

The Family *Halomonadaceae* as a Model of Halophilic Bacteria for Multilocus Sequence Analysis

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Multilocus Sequence Analysis (MLSA) has been proposed as an alternative to DNA-DNA hybridization to genomically circumscribe prokaryotic species and to differentiate them from neighbouring species. Several studies on MLSA in taxonomic groups belonging to the class *Gammaproteobacteria* have been carried out, for instance, concerning the family *Pasteurellaceae* and the genera *Aeromonas*, *Alteromonas*, *Ensifer*, *Pantoea*, *Vibrio* and *Xanthomonas*. However, no studies have been performed within any moderately halophilic group of bacteria. Therefore, we have selected the family *Halomonadaceae*, which includes essentially halophilic bacteria, as a model to test the utility of MLSA with this kind of microorganisms. This family comprises a heterogeneous group of species some of them have suffered a large number of reclassifications. Currently, the family *Halomonadaceae* includes ten validly published genera names, the most prominent being *Halomonas* (with 62 species) and *Chromohalobacter* (which includes 8 species). The genera *Kushneria*, *Salinicola* and *Cobetia* group four, three and two species, respectively. Finally, the genera *Zymobacter*, *Carnimonas*, *Halotalea*, *Modicisalibacter* and *Aidingimonas* only contain so far a single species. In some cases, the phylogenetic relationships between members of this family based on 16S rRNA gene sequence are not clear and a deeply phylogenetic analysis by using several housekeeping genes seems necessary. For that purpose, we have studied the individual and concatenated sequences of six genes: 16S rRNA, 23S rRNA, *atpA*, *gyrB*, *rpoD* and *secA*. Our results have revealed that the family *Halomonadaceae* appears as a monophyletic group within the order *Oceanospirillales*. Five of the six studied genes (16S rRNA, 23S rRNA, *gyrB*, *rpoD* and *secA*) showed a similar evolutionary history. However, *atpA* gene produced a different result; thus, this gene is not useful as a phylogenetic marker within this family. With the exception of the genera *Halomonas* and *Modicisalibacter*, all the other genera included within this family are phylogenetically coherent. We also observed that horizontal gene transfer (HGT) plays an important role in the evolution of members of the family *Halomonadaceae*. Analysis of the six concatenated gene sequences minimized the impact of possible recombination events and phylogenetically is in agreement with the current taxonomic situation of this family. Finally, we conclude that, for future taxonomic studies on MLSA within the family *Halomonadaceae*, 16S rRNA, *gyrB* and *rpoD* genes are the most adequate ones.