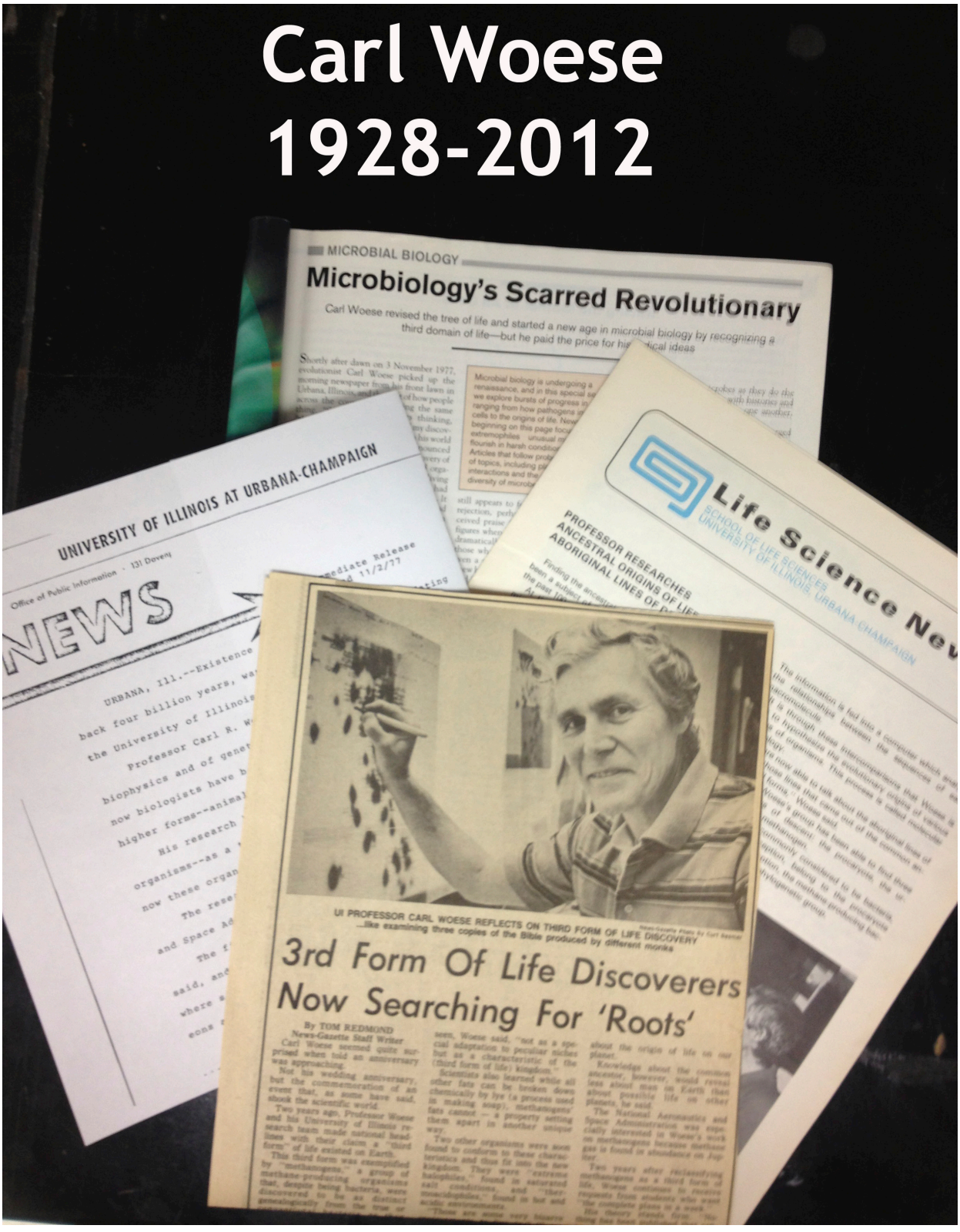
The Bulletin of BISMiS

Published by Bergey's International Society for Microbial Systematics

Volume 4, part 1 – May 2013



The Bulletin of BISMiS

Published by Bergey's International Society for Microbial Systematics

ISSN 2159-287X

Publisher and Editorial Office

Bergey's International Society for Microbial Systematics
Department of Microbiology and Plant Biology
770 Van Vleet Oval
University of Oklahoma
Norman, OK 73019-0390 USA
Email: paul.lawson@ou.edu

On the cover

Collage dedicated in memory of Carl Woese

The Bulletin of BISMiS

Contents of Volume 4, part 1

The Bulletin of BISMiS (2013), Volume 4, part 1, pp 5-5

Carl Woese Remembered

Barny Whitman

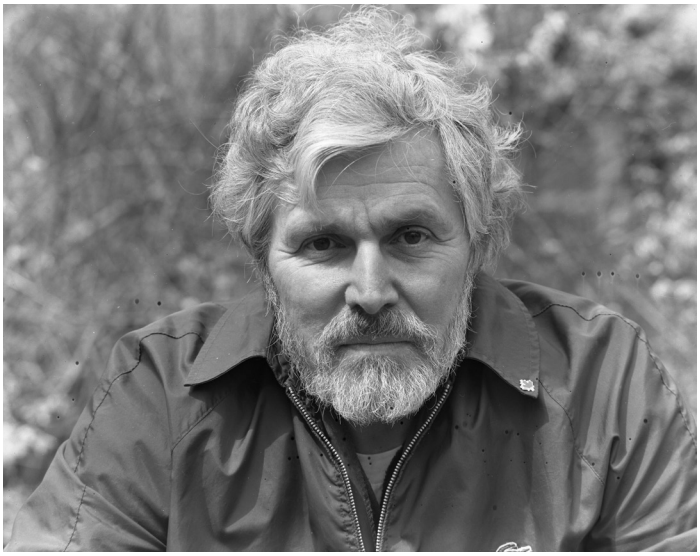


Figure 1: Carl R. Woese (provided by CR Woese)

The inventor of the 16S rRNA classification of life died on Sunday, Dec. 30, 2012, at his home in Urbana, Illinois. A Professor of Microbiology at the University of Illinois, Professor Woese pioneered the use of rRNA sequences to classify life and proposed the three Domain structure of modern life, including the Bacteria, Archaea, and Eukarya. Born July 15, 1928, in Syracuse, N.Y., Woese earned bachelor's degrees in math and physics from Amherst College in 1950 and a Ph.D. in biophysics from Yale University in 1953. He studied medicine for two years at the University of Rochester, spent five years as a postdoctoral researcher in biophysics at Yale, and worked as a biophysicist at the General Electric Research Laboratory in Schenectady, N.Y.

He joined the faculty of the University of Illinois in 1964. Woese received a MacArthur Foundation grant in 1984 and was elected to the National Academy of Sciences in 1988. He was awarded the 1992 Leeuwenhoek Medal by the Royal Netherlands Academy of Arts and Sciences; and he received a National Medal of Science in 2000. In 2003, Woese received the Crafoord Prize in Biosciences, administered by the Royal Swedish Academy of Sciences. Some also considered Woese a candidate for the Nobel Prize. However, in my opinion the Crafoord Prize was much more appropriate. The Crafoord Prize emphasizes evolution and ecology, topics which have become much more important to humankind in the 21st century than those recognized by the Nobel Prize. Moreover, the award in Biosciences is only made once every three or four years.

A memorial was held for Prof. Woese at the University of Illinois on January 26, 2013. A video and guest book are available online at: http://www.igb.illinois.edu/about/archaea.

Figure 2: Buttons made by Professor Woese and his friends around 1980 to celebrate his discovery of the Archaea.

Contact details
Department of Microbiology
University of Georgia
Athens, GA, USA
whitman@uga.edu

The Bulletin of BISMiS (2013), Volume 4, part 1, pp 7-14

What Is A Bacterial Strain? I Will Know It When I See It

Micah I. Krichevsky[1], Paul De Vos[2], Lenie Dijkshoorn[3], Stephen J. Giovannoni[4], Jorge Lalucat[5], Kevin McCluskey[6], Mark Segal[7]

The bacterial species concept is a continuous matter of debate. In the BISMiS Bulletin, the article entitled "What Is a Bacterial Species? I Will Know It When I See It" (Krichevsky, 2011) explored the uncertainty involved in objectively defining the term "species." The current paper addresses the similar problems of establishing the concept of "strain." The basic issue is that there is no judicial commission to codify these terms. Therefore, any definition one uses is, perforce, personal. This is analogous to the process by which a vocabulary evolves. If enough people agree on a particular term's usage, that usage becomes codified in a dictionary. For microbiology, confounding this linguistic evolution are the changes in the science and technology surrounding the subject concepts. The term "strain" is utilized for diverse purposes. This uncertainty in usage stems from this diversity of purpose. How is an isolate determined to be a member of a given strain in practice? The methods and criteria also vary with the reasons for the use of the term.

Introduction

The English philosopher John Locke (August 29, 1632 - October 28, 1704), argued that nature does not make a species. People do, as a mechanism to facilitate communication of a collection of similar ideas under one general term. In addition, Locke stated that the boundaries of species are opinions rather than natural borders. This lack of natural borders, precludes an objective definition of species. This discussion explores whether the same logic on boundaries applies to strains.

The word "strain" has many meanings ranging from separating materials based on size, distortion of materials, describing a level of exercise, etc. The same word is used both as a noun and a verb. In biology, the term generally describes a more or less discrete entity that is uniquely distinct in detail from all other life forms. Cowan (1978) described two meanings for the word: 1. "Descendents of single colony (sometimes a single cell, in which case, clone is appropriate) isolated from a specimen." 2. "Several separate isolations from one or more patients (animals) in an epidemic (epizootic) ….these …strains are assumed to have derived from one source of infection and can be assumed to have similar characters." He states "…when all ascertainable characters are identical, the cultures are best regarded as being of one strain."

Both definitions have inherent uncertainty. In the first definition, it must be assumed that all progeny of the original cultured isolate are unchanged. Major service collections attempt to minimize the chance of mutational change through various strat-

Contact details

[1]Bionomics International, Wheaton, MD, USA

[2]BCCM/LMG bacteria collection, Laboratory for Microbiology, University of Gent, Gent Belgium

[3]Dept. Infectious Diseases, Leiden University Med Center, Leiden, Netherlands

[4]Department of Microbiology, Oregon State University. Corvallis, Oregon, USA

[5]Departament de Biologia, Edifici Guillem Colom, Universitat de les Illes Balears Campus UIB, Palma de Mallorca, Spain

[6]School of Biological Sciences, University of Missouri- Kansas City, Kansas City, Missouri, USA

[7]Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, Washington DC, USA

Corresponding author: Micah I. Krichevsky - micahk@biont.org

egies, e.g., low temperature preservation and storage, large numbers of initial and subsequent replicate cultures to lower the number of serial transfers, etc. Genetic collections assign a new strain designation to a culture with a single point mutation.

The second definition, wherein the various cultures have a set of ascertained characters in common, has a higher level of potential uncertainty than the first definition. Here, the source of the individual isolates cannot be determined to be the ultimate source. The singular original source has to be assumed by inference. In many cases, increase in time and space of organisms sharing the same characteristic, for example *E. coli* O157:H7 or influenza virus H1N1 or *Methicillin Resistant Staphylococcus aureus* (MRSA) might be indicative of an epidemic. Although these characters are flags to mark clinically important organisms, organisms with these features may actually represent distinct strains. Therefore, assessment of their identity at the strain level and identification of their sources and mode of spread would require additional methods including macrorestriction analysis using pulsed field gel electrophoresis, AFLP or rep-PCR fingerprinting or sequence based methods like multilocus sequence typing (MLST) (Struelens et al., 2009). All of these methods help to reinforce the strength of the inference of source.

The following request was sent to a panel of bacteriologists and a mycologist: “First, please contribute your own view of the definition of “strain.” Second, consider the following scenario. You receive two cultures from different culture collections labeled as a type strain of a particular species. What criteria would you use to accept the designation 1) at face value, 2) verify that the two cultures are indeed identical, 3) choose between the two to accept one of them as your exemplar of the species, 4) reject both?”

The responses follow:

De Vos

My vision on the definition of a bacterial strain:

-This is simple: a strain is an isolate – and is composed of all off-spring of that isolate wherever they are.

-Two different subcultures (originating from two different culture collections) of a type strain.

1) Face value: in principle yes but see 2

I would accept the designation as type strain of a species as such because the culture collections are supposed to perform a quality control. Here is the controversy: what is a quality control? In our handling, it controls the viability, and the authenticity -purity.

• Viability: this is relatively simple growth on the recommended medium and controlled colony morphology

• Authenticity: mobility, gram stain, cell morphology, pleomorphism of the cell, 16S rRNA gene sequence (at least the most variable part i.e. mostly the first 500 bases- more than 1% difference is suspect) and eventually other housekeeping gene(s) and if reported specific characteristics (e.g. nitrogen fixation, growth on medium with heavy metals etc.)

• Purity – colony morphology and cell morphology

- However, I perform, before starting experimental work, my own purity check and short authenticity test i.e. colony morphology and light microscopic control of the cell and its gram stain. If this agrees with the overall knowledge on the strain, I accept it as being the envisaged strain.

- If I have insight in the quality check procedures of the culture collections, I would choose that subculture for which the most extended quality control has been performed (often one does not know this, but the quality of the quality control needs to be accepted from the description of the certificate; e.g. ISO norm).

2) Identical cultures

- One cannot prove that two cultures are identical, never. Does one base difference in the genome make them different? Repeating of a sequence

procedure, even on the same DNA, will always result in differences. So it is better to speak about 'EQUIVALENT' cultures i.e. cultures that show the same specific characteristics. A number of growth tests, fatty acid composition etc; if tested under standardized conditions. DNA fingerprint of the overall genome (e.g. AFLP). All of these tests have their shortcomings and characteristics are subjected to changes that may be introduced by cultivating outside the natural ecological niche, preservation procedures etc. These type strains are references in an artificial classification system, and if they do what is expected, it is okay. But this means that the original description of the strain must be very well documented and eventually updated (emended) a shortcoming in many taxa (this is just a comment).

3) Accept the strain as subculture of the type strain between both

- I would take the one that agrees best with the original description more, based on genotypic AND phenotypic indications. If there are major disagreements for each of the two subcultures, I would be very careful and ask for another representative of the type strain, perhaps from a third culture collection. To define major, based on my experience, a difference in one growth test should not be considered differential because readings are often objective, whereas major differences in base composition of rRNA or housekeeping genes are much more reliable. But if this happens, we have a problem and rejection of both is probably the only option. Further research is needed.

The above comments and suggestions are linked with an existing taxonomic system. Often in terms of epidemiological investigation typing at the strain level is needed. Now we talk about another level of differences. Genes, characteristics that are specifically linked with the virulence characteristic, are important to decide whether strains are different or not. This is of course important for elucidating the variability within a species (although only one characteristic is studied i.e. pathogenicity) and therefore limited.

This subject needs further discussion, about which methods are suited for these epidemiological studies.

Dijkshoorn

As for the definition of the term 'strain.' In our lab, we speak about 'strain' without any reference to a species but just meaning a (sub)culture on our desk, disregarding the designation of the species. If we speak about a strain in particular, this is accompanied of course by a precise designation. Dijkshoorn et al., (2000) distinguished two different definitions for strains, the strain in the taxonomic sense and the strain in nature which seem to correspond largely to the two mentioned by Cowan (1978; see above), respectively. The strain in the taxonomic sense descends from a selected, initial single colony and is kept in an artificial culture, and labeled and stored under controlled conditions. The strain in nature can be an organism that has spread, for example, in a human or animal patient, and may be cultured from different body sites. It can even spread to other patients during a chain of infection. Here, the starting point in space and time and the further spread and growth are unknown. The assumption that isolates belong to the strain in nature requires assessment of a number of features including, e.g., the antibiotic susceptibility profile, phenotypic features (e.g. the Vitek profile), high resolution genotypic profile and a possible relatedness in space and time. The genotypic methods may comprise DNA fingerprinting methods like pulsed-field gel electrophoresis, AFLP analysis, rep-PCR typing, multivariable tandem repeat analysis (MLVA), detection of particular resistance genes, or sequence analysis of housekeeping genes.

