Letters to the Editor - Mediation of arsenic oxidation by Thiomonas sp. in acid-mine drainage (Carnoules,France), by Bruneel et al. published in the Journal of Applied Microbiology(2003) 95, 492–499

Fabienne Battaglia-Brunet

Letters to the Editor

Sir—A manuscript entitled 'Mediation of arsenic oxidation by *Thiomonas* sp. in acid-mine drainage (Carnoules, France)', by Bruneel *et al*. was published in the *Journal of Applied Microbiology* (2003) **95**, 492–499.

We feel a number of points that are open to interpretation, coupled with apparent omissions, reduce the quality and scientific equity of this paper.

In the introduction, at the end of the second paragraph, the authors write 'The presence of As-oxidizing bacteria in AMD has been recognized… and one species has recently been identified (Battaglia-Brunet *et al*., 2002). To date, no *Thiomonas* bacteria have been implicated in As oxidation.' The cited reference (Battaglia-Brunet *et al*., 2002), published in the *Journal of Applied Microbiology* **93**, 656–667, is entitled 'An arsenic(III)-oxidizing bacterial population: selection, characterization, and performance in reactors'; the abstract clearly states: 'The CAsO1 consortium contains at least two organisms – strain b3… and strain b6, which is related to the genus *Thiomonas*.'

We find it hard to believe that the authors of the Bruneel *et al*. (2003) paper could cite a reference without having at least read, and taken into consideration, the abstract. The affiliation of the b6 strain to the *Thiomonas* group and the importance of this organism in the As-oxidizing population is clearly explained in the Battaglia-Brunet *et al*. (2002) publication:

i In the chapter 'Bacterial content of CAsO1', we wrote 'Since the lowest divergence between C02, C19 and the closest organism, i.e. *Thiomonas* sp. Ynys3…, was as high as 5·9%, the main organism constituting CAsO1 population may represent a new species.'

ii In the discussion, 'b6 is phylogenetically related to *Thiomonas* sp. Ynys3 [branch of the *β*-Proteobacteria and *Thiomonas* sp. Ynys3 was also isolated

