathway (approx. twice as much as the lactic acid fermentation). Anaerobically, NADH generated by the glycolytic pathway is consumed by alcohol production to maintain redox balance. Under aerobic conditions, in addition to the glycolytic pathway, the pyruvate dehydrogenase complex (PDH) is also involved in producing NADH. The NADH oxidase (Nox)–AhpC (Prx) system receives electrons from the NADH generated in the glycolytic and pyruvate metabolic pathways (PDH; Figure 3A). The Nox–Prx system also contributes to maintain the redox balance by metabolizing oxygen and hydrogen peroxide-scavenging.

B. Family *Bacillaceae*

Amphibacillus xylanus belongs to the family *Bacillaceae* (Figure 1 and Table 2). A key characteristic of the family is its aerobic growth property. However, some genera and species (*Bacillus arseniciselenatis*, *Bacillus infernos*, *Bacillus macyae*, and *Bacillus selenitireducens*) in the family exhibit obligate anaerobic growth characteristics (Logan and De Vos, 2009). The reason for this is that their respiratory chain can not utilize oxygen as an electron acceptor but can utilize organic or inorganic compounds as electron acceptors (Logan and De Vos, 2009). A respiratory chain is found in most genera of the family, except for two of the 19 genera, *Amphibacillus* and *Halolactibacillus* (Ishikawa et al., 2005).

A phylogenetic group called the *Bacillus* HA group contains the halophilic or halotolerant, and alkaliphilic species of the genus *Bacillus* as well as the genus *Amphibacillus* (Ishikawa et al., 2002). Few organisms had been classified in the group when *Amphibacillus xylanus* was isolated. At the time of writing this paper, the family *Bacillaceae* contains 27 genera and 125 species. The phylogenetic relationship of *Amphibacillus* species and its relatives in the *Bacillus* HA group is shown in Figure 2 (Logan and De Vos, 2009). Four species are currently included in the genus *Amphibacillus* (Table 3 and Figure 2). Although most of the species of the HA group are halophilic or halotolerant and alkalophilic, *Amphibacillus xylanus* grows under alkaline conditions but not under halophilic condition.

Amphibacillus xylanus forms endospores. It produces acetic acid under aerobic conditions and formic acid, acetic acid and ethanol under anaerobic conditions. No lactic acid is produced under either condition (Niimura et al., 1989). Recent genomic studies indicated that *Amphibacillus xylanus* genetically lacks lactate dehydrogenase (LDH) for the production of lactic acid. *Halolactibacillus*, a phylogenetic relative of the genus *Amphibacillus*, lacks the ability to form endospores and produces lactic acid as its primary end product (Table 3).

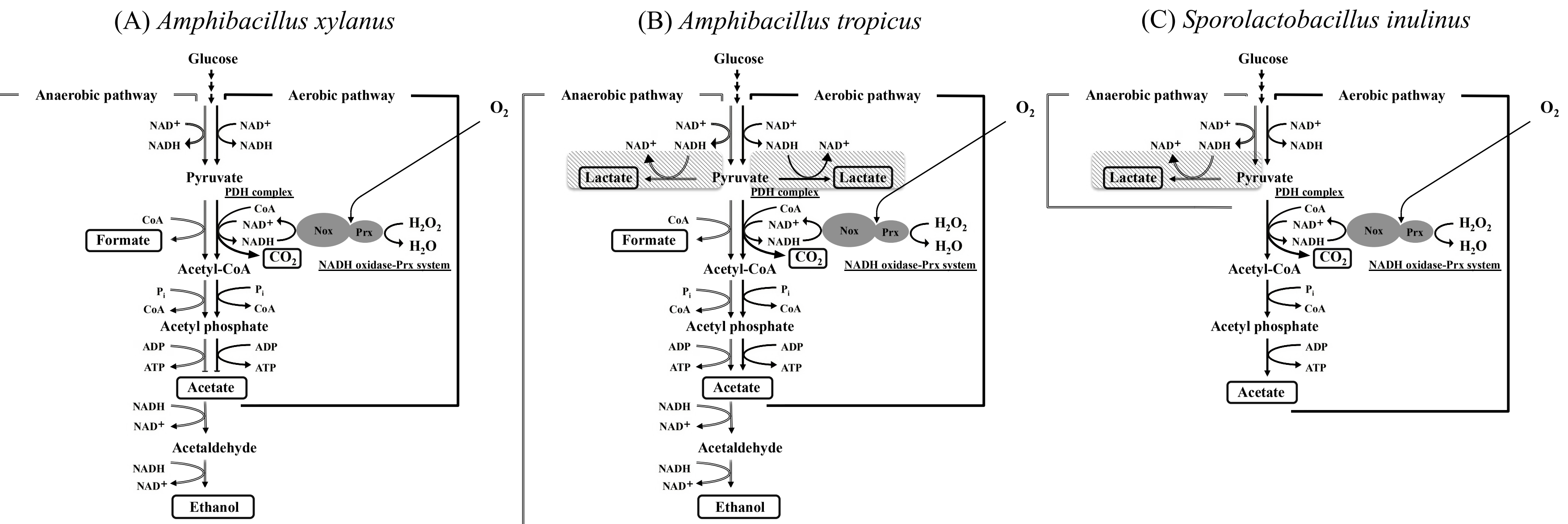


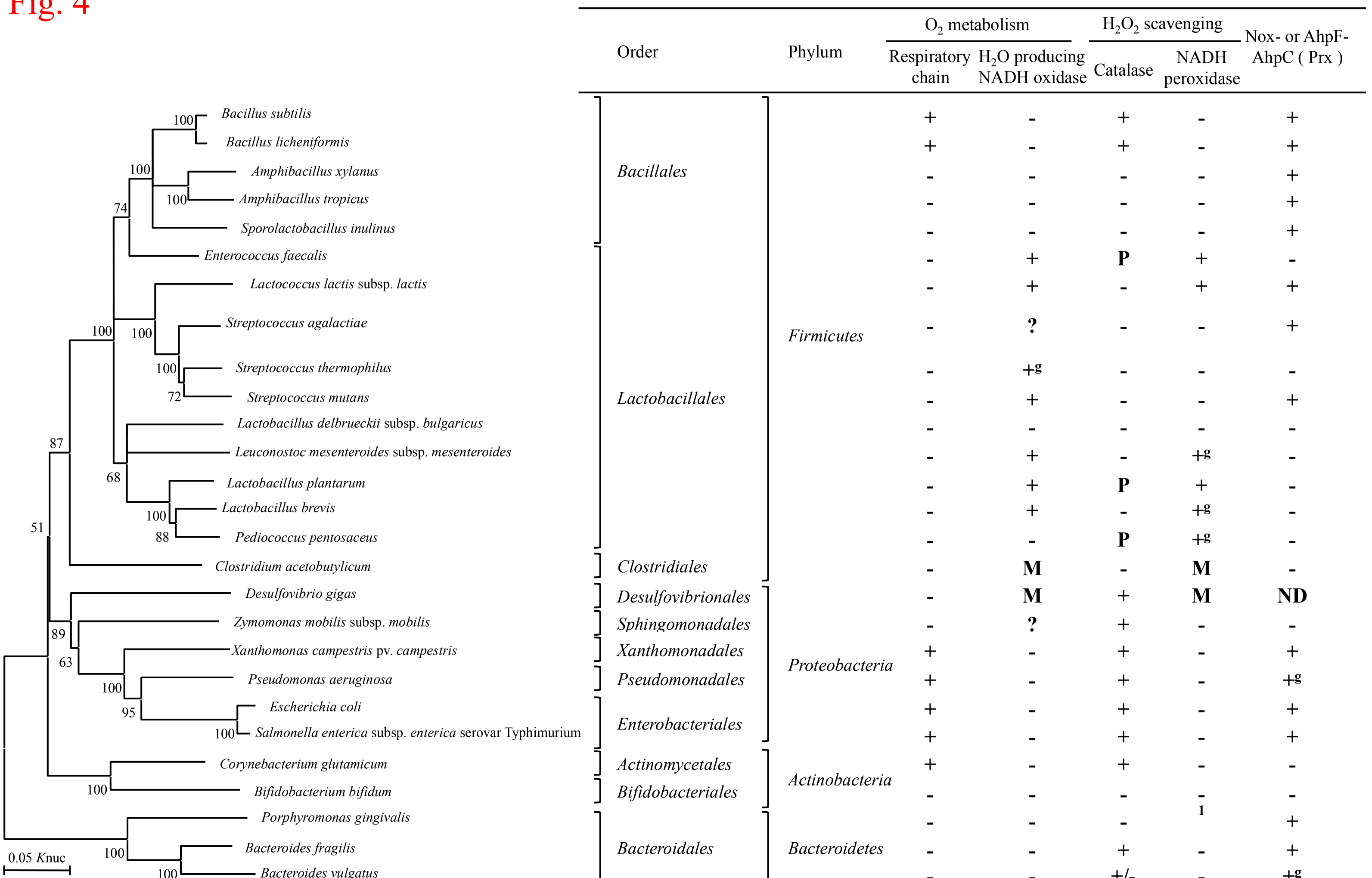
Figure 3. Comparison of proposed metabolic pathways between *Amphibacillus xylanus* and phylogenetically related bacteria. Shadows indicate different parts in metabolic pathways between *Amphibacillus xylanus* and related bacteria. (A) Proposed metabolic pathway in *Amphibacillus xylanus*. Anaerobically, NADH generated by the glycolytic pathway is consumed by alcohol generation to maintain redox balance. Under aerobic conditions, in addition to the glycolytic pathway, the pyruvate dehydrogenase complex (PDH) is also involved in producing NADH. The NADH oxidase (Nox)–AhpC (Prx) system receives electrons from the NADH generated in the glycolytic and pyruvate metabolic pathways (PDH). The Nox–Prx system also contributes to maintain redox balance by oxygen metabolism and hydrogen peroxide-scavenging (Niimura et al., 2000) . (B) Proposed metabolic pathways in *Amphibacillus tropicus, which* produces lactic acid derived from the pyruvate metabolic pathway, so that electrons from pyruvate do not fully contribute to the generation of NADH for the Nox–Prx system (Arai et al., 2008). (C) Proposed metabolic pathway in *Sporolactobacillus inulinus*. *Sporolactobacillus inulinus*, in the family *Sporolactobacillaceae* of the order *Bacillales*, possesses an aerobic metabolic system containing Nox–Prx system, similar to that of *Amphibacillus xylanus* (Nishiyama et al., 1997).

Table 4. Oxygen-metabolizing and hydrogen peroxide-scavenging enzymes in bacteria based on the published biochemical data*

Strain	O_2 metabolism	H_2O_2 scavenging		Nox- or AhpF–AhpC (Prx)	
	H_2O producing NADH oxidase	Catalase†	NADH peroxidase	Present	*t*-Butyl hydroperoxide reductase activity (mU/mg)[1]
Having a respiratory chain					
Obligately aerobic bacteria					
Bacillus subtilis 168	nd	+	nd	+[2]	nd
Xanthomonas campestris pv. *phaseoli*	nd	+	nd	+[3]	nd
Facultatively aerobic bacteria					
Alcaligenes faecalis NRIC 1001[T]	nd	+	nd	nd	14.0
Bacillus licheniformis NRIC 1863	nd	+	nd	+	46.4
Escherichia coli NRIC 1509	nd	+	nd	+[4]	7.4
Pseudomonas aeruginosa NRIC 1114[T]	nd	+	nd	nd	1.1
Salmonella enterica serovar Typhimurium NRIC 1851	nd	+	nd	+[5]	4.0
Lacking a respiratory chain					
Aerotolerant anaerobic bacteria					
Amphibacillus tropicus DSM 1387[T]	−	−	−	+[6]	nd
Amphibacillus xylanus JCM 7361[T]	−	−	−	+[7]	574.7
Lactococcus lactis subsp. *lactis* NRIC 1149[T]	+[8]	−	+[9]	+[8]	1.0
Sporolactobacillus inulinus NRIC 1133[T]	−	−	−	+[10]	8.4
Streptococcus agalactiae NEM316	nd	−	nd	+[11]	nd
Streptococcus mutans NBRC 11713	+[12]	−	nd	+[13]	nd
Enterococcus faecalis ATCC 11700	+[14]	P	+[15]	nd	nd
Lactobacillus brevis DSM 20054	+[16]	−	nd	nd	nd
Lactobacillus delbrueckii subsp. *delbrueckii* NRIC 1053[T]	nd	−	nd	nd	1.1
Lactobacillus plantarum‡	+[17]	P	+[18]	nd	nd
Lactobacillus sanfranciscensis ATCC 27651	+[19]	−	nd	nd	nd
Leuconostoc mesenteroides KSM 1101	+[20]	−	nd	nd	nd
Zymomonas mobilis subsp. *mobilis* NRIC 1158[T]	nd	+	nd	nd	0.8
Obligately anaerobic bacteria					
Bacteroides fragilis 683R	nd	+	nd	+[21]	nd
Bacteroides vulgatus JCM 5826[T]	nd	+/−	nd	nd	0.03
Porphyromonas gingivalis M50	nd	−	+[22]	+[23]	nd
Bifidobacterium bifidum JCM 1255[T]	nd	−	nd	nd	2.0
Bifidobacterium species§	nd	−	+[24,25]	nd	nd
Clostridium acetobutylicum ATCC 824	M[26]	−	M[27]	nd	nd
Clostridium aminovalericum DSM 1283[T]	+[28]	−	nd	nd	1.9
Clostridium butyricum JCM 1391[T]	nd	−	nd	nd	1.6
Desulfovivrio gigas NCIMB 9332	M[29]	+	M[30]	nd	nd

*Symbols: nd, no data; +, positive; −, negative; +/−, some strains are positive, some strains are negative; M, multi-enzyme complex; P, pseudocatalase (Mn-catalase). References: [1]Nishiyama et al. (2001); [2]Antelmann et al. (1996); [3]Mongkolsuk et al. (1997); [4]Seaver and Imlay (2001); [5]Jacobsen et al. (1989); [6]Arai et al. (2009); [7]Niimura et al. (1995); [8]Jiang et al. (2005);[9]Hansson and Haggstrom (1984); [10]Nishiyama et al. (1997); [11]Lechardeur et al. (2010); [12]Higuchi et al. (1993); [13]Pool et al. (2000); [14]Schmidt et al. (1986);[15]Poole and Claiborne (1986); [16]Hummel and Riebel (2003); [17]Jonathan et al. (2011);[18]Murphy and Condon (1984); [19]Riebel et al. (2003); [20]Koike et al. (1985); [21]Rocha and Smith (1999); [22]Diaz and Rogers (2004); [23]Diaz et al (2004); [24]Shimamura et al. (1992); [25]Talwalkar and Kailasapathy (2004); [26]Kawasaki et al. (2009); [27]Kawasaki et al. (2007); [28]Kawasaki et al. (2004); [29]Chen et al. (1993a); [30]Chen et al. (1993b).
†Based on *Bergey's Manual of Systematic Bacteriology*, 2nd edition, Volume 4.
‡ATCC 14917, P5, 10S.
§*B. infantis, B. lactis, B. pseudolongum, B. longum,* and *B. breve* was reported.

