



Short communication

Cultivation of the first mesophilic representative (“mesotoga”) within the order *Thermotogales*[☆]Wagdi Ben Hania^{a,b}, Ramzi Ghodbane^{a,b}, Anne Postec^a, Céline Brochier-Armanet^c, Moktar Hamdi^b, Marie-Laure Fardeau^a, Bernard Ollivier^{a,*}^a Laboratoire de Microbiologie IRD, UMR 180, Aix-Marseille Université, Marseille, France^b Laboratoire d'Ecologie et de Technologie Microbiennes, INSAT, Tunis, Tunisia^c Aix-Marseille Université et Laboratoire de Chimie Bactérienne, CNRS UPR 9043, IFR88, Marseille, France

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ABSTRACT

Cultivated members of the order *Thermotogales* comprise only thermophilic to hyperthermophilic anaerobic microorganisms. However, based on molecular studies, the existence of mesophilic members (“mesotoga”) within this order has been postulated but has not been demonstrated by cultural approaches so far. A “mesotoga” (strain PhosAc3) that belonged to an uncultivated lineage distantly related to the thermophilic *Kosmotoga* genus has now been cultivated in axenic culture. It grew between 30 °C and 50 °C (optimum 40 °C) and oxidized lactate using elemental sulphur as a terminal electron acceptor. Further genomic and physiological characterization of strain PhosAc3 will be important not only for understanding bacterial adaptation to high and moderate temperatures at small evolutionary scales, but also because “mesotoga” might play a crucial ecological role in ecosystems polluted by aromatic compounds.

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Introduction

Thermotogales, the unique order composing the *Thermotogae* phylum, is comprised of eight thermophilic to hyperthermophilic genera [12], including the two recently proposed genera *Kosmotoga* [7,21] and *Oceanotoga* [14] (Fig. 1). Cultivated members of this bacterial order are heterotrophic anaerobes. They inhabit a wide range of hot ecosystems on Earth including shallow and deep hydrothermal vents, terrestrial hot springs, and oilfield ecosystems [12]. Besides their ecological significance, the phylogenetic position of *Thermotogae* is of primary interest for understanding bacterial evolution. Indeed, analyses based on RNA sequences of the small ribosomal subunit (SSU rRNA) have suggested that *Thermotogae*, like *Aquificae*, represent a deep branching lineage within the bacterial domain, which supports the hypothesis that present-day bacteria have arisen from a hyperthermophilic ancestor [1]. However the phylogenetic position of *Thermotogae*, as the nature

(hyperthermophile or mesophile) of the ancestor of *Bacteria*, still remains as a matter of debate [5,6,9,30].

The existence of mesophilic members of this order, informally called “mesotoga”, has been postulated based on analyses of SSU rRNA sequences that have been amplified from many mesothermic terrestrial and subterrestrial environments [18–20,23]. Such sequences were retrieved in particular from anaerobic enrichment cultures on polychlorinated biphenyl compounds inoculated with sediments, but also from microbial communities degrading chlorophenols, polycyclic aromatic hydrocarbons and dimethyl phthalate [11,18–20,27–29]. “Mesotoga” are thus important keys to understand not only (i) biological and molecular mechanisms of adaptation from hot to mesothermic environments, but also (ii) their ecological role within microbial consortia enriched on halogenated aromatic compounds (e.g. polychlorobiphenyl compounds) that are a danger to health as pollutants generated by industries. However, to date, the mesophilic and physiologic features of “mesotoga” have not been provided by cultural approaches, despite the very recent (and brief) report of one “mesotoga” isolate for which no phenotypic or physiologic characteristics or culture conditions have been provided [20]. In this respect, success not only in isolating but also growing mesophilic *Thermotogales* has been presented as a challenge for microbiologists. Here, we report on the cultivation of a mesophilic “mesotoga” (strain PhosAc3) from an anaerobic reactor fed with cheese whey and phosphogypsum.

[☆] The GenBank/EMBL accession numbers for the sequences presented in this study are: FN611033 (SSU rRNA) and FN611034 (LSU rRNA).

