Minutes of the online meeting of the International Committee on Systematics of Prokaryotes [DRAFT of January 28, 2020]

5th January 2020: Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

To the members of the International Committee on Systematics of Prokaryotes

In keeping with Article 4 of the ICSP Statutes, the Editorial Board of the International Code of Nomenclature of Prokaryotes (ICNP) is conducting an open electronic meeting concerning proposals for changes in the ICNP.

The first phase of the meeting will take place from January 5, 2020, until March 1, 2020. It is intended to allow open discussion of the proposals as an email chain among the members of the ICSP and other interested parties. Comments may be made by the 'reply-all' option on your email server. Comments should be less than 500 words in length and should identify the author's name(s) and affiliation(s). Comments should be respectful, and ad hominem comments will be deleted from the record. As comments accumulate, the Editorial Board will transfer them to the ICSP website, and the edited comments will serve as the minutes of the meeting. Please feel free to add interested parties to the email recipient list and solicit comments from interested parties outside the ICSP.

The second phase of the meeting will consist of voting and will take place from March 1 to March 31, 2020. Only members of the ICSP may vote.

The issues for the current discussion are the "Modest proposals to expand the type material for prokarvotes" made bv Whitman (2016; IJSEM 66: 2018-2112; naming of https://doi.org/10.1099/ijsem.0.000980) and a related proposal by Whitman et al. (2019; IJSEM 69: 2174-2175; <u>https://doi.org/10.1099/ijsem.0.003419</u>) concerning granting priority to Candidatus names. To simplify the discussion, the ICSP and contributing colleagues are asked to give particular consideration to the following statements, which represent the central concepts. Should they be passed at the voting stage, other rules will be changed as described in Whitman (2016) and Whitman et al. (2019) to make the remainder of the Code consistent with these changes.

Proposal 1 (Whitman 2016). Extend the nature of the type acceptable for valid publication of a species or subspecies name to allow the use of complete or partial genome sequences as type (Whitman 2016). The new rules would be worded [new text is underlined]:

Rule 18a. The type of a species or subspecies must unambiguously identify the taxonomic group and is a designated strain or other material. Whenever possible, the type of a species or subspecies is a designated strain.

(3) [first section] As from 1 April 2020*, sequences of genomic DNA may also serve as the type when it unambiguously identifies the species. When possible, it should be a high quality draft or better genome sequence.

Rule 30.3.c. [new rule] When a sequence is the type, the accession number in a publically available database or the sequence must be given. It is recommended that, when possible, a sample of the DNA be deposited in at least two publically accessible service collections in different countries and the catalog numbers be indicated.

*The original date of January 2016 proposed in Whitman (2016) is changed to reflect the time necessary to bring this matter to a vote. All of the other proposals in Whitman (2016) will be taken as originally worded.

Proposal 2 (Whitman 2016). Articulates a general concept for what can serve as type for a species.

Rule 18a (3). [second section] <u>As new methods are developed, they may serve as the type</u> material so long as they unambiguously identify the species or subspecies and can be readily archived and compared.

Proposal 3 (Whitman 2016). Allows valid publication of the name of a genus in the absence of a type species if the type is too ambiguous to circumscribe a species.

The rule would be:

"Rule 20a. The nomenclatural type (see Rule 15) of a genus or subgenus is the type species <u>or</u> the sequence of one or more genes that unambiguously identifies the genus or subgenus. The type species is the single species or one of the species included when the name was originally validly published. Only species whose names are legitimate may serve as types."

Proposal 4 (Whitman et al. 2019). Upon acceptance of Proposal 1, the priority of the names of *Candidatus* taxa published before 1 April 2020* which are otherwise in accordance with the rules of the Code will have priority based upon their date of publication in the IJSEM unless a synonymous name already exists based upon deposition of type cultures.

Whitman et al. (2019) also provides a simple nomenclature for identifying the nature of the type material:

'When the type is a culture, the superscript "T" will be used immediately following the name or strain identifier. If the type is a sequence, the superscript "Ts" will be used. If the type is a description, preserved specimen or illustration, the superscript "Td" will be used. If a representative of a taxon is brought into culture, the type strain is then designated as described in Rule 18f. The name may be emended by the new authors, and the superscript "Ts" or "Td" is replaced by the superscript "T".