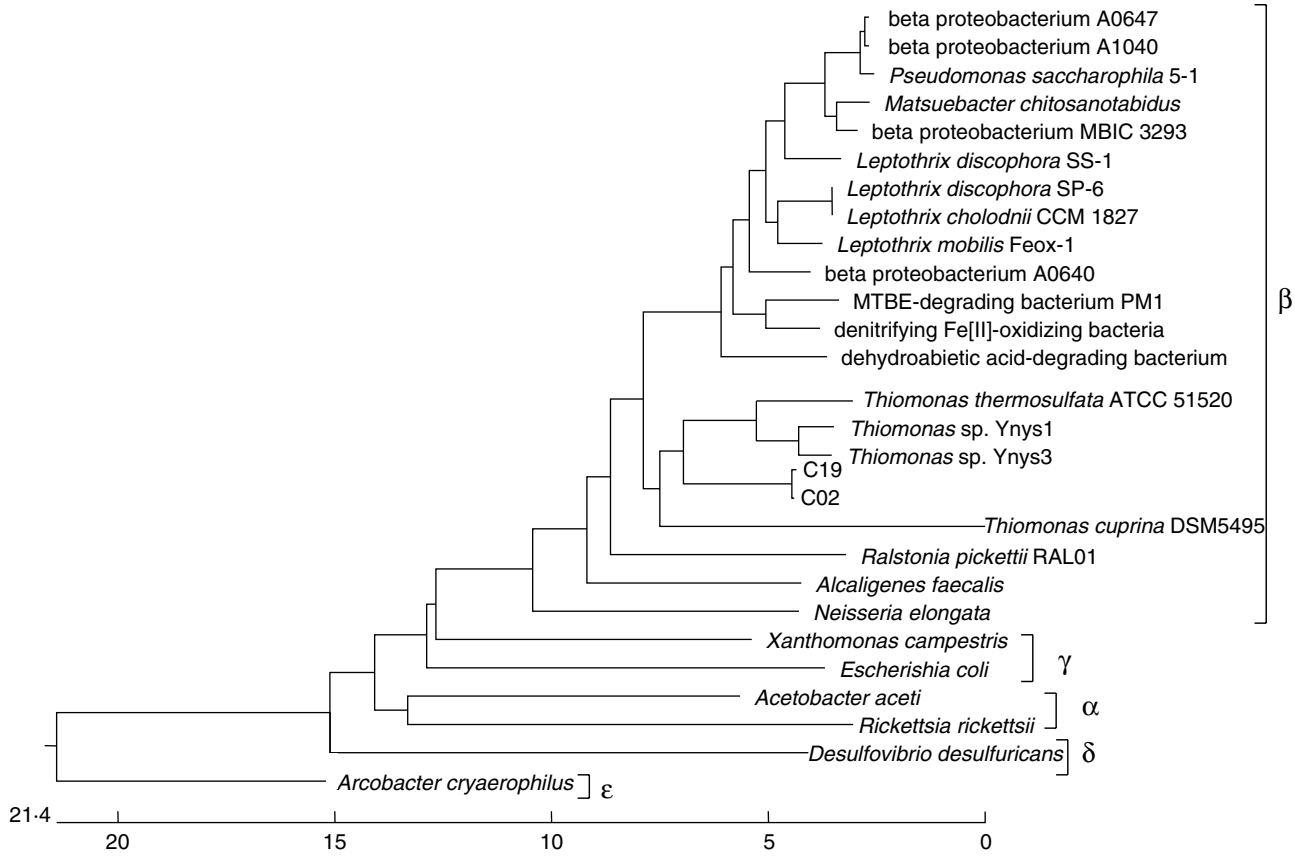

Fig. 6 Clustal-V method tree showing the phylogenetic relationship between clones C02 and C19 and species belonging to the *α*-, *β*-, *γ*-, *δ*- and *ε*-Proteobacteria (from F. Battaglia-Brunet *et al.* (2002), **93**, 656-667)

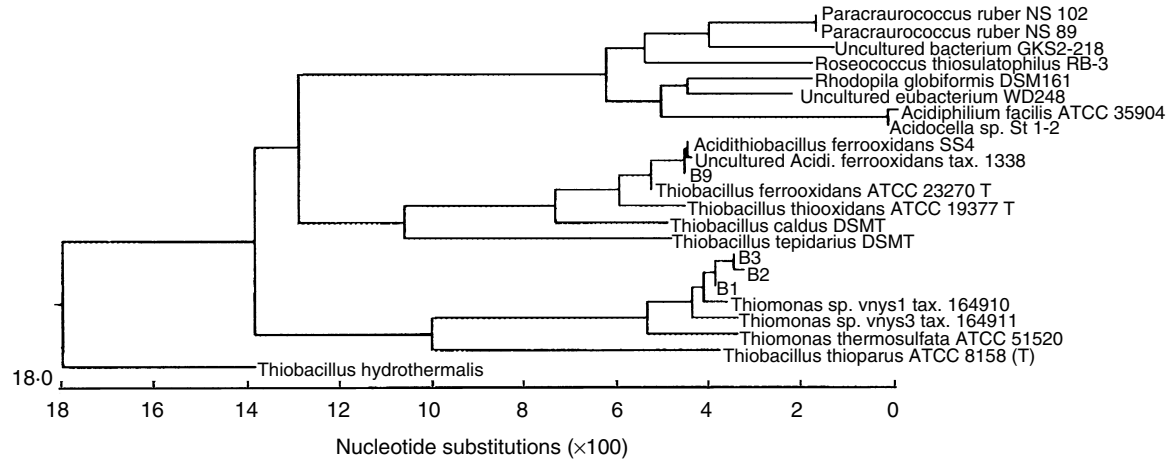

Fig. 3 Phylogenetic tree showing the relationship between the three *Thiomonas* strains B1, B2, and B3 isolated from Reigous Spring and Reigous Creek and other closely related bacteria (from Bruneel *et al.* (2003), **95**, 492–499)

from a mining environment and was able to oxidize Fe(II)].'

Although possibly a new species, the phylogenetic tree given in Figure 6 (Battaglia-Brunet *et al.*, 2002) shows the strain b6 (clones C19 and C02) as belonging to the group composed of *Thiomonas thermosulfata*, *Thiomonas* sp. Ynys 1, *Thiomonas* sp. Ynys3, and *Thiomonas cuprina*. Consequently, it is inappropriate to state 'To date, no *Thiomonas* bacteria have been implicated in As oxidation' [in the Bruneel *et al.* (2003) manuscript] immediately after citing the Battaglia-Brunet *et al.* (2002) reference.

In the results, in the second paragraph, Bruneel *et al.* (2003) present a phylogenetic tree (Figure 3) with their three strains (B1, B2 and B3) close to *Thiomonas* sp. Ynys1, *Thiomonas* sp. Ynys3 and *Thiomonas thermosulfata*. These three bacteria were also the closest neighbouring organisms to the b6 strain in Battaglia-Brunet *et al.* (2002), and whose sequence is available in Genbank (AF460990, cited in Battaglia-Brunet *et al.*(2002).