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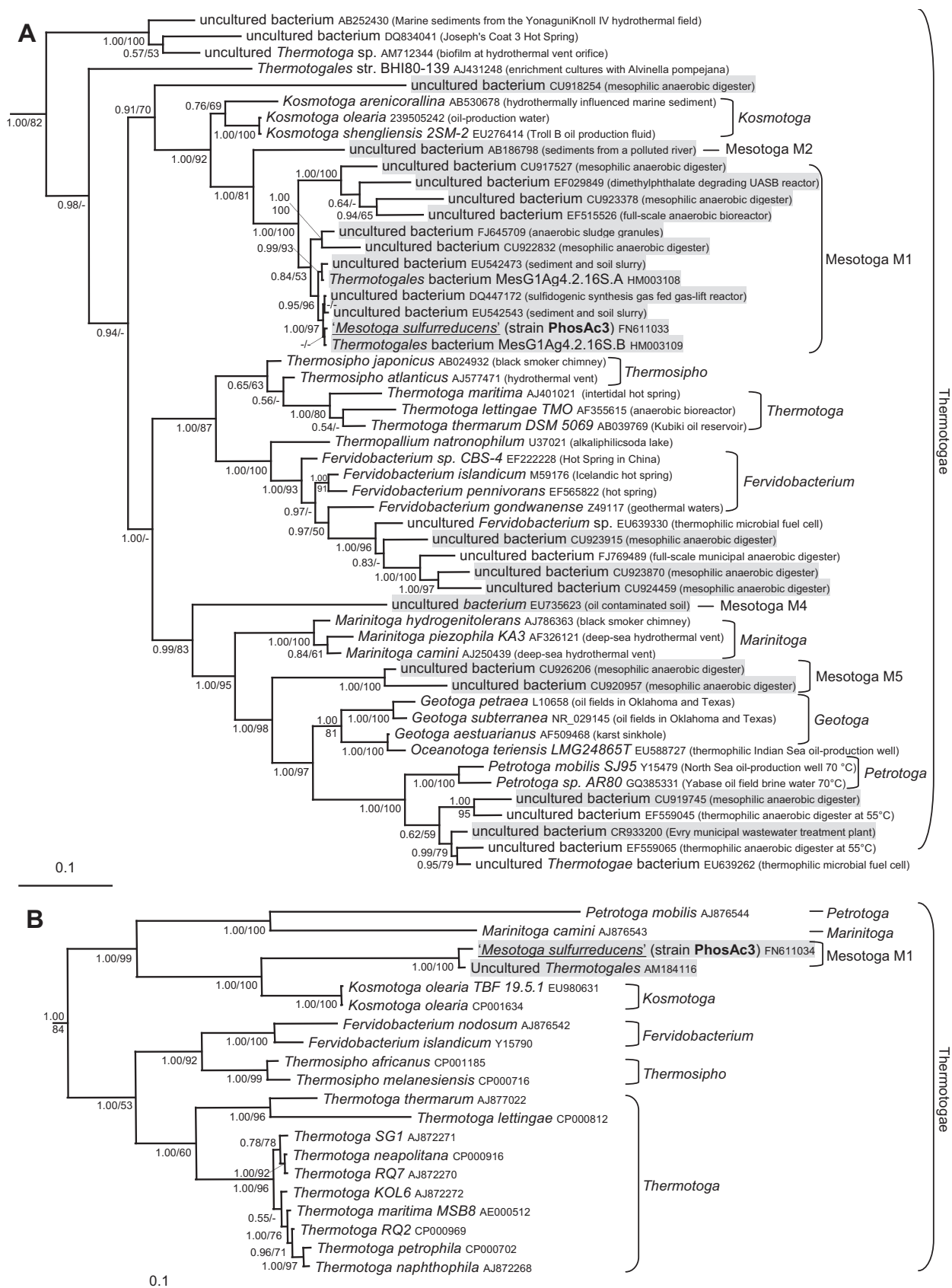


Fig. 1. Rooted Bayesian phylogenetic trees of SSU rRNA (A) and of LSU rRNA (B) (1048 and 2003 unambiguously aligned nucleic acid positions analysed, respectively). For clarity, only the subtrees corresponding to *Thermotogae* are shown (53 SSU and 20 LSU rRNA sequences). Full trees are available as Fig. S2. Numbers at nodes represent posterior probabilities (PP) inferred by MrBayes and bootstrap values (BV) inferred by TreeFinder. Only PP > 0.50 and BV > 50% are shown. Scale bars represent the average number of substitutions per site. *Thermotogales* sequences retrieved from mesothermic environments are shaded.

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