*The original date of 1 January 2020 is changed to reflect the time necessary to bring this matter to a vote.

For further guidance, major publications that discuss these proposals include:

(in favour)

Whitman 2015. Syst. Appl. Microbiol. 38: 217-222 (https://doi.org/10.1016/j.syapm.2015.02.003)

Konstantinidis et al. 2017. ISME J 11: 2399-2406 (https://www.nature.com/articles/ismej2017113)

Rossello-Mora and Whitman 2019. Syst. Appl. Microbiol. 42: 5-14 (https://doi.org/10.1016/j.syapm.2018.07.002)

(against)

Overmann et al. 2019. Syst. Appl. Microbiol. 42: 22-29. (https://doi.org/10.1016/j.syapm.2018.08.009)

Bisgaard et al. 2019. Diagn. Microbiol. Infect. Dis. 95: 102-103. (https://doi.org/10.1016/j.diagmicrobio.2019.03.007)

COMMENTS:

Comments are present in the order they were received and may have been lightly edited. Please email Barny Whitman [whitman@uga.edu] or Lenie Dijkshoorn [L.Dijkshoorn@lumc.nl] for questions, suggestions, errors and omissions.

January 13 Henrik Christensen, Member of Judicial Commission, University of Copenhagen, Copenhagen, Denmark

We have recently published a note that presents a warning (Bisgaard et al. 2019) about the proposal of using DNA sequences as type material to name new species (Whitman). If implemented the proposal to use DNA sequences as type material may have far-reaching consequences for all microbiologists, ID specialists, vets and other specialists dealing with bacterial names, not to speak about the companies that develop species identification tools and strains for biotech production of probiotics, vaccines and enzymes. The risk is an unstable nomenclature violating Principle 1 of the "code" (".. 1) Aim at stability of names, 2) Avoid or reject the use of names which may cause error or confusion 3) Avoid the useless creation of names .. "). I have become involved in this problem as an active scientist working with bacterial taxonomy at the university. I will contact other taxonomic colleagues as well to revive the discussion.

You are of course welcome to contact me for further explanations and discussions of the problem.

January 13

Lenie Dijkshoorn, Executive Secretary ICSP, Leiden University Medical Center, Leiden, The Netherlands

I fully support the letter from Henrik. There is an urgent need for contemplation for workers in the field who use names in daily work.

January 13 William B Whitman, ICSP Delegate, University of Georgia, Athens USA Iain Sutcliffe, Chair of ICSP Northumbria University, Newcastle upon Tyne, U.K. Ramon Rossello-Mora, Vice-Chair of Judicial Commission of the ICSP, Grup de Microbiologia Marina IMEDEA, Illes Balears, Spain

Genome sequencing has revolutionized prokaryotic systematics by greatly improving the identification of species, elucidating the functional properties of taxonomic groups, and resolving many of the ambiguities in the phylogeny of the higher taxa. Following from the principles described in the International Code of Nomenclature of Prokaryotes, gene sequences are also suitable type material for the description of prokaryotic species. As put forth in principle 4 of the Code, the primary purpose of naming is to supply a means of referring to specific prokaryotes. The Code possesses two mechanisms to insure uniqueness and stability of names. First, it gives priority to the earliest name of the entity. Second, each name is associated irrevocably with some type material. The only name that can be used that includes this type material is the name with priority. The relationship of the name to the type material is further determined by the formal description (also called the protologue), which defines how a taxon is delineated in reference to the type material. Gene sequences clearly possess sufficient specificity and information to serve as type material and delineate taxa. In fact, it has been the common practice to differentiate species based upon sequence similarity since the mid-sixties and formally recommended by Wayne et al. (1987).