Two newly described species of the genus *Amphibacillus* (*Amphibacillus tropicus* and *Amphibacillus fermentum*) produce lactic acid in addition to metabolites from the pyruvate metabolic pathway (Zhilina et al., 2001). In an alkaline environment, *Halolactibacillus* species produce formate, acetate, and ethanol in addition to lactate, which is similar to that of *Amphibacillus* species (Table 3; Arai et al., 2009; Ishikawa et al., 2005). On the basis of these metabolites, we predicted that *Amphibacillus tropicus*, *Amphibacillus fermentum* and also *Halolactibacillus* had an oxygen-metabolizing and a hydrogen peroxide-scavening enzyme system equivalent to the Nox–Prx system of *Amphibacillus xylanus*. In fact, proteins related to Nox–Prx system were found in *Amphibacillus tropicus* and *Amphibacillus fermentum* by immunoblot analysis (Arai et al., 2009), and the hydrogen peroxide-scavenging activity of the Nox–Prx was found in cell-free extracts of *Amphibacillus tropicus* (Arai et al., 2009). However, these



Species	Order	Phylum	O_2 metabolism: Respiratory chain	O_2 metabolism: H_2O producing NADH oxidase	H_2O_2 scavenging: Catalase	H_2O_2 scavenging: NADH peroxidase	Nox- or AhpF-AhpC (Prx)
Bacillus subtilis	*Bacillales*	*Firmicutes*	+	-	+	-	+
Bacillus licheniformis	*Bacillales*	*Firmicutes*	+	-	+	-	+
Amphibacillus xylanus	*Bacillales*	*Firmicutes*	-	-	-	-	+
Amphibacillus tropicus	*Bacillales*	*Firmicutes*	-	-	-	-	+
Sporolactobacillus inulinus	*Bacillales*	*Firmicutes*	-	-	-	-	+
Enterococcus faecalis	*Lactobacillales*	*Firmicutes*	-	+	**P**	+	-
Lactococcus lactis subsp. *lactis*	*Lactobacillales*	*Firmicutes*	-	+	-	+	+
Streptococcus agalactiae	*Lactobacillales*	*Firmicutes*	-	**?**	-	-	+
Streptococcus thermophilus	*Lactobacillales*	*Firmicutes*	-	+[g]	-	-	-
Streptococcus mutans	*Lactobacillales*	*Firmicutes*	-	+	-	-	+
Lactobacillus delbrueckii subsp. *bulgaricus*	*Lactobacillales*	*Firmicutes*	-	-	-	-	-
Leuconostoc mesenteroides subsp. *mesenteroides*	*Lactobacillales*	*Firmicutes*	-	+	-	+[g]	-
Lactobacillus plantarum	*Lactobacillales*	*Firmicutes*	-	+	**P**	+	-
Lactobacillus brevis	*Lactobacillales*	*Firmicutes*	-	+	-	+[g]	-
Pediococcus pentosaceus	*Lactobacillales*	*Firmicutes*	-	-	**P**	+[g]	-
Clostridium acetobutylicum	*Clostridiales*	*Firmicutes*	-	**M**	-	**M**	-
Desulfovibrio gigas	*Desulfovibrionales*	*Proteobacteria*	-	**M**	+	**M**	**ND**
Zymomonas mobilis subsp. *mobilis*	*Sphingomonadales*	*Proteobacteria*	-	**?**	+	-	-
Xanthomonas campestris pv. *campestris*	*Xanthomonadales*	*Proteobacteria*	+	-	+	-	+
Pseudomonas aeruginosa	*Pseudomonadales*	*Proteobacteria*	+	-	+	-	+[g]
Escherichia coli	*Enterobacteriales*	*Proteobacteria*	+	-	+	-	+
Salmonella enterica subsp. *enterica* serovar Typhimurium	*Enterobacteriales*	*Proteobacteria*	+	-	+	-	+
Corynebacterium glutamicum	*Actinomycetales*	*Actinobacteria*	+	-	+	-	-
Bifidobacterium bifidum	*Bifidobacteriales*	*Actinobacteria*	-	-	-	-[1]	-
Porphyromonas gingivalis	*Bacteroidales*	*Bacteroidetes*	-	-	-	-	+
Bacteroides fragilis	*Bacteroidales*	*Bacteroidetes*	-	-	+	-	+
Bacteroides vulgatus	*Bacteroidales*	*Bacteroidetes*	-	-	+/-	-	+[g]

ND, no data; +, positive; -, negative; +/-, some strains are positive, some strains are negative
[g], existence of the gene but no its biochemical data; M, multi enzymes complex; P, pseudocatalase (Mn-catalase)
?, contains NADH oxidase but its products are unknown
[1]activity was detected in cell free extract (Diaz and Rogers 2004)

Figure 4. Relationship between enzymes for O_2-metabolizing and H_2O_2-scavenging enzymes and the phylogenetic tree of bacteria based on the 16S rRNA gene sequences. Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 50 % are shown. The bar represents the unit length of the number of nucleotide substitution.

bacteria produce lactic acid derived from the pyruvate metabolic pathway, so that the electrons from pyruvate do not fully contribute to the generation of NADH (Figure 3B). On the other hand, *Amphibacillus xylanus* does not produce lactic acid; instead, the NADH from pyruvate is directly supplied to the Nox–Prx system (Niimura, 1989, 2000). The unusual growth property (up to 80% oxygen atmosphere) of *Amphibacillus xylanus* is likely dependent on the Nox–Prx system and the NADH supplemental systems for its metabolism.

AhpF–AhpC (Prx), which is in the same family system as Nox–Prx, is found in the family *Bacillaceae*, including *Bacillus subtilis*, and *Bacillus licheniformis* (Table 4; Antelmann et al., 1996; Koyama et al., 1998; Nishiyama et al., 2001). These bacteria are phylogenetically distant from the HA group, and can efficiently use oxygen through a respiratory chain. Nox– or AhpF–Prx systems might be widely distributed among other species in the family *Bacillaceae* (details described next and in Section 2).

C. Other bacteria

As a result of studies of the family *Bacillaceae*, the oxygen-metabolizing and hydrogen peroxide-scavenging enzyme system [Nox– or AhpF–AhpC (Prx)] present in *Amphibacillus xylanus* was found not only in aerotolerant anaerobes which lack the respiratory chain, but also in typical aerobic bacteria. Therefore, in this section, its distribution is discussed in a wider variety of bacteria.

The oxygen-metabolizing enzyme, H_2O-producing NADH oxidase has been reported as an alternative metabolic route for respiratory chain-deficient aerotolerant anaerobes and obligate anaerobes (Chen et al., 1993a; Higuchi et al., 1993; Jiang et al., 2005; Kawasaki et al., 2004; Schmidt et al., 1986). Furthermore, NADH peroxidase (Mn-catalase known as non-heme catalase) has been reported as a hydrogen peroxide-scavenging enzyme as well as the Nox– or AhpF–Prx systems (Hansson and Haggstrom, 1984; La Carbona et al., 2007; Parsonage et al., 1993; Poole and Claiborne, 1986; Ross and Claiborne, 1991).

Although the behavior to oxygen is divided into the above-mentioned five different types (see introduction), most of the papers that have reported on these three enzymes studied only four groups: obligate aerobes, facultative aerobes, aerotolerant anaerobes and obligate anaerobes. Therefore, the distribution of Nox– or AhpF–Prx, NADH peroxidase and H_2O-forming NADH oxidase was examined in these four groups in other bacteria (Table 4 and 5).

Nox or AhpF-AhpC (Prx)

AhpF–AhpC (Prx) was first identified as an alkyl peroxide reductase in *Salmonella enterica*, which is a Gram-stain-negative facultative aerobe with a respiratory chain and catalase (Jacoboson et al., 1989). On the other hand, Nox–Prx system in *Amphibacillus xylanus* was identified as a dual enzymic system that functions in oxygen-metabolizing as well as a hydrogen peroxide-scavenging system (Niimura et al., 1995). The reactivity of the AhpF–Prx system to oxygen in *Salmonella enterica* is somewhat lower than that of *Amphibacillus xylanus* (Niimura et al., 1995), because *Salmonella* possesses a respiratory chain, which competes with the AhpF–Prx system for utilization of NADH. In contrast, the Nox–Prx system in *Amphibacillus xylanus* which lacks a respiratory chain possibly operates for both oxygen metabolism and hydrogen peroxide scavenging (Niimura et al., 2000).

Hydrogen peroxide reduction in both Nox–Prx and AhpF–Prx systems has a wide range of substrate specificity for hydroperoxides and high degree of affinity to substrate, leading to possibly the fastest reactivity against hydroperoxide (Niimura et al., 1995, 2000). These systems have been suggested to be important for the oxygen tolerance of some bacteria. The distribution of Nox– or AhpF–Prx systems in various bacteria is summarized in Table 4. Regardless of the Gram reaction of the bacteria studied, these enzyme systems are found in several groups of obligate aerobes and facultative aerobes that have a respiratory chain. *Sporolactobacillus inulinus*, in the family *Sporolactobacillaceae* of the order *Bacillales*, possesses an aerobic metabolic system containing Nox–Prx system, similar to that of *Amphibacillus xylanus* (Figure 3C; Nishiyama et al., 1997). The enzyme system was also found in *Streptococcus mutans* in the order *Lactobacillales* (Pool et al., 2000). Therefore, Nox– or AhpF–Prx systems might be widely distributed among other bacteria (Table 4 and Table 5).

Catalase, NADH peroxidase

Regardless of the Gram-stain reaction in bacteria, catalase

is reported to occur in almost all obligate aerobes and facultative aerobes that possess a respiratory chain with heme-synthetic capacities. Catalase therefore contributes to the reduction of hydrogen peroxide in those bacteria (Schonbaum and Chance, 1976).

On the other hand, Mn–catalase (Kono and Fridovich, 1983), also called pseudocatalase, has been found in some species of lactic acid bacteria that lack heme-synthetic ability. NADH peroxidase is also found in a group of aerotolerant anaerobes lacking a respiratory chain and heme-synthetic ability (Hansson and Haggstrom, 1984; La Carbona et al., 2007; Parsonage et al., 1993; Poole and Claiborne, 1986; Ross and Claiborne, 1991; Shimamura et al., 1992; Talwalkar and Kailasapathy, 2004).

Hydrogen peroxide-scavenging enzyme systems are generally found as a single protein, but obligately anaerobic bacteria, such as *Clostridium acetobutylicum* and *Desulfovibrio gigas* contain a multi-enzyme system for NADH peroxidase (Table 4; Chen et al., 1993a, 1993b; Kawasaki et al., 2004, 2005, 2007, 2009).

H_2O-producing NADH oxidase

H_2O-producing NADH oxidase is found mainly in respiratory chain-deficient aerotolerant anaerobes, but also has been reported in one obligate anaerobe, *Clostridium aminovalericum* (Table 4). H_2O-producing NADH oxidase is an enzyme system derived from a single protein (Chen et al., 1993a; Higuchi et al., 1993; Hummel and Riebel, 2003; Jiang et al., 2005; Riebel et al., 2002; Schmidt et al., 1986), but *Clostridium acetobutylicum* and *Desulfovibrio* also possess multi-enzyme systems for H_2O-producing NADH oxidase as well as NADH peroxidase (Chen et al., 1993a, 1993b; Kawasaki et al., 2004, 2005, 2007, 2009).

Section 2. Oxygen-metabolizing and hydroperoxide-scavenging enzyme systems analyzed by genomics

In this section, we compare the Nox– or AhpF–Prx, H_2O-forming NADH oxidases and NADH peroxidases by analyzing the genes of the enzymes from publicly available genomic databases.

Oxygen-metabolizing and hydrogen peroxide-scavenging enzymes other than catalase use NADH as an electron donor so that an NADH regeneration system is required for the oxidation–reduction balance. NADH is generally supplied from the glycolytic pathway through the pryuvate dehydrogenase complex (PDH) in addition to the TCA cycle. The glycolytic pathway and PDH also operate as an NADH generation system in *Amphibacillus xylanus*, which lacks a TCA cycle (Niimura et al., 1989, 2000). Thus, genes related to PDH are included in this section because most of the obligate aerobes, facultative aerobes, aerotolerant anaerobes and obligate anaerobes have the glycolytic pathway.

In Table 5, we have added genomic analysis data of the same species of the strains shown in Table 4 for which there are no biochemical data. Table 5 shows the distribution of oxygen-metabolizing and hydrogen peroxide-scavenging enzymes from the viewpoint of both biochemical and genomic data.

