A Contrary View of the Proposal To Assign a Neotype Strain for *Methanotherx soehngenii*†

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Arguments are presented for the rejection of the proposal (D. R. Boone, *Int. J. Syst. Bacteriol.* 41:588–589, 1991) that the type strain GP6 of *Methanosaeta concilii* be designated as the neotype strain of *Methanotherx soehngenii*.

It has been proposed (1) that strain GP6^T (= DSM 3671^T = OCM 69^T = NRC 2989^T = ATCC 35969^T), which is the type strain of the type species, *Methanosaeta concilii*^{VP} (*Methanotherx concilii*^{VP}), of the genus *Methanosaeta* (13, 14), be designated as the neotype strain of *Methanotherx soehngenii*^{VP}. The proposal in the Request for an Opinion (1) assumes that strain Opfikon^T, the type strain used for the description of *Methanotherx soehngenii*, was pure when the species was described and that the strain Opfikon^T and strain GP6^T are homologous. I present below arguments as to why we cannot assume that strain Opfikon^T was pure when it was described. I also elaborate on the confusion that would ensue if strain GP6^T were designated as the neotype strain for *Methanotherx soehngenii*.

It is important to consider the background and relevance of the Request for an Opinion (1) to judge its validity. In the early 1970s, many laboratories were competing to isolate acetic acid-utilizing methanogenic bacteria. In most acetic acid enrichment cultures a predominance of fat, rod-shaped, filamentous bacteria was observed. The race was on to isolate this "presumed" acetate-utilizing methanogen. The reported "isolation" of *Methanotherx soehngenii* in 1980 and 1982 (5, 20) was considered to be a significant achievement in the field of methanogenesis. However, the purity of the culture (strain Opfikon^T) has been controversial from the outset, as is summarized in the Request for an Opinion (1) and amplified below.

Exactly half of the respondents of the poll sent to the Subcommittee for Taxonomy of Methanogens voted to assume that strain Opfikon^T had been pure at the time it was originally characterized (1). Several members of the subcommittee who voted to assume that strain Opfikon^T had been pure at the time it was characterized nevertheless expressed personal doubts that this was in fact true (2).

It is not appropriate to assume that strain Opfikon^T was pure, because it is contrary to the information reported in the initial two papers in which the isolation and description of the species was published (5, 20). The 1980 paper (20), in which the characteristics of strain Opfikon were first described, stated that the culture had been enriched to a monoculture and, further, "On agar roll tubes we were never able to obtain entirely contamination-free colonies." This suggests, assuming that the contaminants were not the principal acetate utilizers, that the contaminants were dependent on the acetate-utilizing bacteria for growth. Also, it was stated (20) that small numbers of contaminants were found

by inoculating complex broth media and the contamination "... remained at that level during several transfers." Evidently there were problems of culture purity when strain Opfikon was first described. The 1980 paper (20) also stated, "In the presence of 100 mg/l vancomycin, a concentration which is just not inhibitory for the acetate organism, all contamination tests remained negative even after several transfers." What is the value of contamination tests in which in all likelihood the antibiotic kept the contaminants in check? The culture described in the 1980 paper (20) was stated to be "... in a highly purified stage of culture" by an independent researcher (18), who also stated that pure cultures of acetophilic (i.e., acetate-utilizing) methanogens to that date were represented only by the genus *Methanosarcina*.

The 1982 paper (5), which characterized *Methanotherx soehngenii* on the basis of description of strain Opfikon^T, stated, "The isolation procedure and a preliminary characterization of this 'acetate-organism' has already been published earlier (Zehnder et al. 1980)", which indicates the use of the same suspect culture described in the 1980 paper (20). In 1982 (5) it was stated, "The number of contaminants were calculated using the most probable number (MPN) technique" and, "Since vancomycin (at 0.1 mg/ml) showed no negative effect on growth and methanogenesis it was routinely added to the MS-medium for subculturing and maintenance of *M. soehngenii*." This suggests that there were contaminants to be counted and that antibiotics were always included in the stock culture media to inhibit them. Antibiotic carry-over could have suppressed the contaminants in the purity tests (5, 16, 20), as was also pointed out in the Request for an Opinion (1). The initial isolation and characterization papers (5, 20) still revealed contamination problems in strain Opfikon^T! Since *Methanotherx soehngenii* is based on the description of an impure culture strain, Opfikon^T, it is not validly published as per Rule 31a of the *International Code of Nomenclature of Bacteria* (9).