A stable nomenclature is essential for all scientific disciplines. While this need was met with the adoptions of the Approved Lists in 1980 and the Code of 1990, subsequent changes in 2001 restricted the Code to organisms that can be deposited as pure strains in culture collections. These changes removed the protection of the Code from the names of prokaryotes that cannot be easily cultured. Proposal 1 would restore the original intent of the Code. By allowing gene sequences to serve as type material for prokaryotic species, this simple change will create stability in naming of *Candidatus* taxa, endosymbionts, and many uncultivated prokaryotes. It is already well established that the use of sequence data, increasingly in the form of whole genome sequences, produces reliable and stable classifications. Thus, proposal 1 will meet an important need within microbiology and allow the creation of a unified nomenclature for all prokaryotes, in contrast to the current "International Code of Nomenclature of *Cultivated* Prokaryotes". Proposal 2 states the rationale for Proposal 1. Proposal 4 implements proposal 1 for *Candidatus* taxa and provides a simple system for identifying the nature of the type material.

Proposal 3 recognizes that on some occasions, the sequence data may be of sufficient quality to delineate a genus but not a species. An example might be 16S rRNA sequences, but it is

inevitable that larger amounts of genome sequence data will also be used. In these cases, a genus name may be validly published without designated type species. Because the genus name provides the root for higher taxa, genus names are required creation of stable higher taxonomies.

Wayne et al. 1987. Int J Syst Bacteriol 37:463-464.

January 14 Kostas Konstantinidis, Member of Judicial Commission, Georgia Institute of Technology, Atlanta Georgia, U.S.A.

In response to Christensen and Dijkshoorn:

I do NOT share the same view with you on this issue but before i offer my arguments for this, i would like to ask Henrik (and/or Lenie):

Why you believe the genome/DNA sequence as Type would make for an unstable system OR will make the identification of taxa of medical importance more challenging (since almost all these taxa are known by cultures and, hence, there will be no change to them really if genome sequences are accepted as Type)? Could you offer a couple specific examples to back up these claims?

I would argue that the Bisgaard et al. 2019 paper was vague about these key points, so the underlying rationale is not clear to me yet.

January 15 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

The proposals formulated by Iain (Jan 05) to modify the International Code of Nomenclature of Prokaryotes (The Code) have been percolating for a number of years now. They have been presented in publications and discussed as 'considerations' or 'modest proposals', etc. However, prior to January 5, no formal proposals were presented to ICSP for consideration to vote for adopting. Now, the ICSP Executive Board has formulated formal proposals for consideration – below. This is an essential step forward, which is good to try to resolve the issues, as well as the concerns of the proponents and opponents of the proposals.

My comment here is to the proposed or designated schedule (it is not clear to me) for the 'second phase' of the open electronic meeting, i.e., for voting (March 01-31) on the proposals. I point out that the members of the ICSP are representatives of the various Microbiological Societies of the different countries. As such, our decisions and votes on issues should reflect the considerations or, the consensus – in the best cases, of our respective national Societies. Particularly, this issue of revising The Code warrants informing member microbiologists of the national Societies, and their consideration, as well, rather than only of the individual ICSP members.

In any case, now we have formal proposals to be considered for voting. Unfortunately, the proposed/designated schedule for the 'first phase of the meeting, i.e., open discussion, etc.,' does not allow for consideration by the overall members of the Societies. I know of presentations of some of these issues, as concepts, only in sessions at the last 2 FEMS

meetings. I do not know if the issues have been presented and discussed within the different Society meetings – they have not been discussed before within the Swedish Microbiological Society (SFM).

Now, the problem is that the dates of Societies' meetings, where these proposals could be brought up and debated, are after the proposed 'deadline' for voting on these proposals, in most cases. The annual meeting of the VAAM in Germany is in the second week of March; they do have a Fachgruppe für Systematik und Identifizierung – I do not know if they are considering the proposals in their session. However, the SFM in Sweden is meeting in May; the MS in the UK is meeting in April; the ASM in the USA is meeting in June; the Spanish Society is meeting in July 2021, although a Systematics and Taxonomy meeting is scheduled for April, 2020! These meetings, and the annual meetings of other Societies, as well, are after the 'deadline' for ICSP voting on the proposals.

I propose that the dates of the 'first phase' of the open electronic meeting for open discussion be prolonged, to allow communication of the formal proposals for revising The Code to be circulated to the members of the national Societies. In any case, I commend the ICSP Executive Board for presenting the formal proposals and initiating the open electronic meeting.