Genes corresponding to catalase and Nox– or AhpF–Prx systems are commonly found in obligate aerobes and facultative aerobes that have a respiratory chain. However, the proteins and genes of H_2O-producing NADH oxidase and NADH peroxidase are not seen in these same organisms in the data (Table 5). While the Nox– or AhpF–Prx system shows a high affinity for both hydrogen peroxide and other hydroperoxides (Niimura et al., 1995, 1996), catalase only scavenges hydrogen peroxide, and its affinity for hydrogen peroxide is not as high as Nox– or AhpF–Prx. One of the functions of the enzyme system is thought to be that of a complementary role to compensate for catalase (Seaver and Imlay, 2001).

Obligate aerobes and facultative aerobes with a respiratory chain are represented by the bacteria possessing a TCA cycle. The presence of PDH genes shown in Table 5 agrees with the existence of a TCA cycle in the organisms, suggesting that sufficient NADH is supplied not only to the respiratory chain but also to a Nox or AhpF–Prx system.

Aerotolerant anaerobes lacking a respiratory chain usually lack a heme-catalase but occasionally contain a Mn-catalase. All of the aerotolerant anaerobes lacking a respiratory chain so far studied lack heme-catalase but do possess PDH (protein or its gene) for a NADH generation system. In such organisms, NADH is therefore supplied by

Table 5. Oxygen-metabolizing and hydrogen peroxide-scavenging enzymes in bacteria based on published biochemical and genomic data*

Species	Growth†		O_2 metabolism			H_2O_2 scavenging			Nox- or AhpF-AhpC (Prx)		PDH complex	
	Anaer.	Aero.	Respiratory chain†	H_2O producing NADH oxidase		Catalase†	NADH peroxidase					
				bio	gen		bio	gen	bio	gen	bio	gen
Having a respiratory chain												
Obligately aerobic bacteria												
Bacillus subtilis	−	+	+	nd	−	+	nd	−	+	+	nd	+
Xanthomonas compestris	−	+	+	nd	−	+	nd	−	+	+	nd	+
Facultatively aerobic bacteria												
Alcaligenes faecalis	+	+	+	nd	nd	+	nd	nd	nd	nd	nd	nd
Bacillus licheniformis	+	+	+	nd	−	+	nd	−	+	+	nd	+
Escherichia coli	+	+	+	nd	−	+	nd	−	+	+	nd	+
Pseudomonas aeruginosa	+	+	+	nd	−	+	nd	−	nd	+	nd	+
Salmonella enterica serovar Typhimurium	+	+	+	nd	−	+	nd	−	+	+	nd	+
Lacking a respiratory chain												
Aerotolerant anaerobic bacteria												
Amphibacillus tropicus	+	+	−	−	nd	−	−	nd	+	nd	+‡	nd
Amphibacillus xylanus	+	+	−	−	−	−	−	−	+	+	+§	+
Lactococcus lactis subsp. *lactis*	+	+	−	+	nd	−	+	+	+	+	nd	+
Sporolactobacillus inulinus	+	+	−	−	nd	−	−	nd	+	nd	+||	nd
Streptococcus agalactiae	+	+	−	nd	?	−	nd	−	+	+	nd	+
Streptococcus mutans	+	+	−	+	+	−	nd	−	+	+	nd	+
Streptococcus species	+	+	−	nd	?	−	nd	+/−	nd	+	nd	+
Enterococcus faecalis	+	+	−	+	nd	P	+	+	nd	−	nd	+
Lactobacillus brevis	+	+	−	+	nd	−	nd	+	nd	−	nd	+
Lactobacillus delbrueckii	+	weak	−	nd	−	−	nd	−	nd	−	nd	−
Lactobacillus plantarum	+	+	−	+	nd	P	+	+	nd	−	nd	+
Leuconostoc mesenteroides	+	+	−	+	nd	−	nd	+	nd	−	nd	+
Pediococcus pentosaceus	+	+	−	nd	−	P	nd	+	nd	−	nd	+
Streptococcus thermophilus	+	+	−	nd	+	−	nd	−	nd	−	nd	+
Zymomonas mobilis	+	+	−	nd	?	+	nd	−	nd	−	nd	+
Lactobacillus sanfranciscensis	+	+	−	+	nd	−	nd	nd	nd	nd	nd	nd
Obligately anaerobic bacteria												
Bacteroides fragilis	+	−	−	nd	−	+	nd	−	+	+	nd	−
Bacteroides vulgatus	+	−	−	nd	−	+/−	nd	−	nd	+	nd	−
Porphyromonas gingivalis	+	−	−	nd	−	−	nd	#	+	+	nd	−
Bifidobacterium bifidum	+	−	−	nd	−	−	nd	−	nd	−	nd	−
Clostridium acetobutylicum	+	−	−	M	nd	−	M	nd	nd	−	nd	−
Bifidobacterium species**	+	−	−	nd	nd	−	+	nd	nd	nd	nd	nd
Clostridium aminovalericum	+	−	−	+	nd	−	nd	nd	nd	nd	nd	nd
Clostridium butyricum	+	−	−	nd	nd	−	nd	nd	nd	nd	nd	nd
Desulfovivrio gigas	+	−	−	M	nd	+	M	nd	nd	nd	nd	nd

*Symbols: nd, no data; +, positive; −, negative; +/−, some strains are positive, some strains are negative; P, pseudocatalase (Mn-catalase); M, multienzyme complex; ?, contains NADH oxidase but its products are unknown. Data source: bio, biochemical analysis; gen, genome analysis.
†Based on *Bergey's Manual of Systematic Bacteriology*, 2nd edition, Volume 4.
‡Arai et al. (2009).
§Niimura et al. (1989).
||Nishiyama et al. (1997).
¶*S. dysgalactiae, S. gallolyticus, S. parauberis, S. pasteurianus, S. salivarius*.
#Activity was detected in cell-free extracts but the gene of NADH peroxidase was not found (Diaz et al., 2004a).
***B. infantis, B. lactis, B. pseudolongum, B. longum* and *B. breve* were reported (Shimamura et al., 1992; Talwalkar and Kailasapathy, 2004).

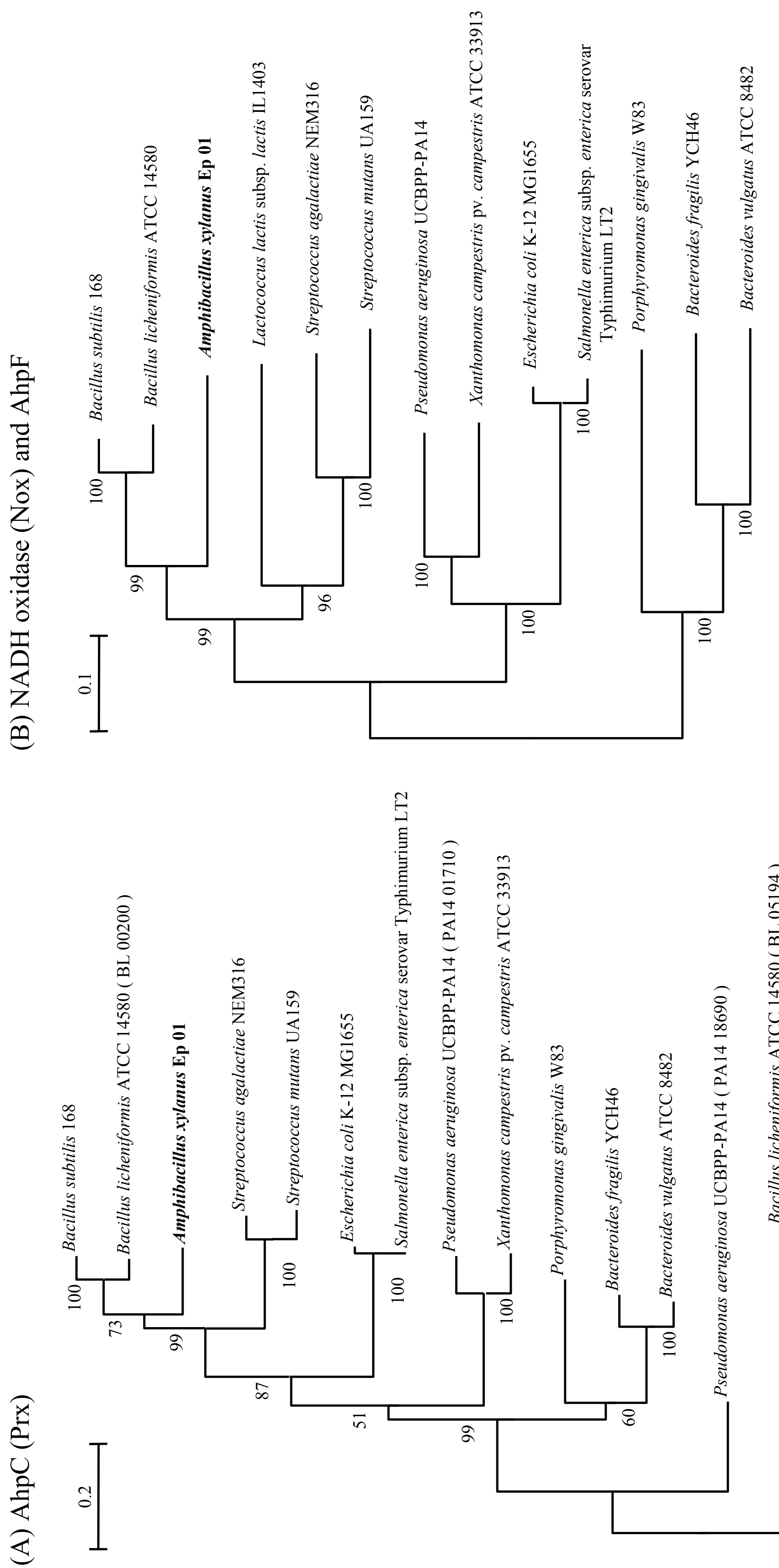


Figure 5. Evolutionary tree of bacteria based on amino acid sequence of (A) AhpC (Prx) and (B) NADH oxidase and AhpF. Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 50 % are shown. The bar represents the unit length of the number of amino acid substitutions.

the glycolytic pathway and PDH for each enzyme, Nox– or AhpF–Prx systems, H_2O-producing NADH oxidases and NADH peroxidases.

On the other hand, although obligate anaerobes have a variety of oxygen metabolic and hydrogen peroxide-scavenging enzymes (H_2O-producing NADH oxidase, NADH peroxidase, Nox– or AhpF–Prx system) and multi-enzymic systems funtional as both NADH peroxidase and H_2O-producing NADH oxidase, no PDH genes have been found from some genome-sequenced obligate anaerobes (Table 5). This fact suggests that NADH is supplied primarily from the glycolytic pathway in obligate anaerobes.

Section 3. Relationship between the distribution of oxygen metabolizing and hydrogen peroxide-scavenging enzymes and the phylogeny of bacteria

We found a distinctive distribution of enzymes in the four groups of bacteria that were categorized based on their physiological behavior in response to oxygen. In this section, we compare the relationship of the distribution of enzymes to bacterial phylogeny based on their 16S rRNA gene sequences (Figure 4).

H_2O-producing NADH oxidase and NADH peroxidase were found in some species in the order *Lactobacillales*, indicating that the distribution of these two enzymes varies at the species level rather than the genus level within the groups of aerotolerant anaerobes and obligate anaerobes that lack a respiratory chain (Table 4).

The distribution of the Nox– or AhpF–AhpC (Prx) system in both aerotolerant anaerobes and obligate anaerobes lacking a respiratory chain, also correlates more to the species level rather than the genus level. Furthermore, the enzyme system (protein or gene) was found in all of the tested strains in the orders *Bacillales*, *Xanthomonadales*, *Pseudomonadales*, and *Enterobacteriales*, and in a few strains in the orders *Lactobacillales*, indicating that the enzyme system is widely distributed across the classes and phyla of obligate aerobes and facultative aerobes with a respiratory chain (Figure 4).

We next tried to create an evolutionary tree based on the amino acid sequences of Nox– or AhpF–Prx system and compare it with that based on 16S rRNA genes, because the enzyme system is widely distributed in bacteria. The enzyme system is composed of two proteins from Prx and Nox or AhpF. Correlation between the evolutionary tree of Prx and the corresponding phylogenetic tree of the bacterial strain was found at the class level but not at the phylum level in the tested bacteria (Figure 5A) However, it is difficult to compare the evolutionary tree of Prx with the phylogenetic tree based on 16S rRNA genes because of the plurality of Prx gene homologs in bacteria. Thus, a comparison between the evolutionary tree based on Nox and AhpF and the phylogenetic tree was conducted, because Nox and AhpF have fewer homologs in single cells. The evolutionary analysis deduced from Nox and AhpF (Figure 5B) produced a similar topology to the phylogenetic tree deduced from 16S rRNA gene sequences at the phylum level (Figure 4). This suggests that Nox and AhpF may have arisen in the early evolution of bacteria and may have had a similar evolutionary history with that of the host organisms.

As Nox, AhpF and Prx are involved in oxygen metabolism in a number of bacteria, the phylogenetic tree of the E3 component of PDH, which exhibits distinct metabolic properties, was compared with its phylogenetic taxonomy. The tree showed a correlation within the family, but not above the family level (Figure 6). Similar results were obtained with both of the other components of PDH and ATP-generating enzymes (data not shown).

Therefore, the strong correlation between the Nox and AhpF evolutionary trees and the 16S rRNA gene phylogenetic tree suggests that these two proteins may be useful in analyzing the evolutionary relatedness among the disparate bacterial groups that contain these enzymes. Furthermore, the analyses presented in this review provide strong phylogenetic evidence to support the hypothesis that oxygen has influenced the evolution of bacteria.