We are surprised that Bruneel *et al.* omitted placing in their phylogenetic tree an As-oxidizing strain closely related to the *Thiomonas* species.

Both the manuscripts described bacteria selected from mining sites. The discussion section in the paper of Bruneel *et al.* (2003) should have included at least a few words to mention this relationship.

In the light of the points discussed above, we feel that the sentence 'These results represent the first reported oxidation of arsenic by *Thiomonas sp.*' (Bruneel *et al.*, 2003, Significance and Impact of the Study), is inappropriate. As a fact, this is the first official publication that demonstrates that *Thiomonas* sp. (and not only a *Thiomonas*-related strain) oxidizes arsenic. However, through this sentence, which appears at a particularly strategic place in the publication, the authors suggest that they discovered this biological phenomenon in the *Thiomonas* group, which is not the case.

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Sir—We read with attention the letter from Dr Battaglia-Brunet. We know that she has patented a process for removing As from water using the bacterial consortium that she and her co-authors described in Battaglia-Brunet *et al.* [*Journal of Applied Microbiology* (2002) **93**, 656–667]. This explains to a large extent her concern. We would like to show there was no ethical breach on our side.

i Phylogenetic tree. When we had to compare our *rrs* gene sequences (16S rRNA gene), we searched GenBank for closest deposited sequences, using BLAST. Then we built a phylogenetic tree with appropriate sequences, i.e. our sequences, the closest sequences in GenBank, and sequences representing the diverse species in the genus. At this stage, displaying all sequences from unidentified organisms or redundant sequences is pointless. Sequences from Battaglia-Brunet *et al.* were not included in the phylogenetic tree we published because they were useless for our identification. Here is a tree showing our strains and theirs (Fig. 1). Our strains B1, B2, and B6 are closely related to *Thiomonas perometabolis* as are organisms Ynys1 and Ynys3. *Thiomonas thermosulfata* is

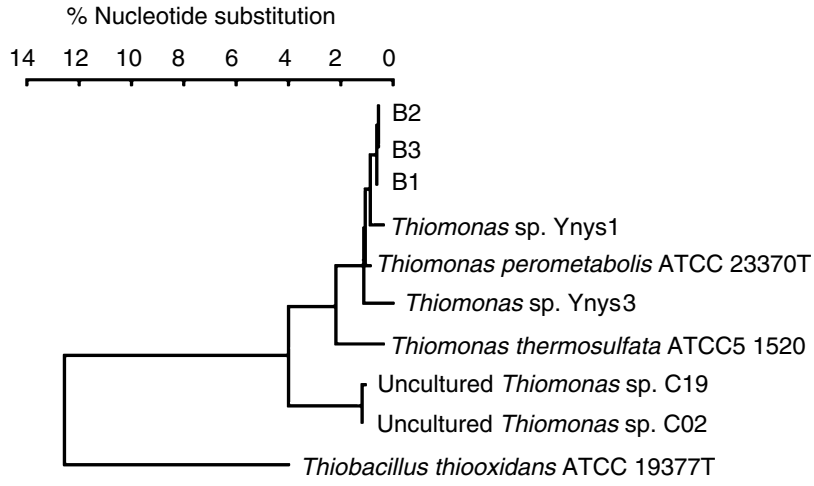

Fig. 1 Phylogenetic tree showing relationship between strains B1, B2 and B3 (Bruneel *et al.* 2003), and those described in Battaglia-Brunet *et al.* 2002

a little more distantly related. Battaglia-Brunet's organisms 'Uncultured *Thiomonas* sp. C19' and 'Uncultured *Thiomonas* sp. C02' (as designated in GenBank) are even less related to our strains and certainly constitute unnamed species. We disregarded sequences below 1000 nucleotides as these blur phylogenetic studies [Murray *et al. International Journal of Systematic Bacteriology* (1990) **40**, 213–215].

ii In the paper by Battaglia-Brunet *et al.*, arsenic oxidation has been demonstrated for a consortium of bacteria (designated as CASO1). From this consortium, *rrs* sequences were obtained after cloning the gene. The resulting clones corresponded to either *Ralstonia* sp. or *Thiomonas* sp. (clones C02 and C19 above mentioned). An isolate (b6) was obtained from the consortium and a short partial *rrs* sequence obtained (similar to C02 and C19). However, no experiment was reported showing the ability of this purified strain to oxidize arsenic. It probably does, but are we supposed to guess what authors do not write?

iii Our command of the English language may have lack of precision. Instead of 'These results represent the first reported oxidation of arsenic by *Thiomonas* sp.' we should have written 'These results represent the first reported oxidation of arsenic by pure cultures of *Thiomonas* sp.' We apologize for such an imprecision.

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