Disregarding whether a subculture is a strain in the taxonomic sense (originally also derived from a strain in nature) or an isolate representing a strain in nature, we usually deal in the microbiological laboratory with cultures (i.e. loopfuls) with large numbers of cells. Bacteria are approximated to have a mutational rate of one in ten to the tenth. Thus, a visual culture even if derived from a single cell has an accumulation of mutations. During its passage in nature or in the laboratory, different selective forces will be exerted and depending on that, variants will develop. Furthermore, horizontal gene exchange with co-existing cultures (strain in

nature) may contribute to diversification. The longer this process lasts, the greater the evolution may be. The mechanisms that maintain strain or species identity are poorly understood.

In this context, we also need to address the term clone. This term is frequently used as a synonym for strain. For example, during an outbreak of an epidemic strain on a ward. However, the term clone is also used in epidemiology to denote relationship between isolates from widely separated geographically areas. This category was described by Ørskov and Ørskov (1983) as follows: '… the word clone will be used to denote bacterial cultures isolated independently from different sources, in different locations, and perhaps at different times, but showing so many identical phenotypic and genotypic traits that the most likely explanation for this identity is a common origin.' Clones falling under this definition may have arisen long ago (centuries, millennia?) given their wide geographic spread, and particular (yet to elucidate) features may contribute to their persistence. A current, widely accepted approach to detect clonal relatedness within species is multilocus sequence analysis of housekeeping genes (e.g. Diancourt et al., 2009; Robinson and Enright, 2004). Within clones evolution continues and different strains can be distinguished by typing methods that are based on less conserved markers than housekeeping genes. It is important to use such typing methods during complex epidemic situations to assess the sources and mode of spread at the strain level. Thus, in these situations, a strain is not synonymous with a clone.

To reply to the question about two cultures:

1. 'Face value': I would indeed first culture the strains to compare the colony morphologies, growth characters and any other observable feature to assess their similarity.

2.- 4. In addition, we would compare the genotypic features of the cultures by e.g. AFLP analysis, a whole genomic DNA fingerprint method with a high resolution. If there would be no observable differences between the two cultures, we might consult the Straininfo site to assess the routing of the organisms and select the one which would likely be the closest to the original strain (assumed shorter routing). In case of discrepancies between the two subcultures (both 'face value' and by AFLP genotyping), there would be the possibility that the organisms belong either to the same species or not. In both cases, a detailed analysis would be required. Whatever the outcome, I would contact the public culture collections and ask for assistance if the strain was obtained directly from such a culture collection. (It is of note that many reference strains that were originally obtained from public culture collections circulate among collaborating institutes in an unofficial circuit).

We had several experiences with confusion of strains including the type strain of *Acinetobacter calcoaceticus* described by Beijerinck. We requested the strain from 10 Public Culture Collections. Two isolates were clearly wrong. Unfortunately, this has never been published.

Giovannoni

I use the terms "strain" and "isolate" interchangeably. I expect most isolates from nature to differ in their genome sequences, and would normally use gene or whole genome sequencing to distinguish between strains.

If I received two cultures ostensibly of the same type strain from different collections, I would choose one and carry on, assuming they were identical. If I had some reason to suspect that they were not identical, and it was important to identify the correct culture, I would look through the literature for DNA sequence data that had a bona fide origin from the type strain. If that data were available, I would amplify and sequence a gene from both strains and compare it to the original sequence.

Although DNA sequence data is what I prefer these days, "I'll know it when I see it" is the principle that got me through the early days of my career, and it applies just as well today. For example, 16S sequence divergence in the SAR11 clade exceeds 10% (Thrash, et al. 2011). So far every one of them looks the same, and they have similar genomes. To a first approximation, they act the same in culture too.

Krichevsky (Adapted from Krichevsky, 2011)

Strain (operational definition)

-Series of isolates or clonal subcultures that are "identical"

-Isolate records compared to establish identity

-Any single isolate the definitive record

-Incidence of strain - two step process

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

-Only the incidence data is further analyzable

The ultimate judgment of identity, based on the methodologies used, is the responsibility of the investigator.

Lalucat

What criteria would you use to accept the designation:

1) At face value - to accept something for what it appears to be rather than studying it more closely.

When a new strain is received in our lab, we always try to verify its identity. The first step is still culturing in a general growth medium to have isolated colonies and to test them for morphological homogeneity. The next step (which is now routine) is the sequencing of the 16S rRNA gene. Other genes can be also very useful, if available. The sequence has to be 100% identical to the sequence reported in the databases, but sometimes differences have been found.

2) Verify that the two cultures are indeed identical, or

As stated before, the sequencing in our hands is the best way to verify if two strains are identical. Several times, we have also applied other molecular typing methods (as RAPDs, ERIC, ARDRA or REP-PCR)

3) Choose between the two to accept one of them as your exemplar of the species.

If we receive the strain from two different collections, we have to track back the history in each collection, to find the depositor in each of them, and we try to consider the "good" strain the one deposited earlier in the collections. If possible, the researcher who isolated the strain is also asked for a culture.

The definitions I use in the classroom are:

Clone: is a group of cells resulting from the division of a single cell. I also use the term "isolate" as synonymous.

Strain: is a clone, or group of clones, which can be differentiated genetically or phenotypically from other clones of the same species.

With respect to the type strains, I assume that the collections have verified the authenticity of the type strain. We have had some problems when comparing gene sequences of type strains received from different culture collections. Therefore, I am discussing now with my colleagues to give a new strain number to all strains that we are culturing in our laboratory, indicating the origin of the strain, but with our own collection number. In subculturing "clones," new clones of the same strain can arise. In our experience, some chromosomally encoded pathways can be deleted from a strain under defined culture conditions, even when the strain is cultured axenically. In this case, a new clone is obtained, but we consider it as a derivative of the same strain.

McCluskey

The relevance of this question to a genetic repository necessarily is of central importance. Implementation is somewhat different from issues impacting taxonomic strains.

In the light of modern whole-genome-sequencing programs currently being undertaken, both strains would be accepted with the indication that both were representatives of the species. Each would be assigned unique accession numbers in our collec-

tion, and additionally, retain the designation from the collection of origin as well as the identity of the depositor. Because they have different histories, they would be treated differently.

This may be more relevant to genetic collections as it manifests itself in a number of ways. In the fungal genetics community, for example, different laboratories investigating virulence in *Cryptococcus neoformans* used a strain known as "H99" as their wild type. They introduced targeted gene deletions into this strain. Because this strain is widely used, versions in different laboratories have different histories and behave differently in experimental evaluation. Researchers need access to the specific strain that was used as the recipient in the genetic manipulations when making comparisons. Similar circumstances exist for isolates of *Candida albicans*, *Aspergillus fumigatus*, *A. niger*, and even *Neurospora crassa* in our collection.

Moreover, recent work with whole genome sequencing has addressed the scale of genetic drift that can impact a strain. The observed frequency of spontaneous mutations in a strain is on the order of 2 x 10^{-5} which translates to approximately 1,000 changes per bacterial genome. If the per-replication rate is even one tenth of this, simply passaging a strain a few times will generate diversity on the order of one change per Kb of DNA.

Finally, as a genetic repository, numerous strains (either subcultures or mutants) may have an identical genotype and be in the same genetic background. At one level, these would be considered to be equivalent. We have just conducted a study of many mutants at the ad-8 locus in *Neurospora* and can show that the strains are not equivalent, although they might be interchangeable with regard to their purpose.

Similarly to how plant pathologists evaluated pathogenic races of pathogens, the strain designation is a way of describing a genotype, and the question of what constitutes a strain changes as one changes one's resolution. At the finest level of resolution, every nucleus is an individual. At a practical level of resolution, all of our ad-8 mutants are the same.

Segal

A strain is a culture whose characteristics, genetic, phenotypic or both, are partially shared by other cultures assigned to a species, but which differ in a discernible way from all the other cultures that are considered strains in the species.

'Strain' seems to be most often used to describe isolates that are circumscribed by the space that defines a species. A visual representation of this for me is the diagram that Elena Kiprianova produced for the *Pseudomonas* species that she studied (Smirnov and Kiprianova 1990). In this exemplar, a strain is a member of a species if it fits within the borders of the 'blob' (defined statistically, of course) that defines the boundaries of the species. An isolate does not necessarily need to immediately fit within a species, if it cannot be found to fit within the space of an existing species. I think of all the transitional 'strains' we have seen. In those cases, I view them as 'strains-in-waiting.' For example, they are isolates in search of a space that is well enough defined to be called a species.

Because of the various ways 'species' can be defined, this will result in strains being defined more-or-less loosely, depending on how the associated species is defined. So in some cases, an isolate will be a strain of a species by one definition, but will be an isolate not associated with a species by a different species definition.

Discussion

The above responses are but a small sample of the microbiological community. Even within this small sample, the responses vary widely in describing a strain as well as how to determine commonality between two isolates. The responses fall in two broad categories based on knowledge of the history of the isolates. The categories basically are those delineated by Cowan. For example, do we know the original clone from which all putative examples were derived or are the isolates from various specimens?

The small sample of views given above of the use of the term "strain," indicates that the stringency in collecting a series of isolates under the rubric of

#90 T, 90 T, A.3.12 T, A3.12 T, ATCC 12633 T, ATCC 23467 T, BCRC 10459 T, BS2 T, CCEB 848 T, CCM 7156 T, CCRC 10459 T, CCT 2357 T, CCTM La 3365 T, CCUG 12690 T, CCUG 2091 T, CECT 324 T, CFBP 2066 T, CIP 52.191 T, CIP 52.191T T, CNCTC 5802 T, CNCTC Ps 161/78 T, DSM 291 T, DSM 50202 T, DSM 50906 T, DSMZ 291 T, DSMZ 50202 T, DSMZ 50906 T, FIRDI 459 T, HAMBI 7 T, HUT-8100 T, IAM 1235 T, IAM 1236 T, ICMP 2758 T, ICMP 3510 T, ICPB 2484 T, ICPB 2693 T, ICPB 2963 T, IFO 14164 T, Jacob PF15 T, JCM 13063 T, JCM 20120 T, JCM 3697 T, K. Yokozawa 40F T, KCTC 1644 T, KCTC 1751 T, KM 1081 T, KM 888 T, Kosako 85004 T, LMAU P60 T, LMD 68.20 T, LMD 72.6 T, LMG 2257 T, NBRC 14164 T, NCAIM B.01157 T, NCAIM B.01444 T, NCAIM B.01445 T, NCAIM B.01447 T, NCAIM B.01634 T, NCCB 68020 T, NCCB 72006 T, NCCB NCCB 68020 T, NCIB 9494 T, NCIB 9528 T, NCIMB 13936 T, NCIMB 9494 T, NCIMB 9528 T, NCTC 10936 T, NZRCC 10269 T, Palleroni # 90 T, Palleroni 90 T, PDDCC 2758 T, R-17431 T, R. Hugh RH827 T, R.Y. Stanier 90 T, R.Y.Stanier 90 T, Stanier 90 T, Stanier A.3.12 T, USCC 2032 T, VKM B-1301 T, VKM B-2187 T, VKPM, B-4589 T, VTT E-92005 T, WDCM 117 T

Figure 1: Type strain designations of copies of *Pseudomonas putida* A.3.12.The letter "T" denotes the type strain. The catalogues of the various collections list both A.3.12 and their own designations as synonyms. Some collections hold multiple examples of the type strain, e.g. DSMZ.

a "strain" varies with the context of the individual series. The epidemiologist, the regulator, the ecologist, the geneticist all likely will have nuanced differences of interpretation. These differences are conditioned by the intended use of the information.

The usual use of the term "strain" denotes that a collection of isolates are identical within the limits of resolution of the methods used to determine identity. The methods may range from an extensive panoply of methods to a single focus on a particular attribute. The driving factor is the aim of the microbiologist. Single attributes may by pathogenicity, toxin production, plasmid directed activity, and others of importance to the individual microbiologist. If the strain loses virulence upon laboratory passage and the virulence returns on the animal or plant passage, it is the same strain?

Culture collections commonly assign new strain designations to cultures obtained from other collections. In the case of type strains, the working assumption is that the new culture is identical to the original collection. For example, a search of http://www.straininfo.net for the type strain designation of *Pseudomonas putida* A.3.12 returned the list of collections listing copies of the strain (Fig 1). However, some collections, and recipients of cultures, adopt the prudent strategy of comparing the new strain to one or more copies of the originally deposited type strain or at least a detailed description (phenotype, macromolecular sequences and patterns, etc.). How many of these strains reflect the originally deposited type strain in all details? If any of the strains are different, how different are they? Does the copy sent to an individual retain the property that motivated the request for the strain?

The working assumption is that the strains have not changed. If it is the motivating characteristic that changed, this would become quickly apparent. Loss or change of an epitope or a virulence factor would preclude effective vaccine production. Pseudomonads and various fungi are used in commercial secondary metabolite production and must conserve the metabolite production.

Klockgether et al., (2010) published on the genomic comparison of the PAO1 strain as obtained from different laboratories, sometimes more than once. They concluded that "*P. aeruginosa* PAO1 shows an ongoing microevolution of genotype and phenotype that jeopardizes the reproducibility of research." Thus, the recipient of a strain, or indeed, the subculture of a strain in one's own laboratory, should

perform quality assurance that the new culture behaves in the desired, expected manner.

The determination of the identity of a new copy or isolate as a member of an existing strain has a more stringent requirement than as a member of a species. Inherent in the species concept is the allowance of variation. How much variation is allowed depends on the method, e.g., within approximately 80% for DNA hybridization, 3% for 16S rDNA. There does not appear to be any comparable guidelines for allowing variation for strains. Determining absolute identity of two isolates is not a practical (or even attainable) result. Informational entropy results in determination errors compounded by changes within a culture (acquiring or losing genes through the various mechanisms of such changes, gene expression during growth, etc.).

Perceived variation exists due to a combination of methodological error and biological change. The microbiologist must judge if the perceived variation is acceptable to denote membership in the original strain. The literature is replete with examples of research performed on strains with the designation of the originating collection. In fact, the strains were derived from a subculture provided by the collection and deserve to have a designation of the recipient laboratory. Including both designations in publications would be helpful. Synonymous designations such as is common in many culture collection catalogues should be the universal practice. The StrainInfo database clearly demonstrates the utility of such synonyms.

Ultimately, the decision as to strain definition and identity is the responsibility of the individual microbiologist. “I will know it when I see it.”

References

Cowan, S.T. 1979. A dictionary of microbial taxonomy. Ed. L. R. Hill. Cambridge: Cambridge University Press.

Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One. *75*(4):e10034.

Dijkshoorn, L. Ursing, B. M. and Ursing, J.B.. 2000. Strain, clone and species: comments on three basic concepts of bacteriology. J. Med. Microbiol. *49*: 397-401.

Klockgether J, Munder A, Neugebauer J, Davenport CF, Stanke Frauke, Larbig KD, Heeb S, Schöck U, Pohl TM.

Krichevsky, M.I. 2011. What is a bacterial species? I will know it when I see it. The Bulletin of BISMiS, Volume 2, part 1, pp. 17–23.

Ørskov F., Ørskov I. 1983. From the national institutes of health. Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the enterobacteriaceae and other bacteria. J Infect Dis. Aug;148(2):346-57.

Robinson DA, Enright MC. 2004. Multilocus sequence typing and the evolution of methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect. *10*:92-7.

Smirnov V. V., and Kiprianova E. A.. 1990. “Bacteria of the Genus *Pseudomonas*.” Kiev Council of Sciences, Kiev, Ukraine. p. 158.

Struelens MJ, Hawkey PM, French GL, Witte W, Tacconelli E. 2009. Laboratory tools and strategies for methicillin-resistant Staphylococcus aureus screening, surveillance and typing: state of the art and unmet needs. Clin Microbiol Infect. *15*:112-119.