Acknowledgements

We are grateful to Dr Ken-ichiro Suzuki at National Institute of Technology and Evaluation for valuable suggestions and critical reading of the manuscript. We also greatly thank Seiichi Sasaki at the United Nations Development Program for valuable assistance. This work was supported by MEXT (Ministry of Education, Culture,

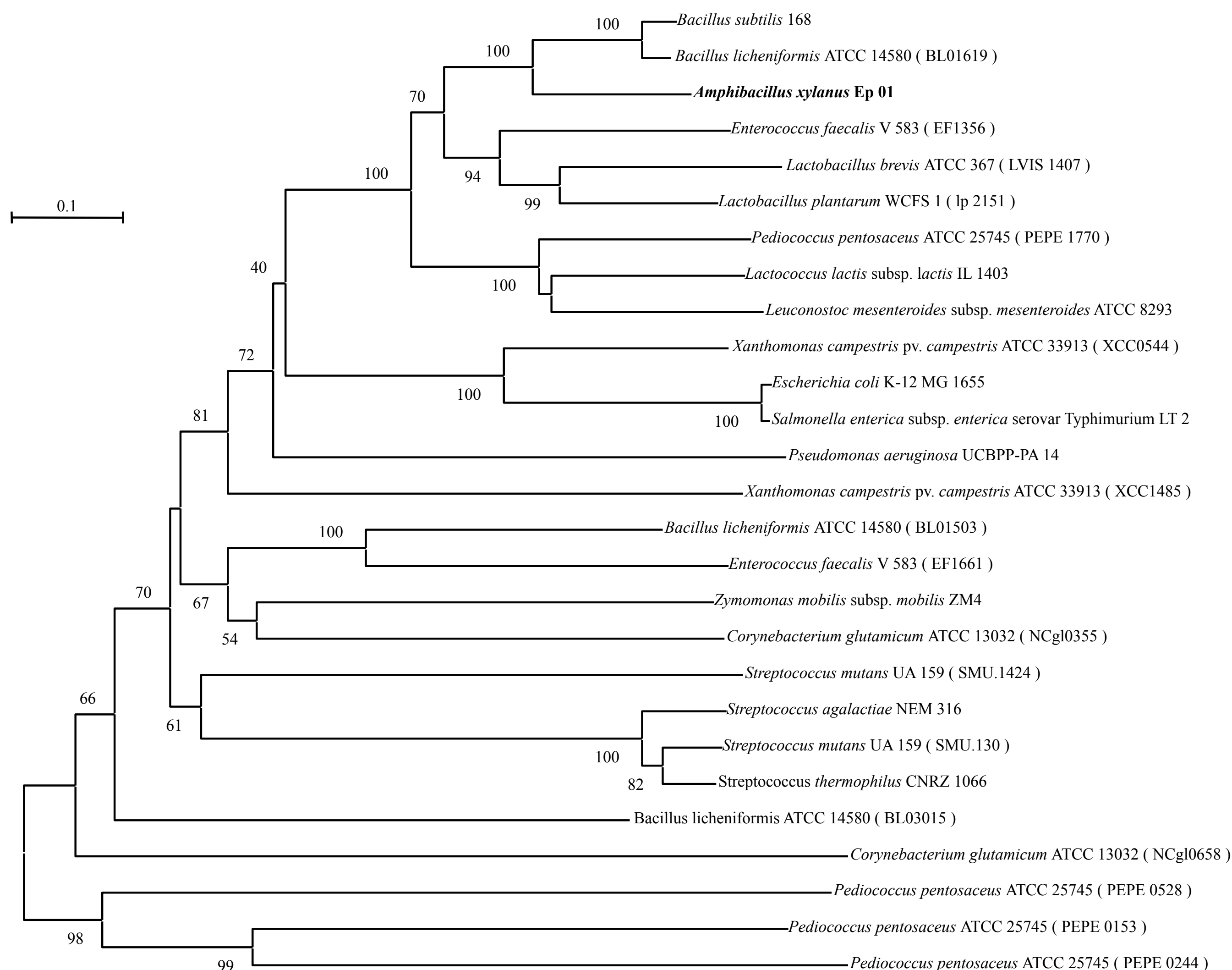


Figure 6. Evolutionary tree of bacteria based on the amino acid sequence of E3 (a component of PDH complex). Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 50 % are shown. The bar represents the unit length of the number of amino acid substitutions.

Sports, Science and Technology)*-Supported Program for the Strategic Research Foundation at Private Universities.

References

Antelmann, H., S. Engelmann, R. Schmid and M. Hecker. 1996. General and oxidative stress responses in *Bacillus subtilis*: cloning, expression, and mutation of the alkyl hydroperoxide reductase operon. J. Bacteriol. *178*: 6571–6578.

Arai, T., S. Yanahashi, J. Sato, T. Sato, M. Ishikawa, Y. Koizumi, S. Kawasaki, Y. Niimura and J. Nakagawa. 2009. Taxonomical and physiological comparisons of the three species of the genus *Amphibacillus*. J. Gen. Appl. Microbiol. *55*: 155–162.

Archibald, F. 1986. Manganese: its acquisition by and function in the lactic acid bacteria. Crit. Rev. Microbiol. *13*: 63.

Chen, L., M.Y. Liu, J. Legall, P. Fareleira, H. Santos and A.V. Xavier. 1993a. Purification and characterization of an NADH-rubredoxin oxidoreductase involved in the utilization of oxygen by *Desulfovibrio gigas*. Eur. J. Biochem. *216*: 443–448.

Chen, L., M.Y.Liu, J. Legall, P. Fareleira, H. Santos and A.V. Xavier. 1993b. Rubredoxin oxidase, a new flavohemo-protein, is the site of oxygen reduction to water by the strict anaerobe *Desulfovibrio gigas*. Biochem. Biophys. Res. Commun. *193*: 100–105.

Diaz, P.I. and A.H. Rogers. 2004. The effect of oxygen on the growth and physiology of *Porphyromonas gingiva-*

lis. Oral Microbiol. Immunol. *19*: 88–94.

Diaz, P.I., P.S. Zilm, V. Wasinger, G.L. Corthals and A.H. Rogers. 2004. Studies on NADH oxidase and alkyl hydroperoxide reductase produced by *Porphyromonas gingivalis.* Oral Microbiol. Immunol. *19*: 137–143.

Hansson, L. and M.H. Haggstrom. 1984. Effects of growth conditions on the activities of superoxide dismutase and NADH oxidase NADH peroxidase in *Streptococcus lactis*. Curr. Microbiol. *10*: 345–352.

Higuchi, M., M. Shimada, Y. Yamamoto, T. Hayashi, T. Koga and Y. Kamio. 1993. Identification of two distinct NADH oxidases corresponding to H_2O_2-forming oxidase and H_2O-forming oxidase induced in *Streptococcus mutans*. J. Gen. Microbiol. *139*: 2343–2351.

Horikoshi, K. 1982. Six enzymes of alkalophilic bacteria, xylanase. *In* Alkalophilic Microorganisms, a New Microbial World (edited by Horikoshi and Akiba), Japan Scientific Societies Press, Tokyo, Springer, New York, pp. 117–121.

Hummel, W. and B. Riebel. 2003. Isolation and biochemical characterization of a new NADH oxidase from *Lactobacillus brevis.* Biotechnol. Lett. *25*: 51–54.

Imlay, J.A. 2008. Cellular defenses against superoxide and hydrogen peroxide. Annu. Rev. Biochem. *77*: 755–776.

Ishikawa, M., K. Nakajima, Y. Itamiya, S. Furukawa, Y. Yamamoto and K. Yamasato. 2005. *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis* sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a phylogenetic lineage in *Bacillus* rRNA group 1. Int. J. Syst. Evol. Microbiol. *55*: 2427–2439.

Ishikawa, M., S. Ishizaki, Y. Yamamoto and K. Yamasato. 2002. *Paraliobacillus ryukyuensis* gen. nov., sp. nov., a new Gram-positive, slightly halophilic, extremely halotolerant, facultative anaerobe isolated from a decomposing marine alga. J. Gen. Appl. Microbiol. *48*: 269–279.

Jacobsen, F.S., R.W. Morgan, M.F. Christman and B.N. Ames. 1989. An alkyl hydroperoxide reductase from *Salmonella typhimurium* involved in the defense of DNA against oxidative damage purification and properties. J. Biol. Chem. *264*: 1488–1496.

Jiang, R.R., B.R. Riebel and A.S. Bommarius. 2005. Comparison of alkyl hydroperoxide reductase (AhpR) and water-forming NADH oxidase from *Lactococcus lactis* ATCC 19435. Adv. Synth. Catal. *347*: 1139–1146.

Jonathan, P.T., J.-I. Hirano, V. Thangavel, B.R. Riebel and A.S. Bommarius. 2011. NAD(P)H oxidase V from *Lactobacillus plantarum* (NoxV) displays enhanced operational stability even in absence of reducing agents. J. Mol. Catal. B-Enzymol. *71*: 159–165.

Kawasaki, S., J. Ishikura, D. Chiba, T. Nishino and Y. Niimura. 2004. Purification and characterization of an H_2O-forming NADH oxidase from *Clostridium aminovalericum*: existence of an oxygen-detoxifying enzyme in an obligate anaerobic bacteria. Arch. Microbiol. *181*: 324–330.

Kawasaki, S., M. Ono, Y. Watamura, Y. Sakai, T. Satoh, T. Arai, J. Satoh and Y. Niimura. 2007. An O_2-inducible rubrerythrin-like protein, rubperoxin, is functional as a H_2O_2 reductase in an obligatory anaerobe *Clostridium acetobutylicum*. FEBS Lett. *581*: 2460–2464.

Kawasaki, S., Y. Sakai, T. Takahashi, I. Suzuki and Y. Niimura. 2009. O_2 and reactive oxygen species detoxification complex, composed of O_2-responsive NADH:rubredoxin oxidoreductase-flavoprotein A2-desulfoferrodoxin operon enzymes, rubperoxin, and rubredoxin, in *Clostridium acetobutylicum*. Appl. Environ. Microbiol. *75*: 1021–1029.

Kawasaki, S., Y. Watamura, M. Ono, T. Watanabe, K. Takeda and Y. Niimura. 2005. Adaptive responses to oxygen stress in obligatory anaerobes *Clostridium acetobutylicum* and *Clostridium aminovalericum.* Appl. Environ. Microbiol. *71*: 8442–8450.

Koike, K., T. Kobayashi, S. Ito and M. Saitoh. 1985. Purification and characterization of NADH oxidase from a strain of *Leuconostoc mesenteroides*. J. Biochem. *97*: 1279–1288.

Kono, Y. and I. Fridovich. 1983. Isolation and characterization of the pseudocatalase of *Lactobacillus plantarum* a new manganese containing enzyme. J. Biol. Chem. *258*: 6015–6019.

La Carbona, S., N. Sauvageot, J.-C. Giard, A. Benachour, B. Posteraro, Y. Auffray, M. Sanguinetti and A. Hartke. 2007. Comparative study of the physiological roles of three peroxidases (NADH peroxidase, alkyl hydroperoxide reductase and thiol peroxidase) in oxidative stress response, survival inside macrophages and virulence of *Enterococcus faecalis.* Mol. Microbiol. *66*: 1148–1163

Leadbetter, E.R. 2002. Section1, 3 Prokaryotic Diversity: Form, Ecophysiology and Habitat. *In* Manual of Environmental Microbiology, 2nd edn.(edited by Hurst, Craw ford, Knudsen, McInerney, Stetzenbach and Walter), ASM Press, Washington, DC, pp. 19–31.

Lechardeur, D., Fernandez, A., Robert, B., Gaudu, P., Trieu-Cuot, P., Lamberet, G., Gruss, A. 2010. The 2-Cys peroxiredoxin alkyl hydroperoxide reductase C binds heme and participates in its intracellular availability in *Streptococcus agalactiae*. J. Biol. Chem. *285*: 16032–16041.

Logan, N.A. and P. De Vos 2009. Genus I. *Bacillus* Cohn 1872, 174[AL]. *In* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 3, The *Firmicutes* (edited by De Vos, Garrity, Jones, Krieg, Ludwig, Rainey, Schleifer and Whitman), Springer, New York, pp. 21–128

Mongkolsuk, S., S. Loprasert, W. Whangsuk, M. Fuangthong and S. Tichartpongkun. 1997. Characterization of transcription organization and analysis of unique expression patterns of an alkyl hydroperoxide reductase C gene (*ahpC*) and the peroxide regulator operon *ahpF-oxyR-orfX* from *Xanthomonas campestris* pv *phaseoli*. J. Bacteriol. *179*: 3950–3955.

Murphy, M.G. and S. Condon. 1984. Correlation of oxygen utilization and hydrogen peroxide accumulation with oxygen induced enzymes in *Lactobacillus plantarum* cultures. Arch. Microbiol. *138*: 44–48.

Niimura, Y. and K. Suzuki. 2009. Genus III. *Amphibacillus* Niimura, Koh, Yanagida, Suzuki, Komagata and Kozaki 1990, 299 emend. An, Ishikawa, Kasai, Goto and Yokota 2007b, 2492. *In* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 3, The *Firmicutes* (edited by De Vos, Garrity, Jones, Krieg, Ludwig, Rainey, Schleifer and Whitman). Springer, New York, pp. 131–134.

Niimura, Y. and V. Massey. 1996. Reaction mechanism of *Amphibacillus xylanus* NADH oxidase alkyl hydroperoxide reductase flavoprotein. J. Biol. Chem. *271*: 30459–30464.

Niimura, Y., E. Koh, F. Yanagida, K. Suzuki, K. Komagata and M. Kozaki. 1990. *Amphibacillus xylanus* new-genus new-species a facultatively anaerobic sporeforming xylan-digesting bacterium which lacks cytochrome quinone and catalase. Int. J. Syst. Bacteriol. *40*: 297–301.

Niimura, Y., E. Koh, T. Uchimura, N. Ohara and M. Kozaki. 1989. Aerobic and anaerobic metabolism in a facultative anaerobe Ep01 lacking cytochrome quinone and catalase. FEMS Microbiol. Lett. *61*: 79–84.

Niimura, Y., F. Yanagida, T. Uchimura, N. Ohara, K. Suzuki and M. Kozaki. 1987. A new facultative anaerobic xylan-using alkalophile lacking cytochrome, quinone, and catalase. Agr. Biol. Chem. *51*: 2271–2275.