The designated type strain, Opfikon^T, was deposited in the German Collection of Microorganisms (Deutsche Sammlung von Mikroorganismen [DSMZ]) as DSM 2139^T (5). The only viable DSM 2139^T culture was assessed to be contaminated as received by the DSM; the contaminants caused blackening of Postgate medium and apparently included a vibrio and a rod-shaped spore-forming bacterium (14). The Request for an Opinion (1) states that "A pure, viable culture of strain Opfikon^T was apparently never deposited in a culture collection, and no pure culture exists today." Why was a pure, viable culture never deposited when this "isolate" was considered so significant to the field of methanogenesis? It is also reported in the Request for an Opinion (1) that

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a third party obtained a *Methanothrix* culture from A. J. B. Zehnder's laboratory and "... thinking it to be strain Opfikon^T, deposited it in the Deutsche Sammlung von Mikroorganismen as strain Opfikon^T (= DSM 2139^T).” If it was mistakenly deposited as DSM 2139^T, why was the DSM not later alerted to this fact? As recently as October 1988, I received from the DSM a culture of DSM 2139^T (which I found to be impure) that was listed as *Methanothrix soehngenii* Opfikon. If the original culture claimed to have been deposited as DSM 2139^T by Huser et al. (5) was not viable, was an authorized replacement culture sent to the DSM? There is no dispute that DSM 2139^T (obtained from A. J. B. Zehnder or the DSM) cultures are impure, since it is accepted that no axenic cultures of *Methanothrix soehngenii* Opfikon^T exist (1).

According to Recommendation 30a of the *International Code of Nomenclature of Bacteria* (9), a culture of the type strain should be deposited in a permanently established culture collection prior to the publication of the name of a new species. Rule 18a stipulates that the type strain should have been maintained in pure culture. I believe that the description of *Methanothrix soehngenii* Opfikon has also been deficient on both of these counts. Recommendation 30a is not an absolute requirement, but the intent is to avoid the very problems discussed here and it cannot be ignored.

The Request for an Opinion (1) recognizes that a few researchers have been successful in isolating this sort of methanogen in pure culture, and then, in contrast to the verifiable facts, we are asked to assume that strain Opfikon^T was pure when it was described. If the culture was pure when described, it should have been possible to use the same protocols as before and repurify the strain Opfikon^T. However, this has not been done in the 10 years since the original publication (1, 14, 17). We can only conclude that the contaminants have been there all along, are difficult to remove, and have been held in low numbers by use of antibiotics.

It would appear that the only “evidence” of culture purity is in a personal communication (1), uncorroborated by information in the isolation and characterization papers themselves (5, 20) or by independent scientists (1, 14) who assessed cultural purity. I therefore disagree with the contention in the Request for an Opinion (1) that, “In the absence of direct evidence that the culture was never pure, I believe that the published report of its purity should be accepted.” Certainly, the onus was on the original authors of the description to provide proof of cultural purity by making available a pure culture to other researchers for verification.

The facts presented in the Request for an Opinion (1) itself and elaborated here do not support the assumption that the strain Opfikon^T was pure when described. It strengthens the conclusion of Patel and Sprott (14) that strain Opfikon^T was never axenic. In view of this information, as suggested by Patel and Sprott (14), *Methanothrix soehngenii* is invalid according to Rules 31a and 18a of *The International Code of Nomenclature of Bacteria* (9).

Almost all research papers up to 1991 (4, 6, 8, 12) describing work done with *Methanothrix soehngenii* list strain Opfikon^T as being equivalent to DSM 2139^T. It is identified in a similar manner in *Bergey's Manual of Systematic Bacteriology* (19). However, after almost 10 years of delay the scientific community is informed (1), “The existence of even a contaminated culture of the original strain Opfikon^T is in doubt,” and “No axenic cultures of *Methanothrix soehngenii* Opfikon^T (T = type strain) exist, nor is any extant contaminated culture known to be derived from

strain Opfikon^T.” So, the culture known as DSM 2139^T (believed up to now to be the type strain for *Methanothrix soehngenii*) and the culture of strain Opfikon^T, on which Huser et al. (5) based the description of *Methanothrix soehngenii* (and which was claimed to have been deposited as DSM 2139^T), may not be the same. Clearly, no one knows for certain what was or is strain Opfikon^T, and independent researchers have judged as impure the cultures provided them as strain Opfikon^T by A. J. B. Zehnder's laboratory or by the DSM (1, 14).

The contention that *Methanosaeta concilii* strain GP6^T (= DSM 3671^T) and *Methanothrix soehngenii* strain Opfikon^T are synonymous is based upon comparison with DSM 2139^T as representing strain Opfikon^T, including the DNA homology studies of Touzel et al. (17) and Ohtsubo et al. (12). Since DSM 2139^T cultures are impure and may not be representative of strain Opfikon^T, I believe that any comparison with pure culture strains is meaningless. Any homology (12, 17) with the pure culture strain GP6^T (= DSM 3671^T) is to some unidentified cells present in a mixed culture “X” (i.e., DSM 2139^T). Therefore, one cannot conclude that strain GP6^T is homologous to *Methanothrix soehngenii*, bearing in mind that the Request for an Opinion (1) has indicated that DSM 2139^T may not even contain any cells of the original strain Opfikon^T described in the two initial characterization papers (5, 20). In light of this, it appears invalid to propose (1) that the type strain GP6^T of the validly published *Methanosaeta concilii* (“*Methanothrix concilii*”) be designated as the neotype strain for *Methanothrix soehngenii*.