Thrash, J. C., Boyd, A., Huggett, M. J., Grote, J., Carini, P., Yoder, R. J., Robbertse, B., Spatafora, J. W., Rappé, M. S., Giovannoni, S. J. 2011. Phylogenomic evidence for a common ancestor of mitochondria and the SAR11 clade. Sci. Reports. 1. doi: 10.1038/srep00013.

Wiehlmann L, and Tümmler B. 2010. Genome Diversity of *Pseudomonas aeruginosa* PAO1 Laboratory Strains. J. Bacteriol. *192*(4):1113. DOI: 10.1128/JB.01515-09.

The Bulletin of BISMiS (2013), Volume 4, part 1, pp 15-17

Embrace The Genome!

Vartul Sangal and Paul A. Hoskisson

We should offer a disclaimer first and foremost by stating that neither of the authors of this piece are systematists, yet, our work is ultimately guided by taxonomy and the over-arching framework that systematics provides is fundamental to make sense of our data.

For many years, bacterial taxonomy relied purely on morphological and biochemical properties of strains until 1970/80s when a polyphasic approach was applied to classification, which added a genetic component to the characterization (Vandamme et al., 1996). The basic taxonomic units, so called species or operational taxonomic units (OTUs), were defined with ≥70% relatedness with ≤5°C difference in the melting temperature (T_m) in DNA-DNA hybridization (Wayne et al., 1987) and >98.7% identity between 16S rRNA sequences (Stackebrandt and Ebers, 2006). Then the phenotypic differences including biochemical properties and/or the variation in surface antigens further subdivided them into biovars or serovars. Now these characteristics certainly have utility in the clinic, but as we begin to look deeper, the fuzzy nature of each of these units grows, and then the reliance of biovar/serovar/pathovar etc. grows; but how reliable are these distinctions?

Salmonella enterica is a classic example of the phenotypic diversity possible within a bacterial species as over 2,500 serovars have been defined based on the variation in the antigenic profiles and biochemical properties (Guibourdenche et al., 2010). The list is ever increasing with many new combinations of these characters getting discovered every year. But, does a serovar represent a group of genetically identical or closely related isolates when compare to other serovars? The answer is not necessarily, as recent studies based on the nucleotide sequences of seven housekeeping genes (Multilocus Sequence Typing, MLST) concluded that isolates within a serovar can be genetically distinct and, conversely, that isolates of different serovars can be closely related at the genetic level (Sangal et al., 2010; Achtman et al., 2012; Tang and Liu, 2011).

Similarly, biotyping has defined four biovars within *Corynebacterium diphtheriae* namely, gravis, mitis, intermedius and belfanti (Tang and Liu, 2011). Again these biovars were found to include wide genetic diversity amongst the isolates (Funke et al., 1997). Then, what are the benefits of assigning genetically diverse isolates to a group and conversely genetically similar isolates into distinct groups using biotyping methods? Indeed some of the traits relied upon, such as carbon utilisation, are precisely the kind we know are moved regularly by horizontal gene transfer. There are, of course, some arguments for biovars/serovars/ecotypes/pathovars. For example, the clinically important *Salmonella enterica* subsp. *enterica*, causes typhoid fever in humans, where isolates are genetically monomorphic according to MLST (Achtman et al., 2012). But why do we need to use a tedious and time-intensive approach that needs specialist experience to define isolates as belonging to a particular serovar when a robust classification could be reached by sequencing the fragments of seven housekeeping genes? Does it really make sense to follow an outdated phenotyping system to taxonomically classify bacteria into biotypes when more advanced genetic approaches are available? Biotyping methods are generally time and labour intensive and needs expertise to interpret the results (or often the results are intrinsically variable). There are also issues related to the reproducibility and reliability of biotyping results from thousands of reference laboratories around the globe. Biotyping has been applied to *Staphylococcus aureus* and several biovars defined (Farfour et al., 2012; Devriese, 1984); but it has long been replaced by MLST and the clonal complexes assigned using MLST, have become popularly used strain nomenclature. Therefore, unreliable phenotypic sub-categorization

Contact details
Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G4 0RE, UK.
paul.hoskisson@strath.ac.uk

should be replaced with more realistic genetic approaches, which have the advantage of being technically straightforward , unambiguous and yielding portable data (i.e. data readily shared through open databases).

Another major concern is the reliance on the similarity of DNA-DNA hybridization and identity of 16SrRNA sequences to assign strains to a species. The classification using DNA-DNA hybridization relies on a four-decade old approach with a largely arbitrary cutoff of ≥70% relatedness. In addition, accurate DNA:DNA pairing requires repetitive comparison of all related strains, which in many cases is neither practical or cost effective: in Streptomyces the cost of doing this with around 700 valid taxa would be prohibitive! The values are dependent on the probes and the target and can be variable for the same set of strains (Coia et al., 1990). Furthermore it is becoming apparent that 16S rRNA sequences lack sufficient resolution to separate all species (Coia et al., 1990), with MLST-like approaches being used more and more to add resolution to the data.

Faced with these limitations, the answer has to be the incorporation of whole genome sequences into the polyphasic approach. Recent advancements in the next generation sequencing technology, along with bioinformatics tools to analyse the data, has resulted in an explosion of sequenced microbial genomes. The numbers of available bacterial genome sequences has grown over from 600 in May 2008 (Coia et al., 1990) to currently 3800 at the NCBI website (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html). Pan-genomic analyses of these data can help define core and accessory genomes, and the average nucleotide identity between orthologous genes between a pair of strains could be used for defining taxonomic units (Achtman and Wagner, 2008) that could replace DNA:DNA pairing. Indeed with the rate that the cost of genome sequencing is falling (~$500 per genome) it may even be cheaper than DNA:DNA pairing!

The International Code of Nomenclature of Bacteria is valuable, but we need to encompass change and not be bogged down in a dogmatic adherence to perceived rules and regulations. We should embrace new technologies and work out ways of replacing difficult or outdated technologies with new, more reliable methods (Whitman, 2011). This all has to be carried out within a robust framework. We are not suggesting an abandonment of the phenotypic and molecular characterization of strains (this is ultimately why we want the taxonomy to be robust too!)Recently, Sutcliffe et al., (2012) proposed a revitalization of the discipline through essentially opening our minds to new approaches and exploring new avenues to cataloguing bacterial diversity, and publishing the data - we have to endorse this.

While it remains to be a matter of debate on how exactly to use genome sequences in species descriptions, the fact is that the genome sequences index entire micro-variation and can help identifying the most reliable boundaries between different operational taxonomic units, because genomes do not lie. Already it could suggested that it is feasible and cost effective to ask taxonomists to consider sequencing the genomes of all new type strains proposed to represent new taxa (Whitman, 2011). Let's embrace the genome and advance our science!

References

Achtman, M. and Wagner, M. 2008. Microbial diversity and the genetic nature of microbial species. Nat Rev Microbiol 6, 431-440.

Achtman, M., et al., 2012. Multilocus Sequence Typing as a Replacement for Serotyping in Salmonella enterica. PLoS Pathog 8, e1002776.

Coia, J.E., Thomson-Carter, F., Baird, D. and Platt, D.J. 1990. Characterisation of methicillin-resistant Staphylococcus aureus by biotyping, immunoblotting and restriction enzyme fragmentation patterns. J Med Microbiol *31*: 125-132.

Devriese, L.A. 1984. A simplified system for biotyping Staphylococcus aureus strains isolated from animal species. J Appl Bacteriol *56*:215-220.

Farfour, E., et al., 2012. Characterization and comparison of invasive Corynebacterium diphtheriae isolates from France and Poland. J Clin Microbiol *50*:173-175.

Funke, G., von Graevenitz, A., Clarridge, J.E., 3rd and Bernard, K.A. 1997. Clinical microbiology of coryneform bacteria. Clin Microbiol Rev. *10*: 125-159.

Guibourdenche, M., et al., 2010. Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. Res Microbiol. *161* : 26-29.

Konstantinidis, K.T., Ramette, A. and Tiedje, J.M. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B. *361*: 1929-1940.

Sangal, V., et al., 2010. Evolution and Population Structure of Salmonella enterica Serovar Newport. J Bacteriol. *192*: 6465-6476.

Stackebrandt, E. and Ebers, J. 2006. Taxonomic parameters revisited: tarnished gold standards. Microbiol. Today *33* : 152-155.

Sutcliffe, I.C., Trujillo, M.E., and Goodfellow, M. 2012. A call to arms for systematists: revitalising the purpose and practises underpinning the description of novel microbial taxa. Antonie van Leeuwenhoek. *101*: 13-20.

Tang, L., Liu, S.-L. 2011. The 3Cs provide a novel concept of bacterial species: messages from the genome as illustrated by Salmonella. Antonie van Leeuwenhoek. *101*: 67–72.

Vandamme, P., et al., 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev. *60*: 407-438.

Wayne, L.G., et al., 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int. J. Syst. Bacteriol. *37*: 463-464.

Whitman, W.B. 2011. Intent of the nomenclatural code and recommendations about naming new species based on genomic sequences. BISMiS Bulletin volume 2, p 135-139.

The Bulletin of BISMiS (2013), Volume 4, part 1, pp 19-41

The Amazing World of Microbes and Microbiologists

Lina V. Vasilyeva

I was born on March 29 of 1941 in Moscow about 2.5 months before the Great Patriotic War broke out. Kapitalina Vasilyeva, my mother, studied in Leningrad at the Lesgaft Institute of Physical Culture, Sport and Health - the oldest sports and educational institution in the world founded in 1896. She came to Moscow for practical training but the War prevented her from returning to Leningrad and probably it was this circumstance that saved her life and mine. Many of her friends died during the siege of Leningrad. Valentin Melikyan, my father, fell in the first days of the War.

My mother married again after the War, and Vasily Alliluev my stepfather (Fig. 1) adopted me. He has become a loved and dear person and has given me a happy childhood (Fig. 2). I often became ill – between the ages of 7 to 12, but I lived in a loving

Figure 1. Lina Vasilyeva's mother and stepfather in 1950.

atmosphere. My father, who was a military pilot in WWII, wanted me to become capable of doing various things in my life. He fostered me to be fearless. Like most children, I was fond of learning. During the War my father's code name was "Sokol" ("Falcon"), therefore he nicknamed me "Sokolenok" ("Falconet") at home. He entrusted me with the car's steering wheel when I could hardly reach the floor pedals with my feet. He also taught me to drive a scooter.

Contact details
Institute of Microbiology
Moscow, Russia
lvasilyeva@mail.ru

Figure 2. (Left) Lina – as a first grade student in 1948. (Right) Lina Vasilyeva in 2011

Unhappily, fate gave him a short life; he departed from this world in 1962. Our mother gave my stepbrother, stepsister, and me swimming and tennis lessons (Fig. 3). Playing piano was a must for us. The last piece of music, which "crowned" my music education, was the Turkish March by Mozart. Dogs have always lived in our home. The first dog of my childhood was a German Sheppard named Ralf, who accompanied me in my walks or cycle drives in the forest.

My childhood dream was to become a pilot when I became an adult. My father never dissuaded me from this idea; once he even seated me at the control wheel of a plane flying on autopilot. From the cockpit, I could see the beauty of the Earth below. It was a Li-2 Soviet transport plane manufactured under the license of the American Douglas DC-3. However, in later years, he discouraged me little by little from the idea of becoming a pilot. By the time I finished school, I was eager to become a geologist. But an incident interfered with that aspiration. Up to the year of 1955, there were separate educational systems for boys and girls in Soviet schools. But our generation was fortunate in that respect. At the age of 13, I was transferred into grade 7 of the boys' school. There were more boys than girls in our class. The boys and girls were on a very friendly footing

Figure 3. First row: Alexander, dog-Ralf; Second row: at the left Lina, Kapitalina, Hadezhda, Valentina (Lina's mother's sister).

and co-education was beneficial to everyone. To this day, we are trying to meet and arrange class reunions at least once a year. Regretfully, several people from our class have already died.

University: 1958-1963

Having finished the school, my friend and I submitted applications to Lomonosov Moscow State University (MSU); she applied for the faculty of Soil Biology and I submitted an application to the geology faculty. She was reluctant to enter the faculty of biology alone so we decided to go there together. At the faculty, we met a 5th year student, Galina El-Registan (nowadays she is also on the staff of the Institute of Microbiology), who told the applicants about the faculty of soil biology, in particular, that the educational program of the faculty had many subjects in common with the program of the geology faculty. Her narrative and the atmosphere of the faculty impressed me greatly, and as a result I applied to the soil department and my friend entered the biology department. During the first two years of our studies, along with studies of physics, mathematics and chemistry, we had lectures and practical work in crystallography, mineralogy, geodesy and cartography.

During summer after the second year, soil students travelled by trucks for a soil zone field trip from Moscow to Yalta (Crimea), with stops in each of the soil zones where we took a soil profile to characterize it. As a result we became knowledgeable about the differences among soil types (sod podzol, brownearth, black soil and others). That fascinating practical experience gave us a good understanding of the difference between soil types of the European part of the USSR in the North-to-South section, starting with sod podzol soils. Regretfully, nowadays, because Crimea is a region of Ukraine, it has become a foreign territory, so students have a reduced zone practice without studying Crimean soils. The memory of that zone field trip will always remain with the students.

As second-year students, we became acquainted with a new subject – medical microbiology. A whole new world of living beings I had known nothing of before opened up to me. Excellent instructors gave us practical lessons about sterility, correct burning of a loop, and being inapprehensive of burner flame. We also learned which diseases are caused by bacteria and which are induced by viruses; we were informed about sources of infection and a lot of other things. I discovered a most interesting book, "Microbe Hunters" by Paul de Kruif, and read it with great interest. That subject foreordained my special field in the future. By the beginning of the third year at the University when every student was expected to choose a specialty I had no hesitation: nothing but microbiology! Therefore, I entered the Department of Soil Biology. Today I can say with assurance that my choice was no mistake. I have devotedly pursued my labor of love throughout my life and I was lucky enough to work side by side with true professionals and enthusiasts infatuated with their subject matter.

When I joined the Department of Soil Biology, it was headed by the founder of the Department, Nikolai A. Krasilnikov (Fig. 4), a global renown scientist. The Department was founded in 1953. Its graduates were full-fledged soil microbiology scientists owing to the fact that the department offered special courses on the structure, taxonomy, physiology and biochemistry of soil microorganisms as well as

Figure 4. Staff of the "Biology of Soils" department, 1957. Left to right in front row: Yu. A. Khudyakova, N. N. Sushkina, N.A.Krasilnikov; second row: T. G. Mirchink, I. P. Babyeva, I. N. Skvortsova, I. A. Aseeva

special courses on soil bacteria, actinomycetes, fungi, yeast, algae, phages, protozoa and a special course on soil enzymes. Under the leadership of N. Krasilnikov, the Department paid special attention to three problems, namely: biology of soil microorganisms; interaction between microorganisms and higher plants; and the ecological significance of physiologically active substances produced by microbes.

My five years at the University were the most interesting and happy of years. We learned many new things which broadened our minds and we recognized that much remained to be discovered. We could hardly imagine how we would be able to leave the University. My graduation paper was devoted to an investigation of gibberellin and was entitled "Formation of gibberellin and gibberellin-like substances with Azotobacter cultures."

After N. Krasilnikov's death in 1973, the Department was headed by his disciple, Prof. D. Zvyagintsev, and since 2009, the Department has been headed by Prof. I. Chernov.

INMI: From 1963 to the Present

In 1963, four students graduated from the Department of Soil Microbiology. A year earlier, in 1962, the Institute of Microbiology of the Russian Academy of Sciences moved to a new building and the Board of Directors of the Institute was interested in expanding the staff of science workers. Hence, a good number of graduates of the MSU faculty of soil biology were enlisted as intern researchers in different departments of the Institute. I was hired for a position in the department of soil microbiology headed by E. Mishustin, a Correspondent Member of the Russian Academy of Sciences.