Niimura, Y., K. Ohnishi, Y. Yarita, M. Hidaka, H. Masaki, T. Uchimura, H. Suzuki, M. Kozaki and T. Uozumi. 1993. A flavoprotein functional as NADH oxidase from *Amphibacillus xylanus* Ep01 - purification and characterization of the enzyme and structural-analysis of its gene. J. Bacteriol. *175*: 7945–7950.

Niimura, Y., L.B. Poole and V. Massey. 1995. *Amphibacillus xylanus* NADH oxidase and *Salmonella typhimurium* alkyl-hydroperoxide reductase flavoprotein components show extremely high scavenging activity for both alkyl hydroperoxide and hydrogen-peroxide in the presence of *Salmonella typhimurium* alkyl-hydroperoxide reductase 22-kDa protein component. J. Biol. Chem. *270*: 25645–25650.

Niimura, Y., Y. Nishiyama, D. Saito, H. Tsuji, M. Hidaka, T. Miyaji, T. Watanabe and V. Massey. 2000. A hydrogen peroxide-forming NADH oxidase that functions as an alkyl hydroperoxide reductase in *Amphibacillus xylanus*. J. Bacteriol. *182*: 5046–5051.

Nishiyama, Y., V. Massey, K. Takeda, S. Kawasaki, J. Sato, T. Watanabe and Y. Niimura. 2001. Hydrogen peroxide-forming NADH oxidase belonging to the peroxiredoxin oxidoreductase family: existence and physiological role in bacteria. J. Bacteriol. *183*: 2431–2438.

Nishiyama, Y., V. Massey, Y. Anzai, T. Watanabe, T. Miyaji, T. Uchimura, M. Kozaki, H. Suzuki and Y. Niimura. 1997. Purification and characterization of *Sporolactobacillus inulinus* NADH oxidase and its physiological role in aerobic metabolism of the bacterium. J. Ferment. Bioeng. *84*: 22–27.

Parsonage, D., H. Miller, R.P. Ross and A. Claiborne. 1993. Purification and analysis of streptococcal NADH peroxidase expressed in *Escherichia coli.* J. Biol.Chem. *268*: 3161–3167.

Poole, L.B. and A. Claiborne. 1986. Interactions of pyridine nucleotides with redox forms of the flavin-containing NADH peroxidase from *Streptococcus faecalis.* J. Biol. Chem. *261*: 14525–14533.

Poole, L.B., M. Higuchi, M. Shimada, M. Li Calzi and Y. Kamio. 2000. *Streptococcus mutans* H_2O_2-forming NADH oxidase is an alkyl hydroperoxide reductase protein. Free Radic. Biol. Med. *28*: 108–120

Riebel, B.R., P.R. Gibbs, W.B. Wellborn and A.S. Bommarius. 2002. Cofactor regeneration of NAD^+ from NADH: novel water-forming NADH oxidases. Adv. Synth. Catal. *344*: 1156–1168.

Riebel, B.R., P.R. Gibbs, W.B. Wellborn and A.S. Bommarius. 2003. Cofactor regeneration of both NAD^+ from NADH and $NADP^+$ from NADPH:NADH oxidase from *Lactobacillus sanfranciscensi*s. Adv. Synth. Catal. *345*: 707–712.

Ritz, D. and J. Beckwith. 2001. Roles of thiol-redox pathways in bacteria. Annu. Rev. Microbiol. *55*: 21–48.

Rocha, E.R. and C.J. Smith. 1999. Role of the alkyl hydroperoxide reductase (*ahpCF*) gene in oxidative stress defense of the obligate anaerobe *Bacteroides fragilis*. J. Bacteriol. *181*: 5701–5710.

Ross, R.P. and A. Claiborne. 1991. Cloning, sequence

and overexpression of NADH peroxidase from *Streptococcus faecalis* 10C1 – structural relationship with the flavoprotein disulfide reductase J. Mol. Biol. *221*: 857–871.

Schmidt, H.L., W. Stoecklein, J. Danzer, P. Kirch and B. Limbach. 1986. Isolation and properties of a water-forming NADH oxidase from *Streptococcus faecalis*. Eur. J. Biochem. *156*: 149–156.

Schonbaum, G.R. and Chance, B. 1976. Catalase, oxidation-reduction, Part C. Dehydrogenases (II), oxidases (II), hydrogen peroxide cleavage. *In* The Enzymes, 3rd edn, vol. XIII (edited by Boyer), Academic Press, New York, pp. 363–408.

Seaver, L.C. and J.A. Imlay. 2001. Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli.* J. Bacteriol. *183*: 7173–7181.

Shimamura, S., F. Abe, N. Ishibashi, H. Miyakawa, T. Yaeshima, T. Araya and M. Tomita. 1992. Relationship between oxygen sensitivity and oxygen-metabolism of *Bifidobacterium* species. J. Dairy Sci. *75*: 3296–3306.

Talwalkar, A. and K. Kailasapathy. 2004. The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium* spp. Curr. Issues Intest. Microbiol. *5*: 1–8.

Yamamoto, Y., M. Higuchi, L.B. Poole and Y. Kamio. 2000. Role of the dpr product in oxygen tolerance in *Streptococcus mutans*. J. Bacteriol. *184*: 3740–3747.

Zhilina, T.N., E.S. Garnova, T.P. Tourova, N.A. Kostrikina and G.A. Zavarzin. 2001. *Amphibacillus fermentum* sp. nov. and *Amphibacillus tropicus* sp. nov., new alkaliphilic, facultatively anaerobic, saccharolytic bacilli from Lake Magadi. Microbiologiya *70*: 711–722.

The Bulletin of BISMiS (2012), Volume 3, part 1, pp. 19–28

The topsy-turvy world of a microbial systematist

Zhiheng Liu

I am deeply honored to be asked to write an account of my career as a microbial systematist (Figure 1). My professional life was not all plain sailing for, like others across the world, I was caught up in dramatic upheavals that were well beyond my control. There is a lot of truth in the quote attributed to the great German philosopher Nietzsche, namely that "what does not destroy us, makes us stronger".

Figure 1. Zhiheng Liu in Haer Bin City, Northeast China in 2011.

Early years

I was born on 9 May 1940 in Fengmu Village, which means "fealty to Mother" village, in Xishua Country, Henan Province, which is about 900 km south of Beijing. My father was a prosperous farmer who was proficient in husbandry and beekeeping. My mother was a loving and gentle person who looked after her four needs of the family and helped my father on the farm. I attended Fengmu Primary School from 1948 to 1953 where I was particularly interested in the sciences and physical education, but I also took part in school plays. It was in my anamnesis that my life of childhood concluded with difficult times in China as ferocious wars were being waged against the invading Japanese army, a situation compounded by the Chinese War of Liberation. It was very difficult to come to terms with these terrible events.

Contact details

Institute of Microbiology, Academia of Sinica, Northern St.2, Ertiao 8#, Zhong-Guan-Cun, Haidian District, Beijing 100190, P.R. China.

zhliu@im.ac.cn

Figure 2. Zhiheng Liu in middle school, 1955.

In 1953, I was enrolled at Xihua No. 1 Middle School (Figure 2), the most prestigious local middle school, having gained high marks in the entrance examination. I studied hard throughout the 3 years I was there and as a result was able to join Zhoukou No. 1 High School without having to sit the entrance examination (Figure 3).

I was fascinated by all of the subjects taught in the high school, but took a particular interest in biology. As a member of the Biology Extracurricular Group I was told about the importance of nitrogen-fixing bacteria in agriculture – this was my first introduction to microbiology. Indeed, since that time I've always been engaged by the applied as well as the pure side of microbiology.

I entered Beijing Agricultural University in 1959 (Figure 4) having gained outstandingly high marks in the entrance examination. I chose microbiology as my main subject and was fortunate to be taught by two famous microbiologists, Professor Dafu Yu, a PhD graduate from Cornell University in Ithaca, New York State, and Professor Jilin

© BISMiS 2012

Figure 3. Zhiheng Liu in high school, 1957

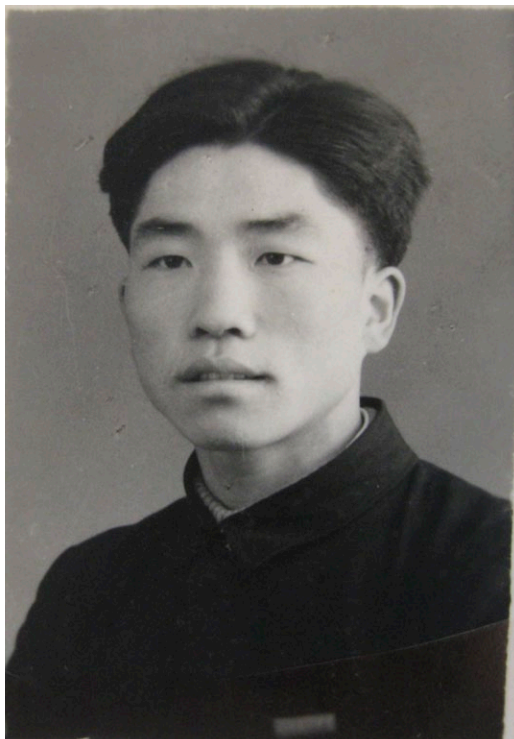


Figure 4. Zhiheng Liu in 1959.

Wu, a soil microbiologist who had been awarded a PhD from Moscow State University. I was also fortunate to be able to carry out my final year project in the Institute of Microbiology of the Chinese Academy of Sciences in Beijing. Here I was introduced to the actinomycetes by Dr Jisheng Ruan, who had obtained his PhD from the Institute of Microbiology of the USSR Academy of Sciences under the supervision of the renowned Professor Nicholai Alexandrovich Krassilnikov. The title of my graduation thesis was *Isolation of Actinomycetes Active Against Gram-negative Bacteria*.

Figure 5. Spending time with farmers in a cotton field at Shihezi farm, Xinjiang Uyghur Autonomous Region, 1967. Zhiheng Liu is at the back, second right.

Out in the cold

After graduation from University in July 1964. I was deemed to be from “prosperous farmer parentage” and to be “insufficiently socialist-minded”. I was sent by the government to the Xinjiang Uyghur Autonomous Region in northwest China over 3000 kilometres from Beijing to be “reformed through labor”. Here, I lived and worked with subsistence farmers as part of the Socialist Education Movement in Rural Areas (Figure 5). Before leaving for the Xinjiang area I got married in my hometown on 9 September 1964. I worked as a farmer in rural areas of Xinjiang for the next 2 years, a situation that was extended when the Great Proletarian Cultural Revolution broke out in June 1966, ushering in years of violent class struggle across China. So like most of the Chinese intelligentsia I had no opportunity to follow my professional interests.

The time I spent in Xinjiang area was very hard, food and clothes were not enough, the days were long, especially

Figure 6. Zhiheng Liu's son and daughter in 1976.

Figure 7. Academician Xunchu Yan (second left), Professor Jisheng Ruan (third left), my classmate Ms Lino Liang (first left) and myself (far right) in the Graduate School of the Chinese Academy of Sciences in Beijing, 1980.

in the hot summers and extremely cold winters. The only break was an annual 3-week visit back to my wife who worked as a nurse in a hospital in Xishua Country, Henan Province, 3000 km distant from Xinjiang. Our son, Liu Xiaotan, was born in 1967, and our daughter, Liu Xiaohui, was born in 1972 (Figure 6).

A new dawn

In May 1972, I was reunited with my wife and family and moved to Urumchi, the capital of Xinjiang Uyghur Autonomous Region. The Great Proletarian Cultural Revolution ended in 1976, ushering in a time of tremendous change in China. At the beginning of 1978, the reintroduction of the Postgraduate Education System provided me with an opportunity to rekindle my interest in microbiology. I was one of several hundred individuals to sit an examination for three MSc places on Actinomycetes taxonomy in the Graduate School of the Chinese Academy of Sciences in Beijing and was fortunate to be awarded one of these places. My luck continued as I was chosen to work under the supervision of Academician Xunchu Yan, an expert in the taxonomy of actinomycetes (Figure 7).

Figure 8. Zhiheng Liu writing his MSc thesis in Beijing in 1980.

As a middle-aged man, I studied hard in order to try and compensate for the 10 years spent in Xinjiang Province (Figure 8). My research assignment was to clarify the taxonomy of a group of nocardioform actinomycetes as these organisms were considered to be a promising source of new antibiotics following the discovery of rifamycin from "*Nocardia mediterranei*" (nov. *Amycolatopsis mediterranei*). My work at the time was greatly influenced by the publication of two books by Academic Press, *Actinomycetes: Characteristics and Practical Importance* (1972; edited by George Sykes and Fred Skinner) and *Biology of*

the Nocardiae (1976; edited by Michael Goodfellow, George Brownell and José Serrano). I completed my thesis *The Isolation and Identification of Novel Nocardioform Actinomycetes Isolated from Soil* and was awarded an MSc in October 1981. A tangible outcome of this work was the publication, in 1983, of two new species, *Nocardia flavorosea* and *Nocardia fusca* in *Acta Microbiologia Sinica*.

Getting back on track

At the end of 1981, I was appointed as an Assistant Professor in the Institute of Microbiology of Chinese Academy of Sciences in Beijing and remained employed there until my retirement in 2005. I was selected to work in the Actinomycetes Laboratory that was run by Dr Jisheng Ruan. The work of my research team (Figure 9) involved the use of chemotaxonomic procedures to improve the classification of diverse filamentous actinomycetes, notably those containing mycolic acids. Several new *Kitasatospora*, *Nocardia*, and *Nocardiopsis* species and one new genus, *Actinoalloteichus*, were described in issues of *Acta Microbiologica Sinica*. Partly as a result of these studies I was appointed as an Associate Professor in August 1987.

Figure 9. Zhiheng Liu (third left) with young scientists and students working in the Actinomycetes Laboratory in 1999.