January 15 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

I should like to reply promptly to these comments since they relate to the timeline & decision making process rather than the scientific issues under discussion.

Firstly, my apologies for any ambiguity in my email of Jan 5th: This is now a 'designated' schedule i.e. voting by ICSP members will begin on March 1st and close on 31st.

Secondly, regarding the timeline and current 'window' for discussion. It is important to stress that the Whitman (2016) proposals were published online on 1st May 2016 i.e. 3 years 8 months ago, which I would have thought is more than sufficient time for interested parties to have encountered these proposals (it is also unambiguous in the original text that these are formal proposals to emend the Code that require an ICSP vote).

Notably, the paper has attracted 26 citations according the IJSEM website (34 by googlescholar), including at least one dedicated commentary outside of the specialist systematics literature (e.g. Bisgaard et al. 2019 in a clinical journal). Moreover, as you note, these issues were highlighted in the last two FEMS meetings and they have also been addressed in specialist meetings (e.g. BISMIS, Bergeys International Society for Microbial Systematics). Thus it is demonstrable that the proposals have had 'reach'.

My personal view is that this is a more than sufficient time for these proposals to have come to the attention and gather responses of the scientific community. Moreover, ICSP members and other interested parties have had the past 44 months to engage in discussions with colleagues and 'gauge the mood'. There are still 12 weeks for further activities of this sort and I am pleased that you have widened the debate by adding recipients to this email trail.

Thus, I believe that the majority vote decision of the ICSP Executive Board to now bring this matter to the vote is the correct one.

January 16 Mei-Chin Lai, ICSP delegate, National Chung Hsing University, Taichung, Taiwan

I agree with "Proposal 1" that genome sequences should be included and suggest that the "completeness" of genome sequences need to be over or around 97%.

January 16 Henrik Christensen, Member of Judicial Commission, University of Copenhagen, Copenhagen, Denmark

In response to Konstantinidis's comments of January 14

Unfortunately there was a space limitation with the paper of Bisgaard et al., and we also wanted to keep the text short. I agree that it would have been relevant to give some examples.

Question:

Why you believe that the genome/DNA sequence as Type would make for an unstable system OR will make the identification of taxa of medical importance more challenging (since almost all these taxa are known by cultures and hence, there will be no change to them really if genome sequence is accepted as Type)? Could you offer a couple specific examples to back up these claims?

I would argue that the Bisgaard et al. 2019 paper was vague about these key points, so the underlying rationale is not clear to me yet.

Answer:

On behalf of my co-authors I will try to give a more extended answer here.

<u>1. Risk for an increase in the number of heterotypic synonyms.</u> A new species B is proposed (validly published) and only one DNA sequence serves as type material and only one sequence is known from the species. B is closely related to an existing well known species A of high clinical importance. This can happen since species of type A have a high diversity at the population level and, in such cases, ANI can be lower that 0.95 for some populations of the species. If only comparisons between A and B are based on type strains (or type DNA material), less than 0.95 ANI can be obtained, and a claim made for a new species. If a medical clinical microbiologist identifies an isolate by whole genomic sequencing as species B, this species is not known to be of clinical importance to him, and he might get confused about the disease associated, how the infection can be treated with antibiotics, and how it can be prevented. The consequence can be a wrong treatment of the patient. The problem already exists with cultured type strains, and it is expected to increase if the proposal of using DNA sequences as type material is adopted.

2. Identification of clinically important streptococci. An example provided by a co-author of the Bisgaard paper is related to the problems in the clinic to differentiate *Streptococcus pneumoniae* (important pathogen) from *S. pseudopneumoniae* and *S. mitis* (commensals). These species are closely genetically related, and their virulence can only be establisbed based on cultivation. This co-author even extents the case to ANY bacteria for which vaccines are being developed. Strain material is essential to test for the specificity of vaccines, for strains of existing species as well as for strains of new species in the future.

A more general statement was made by another co-author: It is only possible clinically to link a name of a culture-positive organism to additional data available through publications, to subjects such as diagnosis, prognosis, and treatment. Allowing for species to be name based upon DNA alone will not be helpful from a clinical standpoint.