Prof. E. Mishustin had been invited to the Institute of Microbiology of the Academy of Sciences of the USSR in 1940 where he founded a Department of Soil Microbiology which he headed for more than 50 years until his death. He laid the foundation for an ecology-geographic branch in soil microbiology and a theory of microbial cenoses that are still of great importance and have recently been substantially advanced.

Perhaps Mishustin's greatest contributions in microbiology were the development of the area of microbiological fixation of atmospheric nitrogen and modern notions of the nitrogen balance in soil and the rational use of mineral and "biologically fixed" nitrogen. In addition, he made important contributions in the field of sanitary microbiology and soil self-purification, evaluation of soil biologic activity and the role of microorganisms for the synthesis and degradation of humic substances and the introduction of microbial populations into soil to enhance fertility.

It was my good fortune to work in the Department led by E. Mishustin. He was a true Master whose leadership enriched the mind immensely. The basic principle of his relationships with his disciples was an organic combination of rigidity and exactingness with much attention given to the scientific efforts of his pupils. A scientist of world fame, he paid genuine and keen interest to the questions we posed him.

I recall E. Mishustin as an easy-going benevolent person of great personal magnetism and a perfect sense of humor who used vivid figural phrases in his speech. These general impressions about Mishustin still remain in my memory.

Kamchatka

In August of 1965, the Institute of Microbiology arranged an expedition to Kamchatka. On the expedition were Mikhail Ivanov, Vladimir Gorlenko and Georgii Zavarzin. It was Zavarzin who suggested that I participate. The trip was marvelous, and the memory of that expedition has always remained with me. The first station of our expedition was volcanologist base "Pauzhetka" on the western coast of Kamchatka. At that time, a hydrothermal field station was under construction at the "Pauzhetka" base, which is in operation now.

After embarking from Petropavlovsk-Kamchatskii

Figure 5. A rest stop on the volcano Kambalny. Kamchatka 1965.

for the sea of Okhotsk, our powerboat was caught in a storm gale. As the storm grew more intense those of us on deck returned to the main cabin. All the passengers in the cabin were lying on the floor in a 'prone position' and we were strongly advised to join them.

When the high seas slightly subsided, a small barge was able to reach our boat during a high tide. We were carefully placed in a herring net and transferred to the deck of the barge. These barges are very unstable and can be easily canted by high waves. We were able to reach the mouth of the river before the tide changed which would have made the passage impossible.

Accompanied by volcanologists from the "Pauzhetka" base, we climbed two volcanoes, Kambalniy and Koshelev (Fig. 5). For the first time in my life, I was given the opportunity to see wonders of Nature such as sulfuric fumaroles, mud pots and geysers. I smelled the scent of hydrogen sulfide and personally experienced, to my chagrin, droplets of sulfuric acid that fell on my face and ate the fabric of my field suit.

At Zavarzin's suggestion, we decided to collect samples at an elevation of 1102 meters at a location that could be easily seen from the base. Having taken the samples and carrying only the samples, a photo cam, and a knife, we came much too close to a female bear with two cubs. Fortunately, she did not notice us because we were standing on the leeward. As I observed her, I could see her in all her beauty when she stood up on her hind legs. It was our good luck that she decided not to abandon her cubs, but the sight of that bear was on my mind for many years. After that encounter, I always tried to follow a guide with a gun as I felt highly reluctant to have another meeting with such a fearful adversary. In remembrance of the meeting with a bear at an elevation of 1102 meters, I assigned this as the strain number for the first pure culture with prosthecae that I described in 1968 - *Prosthecomicrobium polyspheroidum*. Other results obtained through the analysis of samples taken at 1102 meters were published in Zavarzin, et al. (1967).

We also visited the Ebeco volcano on Paramushir Island, in the northern Kuriles, 6 km to the North-West from the town of Severo-Kurilsk. When we came ashore from a power boat in Severo-Kurilsk, we saw the devastation caused by a seismic sea wave (tsunami). Many boats and small ships were scattered at a good distance from the seashore. Houses located far from the shore were empty with smashed windows. Walking on hardened lava, we reached the Ebeco volcano crater and found it filled with water which turned it into an acid lake. From the top rim of the Ebeco volcano, we saw the gorgeous volcano island of Alaid, which is the highest of Kuriles and the outermost northern island

of the string.

The expedition to Kamchatka enabled me not only to see a region of unique beauty and attain deeper insight into the nature of the Earth and the activity of microbes, but also to make a life-long acquaintance with excellent microbiologists and volcanologists.

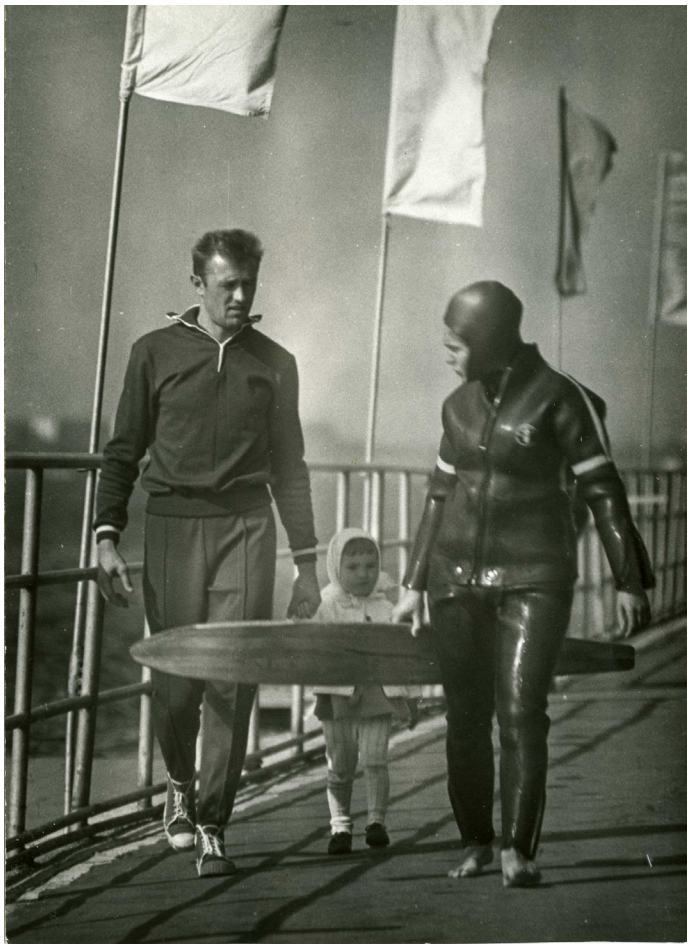


Figure 6. When Lina still participated in water-skiing competitions. A meeting with her daughter and husband, Valeri Ozhogin, after water skiing on one ski at the Moscow Khimki reservoir, 1969.

My Hobbies

When I was a child, I often became ill, so my parents devoted a lot of their time to my physical conditioning. By the time I was in finishing school, I had a second-class senior degree in swimming and during all the 5-years of my studies at the University, I participated in swimming competitions for the Soil Biology faculty team and volleyball competitions.

In the summer after the fourth year at the University I went to Sochi, to the sea. It so happened that in the very first day I made an acquaintance with my future husband who also came to Sochi after a mountain-climbing camp in the Caucasus. At that time he was a post-graduate student at the Kurchatov Institute of Atomic Energy. Those were years when many young people began to go into alpine skiing in the winter and water skiing in summer. In the 1960s, water skiing in our country was virtually in its infancy, and men made many things with their own hands. Alpine skiing equipment was far from perfect too. Thus, thanks to my husband, I became familiar with water skiing during summer weekends (Fig. 6) and in the winter we went alpine skiing near Moscow. Sometimes we managed to go to the Caucasus Mountains, Elbrus region, or to the Kola Peninsula. These two sports were intensively developing in later years involving many young people who had better facilities and equipment. As is well known, the pioneers who lead the way experience many more difficulties than those who follow them – in any kind of human activity.

In the 1980s, we changed from water skiing to surfboarding which makes a sportsman not dependent on a powerboat, but only on the wind. My last trip to the mountains was in 1991 and then I turned to cross-country skiing.

Beginning of professional life

I began work as an intern researcher in the Department of Soil Microbiology in a group headed by Denis Nikitin. At that time Nikitin's attention was drawn basically to the detection of cells of unusual morphology encountered in different soil samples with the use of an electron microscope. Soil is a complicated object for direct microscopic analysis. Nikitin taught me various known methods of soil sample preparation for their subsequent analysis with an electron microscope. In those years, we worked with an electron microscope manufactured in Sumy (Ukraine). A new Japanese microscope appeared at the Institute only late in the 1960s. We were highly dedicated to our work. We analyzed a great number of preparations made of different

soil samples. The electron microscope allowed us to be the first to discover various forms of cells. The results of these investigations are presented in the book "New and rare forms of soil microorganisms" (Nikitin et al., 1966), which provides illustrations of a wide variety of forms including cells with different sorts of appendages. It became clear that the assignment of the discovered forms to the world of bacteria would require their isolation into pure culture for further microbiological investigations.

In our choice of the cultivation medium, we proceeded from the fact that classical methods of seeding soil suspension on typical microbiological laboratory culture media available at the time yielded only of a fraction of microorganisms that were present, namely those whose nutritional requirements correlated with the cultivation conditions. We realized that we needed "poor" medium with lower concentrations of nutrients, which would select against the conventional microflora and increase the relative number of morphologically peculiar organisms. The most frequently encountered organisms in soil suspensions, especially in black soil, hotbed soil and lowland peat were rod-like organisms with spherical appendages. The first to characterize these forms of cells were Nemec and Bystricky (Nemec and Bystricky, 1962), who found them in mixing cultures containing algae. In 1966, the development of similar kinds of cells was noticed in selective nutritional media inoculated with soils from several states of America (Orenski et al., 1966).

With the purpose of isolation of unusual forms from soil, Nikitin suggested the use of various soil media representing agar-type soils or water extracts of soil. The first strain of a "polyspheroid" was obtained from pre-sterilized extracts of black soils from the Krasnodar region, which was inoculated from a humus soil suspension. After a 10-day incubation at 28°C, the enrichment culture was seeded on the same medium, but solidified with 2% agar. Then I seeded the colonies of microorganisms that had developed on the agar-type extract onto the same medium followed by the analysis of cell suspensions from each colony with an electron microscope since the small spherical appendages are not detectable with an optical microscope. Then we isolated the organism into pure culture from the humus soils as strain 1102 (Nikitin and Vasilyeva, 1967).

Figure 7. E. N. Mishustin with the staff of the laboratory of soil microbiology. From left to right: front row – T. Kalininskay, G. Smirnova, E. Gromiko, E. Mishustin; second row – V. Aristarkhova, I. Mishustina, R. Lokhmacheva, T. Lafitskaya, D. Nikitin. (Photo from E. Mishustin's book "My life in science" memoirs. 1997).

Figure 8. Sverdlovsk hall of the Kremlin in Moscow after the State award of the USSR was presented to E. Mishustina for the textbook Microbiology. From left to right: Two members of the staff of the Timiryazevsky Academy, then V. Emtsev, E. Mishustin, R. Lokhmacheva and A. Semenov, 1983.

Since organisms that were identical in their morphology as seen by light microscopy, and shared similar physiological characteristics that had been reported previously to be in the genus *Agrobacterium*, we tentatively ascribed the organism to that genus with the species epithet *polyspheroidum* (Nikitin and Vasilyeva, 1968). This name is indicative of the organism's morphology, and the species name based on their "polyspheroid" morphology was suggested by Orenski (Orenski et

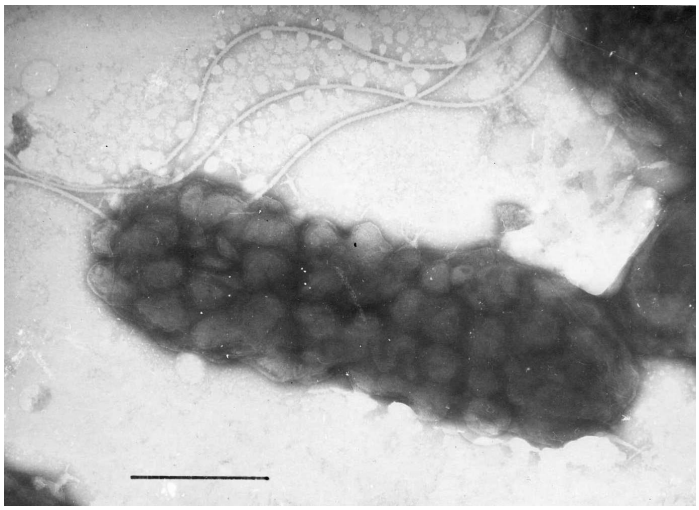


Figure 9. *Prosthecomicrobium polyspheroidum*. Phosphotungstic acid (PTA) negative strain. Bar = 0.5μm

al., 1966). Using an optical microscope, I studied their developmental cycle in microculture and showed that the cells multiply by fission (Vasilyeva, 1969).

My teamwork with Denis Nikitin defined the domain of my further work for many years. I will always be grateful to Nikitin for the time of joint researches and primarily, for the very idea to study new microbes in soil. The diversity of the microbe world, as our experience has revealed, - is infinite, and studying this amazing world is a fascinating and engrossing challenge. After the termination of my traineeship (1963 – 1965) I was enlisted to the Department headed by Academician Mishustin as a junior research scientist (Fig. 7, 8).

My task was to isolate pure cultures of the forms which we observed in soil under an electron microscope. To this end, it was necessary to work out cultivation methods and to investigate the physiologic distinctions of the pure cultures; also, we sought to define the position of this group of organisms in the trophic food-web of soil microbic processes and their interaction with other microorganisms.

In 1968, J. Staley published an article "*Prosthecomicrobium and Ancalomicrobium: new prosthecate freshwater bacteria*" which described bacteria with appendages discovered from aquatic habitats (Staley, 1968). J. Staley was the first to offer the name of "prosthecae" for appendeges with ingrowths of cytoplasm together with the cytoplasmic membrane. Two new genera, *Prosthecomicrobium* and *Ancalomicrobium*, were described. Phenotypic criteria were the basis of assignment of these genera. I was waiting for new publications by J. Staley with great interest since in fact, we were doing in parallel the same kind of investigations on a different continent. The third laboratory that conducted studies of prosthecobacteria, also in an aquatic environment, was located in Germany (Hirsch, and Schlesner. 1981; Schlesner, 1983).

We did not know each other in person, but all of us were "fed" by the information from each other. I was very much surprised and grateful to Prof. Schlesner when he sent me a copy of his dissertation in 1983. I always regarded it as an example of the accurate execution of a scientific paper and I always demonstrated it to my students and post-graduates.

1965 - 1980

In cooperation with Tatyana Lafitskaya, I studied the morphological and cytological features of a "polyspheroid" as well as the physiological characteristics of newly isolated prosthecobacteria. We used media of various compositions for growing a "polyspheroid," and we discovered the degree of appendage development on a cell to be dependent of cultivation conditions and most notably of the medium composition. When grown on poor medium, appendages were well expressed and were arranged spirally on a cell. An excess of organic matter resulted in their loss and appearance of branched forms. It was necessary to find out if these appendages were prosthecae and what role they played for the organism. Ultrathin sections of polyspheroid cells of typical morphology allowed me to find cytoplasm and cytoplasmic membrane growing into the appendages, which provided us the basis to consider them as prosthecae. Therefore, we assigned the organism which was preliminarily designated as *Agrobacterium polyspheroidum* (Fig. 9) to the genus *Prosthecomicrobium*, and the trivial name of the organism, "polyspheroid," was retained as a species epithet - *Prosthecomicrobium polyspheroidum* (Vasilyeva and Lafitskaya, 1976).