It was my privilege to succeed Jisheng Ruan as Director of the Actinomycetes Laboratory in March 1994. The next few years were very exciting ones as we received generous support from the Natural Science Foundation of China, which allowed us to introduce a range of molecular systematic procedures into our polyphasic taxonomic studies (Figure 10). This support paid rich dividends as over the next 15 years we assigned over 70 new species to 19 genera. In addition, we proposed the genus *Yuhushiella* after a dear friend from my Xinjiang days, Yuhu Shi, in recognition of his pioneering work on the exploitation of microbial resoruces of the Xinjiang Uyghur Autonomous Region of China. Many of the papers derived from these studies were published in the *International Journal of Systematic and Evolutionary Microbiology*. We remain grateful to all of those who helped translate our "Chinese English" into a form that was acceptable to the scientific community.I was given new responsibilities in 1996 when I was appointed Director of the Chinese General Microorganism Culture Collection Centre (CGMCCC) in the Institute of Microbiology. My responsibilities were twofold as I was expected to improve the quality of the collection, notably by ensuring that strains were correctly classified, and to establish links with key service collections across the world, such as the BCCM (Belgium), DSMZ (Germany) and the JCM (Japan). The collection is now housed in excellent facilities in the new Institute of Microbiology in Beijing. I'm pleased that I was able to play a role in the development of this major microbial resource.

Figure 10. Zhiheng Liu (front row, third left) together with young scientists and students of the Actinomycetes Laboratory in 2003. Ying Huang, who succeeded me as Director, is second on the left.

Expanding horizons

When China opened up to the outside world in 1979 it became possible for us to develop links with fellow microbiologists across the world. Between March 1989 and November 1991 I spent two 6-month periods working with Professor Marian Mordarski, the Director of the Institute of Immunology and Experimental Therapy in Wroclaw, Poland, on a project funded by the Chinese Association for Science and Technology and the Polish Academy of Sci-

Figure 11. Together with colleagues from the Microbial Genetics Laboratory of the Institute of Immunology and Experimental Therapy in Wrocław in 1989 (Zhiheng Liu is on the right of the back row with Marian Mordarski).

ences (Figure 11). In Wroclaw, I learnt several molecular systematic techniques which were used to help improve the classification of the genus *Nocardiopsis*. The resultant work was published in *Actinomycetes* and in the national journal, *Chinese Biodiversity*. I was able to introduce the new techniques to the Actinomycetes Laboratory on my return to Beijing.

My next trip abroad was also succesful as it helped me to further develop my career and gave me the confidence to develop the Actinomycetes Laboratory following the retirement of Jisheng Ruan in March 1994. I was fortunate to be included in the latter stages of a joint international research project supported by the Chinese Academy of Sciences and The Royal Society and directed by Michael Goodfellow and Jisheng Ruan. From September 1994 to March 1995 I worked in the Microbial Resources Centre at Newcastle University (Figure 12). This was a wonderful time to be in Newcastle as the laboratory was full of talented PhD students, some of whom, like Jongsik Chun, Mohamed Hamid, Nevzat Sahin, Wasu Pathom-aree and Martha Trujillo, have gone on to excel as microbial systematists. Consequently, I was able to return to Beijing with a complete understanding of the theory and practice of polyphasic taxonomy with particular reference to the actinomycetes.

Figure 12. With Wasu Pathom-aree (far left) and Hassan Shojaei and Kamil Isik (second and far right) in the Microbial Resources Laboratory at Newcastle University in 1994.

Figure 13. Together with members of the Microbial Resources Centre, Newcastle University, 2003.

The award of a 3-year Royal Society Joint Project Grant [China–UK (1407/Q814)] to Michael Goodfellow and myself took me back to Newcastle for a year beginning in November 2002. The Microbial Resources Centre, as ever, was an exciting place to be as it was full of PhD students from across the world with Koreans and Mexicans in the ascendancy (Figure 13). The work I started in Newcastle on this project was further developed in Beijing and Newcastle, notably by Ying Huang and by Liming Wang both of whom spent time in Newcastle. Liming returned to Newcastle to do a PhD in stem cell research! The joint project went well as we were able to show that acidophilic actinomycetes (growth range pH 4.0–5.5) were common in acidic soils in China and the UK (Figure 14), that they

Figure 14. Sampling the acidic soils of Thrunton Wood, near Newcastle with Ros Brown, Michael Goodfellow and Gail Payne (left to right), 2002.

Figure 15. Together with Ying Huang (my right) and Alan Bull (my left) during his visit to the Actinomycetes Laboratory, 2005.

formed a taxonomically tight group on the basis of 16S rRNA gene sequence data, and were a source of novel antibiotics. Tangible outcomes of the project included the publication of several new species of *Kitasatospora*, *Nocardia*, *Streptomyces*, and *Streptacidiphilus* species.

I'm really pleased that the mutually beneficial research collaboration between Beijing and Newcastle that was started by Jisheng Ruan and developed during my tenure as Director of the Actinomycetes Laboratory is being continued by Ying Huang. Indeed, the collaboration between the two centers has extended beyond the original two research groups given the award of a joint project grant "Cryptic Biosynthetic Gene Clusters from Marine Bacteria" awarded to Professor Lixin Zhang and Dr Jem Stach from the Chinese Academy of Sciences and the Royal Society. Indeed, at this very moment Dr Dylan Wang from Lixin Zhang's group is working in Newcastle on the genetics and systematics of the genus *Verrucosispora* together with Michael Goodfellow and Jem Stach.

The successful interaction between Beijing and Newcastle took on a new dimension when a joint project "Marine Actinomycete Diversity as a Source of New Drugs" was funded by the Chinese Ministry of Science and Technology (MOST). The results of this project have been the subject of many original publications and have been presented at several national and international symposia by Lixin Zhang. This project has also involved Professor Alan Bull (University of Kent, UK) who visited the Actinomycetes Laboratory in 2005 (Figure 15).

It was also my good fortune to be involved with three other international collaborative projects designed to further our understanding of the systematics of filamentous actinomycetes of clinical, ecological and industrial importance. The first with Professor Jean Swings of the Department of Biochemistry, Physiology and Microbiology at Ghent University (Figure 16) was supported through a Belgian–Chinese Exchange Programme "Classification and Identification of Actinomycetes, specifically Bioactive *Streptomyces* Strains Isolated from Chinese Soils" (1998–2004). The second with Marian Mordarski and Andrzy Gamian of the Institute of Immunology and Experimental Therapy in Wroc□aw was funded by the Polish Committee of Scientific Research and MOST focused on the classification and identification of clinically significant aerobic actinomycetes belonging to the genera *Amycolatopsis*. Finally, the work with Miroslav Petricek of the Institute of Microbiology in Prague, (2002–2004), which was supported through the Czech and Chinese Academy of Sciences Exchange Scheme, was designed to foster our understanding of the biosynthesis of antibiotics by actinomycetes.

All of these collaborative projects lead to significant improvements in the classification of actinomycetes and our understanding of how these organisms could be used as microbial resources in China. Much of the work with Ghent was subsumed into a large scale International Collaborative Project led by David Labeda (United States Department of Agriculture in Peoria) and designed to improve the classification of the most complex of prokaryotic taxa, the genus *Streptomyces*. The results and conclusions

Figure 16. Together with Jean Swings (center) and his colleagues at Ghent University, 2001.

Figure 17. Zhiheng Liu (fifth from left) together with members of the Joint Laboratory and HISUN-IMCAS, Taizhou – Zhejiang Province, 2007.

drawn from this epic study have recently been published in *Antonie van Leeuwenhoek* and hopefully will prove invaluable for the *Streptomyces* research community.

Putting micro-organisms to good use

Since my time at Zhoukou No. 1 High School I've been interested in how micro-organisms can be used for agricultural and industrial purposes. Over the years I've worked closely with colleagues in pharmaceutical companies, notably by setting up the Joint Laboratory for Biopharmaceutical Research HISUN Pharmaceutical Company and the Institute of Microbiology of the Chinese Academy of Sciences) (Figure 17). I've also provided advice on the selective isolation and screening of rare actinomycetes to colleagues working at the New Drug Research and Development Center of North China Pharmaceutical Corporation in Shijiazhuang, Hebei Province, 300 kilometres south of Beijing. In addition, I acted as an advisor to the "Good Earth Group" on how micro-organisms can be used to produce innovative dairy products.

Astrobiology

In addition to microbial diversity I've always been interested in the origin of life on this planet and the prospect of lifeforms on other planets, hence my involvement in astrobiology. On the experimental side I played a part in establishing the behavior of selected micro-organisms in reversible satellites as part of the Chinese Space Programme. We were able to show, for instance, that edible

Figure 18. Zhiheng Liu at COSPAR' 2008 in Montreal, Canada, with astrobiologists (from left) Professor Gerda Horneck (German Institute of Astromedicine, Germany) and Professor Raulin Francois (University of Paris).

fungi, such as *Pleurotus ostreatus* grew faster under space flight conditions. Similarly, *Bacillus subtilis* and *Streptomyces ansochromogenus* gave significantly higher yields of superoxide dismutase (SOD) and Nikkomycin (an antibiotic used in farming), respectively. The results of these and several other experiments were published in the Chinese journal Space Medicine and Medical Engineering.

I've also had a role in the management and promotion of the Chinese Space Programme. I was, for instance, elected onto the Standing Committee of the Space Society of China. In addition, I've participated in many national and

international conferences on Astrobiology (Figure 18), notably at the International Conference on the "Origin of Life" that was held in Beijing in 2005, and the International Astrological Association (IAA) "Humans in Space Symposium" that took place in Beijing in 2007. I've also participated in the national program organized by the Chinese Academy of Sciences, Space Science and Technology in China: A Roadmap to 2050.

Teaching and supervision

I have always enjoyed teaching and supervising undergraduate and postgraduate research projects, including students from other countries such as Belgium, Mongolia and Switzerland (Figure 19). I was thrilled to be deemed an excellent supervisor of postgraduate students by the Chinese Academy of Sciences (1996), to be awarded the Huawei Teaching Bonus of the Chinese Academy of Sciences in 1999 and to receive the first teaching bonus from the Institute of Microbiology in that year. I have taught in many universities and research institutes across China, and served as a Guest Professor in the College of Life Sciences at Beijing Normal University (2008), at the Graduate School of the Chinese Academy of Agriculture and Sciences (1994, Beijing), in the Biological School at Hebei University (1999) and at the Open Laboratory of the Microbial Resources at Yunnan University in Kunming (2000).

I have also tried to serve the microbiological community by writing books, notably *Taxonomy and Applications of Actinomycetes* (Science Press, Beijing, 1990), *Modern Actinomycete Biology and Biotechnology* (Science Press, Beijing, 2004), *Systematics of Actinomycetes* (Science Press, Beijing, 2006) and *Current Microbiology* (Science Press, Beijing, 2002, 2008). I have written many chapters for books and have published over 150 original papers in both Chinese and international journals.

Life beyond retirement

I formally retired from the Institute of Microbiology in May 2005 after serving as Director of the Actinomycetes Laboratory for 10 years. It was wonderful to have more time to spend with my family and friends though I was also determined to maintain and develop my microbiological interests. Once again I had a stroke of good luck as I was invited to join the research group of Lixin Zhang, who had recently joined the Institute of Microbiology bring-

Figure 19. Zhiheng Liu (first left) together with Guangzheng Meng (middle), Director of the Institute of Microbiology, who is awarding an MSc certificate to my Swiss student, Lukas Wick, in 1998.

ing with him a wealth of research experience and business acumen. So, I became a member of Lixin's Microbial Diversity for Drug Discovery Unit (Figure 20). My primary responsibility was to set up a Microbial Strain Bank to be used for High Throughput Screening for Drug Discovery. This task involves me in the selective isolaton of actinomycetes from environmental samples and the selection of novel isolates for screening purposes. I also have time to pursue my interests in innovative microbial biotechnology and research. It is a great privilege to be able to contribute in these ways.

Some reflections on a topsy-turvy career

My career has had its ups and downs but even when life was most difficult I've had unqualified support from my wife, Ms Yuying Zheng, and our two children, Xiaodan Liu (son) and Xiaohui Liu (daughter), from Hongqing Jia (son-in-law) and Jing Liu daughter-in-law (Figure 21), as well as from good friends, notably Yuhu Shi, Zhuyin Wu, Tiancheng Tang, Jisheng Ruan, Guangzhen Meng, George Fu Gao, Chenglin Jiang, Zhongze Zhang, Lihua Xu, Lixin Zhang, Li Ping Zhang, Wenju Li, Ying Huang and many friends from abroad, such as Marian Mordarski, Michael Goodfellow, Dave Labeda, Jean Swings, Philip Desmeth, Reiner Kroppenstedt, and Kazunori Hatano. Indeed, I consider myself lucky that my contributions to prokaryotic systematics has brought me into contact with such wonderful colleagues.

Figure 20. Me (front row, fourth right) together with Lixin Zhang (third right) and his research group, 2008.

Figure 21. Celebrating my 70th Birthday together with my extended family in Beijing, May 2010.

Figure 22. Announcement by Michael Goodfellow (center) at the closing ceremony of the inaugural meeting of Bergey's International Society of Microbial Systematics on May 22, that Bergey's Manual Trust had awarded this year's Bergey Medals to Jisheng Ruan (left) and myself.

It has also been my good fortunate to have had positions in the Institute of Microbiology that have allowed me to contribute to pure and applied microbial systematics over many years. Microbial systematics in China has come a long way since my early years in the field. This was demonstrated by the excellent talks and posters presented by young Chinese microbiologists at the Inaugural Meeting of Bergey's International Society for Microbial Systematists that was held in Beijing in May 2011. Those of us on the Local Organising Committee felt amply rewarded when the meeting was considered to be a great success by colleagues and friends from home and abroad. Most unexpectedly this meeting turned out to be a momentous occasion for me as I had the honor to be awarded the Bergey Medal by Bergey's Manual Trust for my contributions to microbial systematics (Figure 22). I was overjoyed to be recognized in this way and would now like to take this opportunity to thank all of my co-workers who have contributed to this and other awards that have come my way.