An even more general statement made by the same co-author reaches beyond the clinical field relates to the scientific demand for reproducibility of experiments. The deposit of genomic DNA or, worse, simple submission of wgs data to a public database does not allow reproduction and confirmation of the conclusions of the authors about the taxonomic status of new isolates and strains simply because there will be no proof that the wgs data are coming from the proposed species.

January 17 Edward Moore, ICSP delegate, University of Gothenburg, Gothenburg, Sweden

In response to Lai's comments of January 16

Thank you for your mail and your comment.

Please note: The proposals would establish new rules that would **<u>substitute</u>** whole genome sequence data for the strain as the type material required for the valid publication of names of new bacterial species. IJSEM already requires including whole genome sequence data for the valid publication of new species names, since 2018.

The proposed rule changes do <u>**not**</u> make a proposal for the coverage or the quality of the whole genome sequence data that would serve as the type material.

Question: Why do you propose 97% 'completeness' or coverage of genomes; why not 20%, i.e., the amount necessary for ANI analyses, or complete genomes, including plasmids?

Thank you in advance for your consideration.

If proponents of the new rules proposals disagree with my assessment, please correct my response to Mei-Chin.

January 17

Frans Reubsaet, Diagnostic Laboratory for Bacteriology and Parasitology (BPD), Center for Infectious Disease Research, Diagnostics and laboratory Surveillance, National Institute of Public Health and the Environment (RIVM), The Netherlands

In response to Moore's comments of January 17

At this moment most genomes are analyzed by Illumina platforms. We experienced that pollution with other DNA is no hypothetical. Second, even the de novo created sequences are artificial. So if the decision is made in favour of whole genome sequences, sooner or later it will become clear that poor data will not prevail.

January 17 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

Regarding the discussion between Ed and Mei-Chin, I'd like to clarify a few points regarding proposal 1, which would allow gene sequences to serve as type. If passed, strains would still remain the preferred type [see highlighted text below]. Thus, sequence data would only substitute for strains when strains are unavailable.

Rule 18a. <u>The type material of a species or subspecies must unambiguously identify the</u> taxonomic group and is a designated strain or other material. Whenever possible, the type of a species or subspecies is a designated strain.

This proposal also does not require a whole genome sequence but only enough sequence to unambiguously identify the species. This wording was chosen to allow naming of endosymbionts where the whole genome sequence is not available. There are many examples of this in IJSEM, but a recent one describes a *Borrelia* species, Loh et al. 2017 [doi.10.1099/ijsem.0.001929] where the diagnosis was made on the basis of the sequences of five genes: 16S rRNA, *flaB*, *groEL*, *gyrB* and *glpQ*.

(3) <u>As from April 2020, sequences of genomic DNA may also serve as the type material when it</u> <u>unambiguously identifies the species.</u> <u>When possible, it should be a high quality draft or better</u> <u>genome sequence.</u>

The second sentence [highlighted] constitutes a recommendation stating a preference for genome sequences. There is substantial precedence for the Code to make recommendations as well as rules. For instance, the 1990 Code recommended deposition of strains as type material. The current Code recommends the descriptions should conform to the minimum standards for the group (Recommendation 30). Because minimum standards for whole genome sequences were proposed in IJSEM in 2018 [Chun et al. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68: 461-466. doi.org/10.1099/ijsem.0.002516], clear directions regarding implementation of this recommendation already exist.

January 18 Kostas Konstantinidis, Member of Judicial Commission, Georgia Institute of Technology, Atlanta Georgia, U.S.A.

In response to Christensen's comments of January 16

In my view, there are two distinct issues, one is the concept of using the DNA as an alternative type material and the other is the technical aspect on what the minimum standards will be for this. My read of Whitman's comment of January 17 is: no change in how we do business for cultured organisms (that is, depositing an isolate to 2 culture collections is the recommended way to name/describe new taxa) and genome sequence becomes an equally appropriate alternative type material for uncultured taxa and fastidious organisms that are difficult to be maintained in culture collections or get lost (if i have misread this, please somebody to correct me!). This way, we will be able to start describing the "uncultivated majority" using similar standards to those used for cultures, and i would argue that this would even promote the culturing of the important uncultivated taxa because of more interest/attention to them once they are described taxonomically. Karthikeyan et al. 2019 is a great recent example of this from my

group (we are now in the process of depositing the isolate to culture collections); i am aware of several similar examples if you want to see more. I short, i see this as a win-win situation for all of us and no threat whatsoever for the culture collections. To the contrary, I think allowing genome sequence to serve as type material may further promote culturing efforts! So, I am in favor of Whitman's proposal personally as I understand the proposal.