When we found that prosthecae were missing on

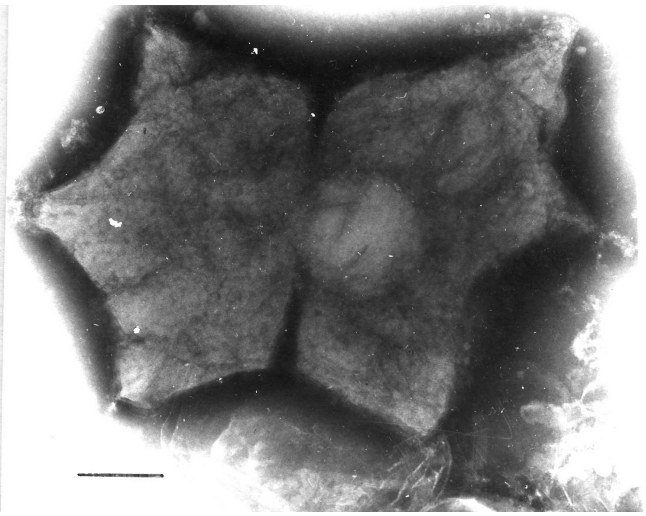


Figure 10. *Stella humosa* type strain AUCM B-1337. PTA negative strain. Bar = 0.5μm.

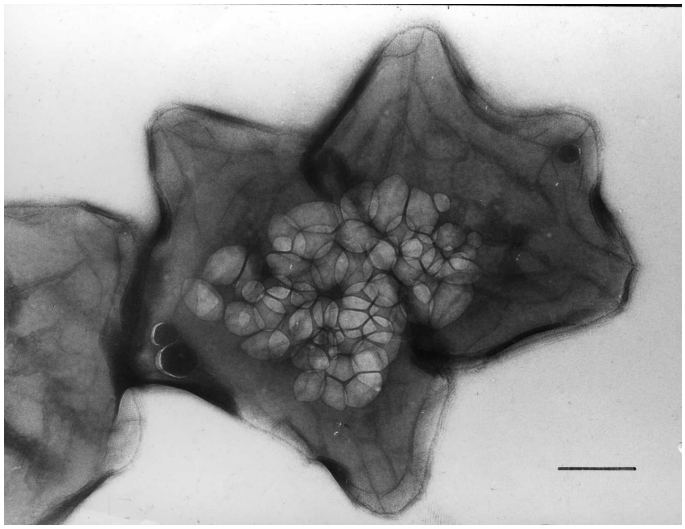


Figure 11. *Stella vacuolata*. Bright areas in cytoplasm are gas vesicles. Uranylacetate-negative stain. Bar = 0.5μm.

whole cells and ultrathin sections grown under excessive organic matter, we assumed the original morphology of a polyspheroid to be a result of the organism's adaptation to a low organic content in the medium of less than 0.1% total organic concentration. Prosthecae, by their enlargement of the cell surface area, enhance the ability of bacteria to capture nutrients at low concentrations under conditions of oligotrophic nutrition.

Our joint research work with E. Krasilnikova and N. Aleksandrushkina from MSU revealed that the physiological peculiarities of "polyspheroid" strains 1102 and 1103 isolated from different types of soils ensured low hydrolytic activity, indicating that the organism cannot directly utilize polymeric compounds. Therefore, the polyspheroid bacteria in natural environments depend on other microorganisms which are able to decompose polymeric compounds.

The "polyspheroid" organisms metabolize mono- and disaccharides using an oxidizing pathway, either the pentose-monophosphate pathway or the Entner-Doudoroff pathway. They utilized lower alcohols, the majority of organic acids and cyclic compounds. Among the amino acids, the most easily assimilated ones were glutaminic acid, aspartic acid, cystine, histidine and oxyproline as sources of nitrogen and carbon for growth. The organism did not grow without additional growth factors which turned out to be vitamins - thiamine and riboflavin (Lafitskaya, et al., 1976). In these experiments, we realized for the first time, that cultures of the "polyspheroid" possess properties correlating with representatives of the "dispersal microflora" - a term suggested by G. Zavarzin (Zavarzin, 1970) - and can be regarded as typical representatives of this ecological grouping.

At the Institute of Microbiology, G. Zavarzin always took a keen interest in research aimed at the isolation and investigation of new microorganisms. When he dropped by Mishustin's Department of soil microbiology, he always used to ask me and Tatyana Lafitskaya "What's new?" and if there was a new microbe reported, he always asked us to show it to him under a microscope. He was a brilliant microscopist. For us, it was always very interesting and useful to discuss new results with him, and for him, it always provided new thought-provoking information.

I have found that a medium which contained fulvic acids as the basic substrate ensured the growth of a large variety of organisms with a specific morphology. Using an electron microscope, I checked for the presence of the sought-for forms in the fluid medium after 7-10 days of incubation at 28°C in the enrichment culture. When they were observed, I seeded the enrichment culture on the same medium, but in its agar form. In most cases colonies of conventional forms were found growing with cells of the organisms of interest to me being present as admixtures discernible under an optical

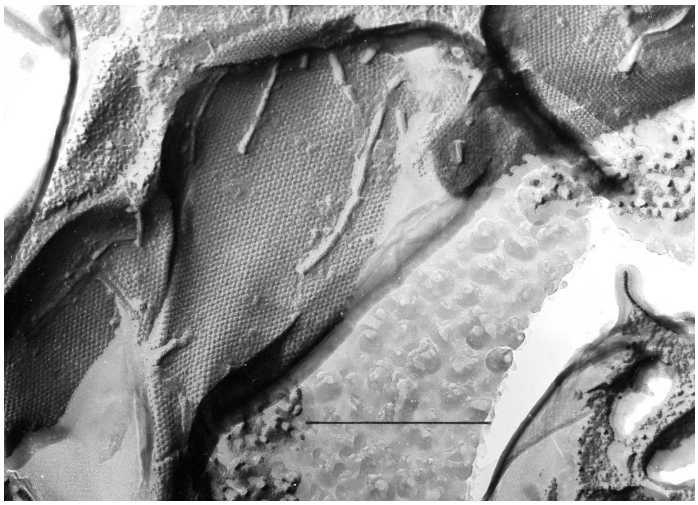


Figure 12. External cell wall layer of *Stella humosa*. Bar = 0.2µm

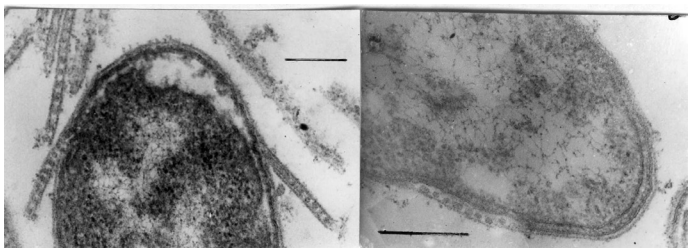


Figure 14. Ultrathin sections cell of the *Stella humosa* from MPA. Bar = 0.25µm

microscope.

In order to identify the strain of interest, I pressed a sterile cover glass to the agar surface on a Petri dish, which I viewed under an optical microscope. When I found a spot with the desired form from the impression, I took microorganisms from this spot with a capillary tube and transferred them to fresh agar. After growth of organisms could be seen near a capillary fragment, the bacteria were washed off and streaked on a Petri dish with fresh medium, while monitoring the microflora composition with optical and electron microscopes. The latter two operations were repeated until a morphologically homogeneous culture was obtained. The application of this method of extraction allowed us to obtain a number of pure cultures, the first representatives of genus *Stella*, *Prosthecomicrobium*, and *Angulomicrobium* whose cells featured various prosthecae and were easily identified in Nature.

In May of 1975, I defended my PhD thesis, "Soil Prosthecobacteria" which was concerned with the description of the representatives

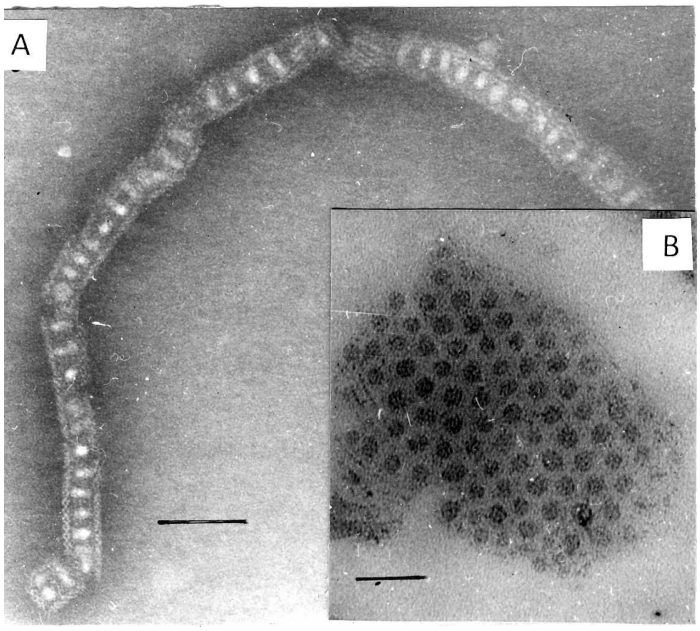


Figure 13. A. General view of a "supporting filament" of *Stella humosa*. PTA stain. Bar = 0.1µm. B. Ultrathin section of showing the external layer of the cell wall of *Stella humosa*. Bar = 0.1µm

of soil prosthecobacteria isolated from the chernozem soil of the Krasnodar Region including *Prosthecomicrobium polyspheroidum*, strain 1102 and 1103; *Stella humosa*, strain VKM B-1137; *Prosthecomicrobium* sp., strain VKM B-1138. The thesis provided a description of the morphology, ultrathin structure and physiological characteristics of bacteria with different types of prosthecae.

Genus *Stella*

Strain VKM B–1137 was the first organism I isolated whose star-shaped morphology was completely unique for bacteria (Fig. 10). This feature facilitated its easy detection with phase-contrast microscopy under natural conditions. The existence of radial symmetry among prokaryotic organisms was verified with the isolation of this bacterium, which I named *Stella humosa* (soil star) (Vasilyeva, 1970). The form of *Stella* cells resembles a starfish, or a hexagram "Star of David" (Vasilyeva, et al., 1974). All the bacteria of genus *Stella* are nonmotile, and found as solitary or paired cells.

The organism multiplies by fission. The fission plane is perpendicular to the plane of the cell. The septation occurs between the prongs in the narrowest part of the cell. Cell fission begins with

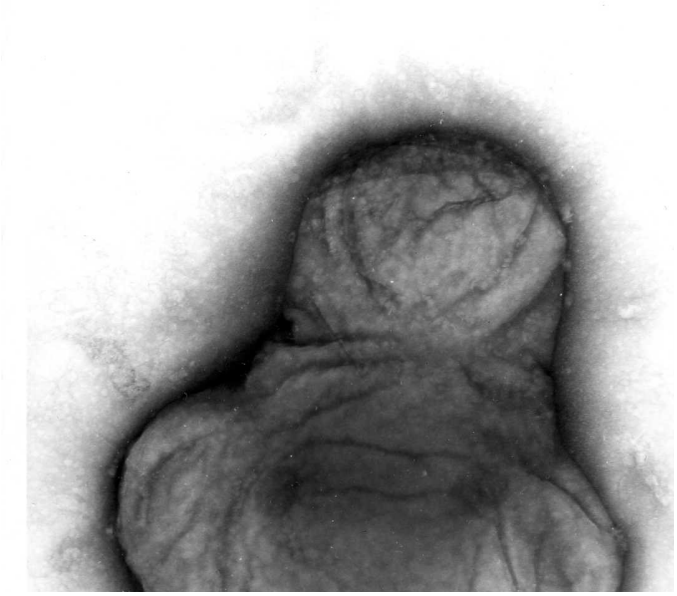


Figure 15. *Angulomicrobium tetraedrale*. Cell of type strain VKM B-1335. Phosphotungstic acid stained. Bar = 0.5µm.

the in-growth of the septum and finishes by the formation of a constriction midway between the poles. The apexes of two new protrusions resulting from transverse fission are situated at the initial in-growths of the septum while the third apex is formed at the constriction place. When division is complete, the cells produce the three missing prosthecae and are then ready for a new fission (Vasilyeva, 1972a). Flat star-like bacteria were first found in soils (Nikitin et al., 1966) and in aqueous samples (Staley, 1968; Hirsch and Schlesner, 1981).

By the early 1980s, when the former fulvic acid based medium was replaced by a medium containing horse dung extract, we succeeded in isolating and making a collection of bacteria of the genus *Stella*. The cells of strains from different sources varied in size and differed in presence/absence of gas vesicles. The gas vacuolate strains have gas vesicle subunits which appear as 150-nm wide cylinders with cones at both ends. These strains were assigned to a new species named *Stella vacuolata* (Vasilyeva, 1985). A type strain of the VKM B–1552 (Fig. 11) was isolated from activated sludge (of a hog farm), and two other strains of the collection were isolated from horse dung (Vasilyeva and Semenov, 1986).

The appendages of *Stella humosa* are prosthecae. Ultrathin sections show cytoplasm with ribosomes and a cytoplasmic membrane completely filling the appendages. The cell wall profile is similar to that of other Gram-negative bacteria. A distinctive feature of *Stella* cells is an external layer of cell wall (Fig. 12), which consists of repeated polygonal formations of 250 Å in diameter and with center-to-center intervals of 400 Å. These were observed on negatively stained fragments of the external layer (Fig. 13 B) and in ultrathin sections. These subunits are arranged in a chessboard order. On Phosphotungstic acid (PTA) staining of *Stella humosa*, whole organisms of typical morphology, I found that a cell is bound by filaments that emanate from the appendages. I named these "supporting filaments" (Fig.13 A). Ultrathin sections through the "supporting filaments" (Fig. 14) reveal polygonal formations that are arranged in a single row (Vasilyeva, 1972a).

In order to find structures responsible for the unique shape of *Stella*, I investigated the comparative morphology of *Stella* cells that were grown on different media. When cultivated on rich medium (meat peptone agar – MPA), the organism lost its characteristic shape, the external layer of the cell and "supporting filaments" were exfoliated (Fig. 13) and the cell acquired a rounded form (Vasilyeva, 1972a).

A study of nutrient requirements of strains of genus *Stella* revealed that none of the 34 tested carbohydrates was utilized. The obligatory condition for the growth of the organism is the addition of yeast extract (0.01 %) to the medium, and its concentration cannot exceed 0.025 % since otherwise it would result in morphological changes and growth depression. All the strains obtained in the collection utilized L-glutamic acid, and the majority of the strains utilized pyruvate, malate, succinate, α-ketoglutarate, lactate and the fatty acids formate and acetate.

The melting point analyses of the DNA of bacteria from the genus *Stella* have shown that all formed a sufficiently homogeneous group, with a high content of G+C bases ranging from 69.3 - 73.5 mole % (Lysenko et al., 1984). Pre-classification of the

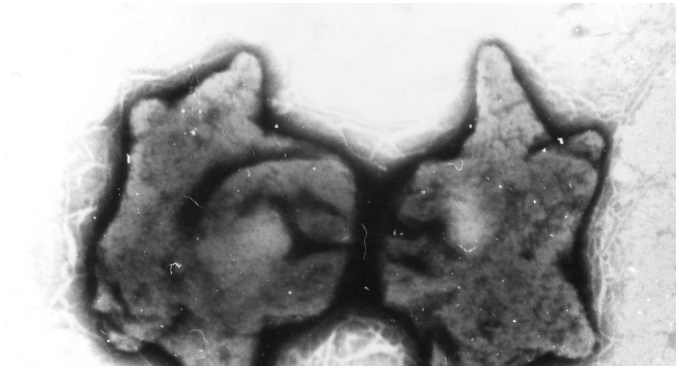


Figure 16. *Labrys monachus*. The "double axe" shape of the figure formed by the mother and daughter cell. Uranyl acetate, negative stain, at a concentration of 0.03%. Bar = 0.5μm.

strains of genus *Stella*, based on the morphological

characteristics, was confirmed by a narrow width of DNA melting. All the strains of the genus *Stella* from our collection feature exhibited close values of the hyper-chromic effect (27-32%), and the data of the temperature range below 3-4 are indicative of the absence of heterogeneity of the nucleotide composition in linear molecules of their DNA (Lysenko et al., 1984).