So what of the future? I plan to spend more time with my family, especially my grandchildren Shikun Liu (grandson) and Yuhan Jia (granddaughter) and my friends. However, I also hope to make modest contributions to the research being conducted by Lixin Zhang and his group. One exciting project, which involves old and new friends in Newcastle, is to isolate and screen novel actinomycetes from environmental samples collected from extreme hab-

Figure 23. Investigating microbial resources in an arid, ancient poplar forest in Southern Xinjiang Province, China July, 2011.

itats. This project has allowed me to return to Xinjiang Province in much happier circumstances (Figure 23.). So, I'm inclined to go along with William Shakespeare that "All's well, that ends well".

Acknowledgements

I'm very grateful to Professor Michael Goodfellow for helping me to tidy up this autobiography, many thanks to Dr Dylan Wang for helping me to write the first draft, and Professor Lixin Zhang for helping with revision of this article.

The Bulletin of BISMiS (2012), Volume 3, part 1, pp. 29–37

Sailing through the scientific ocean – my research on the systematics of actinomycetes

Ji-Sheng Ruan

I am deeply honored to have been asked to write about my life with the actinomycetes for *The Bulletin of BISMiS*. My involvement with the systematics of actinomycetes began over 50 years ago and continues to the present day (Figure 1). Over this time my research work has been focussed on the classification of filamentous actinomycetes of academic, ecological and industrial importance. My long association with the actinomycetes has taken me to interesting places and allowed me to interact with many talented individuals involved in the biology of these fascinating organisms. It has also been my good fortune that several of these professional relationships have evolved into cherished personal friendships. So, let me now begin at the beginning.

Early interest in plant pathology

I was born in 1926 in a small village in the Fongren District of Tangshan City in Hebei Province, which lies 260 km north-east of Beijing. My parents were farmers, but my father also had a part-time business in the village. He was one of the few in our village who was educated and he expected me to study hard and become a scientist. From an early age I loved plants very much and it was for this reason that I decided to specialize in biology. I went to the Chinese Agricultural University in 1949 after attending the famous Tangshan First Middle School from 1942 to 1948. I majored in plant pathology at university and was awarded a BSc in July 1953. I was then sent by the Chinese Agricultural University to the Institute of Agricultural Sciences in the Neumenggu Autonomous Region, which is 750 km north of Beijing, as a Research Assistant in plant pathology.

In March 1954, I was transferred by the Personnel Ministry of the National Council of the People's Republic of China to the Laboratory of Mycology and Plant Pathology of the Chinese Academy of Sciences in Beijing. Here I was fortunate to work under the guidance of Professor Wei-Tan Qiu, a celebrated plant pathologist. Over the next 3 years I worked on soft rot disease of cabbage caused by *Erwinia aroideae*. This research lead to the publication, in Chinese, of four papers in Plant Pathology. The method I developed for controlling soft rot disease of cabbage led me to be awarded the Second Achievement Prize by the Chinese Academy of Sciences in 1956.

Figure 1. Ji-sheng Ruan in 2005.

Falling in love with the systematics of actinomycetes

My next break occurred in May 1957 when I was seconded from work for 6 months to learn Russian in Beijing. In November of that year I was chosen to become a graduate student at the Institute of Microbiology of the USSR Academy of Sciences in Moscow.I intended to study bacterial plant pathogens, but as there were no plant pathologists in the institute it was agreed that I should work on actinomycetes under the supervision of Professor Nikolai Aleksandrovich Krassilnikov. I was particularly fortunate

Contact details
State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P.R. China.
jishengruan@yahoo.com.cn

© BISMiS 2012

to join Krassilnikov's Laboratory as he was recognized at that time as a world leader on the biology of actinomycetes. It was also around this time that actinomycetes became known within the scientific community as a source of therapeutic antibiotics in the wake of the discovery of streptomycin from a *Streptomyces griseus* strain by Waksman and Schatz. So, my work with the actinomycetes started by chance!

In Moscow, I was rigorously trained in the theory and practice of prokaryotic systematics and introduced to the actinomycetes by Krassilnikov (Figure 2). My project involved the selective isolation of orange-colored actinomycetes and their subsequent classification using biochemical, cultural, morphological and physiological procedures which held sway at that time. I enjoyed working with the actinomycetes, completed my thesis, in Russian, in May 1961 and was awarded a PhD later that year. I published six papers with Krassilnikov in *Mikrobiologiya*. Professor Krassilnikov, a hard taskmaster, was very pleased with my work and was fond of saying to visiting Chinese scientists that "Ji-sheng will become the leading taxonomist in your country on the systematics of actinomycetes". It was my aim on returning to China to do just that.

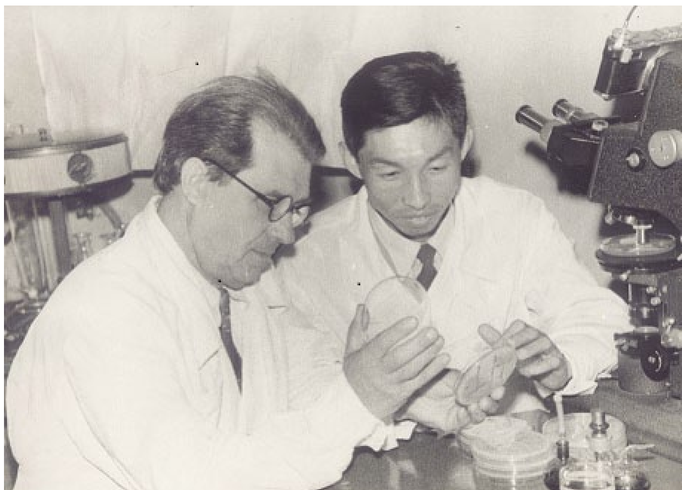


Figure 2. Studying the systematics of actinomycetes under the direction of Professor N.A.Krassilnikov (left) (1957).

Developing actinomycete systematics in China

The experience I gained in Moscow helped me stand out against other young Chinese microbiologists when I returned to Beijing in October, 1961. I was appointed as an Assistant Professor in the newly established Institute of Microbiology of the Chinese Academy of Sciences in Beijing and was fortunate to be assigned to the group of Professor Xunchu Yan to work on the taxonomy of the genus *Streptomyces*. Since these early years I've tried to keep up with conceptual and technological developments in actinomycete systematics and to spread the resultant good practice throughout China. My research interests have tended to reflect the major technological changes that have driven developments in the classification and identification of actinomycetes over the past 50 years.

(a) Use of electron microscopy

I've always been interested in the application of new technologies and was the first microbiologist in China to use electron microscopy for taxonomic purposes. It was not easy to do such work as I had to go to the Metal Institute of the Chinese Academy of Sciences in Shenyang as we did not have an electron microscope in our institute. In an extensive series of studies I examined the spore surface ornamentation of about 100 Streptomyces species and found that strains with spiral spore chains had hairy, spiny or warty surfaces whereas those with spores in straight to flexuous chains had smooth surfaces. The results of these studies lead to a number of publications in *Acta Microbiologica Sinica* in 1964 and thereby helped to establish the importance of spore surface ornamentation in the classification and identification of actinomycetes, a property that is still widely used in the taxonomy of filamentous actinomycetes, notably streptomycetes.

(b) Morphological observations using coverslip cultures for the recognition of actinomycete genera

It was a problem in the early days to distinguish between aerial and substrate mycelia by conventional light microscopy. It was, for instance, difficult to separate nocardiae which produced mycelia that underwent fragmentation and streptomycetes which formed aerial hyphae that differentiated into spore-like elements. In 1963, I developed a method to distinguish between aerial and substrate hyphae which involved the insertion of sterile coverslips into media inoculated with the test organism. The coverslips were removed after incubation for 1, 3, 7, 14, and 30 days, the attached growth fixed and observed under a light microscope. Aerial hyphae were found to be dark colored and one to two times wider than the corresponding light-colored substrate hyphae. The coverslip technique, which is still widely used in China to detect the presence of spores on aerial and substrate mycelia, was published in

Acta Microbiologica Sinica (1974, 1982).

(c) Isolation of rare actinomycetes

It had become apparent by the early 1960s that filamentous actinomycetes other than streptomycetes were a rich source of novel, commercially significant antibiotics. Consequently, in 1963, the Director of the Institute of Microbiology, Professor Fang Lan Dai, asked me to undertake a project designed to isolate "rare actinomycetes" from natural habitats, a catch-all term at the time which embraced all actinomycetes other than streptomycetes. It was not clear at the onset of this project how novel procedures might be designed to isolate non-streptomycete taxa. However, once again, a valuable discovery arose by sheer luck!

One day, much to my surprise, isolation plates left on the bench for 2 weeks by students, who had isolated streptomycetes from them, were also found to support the growth of small filamentous colonies which I was able to divide into three groups based on colonial and morphological criteria. The first group contained small, orange colonies which lacked aerial hyphae but produced motile spores; the second group, small yellow colonies, with or without aerial hyphae, which fragmented into rod- and coccus-like elements, and a third group composed of small dark-colored colonies, which lacked aerial hyphae but formed grape-like spores on the substrate mycelium. At the time, it was difficult to obtain reference strains for comparative purposes, but from the limited available literature I was able to conclude that the three groups were composed of organisms belonging to the genera *Actinoplanes*, *Nocardia* and *Micromonospora*, respectively.

The discovery that small, slowly growing, filamentous actinomycetes grew on isolation media designed for the recovery of streptomycetes from natural habitats gave us the confidence to try out a range of potentially innovative approaches for the isolation of rare actinomycetes from diverse soil samples collected across China. Between 1964 and 1966 over 200 strains of rare actinomycetes were isolated and maintained on diferent media. A range of approaches were used to isolate these organisms including (a) heat pretreatment of dried soil at 80°C for 30 seconds to isolate *Nocardia*; (b) heat pretreatment of dried soil at 120°C for an hour to isolate other rare filamentous actinomycetes followed by sonication and treatment with phosphate buffer; (c) incubation of isolation plates for 30 days, as opposed to the 7 used to recover streptomycetes; (d) use of different media formulations, such as glucose-asparagine, soil extract and water agars; (e) addition of antibiotics to media formulations, such as the use of novobiocin (25 g/ml) for the isolation of Actinoplanes and *Micromonospora* strains, and rifamycin (25 g/ml) for the isolation of *Actinomadura* strains, and (f) the addition of vitamins to media for the selective isolation of *Microbispora* strains. In all cases we paid particular attention to small or unusual, slowly growing colonies that grew on the isolation plates. Such colonies were examined under the light microscope in order to detect and record, distinctive morphological characteristics. These strategies allowed us to isolate a diverse collection of rare actinomycete from several natural habitats.

Difficult times

I was in high spirits following the isolation of so many interesting organisms and was looking forward to classifying them when events were overtaken by the onset of the Great Cultural Revolution. In May 1966, I had to abandon my beloved actinomycetes as I was obliged to study political affairs and the quotations of Chairman Mao. In July 1970 I was sent to the Qan jang 5.7 Labor School in Hubei Province, l200 km sourth of Beijing, far away from my wife and two small daughters. I was responsible for managing a large peach orchard, a task which involved weeding, watering and fertilizing the soil and harvesting the peaches. The hard work involved made me stronger, but throughout this time I spent time planning my future research activities.

Back to Beijing to resume my career

In November 1971, I was reunited with my wife and daughters in Beijing and resumed my career at the Institute of Microbiology. I was eager to make up for lost time and quickly set about reviving my precious collection of rare actinomycetes which had been preserved at □20°C. The subsequent taxonomic studies were not only based on morphological criteria but also on the results of chemotaxonomic procedures that were being developed at the time by Hubert and Mary Lechevalier in the Waksman Institute at Rutgers State University in New Brunswick.The cultures were examined for key chemical markers, such as the isomers of diaminopimelic acid, whole-cell sugars and mycolic acids, and the G+C contents of DNA preparations estimated. It is often forgotten now that many of the major

developments in the classification of actinomycetes were based on the use of chemical and morphological markers.

Our painstaking studies on the rare actinomycetes paid rich dividends as we were able to describe 45 new species belonging to six rare taxa, namely the genera *Actinoplanes* (including *Ampullariella*), *Micromonospora*, *Nocardia*, *Nocardioides*, *Nocardiopsis*, and *Streptosporangium*. Descriptions of these novel taxa were published in a series of papers in *Acta Microbiologica Sinica* in 1974, 1976, 1979, 1981, 1982, 1983, and 1984. These studies revealed for the first time the extent of actinomycete diversity in natural habitats in China and helped close the gap between the quality of taxonomic work being undertaken in China and the West. Partly as a result of this extended project on rare actinomycetes I was promoted to the rank of Associate Professor in March 1978.

Next stop the USA

In 1981, I was invited by Hubert Lechevalier to take up a 2-year postdoctoral fellowship at the Waksman Institute (Figure 3). Once again, I was fortunate to join a talented and dedicated group of microbial systematists. Initially, I was asked by Mary Lechevalier to study a number of *Nocardia* strains from a chemotaxonomic perspective. Much to my surprise, I found that the type strains of three species, *Nocardia mediterranei*, *Nocardia orientalis*, and *Nocardia rugosa*, lacked mycolic acids, key components of nocardial cell envelopes. I repeated the experiment three times and obtained the same result on each occasion. Hubert and Mary Lechevalier were very pleased with my results

Figure 3. The two most famous chemotaxonomists in the world (1981), Hubert Lechevalier (right) and Mary P. Lechevalier (left), with Ji-sheng Ruan (middle).

as they provided conclusive evidence for the transfer of the three species to two new genera, notably to the genus *Amycolatopsis*. The paper proposing the two new genera was the first of several articles we had published in the *International Journal of Systematic Bacteriology*. I was also able to show that cultures brought from China represented new species of *Actinoplanes* and *Streptomyces*.

Support from the Charles and Joanna Busch Foundation allowed me to isolate and classify over a hundred Frankia strains from non-leguminous plant nodules. This work lead to the publication of several putatively new *Frankia* species in *Plant and Soil* (1984) and *Physiologia Plantarum* (1987). I learnt a lot of what were then advanced chemotaxonomic and molecular systematic procedures during my time at the Waksman Institute and was fortunate to become a close friend of Hubert and Mary Lechevalier, friendships which remain as strong as ever.