As far as the technical standards, be sure that all (or most, at least) of us that would like to have genome sequence as type material, do NOT want to do this with lower standards. We want to have as high standards as the isolate genomes, if not higher. I do believe it is doable. I explain a bit more below for those that want to read more on the technical issues and then address your specific concerns further below. I will also try to publish officially the points below in peered review press so you can refer to them and offer your arguments in favor or against toward helping to establish, hopefully, the standards that we can all adopt and use in practice soon! But the essence of what i am writing below can also be found in the paper cited by Sutcliffe above, Konstantinidis et al. 2017 (https://www.nature.com/articles/ismej2017113).

On the technical standard issue:

Several scientists have argued that the MAG and SAG information is not of similar quality to the information derived based on isolate-based experiments in the lab and thus, does not represent well the organisms under investigation (Bisgaard 2019, Overmann 2019). While this is, at least partly, true, it is not critical enough to prevent progress towards cataloguing the taxonomic diversity of uncultivated organisms, for several reasons. First, prokaryotic taxonomy has always relied on imperfect methods; MAGs/SAGs are not an exception to this. Take, for instance, the DNA-DNA hybridization (DDH) method, the "golden standard" for species demarcation. The genome-aggregated average nucleotide (ANI) value of shared genes among two related genomes (Konstantinidis and Tiedje 2005) has been shown to correlate well with their DDH values, and deviations in the values were common and largely attributable to the experimental noise of the former as opposed to the latter method (Goris 2007). Second, there are approaches to assess guality beyond reasonable doubt such as visual examination of read-recruitment pots (Rodriguez-R 2016) in combination with the quality checking pipelines (Parks 2015, Rodriguez 2018), and in our view only genomes of high enough quality based on these tests should be taxonomically described (Konstantinidis 2017). Third, the standards to use have been outlined already previously by us (Konstantinidis 2017) and others (Bowers 2017), and are of similar stringency to those used for isolate genomes. Further, long-read sequencing for routine taxonomic descriptions, even on environmental samples, is coming up soon [e.g., (Andersen 2019)], and is strongly expected to circumvent several of the low quality issues reported for MAGs and SAGs in the literature, e.g., identify and fix genome sequences that may be chimeric. It has been argued that when DNA sequence type material is replaced by new versions due to new sequencing technologies and/or tools for genome assembly, the species descriptions would have to be consequently revised, resulting in an unstable classification (Bisgaard 2019). However, this is unlikely to be true for most -if not all- taxa because such new versions will mostly affect only a small number of genes or nucleotide substitution positions in the genome as analysis of mock datasets of known composition has revealed (Sczyrba 2017) or the sequencing of the isolated Candidatus Macondimonas diazotrophica that was almost identical to its corresponding MAG (e.g., ANI >99.9%) (Karthikeyan 2019). It is even less likely that the affected genes by new genome versions would represent the species-diagnostic traits because these genes are often the hypothetical, mobile or prophage-associated genes found in multiple copies (and short contigs) in the genome (Pena-Gonzalez 2019). Hence, the genealogy of the genome and thus, its nomenclature and classification, will remain unaffected in the great

majority of cases where new versions of the genome become available. In a few cases that the new genome version will include major changes in gene content, the old version could be replaced by the new version in a process analogous to replacing the (usually lost) type strain of a (named) species by a neotype strain for isolated organisms.