The strains with cells that had gas vesicles and other

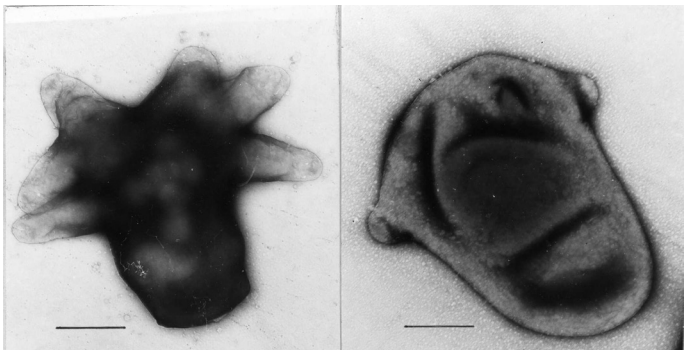


Figure 17. Influence of concentration of glucose on morphology of the cells of *L. monachus*. Cell on the left - 0.01%; on the right – 0.25%. Bar = 0.5μm

phenotypic differences featured the highest value of G+C bases (70-73.5 mole %) and manifested a high degree of homology. Other strains of the collection formed a group of organisms with the homology level in DNA from 3 to 20% (Chernykh et al., 1988). Such a range of affinity usually corresponds to distances between species within a genus. Actually these strains, despite their similar cell morphology, should

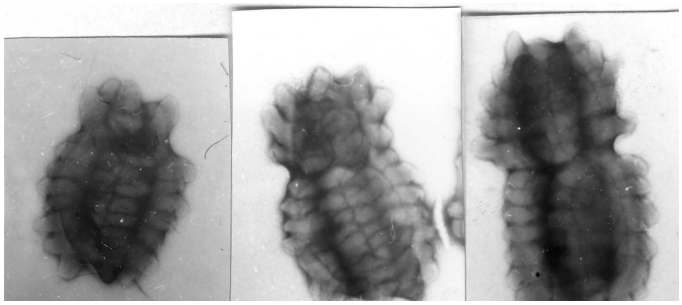


Figure 18. *P. consociatum*, strain 11 showing different stages of reproduction. Uranyl acetate, negative stain. Bar = 0.5μm.

be regarded as independent species. The same conclusions were made by Schlesner (Schlesner, 1983) for strains IFAM 1312 (*S. aquaticus*) and IFAM 1320 (*S. pusilla*) during an investigation of a collection of strains of the genus *Stella* isolated from water media from their habitats.

Genus *Angulomicrobium*

In 1976, a new unusual organism, strain B-1336, with cells of a flat triangle form was obtained from a sample of peat bog and characterized. The organism does not belong to the Prosthecobacteria, but like *Labrys*, it exhibits radial symmetry and multiplies by budding (Lafitskaya and Vasilyeva, 1976). Prof. Zavarzin suggested that Lafitskaya and I compare two strains, that is, budding bacterium strain B-1336 and mushroom-shaped bacterium strain B-1335 (Fig. 15) extracted from a methane oxidizing microbial biocenosis (Namsaraev and Zavarzin, 1972).

A comparison of the triangular bacterium strain B-1336 with a representative of the polygonal bacterium B-1335, designated as "tetrahedron" and similar to one described as a mushroom-shaped bacterium" (Whittenbury and Nicoll, 1971; Stanley et al., 1976), led us to conclude that these organisms differ slightly both in morphology and physiology, but nonetheless fall into the same group of polygonal bacteria and, hence, they were classified as a separate genus named *Angulomicrobium*, with the specific name *Angulomicrobium tetraedrale* and the type strain VKM V-1335 (Vasilyeva et al., 1979, Vasilyeva, 1989). In 2004, Ingo Fritz with co-authors isolated a new strain VKM V-1336 and described it as a new species - *Angulomicrobium amanitiforme* (Fritz et al., 2004).

1980 - 1998

In 1980, Alexander Semenov came to the Department of soil microbiology after 2 years as a "trainee researcher" from another laboratory to take the position of Lafitskaya, who had left the Institute. Years of our teamwork from 1980 to 1989 were very fruitful. Alexander was a surprisingly hardworking researcher capable of handling chemostatic flow-through cultivation. Another scientific worker who came to work with our small team at that time was Alla Giniyatulina. Our joint effort allowed us to study distinctive features of physiology of the expanded collection of Prosthecobacteria cultures.

Genus *Labrys*

While studying an enrichment culture from a sediment sample from Lake Mustjarv (Estonia) I found cells which had the form of a flat triangle with appendages on two apexes. The morphology of such cells is easily discernible under an optical microscope. Cells of strain 42 were in the form of a flat triangle (sized 1.2-1.3 × 1.3-1.45 μm) and were nonmotile. Two to three peaked prosthecae of up to 0.6 μm long extended from each of two apexes of the triangle (Fig 16). Ultrathin sections of the isolated bacterium revealed that the appendages were prosthecae.

The cells multiplied by budding. The bud growth began at a triangle apex free of prosthecae. In so doing, the free extremity of the cell elongated and a bud was formed on it, which enlarged gradually to reach the dimension and the shape of the mother cell; at this stage of reproduction, it resembled a double-edge axe ("labrys"). The formation of new buds of the maternal and filial cells occurred at the place of cell division. This extraordinary bacterium is a combination of three rare taxonomic characters: radial cell symmetry, presence of prosthecae, and multiplication by budding (Vasilyeva, 1980). Owing to its resemblance to the form of a double-edge axe or hatchet, the bacterium we proposed the genus name *Labrys*, with the type species *Labrys monachus* (Vasilyeva and Semenov, 1984).

Labrys monachus primarily utilizes monosaccharides and sugar alcohols and only maltose and trehalose as disaccharides. It did not utilize polysaccharides and amino acids. The only organic acids utilized were pyruvate, lactate and gluconate. The cells responded to a rise of substrate concentration by a reduction of prosthecae length accompanied by their widening at the base, thereby making the cell more roundish (Fig. 17). This could be readily observed at a substrate concentration of 0.03%.

The genus *Labrys* with the type species *L. monachus* has a G+C content of 67.9 mole % and occupies a position between the budding bacteria with radial cell symmetry without prosthecae, i.e., *Angulomicrobium* (63.3 mole %) and the prosthecate genus *Stella* (69.3 - 73.5 mole %). The lack of DNA homology of *Labrys* with all strains of *Stella* was published by Lysenko et al. (1984) and confirmed the legitimacy of assignment of *Labrys* to a separate genus on the basis of morphology and developmental cycle. In 2005, Miller and co-authors (Miller, et al., 2005) described a new species of genus *Labrys* - *L. methylaminiphilus sp.nov.* and others have added to the list of species in the genus.

Genus *Prosthecomicrobium*

In addition to the "polyspheroid," I isolated the first soil strain of the genus *Prosthecomicrobium* from chernozem soil from the Krasnodar region; these cells had appendages all over their surface that fanned out in all directions.

From the viewpoint of morphology, strain VKM B-1138 is identical to a type strain of species *Prosthecomicrobium enhydrum*, described by James Staley (1968). Ultrathin sections of typical cells of the strain have shown that their appendages are prosthecae. According to the microfilming data, they multiplied by budding with the formation of a sessile bud (Vasilyeva, 1972). At late stages, such multiplication might be wrongly taken for fission. In 1984 James Staley (Staley, 1984) reported the occurrence of budding in the genus *Prosthecomicrobium,* indicating that members of this genus, like *Ancalomicrobium*, also divide by budding.

By the early 1980s, our group had a collection

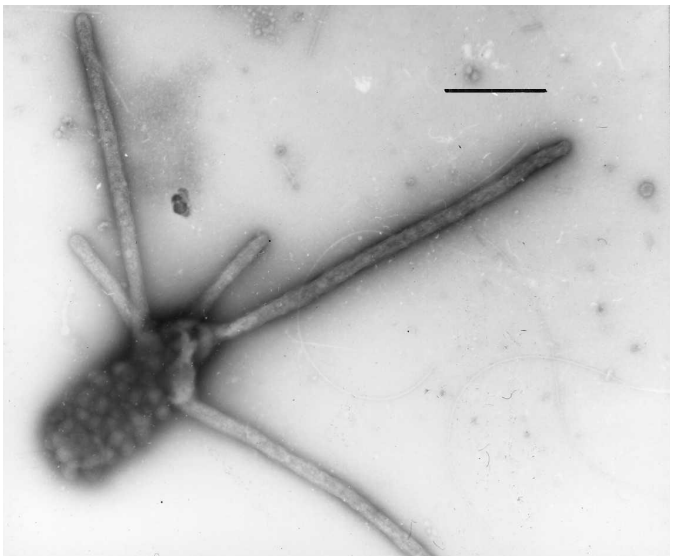


Figure 19. *P. hirschii*, strain 22. PTA, negative strain. Bar = 1.0μm

of strains of prosthecobacteria obtained from different habitats. Cells of all strains had numerous prosthecae which differed in number and length. We discovered that the organisms in our collection exhibited numerous variations from the type-species of the genus *Prosthecomicrobium* and could not be assigned to any of the known species of the genus. Not only did they differ in morphology, but they showed no DNA-DNA homology with the type strains of the species. All the analyzed strains of the collection comprised a morphologically continuous range of forms (Vasilyeva et al., 1991).

Determination of the mol% G+C of DNA in all the prosthecobacteria strains of the genus *Prosthecomicrobium* in our collection revealed that all of them are characterized by a high G+C-base content of 62.2-70.4 mole % that lie within the range of values typical for other described species of the genus *Prosthecomicrobium*.

Determination of the degree of DNA - DNA hybridization of the investigated cultures revealed a strain grouping which permitted a comparison of these hybridization data with the phenotypic characteristics of the groups (Chernykh et al., 1990). Strains were grouped according to their morphological features as follows.

The first group of bacteria included strains 11 (Compost), 16 (yellow ocher), and 26 (Hotbed soil), where the prosthecae were aligned down the cell in five solid rows, and were well distinguishable under the light microscope, their dimensions being 0.2-0.25 μm. The cells of these strains were short rods sized 0.5-1.0 × 0.8-1.35 μm and were nonmotile (Fig.18).

The study of the physiological features of the first group of bacteria revealed that strains 11 and 16 utilized only certain monosaccharides and sugar alcohols. Lactose alone was utilized (by strain 11) of all the disaccharides tested. Strains 16 and 26 utilized methanol, and strain 26 also utilized methylamine. None of the amino acids were utilized by any member of this group. The GC-content of DNA was about 66.0-68.5 mole %.

Despite the phenotypic identity of the bacteria in the first group, strains 11 and 16 produced 43 % hybridization with each other, while strain 26 showed only 30 % hybridization with the other two (Chernykh et al., 1990). For this group of strains, we proposed a new species of the genus Prosthecomicrobium - P. consociatum sp. nov. The type strain of the species is strain 11.

The second group of prosthecobacteria encompassed strains 25 (horse manure), 17 (yellow ocher), and 18 (hotbed soil), in which the prosthecae were somewhat longer than those of the strains in the first group. All the strains produced a yellow pigment. When inoculated onto potato-agar, they dissociated by producing variations that were either unpigmented (strains 25 and 17) or produced pigments different from the pigmented variants of the original strain 18. The cells of strain 25 were short nonmotile rods. Strain 17 was similar in its morphology to strain 25. The characteristic feature of these two strains was random distribution of prosthecae shorter than 0.30 μm. The prosthecae of strain 18 cells were sized 0.4-0.65 μm.

All the strains of the second group utilized a large variety of substrates: mono- and disaccharides and certain sugar alcohols. A distinctive feature of this group was their ability to utilize organic acids, those of the Krebs cycle, with some differences between the strains. Certain amino acids were also utilized, but not by all strains.

The degree of the DNA-DNA hybridization among the dissociated variants of each strain was 100% and hybridization between initial strains 25 and 17 ranged from 80 to 95 %. Strains 18, when compared against strains 25 and 17, showed 30-50 % hybridization. The prosthecobacteria of the second group showed some relationship to those of the first group, in the range of 18-30%. Then we proposed a new species of genus *Prosthecomicrobium* - *P. mishustinii* sp. nov. The type strain of the species is Strain 17. The GC content of DNA is from 63.7 to 65.2 mole %.

In 2010, Staley with co-authors reclassified the polypheletic genus *Prosthecomicrobium* to form two novel genera, *Vasilyevea* gen. nov. and *Bauldia* gen. nov., with four new combinations: *Vasilyevaea enhydra* comb.nov., *Vasilyevaea mishustinii* comb. nov., *Bauldia consociatum* comb. nov. and *Bauldia litoralis* comb. nov. (Yee, et al., 2010).

Two strains of VKM B-1138 and strain 20 produced a red pigment. They were morphologically related to each other, as well as to the typical strain *P. enhydrum* VKM B -1376, which produced a yellow pigment. The prosthecae of all these strains were about 0.5 μm long, while in strain 20 their length reached 1.0 μm.

The spectrum of substances utilized by the bacteria of this group differed from that of strain VKM -1376. They did not utilize polysaccharides nor tri-, di-, or monosaccharides. Sugar alcohols that were used by strain 20 included arabitol, adonitol, sorbitol and mannitol.

DNA-DNA hybridization between strains VKM B -1138 and 20 reached 55 %, and only this group of bacteria showed hybridization with *P. enhydrum* VKM B-1376 of 30 %, with which they also manifested close morphology.

Strain 21 stood apart from all the rest of the bacteria examined, its cells being of coccal form, with short prosthecae of varying length (from 0.45 to 1.0 μm). Occasionally one of these prosthecae reached 3.5 μm in length. The cells were nonmotile.

The cells of strain 21 utilized only the ribose and xylose of all the monosaccharides tested. The only disaccharide they used was mellibiose. As to organic acids and alcohols tested, they utilized α-ketoglutarate, malate, butyrate, acetone, ethanol, methanol, propanol, and monomethylamine. Their physiological features, as well as the absence of DNA-DNA hybridization with two typical strains from the species of the genus *Prosthecomicrobium* and also with strains VKM B-1138 and 20, prompted us to view this culture separately.

Figure 20. Vegetative experiments on use of rice straw and compost on alkaline takyrovidny soils of Southwest Pribalkhashya. Sampling together with the staff of Institute of AN soil science KAZSSR. Kazakhstan, mid-1980s

Strain 22 was related by its morphology to *P. hirschii*. Its characteristic feature was the presence of two morphologically different cells: one type had multiple, short (0.35 μm) prosthecae, the other had long ones (over 2 μm). Some cells displayed both long and short prosthecae. Most of the motile cells had short prosthecae (Fig. 19).

Strain 22, like the typical strain *P. hirschii* (Staley, 1984) utilizes most monosaccharides, but not di-, tri-, or poly-saccharides. A check for the utilization of organic acids, alcohols, methylated amines, revealed that the cells were able to utilize almost all of the Krebs cycle acids and the volatile fatty acids. The strain utilized also C-1 compounds, such as formate, methanol and monomethylamine. Of the amino acids tested: glutamate, glutamine, aspartate, asparagine, alanine, proline, serine, histidine and hydroxyproline were used. The similarity in the morphological, physiological, and biochemical features between strain 22 and the typical strain *P. hirschii* allowed us to identify it as

P. hirschii, strain 22 (Vasilyeva, et al., 1991).

Heterotrophic prosthecobacteria, though varying in morphology, are quite similar in physiology. Each organism utilizes a limited set of simple organic compounds, and each genus of bacteria prefers to utilize one or two groups of compounds.

A species or a group of strains differed in a set of substrata, i.e., there was substrate specificity of these bacteria. The prosthecobacteria studied are not able to grow anaerobically or to ferment. Also, they are unable to hydrolyze polymers. Therefore, they depend on the activity of other physiological groups of bacteria in the community, capable of decomposing polymeric and cyclic compounds. Their utilization of only simple organic substances places the prosthecobacteria at the end of trophic chains of carbon decomposition, i.e., at the final stages of organic substance mineralization.