Introduction of chemotaxonomic and molecular systematic procedures to China

My stay in New Brunswick, New Jersey, was not only important for my own career but also for the promotion of modern prokaryotic systematics in China. In April 1983, soon after my return to Beijing I decided to split my research team into three groups, each led by one of my senior colleagues. Zhiheng Liu was responsible for the analysis of phospholipids of representatives of genera classified in the family *Nocardiaceae*; Xiaotao Lu for determining the menaquinone profiles of *Actinoplanes* species, and Yanlin Shi for the isolation and molecular classification of *Frankia* strains derived from the nodules of plants growing in Xishuannbanna in Yunnan Province. Using a combination of chemotaxonomic and morphological features and molecular data we were able to distinguish between genera classified in the families *Actinoplanaceae* and *Nocardiaceae* and to clarify the taxonomy of the genus *Frankia*. The results of these studies were presented in several publications, notably in *Acta Microbiologica Sinica* and the journal *Actinomycetes*; they were also presented at various international meetings and were the subject of several book chapters. These achievements represented a dream come true as I had succeeded in significantly contributing to the establishment of modern actinomycete systematics in China. In March 1985, I become a full professor and soon thereafter I was appointed as Director of the Actinomycetes Laboratory in the Institute of Microbiology. Next,

Figure 4. National Key Research Project team (1990-1994). Ji-sheng Ruan (front row, first left); the president of NSFC, Dongcai Liang (front row, fifth from left) .

I was invited to lead a national research project.

National Key Research Project

In November 1989, I was appointed by the National Science Foundation of China (NSFC) to be the principal investigator of a 5-year National Key Research Project (January 1990–December 1994) (Figure 4) designed to establish the distribution and commercial significance of different kinds of actinomycetes in diverse habitats in Yunnan Province, a hot spot region in Southwest China that contains many endemic animal and plant species. This was an exciting and huge undertaking as I had to coordinate the activities of 71 research workers (including four full professors) located in five institutions (Institute of Microbiology of the Chinese Academy of Sciences in Beijing; Institute of Pharmacology of the Chinese Academy of Sciences in Shanghai; Institute of Medicinal Biotechnology of the Chinese Academy of Medical Sciences in Beijing; Hebei University in Baoding; and the Institute of Microbiology at Yunnan University in Kunming).

Initially, rare filamentous actinomycetes were isolated from a range of habitats across Yunnan Province using previously developed selective isolation procedures. Representative strains selected from around ten thousand isolates were the subject of chemotaxonomic and molecular systematic procedures, the latter included 23S rRNA gene sequencing and DNA–DNA pairing. Special attention was paid to *Frankia* isolates which were assigned to groups based on 16S–23S rRNA intergenic and 5¢ terminal 23S rRNA gene sequences. The taxonomic data were written up for publication, notably in *Actinomycetes* (1991–1992), *Acta Ecologica* (1992) and the *International Journal of Systematic Bacteriology* (1994). Screening studies carried out on representatives of novel taxa lead to the discovery of twelve bioactive compounds active against cancer, fungi and viruses. Two novel antibiotics, yunanmycin and yugumycin were patented. The success of the project was recognized by the NSFC Inspection Team who deemed the work to have been of an “international standard”.

The international dimension

When working at the Waksman Institute, I was given the opportunity by Hubert Lechevalier to participate in the 5th International Symposium on the Biology of the Actinomycetes which was held in Oaxtepec, Mexico, in 1982. This proved to be an important meeting for me as I was able to meet key players in most facets of the biology of actinomycetes, including Dwight Baker, Tom Cross, Mike Goodfellow, Reiner Kroppenstedt, Marian Mordarski, Beth Mullin, and Stan Williams. I was elected to the Taxonomic Subcommittee on the Actinomycetes, which worked under the auspices of the International Committee of Systematic Bacteriology (ICSB) of the International Union of Microbiological Societies (IUMS). I subsequently become Chairman of the ICSB Subcommittee on the Taxonomy of *Frankia*.

The meeting in Oaxtepec opened up a whole new world for me. I subsequently reported our research findings and chaired sessions at the 6th (Debrecen, 1985), 7th (Tokyo, 1988), 9th (Moscow, 1993), 14th (Newcastle upon Tyne) and 15th (Shanghai, 2009) ISBA conferences. I also participated in conferences on Frankia and Actinorhizal Plants, including the 7th (Storrs, CT, USA, 1988) and 9th (New Zealand 1992) conferences. I also played a role in the International Workshop on the Application and Control of Microorganisms in Asia (Tokyo, 1994).

International research projects

My involvement in ISBA meetings brought me into contact with other microbiologists interested in the systematics of actinomycetes for exploitable purposes. This, in turn, paved the way for three international collaborative research projects designed to promote actinomycete systematics. The first, with Marian Mordarski of the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw (1985–1992), was supported by the

Figure 5. Signature of China–Poland Collaboration Project (1985–1992). M. Mordarski (second from right) and his wife, Anna Przondo-Mordarski; Ji-sheng Ruan (first on left) and his wife, Ye Li.

Figure 6. Signature of China–USA Collaboration Project (1990–1992). Dwight Baker (front row, second from right) and Ji-sheng Ruan (front row, second from left).

Figure 7. Signature of China-UK Collaboration Project (1992-1994). Mike Goodfellow (fourth from left) and Ji-sheng Ruan (third from left).

Figure 8. Celebration of a collaboration (1992). Ji-sheng Ruan (third from right), and Punita, Lena, Mike and Maya Goodfellow (from left to right).

Chinese Association for Science and Technology and the Polish Academy of Sciences (Figure 5); the second with Dwight Baker of Yale University (1990–1992) was funded by the National Science Foundation of the USA and the Chinese Academy of Sciences (Figure 6), and the third with Mike Goodfellow of the University of Newcastle upon Tyne (1992–1994) was funded through an Exchange Programme between the Chinese Academy of Sciences and The Royal Society (Figures 7 and 8). These collaborative programmes not only led to significant improvements in the systematics of actinomycetes, as witnessed by publications in *Acta Ecologica* (1992), *Actinomycetes* (1991, 1992), and the *International Journal of Systematic Bacteriology* (1998), but also to the training of a generation of young Chinese microbiologists in the concepts and practices of prokaryotic systematics. It was through these projects that my professional relationships with Dwight Baker, Mike Goodfellow, and Marian Mordarski evolved into close, lasting friendships; all three were frequent visitors to my laboratory. I will always cherish the memories of the wonderful times we spent together, not least social occasions at home with my wife, Ye Li (Figure 9).

Educating young scientists

I have always been interested in education and have tended to seize opportunities to introduce young microbiologists to the wonderful world of the actinomycetes. Shortly after graduate education was established in China, Professor Xunchu Yan and I were the first to be appointed as supervisors in the Taxonomy of Actinomycetes (1978). Over

Figure 9. Welcoming our great friends to our home (August 2011). Mike Goodfellow and his wife Punita (in the middle), flanked by Ji-sheng Ruan and his wife Ye Li.

Figure 10. Chinese Academy of Tropical Agricultural Sciences (July 2006). Ji-sheng Ruan (fourth from left), Mike Goodfellow (third from left), and Kui Hong (first from left).

the years, I've supervised 12 graduate students, a post-doctoral fellow and visiting scientists from Iraq and Vietnam. Some of the graduate students became established in their own right, including Zhiheng Liu, who succeeded me as Director of the Actinomycetes Laboratory in 1996, Chengling Jiang, who promoted actinomycete systematics at the Institute of Microbiology at Yunnan University in Kunming, and Liping Zhang, who did similar work at Hebei University.

It has also been my privilege to teach students at Hebei University (1980–1986) and in the Institute of Microbiology in Kunming (1985–1990) as a Visiting Professor. I've also trained over 150 young scientists in chemo- and molecular systematics at workshops held in Liaoning University (1973), Xichuan University (1976), Guangxi University (1985) and in the Institute of Microbiology in Beijing (1993), the latter with Mike Goodfellow. I was also invited to give seminars in 1994 at the South East Asia Regional Training Workshop organized by UNESCO on Rapid Methods in Microbiology and Biotechnology (Bangkok, Thailand) and at the Training Course on Biotechnological Utilization of Tropical Resources (Haikou, China, 2008, 2010).

It is also a pleasant duty to find time to write and edit books so that the next generation of graduate students can be introduced to new concepts and cutting edge techniques used in the classification and identification of actinomycetes. Together with colleagues, I have prepared six monographs, including the *Taxonomic Handbook of Streptomyces* (Science Press, China, 1975), the *Taxonomic Basis of Actinomycetes* (Science Press, China, 1977) and *Research and Application of Actinomycetes* (Science Press, China, 1990). I have also published over 120 original papers.

Retired but far from finished

I retired from the Institute of Microbiology in March 1994 after 10 years as Director of the Actinomycetes Laboratory. Shortly thereafter I became a Visiting Professor at the Institute of Microbial and Cell Biology at the National University of Singapore where I was to work for the next few years with the Director of Microbial Resources, Ye Wang, an expert molecular biologist. My responsibility was to isolate and classify rare actinomycetes from local habitats.

When I started my new task I was surprised to find that I was the only taxonomist in an institute which did not contain a single actinomycete culture hence together with my three colleagues I had to start the project from scratch. We used a range of selective procedures to isolate taxonomically diverse, filamentous actinomycetes from a range of tropical rainforest soils and then set about classifying them using chemotaxonomic, morphological and molecular systematic methods, including 16S rRNA gene sequencing. The isolates were assigned to 36 genera, including four new ones, *Actinopolymorpha*, *Nonomuria* (later *Nonomuraea*), *Thermobifida*, and *Thermobispora*. Given the comprehensive nature of our studies we were able to improve the classification of several taxa, including the genera *Actinomadura*, *Microbispora*, and *Micro-*

Figure 11. The Group of Actinomycete Systematics and Resources, Institute of Microbiology, CAS (2009). Ji-sheng Ruan (fourth from left) and Ying Huang (3rd from left).

Figure 12. Ji-sheng Ruan's family (1998). From left: Peixin Ruan (younger daughter), Peihua Ruan (older daughter), Ji-sheng Ruan, and Ye Li (wife).

tetraspora, as well as reviving the genus *Kitasatospora*. Several publications arose from this work, including an overview paper in the *Journal of Industrial Microbiology and Biotechnology* and articles in the *International Journal of Systematic and Evolutionary Microbiology*.

Together with my family I returned to Beijing in October, 1998. Since that time I've continued to foster my interest in the actinomycetes, notably in collaboration with Kui Hong in Haikou, Hainan Province and Ying Huang, in Beijing. I worked closely with Kui as a part-time Visiting Professor at the Institute of Tropical Bioscience and Biotechnology of the Chinese Academy of Tropical Agricultural Sciences (2004-2010) (Figure 10). Together we trained over 30 graduate students in actinomycete systematics and studied actinomycete diversity in mangrove swamps throughout South-East China. We described several new species, mainly in the *International Journal of Systematic and Evolutionary Microbiology*. In addition, we discovered several new interesting bioactive compounds, such as the benzamides and quinazolines isolated from a *Streptomyces* strain, ten new azalomycin macrocyclic lactones isolated from another mangrove actinomycete, *Streptomyces* strain 2117263, and a new sesquiterpene isolated from *Streptomyces* strain 0616208.

I was invited back to my old laboratory at the beginning of 2006 as a part-time consultant to work with Ying Huang, the new Director of the Actinomycetes Systematics and Resource Group and a highly trained molecular biologist. Ying developed and used several new molecular fingerprinting techniques, especially Multilocus Sequence Analysis (MLST), to help unravel the complex taxonomy of the genus Streptomyces. Together we have supervised 10 graduate students and described several new species of the genera *Microbacterium*, *Micromonospora*, *Nonomuraea*, *Verrucosispora*, and *Streptomyces* in the *International Journal of Systematic and Evolutionary Microbiology*. We have also published the book *Rapid Identification and Systematics of Actinobacteria* (Science Press, China, 2011) (Figure 11).

Looking backwards and forwards

When looking back I have no regrets that I devoted my scientific career to understanding the intricate taxonomic relationships that exist between my beloved friends, the actinomycetes. At times my journey has been difficult, but I've always been sustained by many wonderful colleagues and by the unstinting support by my wife, Ye Li, and our daughters, Pei Hua and Pei Xin (Figure 12). I have never wished for anything beyond this though I have to admit that I've been quietly pleased that my scientific work has been recognized in China and beyond, notably by the recent award of the Bergey Medal by Bergey's Manual Trust. I was also thrilled that Ying and her colleagues named the new genus *Ruania* after me (Gu *et al.*, 2007); this taxon together with the genus *Haloactinobacterium* forms the new family *Ruaniaceae* (Tang *et al.*, 2010). Kui Hong and her colleagues also named a new genus after me, namely *Jishengella* (Ji.sh.eng.ell'a. N.L fem. n. *Jishengella* from Jisheng, named after Jisheng Ruan, the Chinese microbiologist).

The announcement that I was to receive the Bergey Medal

was made by Mike Goodfellow at the Inaugural Meeting of Bergey's International Society for Microbial Systematics which was held in Beijing last May. It gave me pleasure from the bottom of my heart to see so many young Chinese microbiologists at this meeting and to know that some of them will play an important role in furthering our understanding of the kinds and diversity of actinomycetes present in natural habitats and show how we might use such organisms for the good of humankind. Although I'm now advanced in years I intend to contribute to and keep abreast of changes in actinomycete systematics, developments that will increasingly be shaped by advances in molecular systematics.

Finally, I would like to take this opportunity to pay tribute to my many friends and colleagues at home and abroad for sailing with me on a fascinating voyage of discovery across a tiny speck of the scientific ocean and to thank the Chinese Academy of Sciences for providing a home for me at the Institute of Microbiology in Beijing as well as my family's great support during my career.