References cited: Andersen et al. 2019. <u>Syst Appl Microbiol</u> **42**: 77-84. Bisgaard et al. 2019. <u>Diagn Microbiol Infect Dis</u> **95**: 102-103. Bowers et al. 2017. <u>Nat Biotechnol</u> **35**: 725-731. Goris et al. 2007. Int. <u>Syst Evol Microbiol</u> **57**: 81-91. Karthikeyan et al. 2019. <u>ISME J</u> **13**: 2129-2134. Lawrence and Ochman. 1998. <u>Proc Natl Acad Sci U S A</u> **95**: 9413-9417. Overmann et al. 2019, <u>Syst Appl Microbiol</u> **42**: 22-29. Parks et al. 2015. <u>Genome Res</u> **25**: 1043-1055. Pena-Gonzalez et al. 2019. <u>Appl Environ Microbiol</u> **85**(24). Rodriguez et al. 2018. <u>Nucleic Acids Res</u> **46**(W1): W282-W288. Rodriguez-R and and Konstantinidis. 2016. <u>PeerJ Preprints(e1900v1)</u>. Sczyrbaf et al. 2017, <u>Nat Methods</u> **14**: 1063-1071.

January 20

Ramon Rossello-Mora, Vice-Chair of Judicial Commission of the ICSP, Grup de Microbiologia Marina IMEDEA, Illes Balears, Spain

In response to Christensen's comments of January 16

I recall Cowan 1965 [3]: The adequacy of characterization of a bacterium is a reflexion of time; it should be as full as modern techniques make possible. Unfortunately, one now regarded as a adequate is likely, in ten years time, to be hopelessly inadequate. I think taxonomy must adapt to the modern times. To Christensen concerns:

1. Risk for an increase in the number of heterotypic synonyms.

This is independent of genomes as type material. Close to 90% of the species descriptions in IJSEM are single strains [18], mostly without genome provided, nor DDH, using 98.7% 16S rRNA threshold. The use of strict or narrow values (e.g. 70% DDH) has been incorrectly used to force unnecessary classifications [13, 14]. I anticipate that with the genome sequencing, the recognition of heterotypic synonyms will increase. However, genome sequence as a reference will provide a much more stable framework than the simple use of 16S and API strips. The evidences of an evolutionary gap between species ([7, 17], will facilitate circumscriptions as the database grows.

Diseases are not always linked to species identity. Just looking to e.g. Bacillus cereus group [8], some traits are linked to a strain and could even be horizontally transferable (e.g. cry genes diagnostic of B. thuringiensis). Other clinically relevant traits as e.g. hemolysin or enterotoxin genes could be genus widely distributed [9]. For instance, sequencing B toyonensis genome allowed the (i) detection of clinically relevant genes and (ii) understanding of their non-functional nature. This is a good example of the contrary of what is mentioned.

There are many other cases in where it is clear a strain-specific and not species-specific virulence factors e.g. Legionella pneumophila [2], Vibrio toranzoniae [11], Pseudomonas aeruginosa [4], Streptococcus uberis [20], Ralstonia solanacearum [21], and so on...

Treatments against clinical infections are mostly done using antibiotic treatment, and sensitivity may be (i) strain specific, (ii) susceptible of horizontal gene transfer and/or (iii) susceptible of spontaneous mutation. Unstable characters, as linked to plasmids (e.g. degradation of naphthalene; [15]) have always been considered not suitable for taxonomic purposes. It is known that characters like phage sensitivity, immunoreactivity [16] and antibiotic susceptibility are often strain-specific and, may be of a lot of relevance for clinical issues but not for taxonomy.

2. Identification of clinically important streptococci bacteria for which vaccines are being developed.

I agree that for vaccine development living material is needed, but immunoreactivity may be strain specific, and virulence factors that can be horizontally transferred. I doubt that clinical microbiologists will abandon cultivation just because the reasons to isolate an organism are very distant from those of the classification purposes.

It would be good to check how many new descriptions in IJSEM are related to clinical cases and with medical relevance. And how many of them have their virulence factors elucidated. I anticipate that if any, very few.

We never underestimated the value of cultivation and evaluation of clinical relevant traits, but the investigation in infection and disease's research is significantly different from classification. I trust that if a study reveals a clinically relevant yet uncultivated organism, this will lead to focus efforts in obtaining pure cultures as occurred with Salinibacter [1], Macondimonas [10]; and many more examples of ecologically relevant organisms [6, 7, 12, 19].