These physiology distinctions allowed us to regard all the studied prosthecobacteria as typical representatives of the ecological group known as the "microflora of dispersal" as proposed by Prof. G. Zavarzin, (Zavarzin, 1970). Later, in 1995, Prof. Zavarzin and I proposed the use of a new term which stresses the ability of prosthecobacteria to utilize substrates dissipating from the sites of their origin and to call this group "dissipotrophic bacteria" (Vasilyeva and Zavarzin, 1995).

One of the characteristic peculiarities of prosthecobacteria is a reaction of an organism to variations of substrate concentrations in the medium. Cells of bacteria have a typical morphology on media with low substrate concentration (below 0.25 %) in pure laboratory culture, i.e., all of them are oligotrophic organisms. When using media with increased content of nutrients for culture growth, the morphology of cells changed which manifested itself in a loss of prosthecae.

In the beginning of my work, Prof. G. Zavarzin and I discussed the kinetic problems of oligotrophic bacterial growth and came to the conclusion that there exists a specific group of bacteria which is adapted to the utilization of organic substances dissipating from the formation sites during polymeric

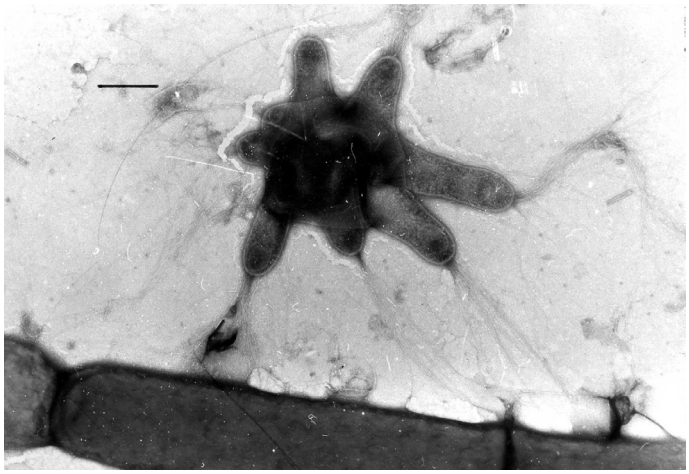


Figure 21. Cellulose decomposing bacteria found during compost decomposition in the takyr-like soil. Herpetosiphon and a prosthecobacterial cell. Bar = 0,5μm

substance hydrolysis and to dissipation from the sites of anaerobic decomposition. All these bacteria should have similar kinetic characteristics. They should be substrate specific and they should have a high affinity for the substrate at a low concentration.

These experiments were staged in cooperation with the research worker of our laboratory A. Semenov for three different species of prosthecobacteria (*Stella vacuolata*, *Prosthecomicrobium hircshii* and *Labrys monachus*). The results of these researches revealed that prosthecobacteria possess the following distinctions: low growth rate and very high substrate affinity (Ks) (Semenov and Vasilyeva, 1985a, 1986, 1987). The Ks values are one or two orders of magnitude lower than those for many other bacteria utilizing the same substrate. The substrate is completely utilized by prosthecobacteria. In spite of low growth rates, prosthecobacteria have an advantage at the limiting concentrations of substrate. But this high affinity at low concentration correlates with the low growth rate, so this group of prosthecobacteria is represented by slowly growing bacteria that are specific to the substrate but that enables them to grow at a very low concentration (Semenov et al., 1986 a, b). The high affinity to the substrates is due to a highly efficient transport system. For instance, *L. monachus* has two systems for glucose transport, one of which has an extremely high affinity (Semenov et al., 1986 b).

Figure 22. Discussion of data with the Doctor F. Kuntz. Prague in the mid-1980s.

Figure 23. Meeting in Budapest with Professor K. Domsh, ten years after his visit to the laboratory of soil microorganisms at the Institute of Microbiology in 1975.

While working with enrichment cultures from different habitats, I came to the conclusion that bacteria with unusual morphology are found in places of active decomposition of organic substances. It was logical to expect on this basis that heterotrophic prosthecobacteria would be observed in greater concentrations in systems where there is vigorous decomposition of organic substances while existing as solitary cells where decomposition occurs at a reduced rate.

Prosthecobacteria as indicators of soil processes

Kazakhstan

In order to establish regularities of prosthecobacteria development in soil microbial communities, I chose conditions where there was a one-time supply of an organic substance. We used soils that were poor in organic substance and where this material is completely decomposed during one vegetative season.

These soils were the subject of joint researches between the scientists of the Institute of Soil Sciences of the Academy of Sciences of KazSSR and the Institute of Microbiology of the Academy of Sciences of the USSR. The work continued from the beginning of 1980 until 1990. I took advantage of the experiments on the application of rice straw and straw-fertilizer compost for rice cultivation on alkaline takyr-like soils of South-West areas near the Lake of Balkhash. The experiments served as a model for clarification of a niche for prosthecobacteria in the microbial community of soils. This work was carried out in cooperation with Sergei Nelidov and the staff of the laboratory of biochemistry of saline soils (Fig. 20).

With the aim in view of using prosthecobacteria as a tracer group, I took advantage of a system of indicators developed

Figure 24. Left to right: Jim Staley, Lina, David Teakle, visiting from the University of Queensland, Australia and his wife Kathy, at the Staley home in Seattle, 1991.

by Academician Mishustin that was widely used with associations of soil microorganisms in the Soviet Union. Simultaneous research projects were carried out that were focused on the composition of organic substances in soil and on the microbiological analysis

of the basic groups of microorganisms in the same horizons and over the same time intervals.

Academician Mishustin suggested the use of nonspore-forming bacteria as indicators for the first stage of decomposition of fresh organic substance, while at later stages, spore-forming becomes prevalent. Making a morphologically distinctive group, the prosthecobacteria offered a rare opportunity because they could be detected directly in soils and compared with their physiological functions which had been found in investigations of their pure cultures.

We applied a sequential dilution method and, by identifying prosthecobacteria on the basis of morphological characteristics, determined the concentrations of the different species over time (June, July, September, October) and depending on the extent of straw and compost mineralization in the soils.

The introduction of organic fertilizers caused a sharp increase in the number of prosthecobacteria in later periods of observation, while no increase of prosthecobacteria development was observed in the control set. The experimentally obtained data on the abundance of prosthecobacteria indicated that, compared to straw, compost adds a sufficient amount of easily utilized organic substances to soil. The development of bacteria of the genus *Prosthecomicrobium* on straw in June and especially in July, was poorer than on compost; the compost where plant residues were exposed to anaerobic pre-treatment contained lactic, acetic, oil, etc. These compounds were a much more productive substrate than straw. The development of the saprotrophic microflora on soil fertilized with compost was higher with regard both to the course of succession and to the soil profile in the first two months.

By the end of rice vegetation (August, September), the activity of the saprotrophic microflora dropped on compost, but it was still higher than in the control set and in the straw. The inventory of the number of different types of prosthecobacteria showed that organic substance introduction increased the number of prosthecobacteria in soil by the end of the period of its decomposition in the soil, and

Figure 25. Staff of the Department of Microbial Communities before it was sub-divided into three laboratories, led by academician G. Zavarzin. Mid-1990s.

Left to right (sitting): L. Gerasimenko, A. Nozhevnikova, G. Zavarzin, E. Bonch-Osmolovskaya, M. Pusheva; Middle row, left to right (standing): V. Nekrasova, L. Vasilyeva, N. Savelyeva, E. Detkova, T. Zilina, R. Miroshnichenko,

Back row, left to right (standing): V. Orleansky, O. Kotsyurbenko, V. Kevbrin, N. Chernykh, E. Pikuta, A. Lysenko, A. Slobodkin.

the greatest number of prosthecobacteria was observed on soils amended with acidulous compost as compared to straw (Nelidov et al., 1986). The products of compost decomposition penetrated deeper into soil stimulating the development of prosthecobacteria (*Stella* and *Prosthecomicrobium* species), while straw worked mainly in the upper layer.

The increase in the number of prosthecobacteria by the end of the period of organic matter decomposition proved their role as indicators. Prosthecobacteria did not appear to be representatives of the autochthonous microflora, but they characterize the final stage of the development of the zymogenic soil microflora. On the basis of a correlation with typical representatives of the zymogenic microflora, we concluded that prosthecobacteria belong to a set of microorganisms comprising a zymogenic community and may be regarded as indicators of the final stage of organic substance decomposition.

We verified our idea that aerobic cellulose decomposition is the main pathway in which prosthecobacteria participate. Careful examination

of colonies on the filter paper reveals the presence of prosthecobacteria at a certain distance in front of the gliding cellulose decomposing bacteria. With regard to the qualitative composition, cellulose-decomposing bacteria of the takyr-like soil primarily comprised of species of *Cytophaga* and multicellular trichome bacterium *Herpetosiphon* (Fig. 21).

Prosthecobacteria, including those with radial cell symmetry, are a constant component of these mixed colonies. Thus, the primary food chain in the community was identified. On application of organic fertilizers into soil, we observed a sharp numerical increase, not only of cellulose-decomposing organisms, but also of representatives of other physiological groups, prosthecobacteria among them.

Krasnodar

In cooperation with a post-graduate student from the Timiryazev Agrarian Academy, Genrietta Blagoveshchenskaya, we confirmed the main regularities discovered when studying takyr-like soils through the example of the meadow-chernozemic soil with application of green manure. In the course of green manure decomposition in the soil, colonies of cellulose-decomposing bacteria contained the following prosthecobacteria: *Caulobacter*, *Asticcacaulis*, *Hyphomicrobium*, *P. polyspheroidum*, *P. consociatum*, and *Stella*. An unidentified *Prosthecomicrobium* sp. with short prosthecae with pili at the apex was also present. The species composition and the number of prosthecobacteria depended on the stage of green manure decomposition.

In the 1980s, in conformity with the project of bilateral cooperation between INMI of the Czech AS, Prague, and INMI of the AS of the USSR, Moscow, in the field of "Microbiological processes in soil and rhizosphere," at the Department of ecology of microorganisms (headed by Dr. V. Vanchura) of the Institute of Microbiology in cooperation with Dr. F. Kuntz and post-graduate student Mashkova, we studied microbial associations in the course of cellulose (0.1%) decomposition in a system during its continuous flow through chernozem soil under aerobic and anaerobic conditions.

We determined the number of various groups of microorganisms in dynamics, and searched for prosthecobacteria inherent in the chernozem soil depending on a layer under the experimental conditions (Fig.21). After a month of carboxymethyl cellulose flow through the chernozem soil in aerobic conditions, the following prosthecobacteria were found: *Caulobacter* sp., *Hyphomicrobium* sp. and *P. polyspheroidum*. The results of this work were presented in our report "Cellulolytic activity of soil continuously enriched with carboxymethyl cellulose in aerobic conditions and nitrogen" submitted at the 16th International Symposium of FEBS.

The 1980s were very fruitful for me. In 1982, I reported on the results of prosthecobacteria investigations in Alma-Ata at the 7th Congress of AUMS. As a member of the delegation from our laboratory, I participated with a report in the IX International Symposium on Soil Biology in Budapest in 1985 (Fig. 23).

Another report, "New Unusual Bacteria with Radial Symmetry," was delivered at the IVth International Symposium on Microbial Ecology in Ljubljana in 1986 (Perspectives in Microbial Ecology, 1986).

In 1985, we prepared papers on the characterization of prosthecobacteria of species *Stella*, *Labrys*, *Prosthecomicrobium* (*P. polyspheroidum*) and *Angulomicrobium,* which we published in the First Edition of Bergey's Manual of Systematic Bacteriology.

During all the years of research of prosthecobacteria, I followed the papers by J. Staley with keen attention, but it was only in 1990 that we, for the first time, managed to shake hands in Moscow. Owing to Staley's invitation, I, for the first time, came to America, to Seattle, to see the University and deliver a report ("The role of Prosthecobacteria in carbon cycling in soil") at the laboratory seminar. I got an opportunity to work with a microscope which was a "help-mate" in so many discoveries. That was a great time (Fig. 24).

The investigation of a collection of strains of the genus Prosthecomicrobium was the last work of our team in the Department headed by Ac. E. Mishustin. In 1990, I defended my doctorate thesis,

"Prosthecobacteria in soil."
Ac. E. Mishustin's age and illness caused a reorganization of the Department of Soil Microorganisms. E. Mishustin recommended me to join G. Zavarzin's laboratory. Starting with 1991, I became a researcher at the Laboratory of Relict Microbial Communities headed by G. Zavarzin.

1991-To Present

I first met G. Zavarzin as a 5-year student of the MSU when he, the youngest chief of the Laboratory of "Lythotrophic Microorganisms" of the Institute of Microbiology, lectured to us at a seminar of the Department of Soil Biology at the University. By the mid-point in his talk, my course-mate (T. Dobrovolskaya) and I could no longer understand everything he was saying. As it turned out, we were not the only ones. During the expedition to Kamchatka, having read a lecture to volcanologists, G. Zavarzin asked me whether everything was clear in his report or whether it was too difficult. He knew his "sore point" and was always delighted to hear that "yes, it was clear." In later years, I learned that the staff of the Institute joked saying that when you read Zavarzin's article or book you need to have a dictionary at hand and a reader could not be always sure that he would understand everything in what he had read. Repeated readings were often required. Zavarzin's train of thoughts and writing style were always nontrivial.

Regretfully, any reorganization "throws one off the working course." I faced a workplace rearrangement, change of subject matter and a working team. Besides, the period from 1991 was hard both for the country in general and for science, in particular. I had to change over to investigations of other groups of microorganisms, to master research methods of novelty for me.

Zavarzin focused my attention to my work with prosthecobacteria cultures, and our discussions of the results obtained have greatly helped me to become integrated with the laboratory staff without much difficulty and to become the head of a group (Fig. 25). In cooperation with post-graduate student Marina Omelchenko and Nina Savelieva, we participated in the program "Global Changes of Environment and Climate." Hotbed gas emissions from northern boggy terrains were thought to make an important contribution into the state of the global atmosphere, and psychrophilic bacteria responsible for these processes remained unknown.

The first psychrophilic obligate methanotroph was isolated from tundra soil in the Polar Urals (Omelchenko et al., 1993). The organism had an optimal temperature range of 3.5-10º C, but at 20º C its growth is minimal. The organism was assigned to a new genus named *Methylobacter* with the formation of new species *M. psychrophilus* (Omelchenko et al., 1996). Psychrophilic methane oxidizing processes are of importance for elucidation of the mechanism of methane emission reduction from the northern territories of Russia. Two new species of hydrogen-reducing bacteria, *Arthrobacter crygenae* sp. nov. and *Acidovorax psychrofacilis* sp. nov., were isolated from the same sample of soil (Vasilyeva et al., 1998) acting as representatives of a bacterial filter impeding gas emission into atmosphere in the permafrost zone. Prosthecobacteria were also studied. Isolated were psychrotolerant bacteria of the genus *Caulobacter* (Berestovskaya et al., 2006) and a new species - *Asticcacaulis benevestitus* sp. nov. (Vasilyeva et al., 2006).

Three strains of Gram-negative, aerobic, motile bacteria with bipolar flagella were isolated from different acidic tundra wetland soils. The new strains were moderately acidophilic and psychrotolerant, capable of optimum growth at pH=5.5-6.0. These bacteria were facultative methylotrophs that utilized methanol, methylamines and a wide range of other sources of carbon and energy, such as sugars and polysaccharides, ethanol and amino acids. On the basis of a polyphasic analysis of their phenotypic and genotypic properties, novel strains were proposed as representing a new genus and novel species, named *Methylorosula polaris* (Berestovskaya et al., 2012).