It should also be noted, bearing in mind the questionable status of DSM 2139^T as representative of strain Opfikon^T, that Macario and Conway de Macario (10) reported that strain Opfikon^T was antigenically distinct from strain GP6^T. Roustan et al. (15) showed that strain GP6^T was susceptible to a lytic phage but that strain Opfikon^T was not. Physiological differences are as presented in earlier papers from my laboratory (13, 14).

Besides strain Opfikon^T, there are serious problems with other “strains” that have been classified as *Methanothrix soehngenii* by the various authors who first described these cultures as such. Strain FE (= DSM 3013) was also an impure culture, containing a vibrio, as acknowledged by Touzel et al. (17) in their own paper, in which they first described it as a strain of *Methanothrix soehngenii*. No axenic culture of strain FE had been deposited with the DSM by the end of 1989 (14). Another culture, described as *Methanothrix soehngenii* VNBF, was always maintained in medium containing 0.5 mg of penicillin G per ml or 0.1 mg of D-cycloserine per ml (3) and it was not deposited in a permanently established culture collection (3, 17). There is no record that the culture purity was verified by independent scientists, and an axenic strain VNBF may not be available today. This clearly illustrates that “strains” ascribed to *Methanothrix soehngenii* to date, by authors describing these as such, have questionable taxonomic validity. Unfortunately, *Bergey's Manual of Systematic Bacteriology* (19) does not draw attention to the lack of axenic cultures of strains Opfikon^T and FE, and this is true of almost all publications using strain Opfikon^T (DSM 2139^T) for studies on genetics, biochemistry, and enzymology. This has created a mistaken impression of taxonomic authenticity for *Methanothrix soehngenii*.

The description of *Methanosaeta thermoacetophila* as a new combination (14) may indeed be invalid, because the original characteristics of *Methanothrix thermoacetophila*

(11) were based on an impure culture called strain Z-517, even if it was purified later as DSM 4774^T. However, rejecting *Methanosaeta thermoacetophila* as an invalid name (1) has no bearing on the validity of the genus *Methanosaeta* because the description of the genus is based on the description of *Methanosaeta concilii* as the type species, with strain GP6^T as the type strain.

The name *Methanothrix soehngenii* is associated with contaminated cultures. A culture either is axenic or is not. The continued use of this contaminated strain (DSM 2139^T) has contributed to confusion in the published literature with respect to the "isolation" and identification of similar methanogenic bacteria. For example, knowing that the culture they described as strain FE was not pure, Touzel et al. (17) still felt that it was valid to designate it as a strain of *Methanothrix soehngenii*. Similarly, although Kamagata and Mikami (7) acknowledged that all six of their cultures were impure, they designated these as specific strains of *Methanothrix soehngenii*. It appears to be acceptable to assign contaminated cultures in this fashion, and this practice should not be tolerated. Kamagata and Mikami (8) caused further confusion in the nomenclature issue by mistakenly suggesting that *Methanothrix soehngenii* Opfikon (DSM 2139) could be called *Methanosaeta concilii* Opfikon on the basis of the proposals of Touzel et al. (17) and Patel and Sprott (14).

On the basis of the facts, it is my contention that the preservation of the name *Methanothrix soehngenii* will further add to the confusion regarding the nomenclature of acetoclastic, mesophilic, rod-shaped methanogens. Therefore, even if one assumes that strain Opfikon^T was pure when described, the request to designate strain GP6^T as the neotype strain of *Methanothrix soehngenii* (1) should be rejected. Serious consideration should also be given to place the name *Methanothrix soehngenii* on the list of rejected names.

Methanosaeta concilii GP6^T (= NRC 2989^T = DSM 3671^T = ATCC 35969^T = OCM 69^T), formerly called "*Methanothrix concilii*" (13, 14), was the first independently verified, axenic culture representing mesophilic, rod-shaped, methanogenic bacteria using acetic acid as the sole carbon and energy source. Strain GP6^T has been pure since the first report in 1984 (13), has always been retained in pure culture, and has been deposited in several culture collections. There is no confusion about its authenticity. I believe that we followed the options available to us under the rules of *The International Code of Nomenclature of Bacteria* (9) when we proposed the name *Methanosaeta* (14). Therefore the name should be allowed to remain as the valid name. Whether *Methanothrix concilii* should have been transferred into *Methanosaeta* as *Methanosaeta concilii* (as a new combination), as suggested in the poll (2) sent to the Subcommittee for Taxonomy of Methanogens, or described as "*Methanosaeta concilii* gen. nov., sp. nov. . . ." (14), is a point of interpretation of the rules of nomenclature (9). This should not have a bearing on the scientific validity and priority of the name *Methanosaeta concilii*.

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