References:

- 1- Anton et al. 2002. IJSEM. 52:485-491
- 2- D'Auria et al., 2010. (<u>https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-11-181</u>)
- 3- Cowan. 1965. J Gen Microbiol 39: 143-153
- 4- Choi et al, 2002 (https://jb.asm.org/content/184/4/952)
- 5- Harbison et al., 2016 (https://academic.oup.com/femsle/article/363/15/fnw151/2197705)
- 6- Henson et al., 2018 (https://www.nature.com/articles/s41396-018-0092-2.pdf)
- 7- Jain et al., 2019 (https://www.nature.com/articles/s41467-018-07641-9)
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January 20 Anne Willems, Ghent University, Ghent, Belgium

In response to Christensen's comments of January 16

I have a question regarding what would be the consequences of the newly proposed rules in the following situation: In case a species would be described with a genome sequence as type material, for example in the absence of a culture or in case of a MAG, and later on cultures belonging to that species do become available: can and should a type strain be designated then for that species even though it's genome sequence may not perfectly match with the one first proposed? Would the type strain replace the type genome that was first proposed?

January 20 Iain Sutcliffe, Chair of the ICSP, Northumbria University, Newcastle upon Tyne, U.K.

In response to Willem's comments of January 20

This circumstance is directly addressed in the Whitman (2016) proposals, by minor amendment to Rule 18f. which would allow for the replacement of a type sequence of genomic DNA with a type strain (see text in blue below).

This is one of the ancillary changes referred to in my original email. Apologies for not being clearer.

Rule 18f. If a <u>sequence of genomic DNA</u>, description or illustration constitutes, or a dead preserved specimen has been designated, the type of a species [Rules 18a(1) <u>and 18a(3)</u>] and a later strain of this species is cultivated, then the type strain may be designated by the person who isolated the strain or by a subsequent author. This type strain shall then replace the <u>sequence of genomic DNA</u>, description, illustration or preserved specimen as the nomenclatural type. The designation of a type strain in this manner must be published in the IJSEM, the authorship and date of priority of publication being determined by the effective and valid publication of the name by the original authors (Rule 24b). (underlined text are new additions to the current rule)

January 21 Pierre-Edouard Fournier, ICSP Delegate, UMR VITROME, Marseille, France

As a clinical microbiologist, I have been using partial, and then complete genomic sequences for bacterial identification on a routine basis for diagnostic purposes for many years. As a consequence, I support the proposal to use genomic sequences as type material for new taxa when a culture cannot be obtained.

However, and as discussed recently with lain, I have a few concerns that include:

- Defining quality criteria that will be applied to DNA sequences prior to being used as type material is crucial and may be very difficult for metagenomic data. There are few sequencing systems commercially available currently, but so many sequence analysis softwares and strategies...

- There is a risk of discouraging culture efforts, and notably the deposit in two type culture collections, of strains of previously described *Candidatus* species whose type material is a DNA sequence. There is a risk that microbiologists who cultivate strains belonging to previously described *Candidatus* species only deposit them in a single culture collection, as requested for publishing in most journals, and do not make the effort to publish them as type strains in IJSEM as described in Iain's message below. To avoid this, maybe the cultivators' names should be added to validation lists, not as "discoverers" of the new species but as the first "cultivators".

- New *Candidatus* species will be proposed mainly on the basis of DNA sequence data, as no strain will be available at the time of description. Currently, many new species descriptions use overall genome relatedness indexes and "universal" thresolds such as 70% for dDDH and 95-96% for OrthoANI. However, these thresholds do not apply to all taxonomic groups and may, therefore over- or underestimate the biodiversity of some groups of prokaryotes. When cultivable strains are available, phenotypic data may help with a more precise classification. With a reduced number of phenotypic characteristics evaluable, which will be the case with uncultivated species, this may not be possible.

January 21 William B Whitman, ICSP Delegate, University of Georgia, Athens USA

In response to Fournier's comments of January 21

With regard to Pierre-Edouard's comment about recognition being given to the cultivators, a mechanism already exists to do just that. Changing the type from a sequence to a strain should be recognized as a change in the species circumscription, which would be recognized by an emendation of the species description [see Rule 35]. Emendations are indicated in the defining publication that accompanies the species name. This has already been done for at least one species whose type was a description.