During the late 1990s and especially at the beginning of the new millennium, times were hard for me since my mother aged 86, was in need of daily help. First, she had poor eyesight, and three years before her death she went blind. Unfortunately, she rejected any help but mine. Of course, it affected

my work greatly, but after my mother's death in 2006, my work became a medicine for me. G. Zavarzin, perceiving my state, offered me to work on a problem which was of interest for him. He had suggested a pattern of trophic relationships of microorganisms in the course of wood decomposing by xylotrophic fungi which required verification. The process of wood destruction by xylotrophic fungi and the participation of bacteria in this process are understudied. Nowadays, it is not known what groups of ombrophilous (Zavarzin, 2009) dissipotrophic bacteria are immanent for ultrafresh acidic dystrophic waters of forest-marsh habitats at the initial stage of wood decomposing by xylotrophic fungi when the concentration of readily available nutrients is low.

The analysis of bacterial diversity of the xylotrophic myco-bacterial community of ultrafresh dystrophic waters has allowed us to reveal new species of microorganisms in its composition that belong to known genera. In cooperation with post-graduate student M. Zaychikova and Yu. Berestovskaya, I isolated a range of bacteria from acidic (pH=4.3) dystrophic (140 μS) waters in experiments on pinewood being degraded by xylotrophic fungi. On the basis of molecular-genetic and phenotypic features, we described pure cultures of indicator forms of new representatives of ombrophilous bacteria: *Xanthobacter xylophillus* sp. nov. (Zaychikova et al., 2010), *Ancylobacter abiegnus* sp. nov. (Zaychikova et al., 2010), *Singulisphaera mucilagenosa* sp. nov. (Zaychikova et al., 2011). Eco-physiological features of the isolated strains characterize these new species as representatives of the group of oligotrophic dissipotrophic organisms, belonging to an acidotrophic family and related to the xylotrophic fungi community.

M. Zaychikova was the last post-graduate student to defend her PhD thesis in Ac. G. Zavarzin's laboratory at the Scientific Council in 2011. Over the years of work under the guidance of Ac. G. Zavarzin, I came to appreciate more fully the scale of personality of this charismatic scientist. He was able to perceive and generalize the results of scientific researches at a biospheric level. These are not merely fine words. G. Zavarzin was able to see regularities and their global consequences behind factual evidence. No wonder it was G. Zavarzin who was in charge at the Academy of Sciences of such large scale projects as "Evolution of the Biosphere," "Global Changes of Environment and Climate" and others.

Not a single article written by researchers of his lab was submitted for publication without having been read by G. Zavarzin. His criticism was always specific and highly helpful for his colleagues.

Until his last days, Zavarzin took a sincere interest in any, even seemingly insignificant, scientific results. Every day he made a round of the researchers of the laboratory and addressed them his favorite question: "What's new?" In case a researcher reported on something new, Zavarzin became enthusiastic about the idea himself, discussed it with other researchers, and this discussion often resulted in an interesting view of the problem and its further resolution. It was interesting, and it attracted the staff of the laboratory to work with Zavarzin. G. Zavarzin belonged to a group of scientists who are faithful to their work; he could not see his life without microbiology.

Having lived my life anew on these pages, I realized that I am a very happy person. I am happy because I managed to choose an amazing profession which is dear to my heart and my mind. Thanks to this profession, I met remarkable and interesting people with true scientific zest.

Acknowledgement

I am grateful to Jim Staley for his constructive remarks to the manuscript.

References

Berestovskaya, Ju.Ju., A.M. Lysenko, T.P. Tourova and L.V. Vasilyeva. 2006. Psychrotolerant *Caulobacter* from transpolar tundra soil of Russia. Mikrobiologiya. *75*: 377-382.

Berestovskaya, Ju.Ju., O.R. Kotsyurbenko, T.P. Tourova, T.V. Kolganova, N.V. Doronina, P.N. Golyshin and L.V. Vasilyeva. 2012. *Methylorosula polaris* gen.nov., sp. nov., an aerobic,

facultatively methylotrophic psychrotolerant bacterium from tundra wetland soil. Int. J. Syst. Evol. Microbiol. *62*: 638-646.

Chernykh, N.A., L.V. Vasilyeva, A.I. Giniyatullina, and A.M. Semenov. 1990. DNA-DNA Hybridization of new *Prosthecomicrobium* strains. Mikrobiologiya. 59: 127-132.

Chernykh, N.A., L.V. Vasilyeva, A.M. Semenov and A.M. Lysenko. 1988. DNA homology in Prosthecobacteria of *Stella* genus. Izv. Akad. Nauk SSSR, Ser.Biol. *5*: 776-779.

Fritz, I., C. Strompl and W-R. Abraham. 2004. Phylogenetic relationships of the genera *Stella, Labrys* and *Angulomicrobium* within the '*Alphaproteobacteria*' and description of *Angulomicrobium amanitiforme* sp.nov. Int.J.Syst.Evol.Microbiol. *54*: 651-657.

Hirsch, P. and H. Schlesner. 1981. The genus *Stella*. In *The Prokaryotes* (M.P. Starr, H. Stolp, H.G. Truper, A. Balows, and H.G. Schlegel ,eds.). Springer-Verlag, Berlin. pp. 461-465.

Lafitskaya, T.N. and L.V. Vasilyeva. 1976. A new triangle bacterium. Mikrobiologiya. *45*:812-816.

Lafitskaya, T.N., L.V. Vasilyeva, E.N. Krasilnikova and N.I. Aleksandruskina. 1976. Physiology of *Prosthecomicrobiun polyspheroidum*. Izv. Akad. Nauk SSSR, Ser.Biol. *6*: 849-857.

Lysenko, A.M., A.M. Semenov and L.V. Vasilyeva. 1984. DNA nucleotide composition of prosthecate bacteria with radial cell symmetry. Mikrobiologiya. *53*: 859-861.

Miller, J.A., M.G. Kalyuzhnaya, E. Noyes, J.C. Lara, M.E. Lidstrom and Chistoserdova, L. 2005. *Labrys methylaminiphilus* sp. nov., a novel facultatively methylotrophic bacterium from a freshwater lake sediment. Int.J.Syst. Evol. Microbiol. 55: 1247-1253.

Namsaraev, B.B. and G.A. Zavarzin. 1972. Trophic links in the methane oxidizing culture. Microbiologiya. *41*: 999-1006.

Nelidov, S.N., L.V. Vasilyeva and E.N. Mishustin. 1986. Application of crop residues for rice yield increase on alkaline soils under amelioration. Izv.Akad. Nauk, Ser. Biol. *1*: 43-57.

Nemec, P. and V. Bystricky. 1962. Peculiarity of some microorganisms accompanying diatomaceae, Preliminary report. J. Gen. and Appl. Microbiol. *8*: 121-129.

Nikitin, D.I. and L.V. Vasilyeva. 1967. Rod-shaped organisms with spherical swellings. Izv Akad. Nauk SSSR, Ser Biol. *2*: 296-301.

Nikitin, D.I. and L.V. Vasilyeva. 1968. The new species of soil organism *Agrobacterium polyspheroidum*, nov. sp. Izv.Akad.Nauk SSSR, Ser. Biol. *3*: 443-444.

Nikitin, D.I., L.V. Vasilyeva and R.A. Lokhmacheva. 1966. New and rare forms of soil microorganisms. "Nauka," Moscow. p. 70

Omelchenko, M.V., L.V. Vasilyeva and G.A. Zavarzin. 1993. Psychrophilic methanotroph from tundra soil. Current Microbiology. *27*: 255-259.

Omelchenko, M.V., L.V. Vasilyeva, G.A. Zavarzin, N.D. Savelyeva, A.M. Lysenko, L.L. Mityushina, V.N. Khmelenina and Yu.A. Trotsenko. 1996. A novel psychrophilic methanotroph of genus *Methylobacter*. Mikrobiologiya. *65*: 339-343.

Orenski, S.W., V. Bystricky and K. Maramorosch. 1966. Polyspheroids from American soils. Nature. *210*: 221.

Schlesner, H. 1983. Isolierung und Beschreibung knospender und prostekater Bakterien aus der Kieler Forde. Ph. D. Thesis, Christian-Albrechts-Universitat, Kiel, Germany.

Semenov, A.M. and L.V. Vasilyeva. 1985. Morpho-phisiological characteristics of budding prosthecobacterium *Labrys monachus* with a radial symmetry of cell under periodical and

uninterrupted cultivation. Izv. Acad. Nauk SSSR, Ser. Biol. *2*: 288-293.

Semenov, A.M. and L.V. Vasilyeva. 1987. *Stella vacuolata* growth under periodical and continuous cultivation. Izv. Acad. Nauk SSSR, Ser. Biol. *2*: 307-311.

Semenov, A.M. and L.V.Vasilyeva. 1986a. Morphological and physiological characteristics of the oligotrophic prosthecobacterium *Prosthecomicrobium hirschii* grown under conditions of batch and continuous cultivation. Mikrobiologiya. *55*: 248-252.

Semenov, A.M., L.A. Okorokov and L.V.Vasilyeva. 1986 b. Discovery of extremely high affinity to substrate of prosthecobacteria. Rep. USSR Acad.Sci. *291*: 225-227.

Semenov, A.M., L.V. Vasilyeva and C.D. Varfolomeyev. 1986 a. Kinetic parameters of some prosthecobacteria growth. Izv. Acad. Nauk SSSR, Ser. Biol. *1*: 131-134.

Staley, J. 1984. *Prosthecomicrobium hirschii*, a new species in a redefined genus. Int. J. Syst. Bacteriol. *34*:304-308.

Staley, J.T. 1968. *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. J. Bacteriol. *95*:1921-1942.

Stanley, P.M., R.L. Moore and J.T. Staley.1976. Characterization of two new isolates of mushroom-shaped budding bacteria. Int. J. Syst. Bacterial. *26*: 522-527.

Vasilyeva L.V. 1989. Genus *Angulomicrobium* (Vasilyeva, Lafitskaya and Namsaraev, 1979, 1037). In *Bergey's Manual of Systematic Bacteriology* vol. 3 (J. G. Holt and J. T. Staley, eds). Williams and Wilkins, Baltimore, pp. 1969-1971.

Vasilyeva, L.V. 1969. Ultrastructure and cycle of development of *Agrobacterium polyspheroidum*. Izv. Akad. Nauk SSSR, Ser Biol. *5*: 780-781.

Vasilyeva, L.V. 1970. A star-shaped soil microorganism. Izv. Akad. Nauk SSSR, Ser. Biol. *2*: 308-310.

Vasilyeva, L.V. 1972a. Peculiarities of ultrastructure and the cycle of development of the bacterium *Stella humosa*. Izv. Akad.Nauk SSSR, Ser.Biol. *5*:782-785

Vasilyeva, L.V. 1972b. On the cycle of development and cytological properties of a new soil microorganism possessing prosthecs. Izv. Akad.Nauk SSSR, Ser. Biol. *6*: 860-864.

Vasilyeva, L.V. 1980. Morphological grouping of prosthecobacteria. Izv.Akad. Nauk SSSR, Ser. Biol. *5*: 719-773.

Vasilyeva, L.V. 1985. *Stella*, new genus of soil prosthecobacteria, with proposals for *Stella humosa*, sp. nov. and *Stella vacuolata*, sp. nov. Int. J. Syst. Bacteriol. *35*: 518-521.

Vasilyeva, L.V. 1986. New unusual bacteria with radial symmetry. In *Perspectives in Microbial Ecology*. pp. 147-152. IV International Symposium on Microbial Ecology, Ljubljana, Slovenia.

Vasilyeva, L.V. and A.M. Semenov. 1984.New budding prosthecate bacterium *Labrys monahos* with radial cell symmetry. Microbiologiya. *84*: 68-75.

Vasilyeva, L.V. and A.M. Semenov. 1986. Prosthecobacteria, genus *Stella* and description of a new species of *Stella vacuolata*. Izv.Akad. Nauk SSSR, Ser.Biol. *4*: 534-540.

Vasilyeva, L.V. and G.A. Zavarzin. 1995. Dissipotrophs in the microbial community. Microbiology *64*:239-244.

Vasilyeva, L.V. and T.N. Lafitskaya. 1976. Assignment of *Agrobacterium polyspheroidum* to the genus *Prosthecomicrobium polyspheroidum* nov. comb. Izv. Akad. Nauk SSSR Ser. Biol. *6*:919 – 922.

Vasilyeva, L.V. Genus *Stella* (Vasilyeva 1985, 521). 1989. In *Bergey's Manual of Systematic Bacteriology* Vol. 3 (J.T.Staley, M.P.Bryant, N.Pfenning and J.G.Holt, eds.). Williams and Wilkins, Baltimore, pp. 1921-1924

Vasilyeva, L.V., A.M. Semenov and A.I. Giniyatullina. 1991. New soil bacterial species belonging to the P*rosthecomicrobium* genus. Mikrobiologiya. *60*: 350-359.

Vasilyeva, L.V., N.D. Savelyeva, M.V. Omelchenko, A.M. Lysenko, M.A. Pusheva, Yu.A. Trotsenko and G.A. Zavarzin. 1998. *Arthrobacter crygenae* sp.nov. and *Acidovorax psychrofacilis* sp.nov., Psychrophilic hydrogen bacteria from tundra Soil. Mikrobiologiya *67*: 237-243.

Vasilyeva, L.V., T.N. Lafitskaya and B.B. Namsaraev. 1979. A new genus of budding bacteria, *Angulomicrobium tetraedrale*. Mikrobiologiya. *48*: 1033-1039.

Vasilyeva, L.V., T.N. Lafitskaya, N.I. Aleksandruskina and E.N. Krasilnikova. 1974. Physiologo-biochemical peculiarities of prosthecobacteria *Stella humosa* and *Prosthecomicrobium* sp. Izv. Akad.Nauk SSSR, Ser.Biol. *5*: 699-714.

Vasylieva, L.V., M.V. Omelchenko, Yu. Yu. Berestovskaya, A.M. Lysenko, W.-R. Abraham, S.N. Dedysh and G.A. Zavarzin. 2006. *Asticcacaulis benevestitus* sp. nov. a psychrotolerant, dimorphic, prosthecate bacterium from tundra wetland soil. Int. J. Syst. and Evol. Microbiol. *56*: 2083-2088.

Whittenbury, R. A. and J. M. Nicoll. 1971. A new, mushroom-shaped budding bacterium. J. Gen. Microbiol. *66*: 123-126.

Yee, B., Oertli, G.E., Fuerst, J.A. and J.T. Staley. 2010. Reclassification of the polypheletic genus *Prosthecomicrobium* to form two novel genera, *Vasilyevea gen. nov.* and *Bauldia gen. nov*. with four new combinations: *Vasilyevaea enhydra* comb.nov., *Vasilyevaea mishustinii* comb. nov., *Bauldia consociatum* comb. nov. and *Bauldia litoralis* comb.nov. Int. J. Syst. Evol. Microbiol. *60*: 2960-2966.

Zavarzin, G.A. 1970. The notion of microflora of dispersion in the carbon cycle. J.Gen. Biol. Izv. Akad. Nauk SSSR. *31*:386-393.

Zavarzin, G.A. 2009. Ombrofila - inhabitants of plains. Nature *6*: 3-14.

Zavarzin, G.A., L.V. Vasilyeva, and V.V. Trukova. 1967. On the participation of microorganisms in post-volcanic processes. Izv. AN SSSR, Ser. Biol. *4*: 605-611.

Zaychikova, M.V., Ju.Ju. Berestovskaya, V.N. Akimov, A.K. Kizilova and L.V. Vasilyeva. 2010. *Xanthobacter xylophilus* sp.nov., a member of the xylotrophic mycobacterial community of low-mineral oligotrophic waters. Microbiology *79*: 83-88.

Zaychikova, M.V., Ju.Ju. Berestovskaya, V.N. Akimov, A.K. Kizilova and L.V. Vasilyeva. 2010. *Ancylobacter abiegnus* sp.nov., an oligotrophic member of the xylotrophic mycobacterial community. Microbiology. *79*: 483-490.

Zaychikova, M.V., Ju.Ju. Berestovskaya, V.N. Akimov, N.A. Kostrykina and Vasilyeva. L.V. 2011. *Singulisphaera mucilagenosa* sp.nov. – a new acido-tolerant representative of *Planctomycetales*. Microbiology *80*: 105-111.