

PqqE from *Methylobacterium extorquens* AM1: a radical S-adenosyl-L-methionine enzyme with an unusual tolerance to oxygen

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Supplementary Data

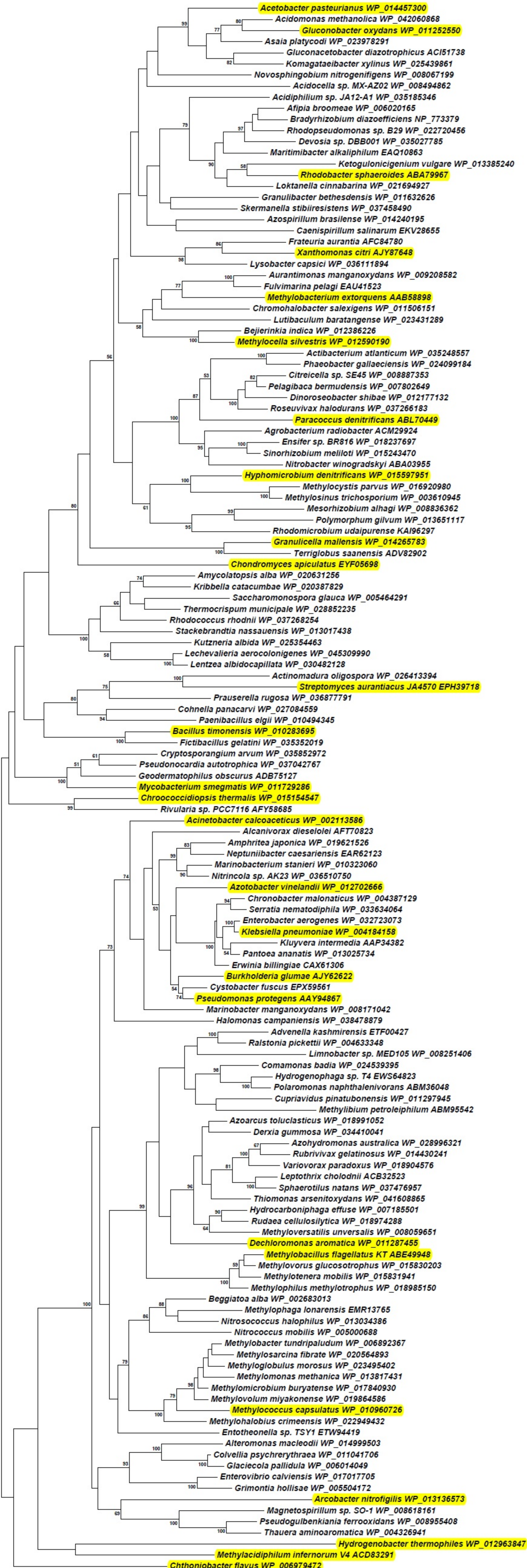
Supplementary method

Construction of phylogenetic tree of PqqE homologs

A total of 141 amino acid sequences retrieved from the list of PqqE homologs in the structure-function linkage database (1) were subjected to multiple sequence alignment with a MEGA6 software (2) using the MUSCLE algorithm (3), and the alignment obtained was used for phylogenetic analysis in MEGA6 with the maximum-likelihood method using 100 bootstrap replications for branch support estimations. Phylogenetic distances for construction of the tree were evaluated using the substitution model reported by Le and Gascuel (4).

Supplementary references

1. Akiva, E., Brown, S., Almonacid, D. E., Barber, A. E., Custer, A. F., Hicks, M. A., Huang, C. C., Lauck, F., Mashiyama, S. T., Meng, E. C., Mischel, D., Morris, J. H., Ojha, S., Schnoes, A. M., Stryke, D., Yunes, J. M., Ferrin, T. E., Holliday, G. L., and Babbitt, P. C. (2014) The structure-function linkage database. *Nucleic Acids Res.* **42**, D521–D530
2. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729
3. Edgar, R.C. (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797
4. Le, S. Q., and Gascuel, O. (2008) An improved general amino acid replacement matrix. *Mol. Biol. Evol.* **25**, 1307–1320



Supplementary Fig. S1 Molecular phylogenetic analysis by the maximum likelihood method. The phylogenetic tree constructed for 141 PqqE sequences with the highest log likelihood (-41230.3329) is shown. The percentage of trees in which the associated PqqE homologs clustered together is shown next to the branches. Initial tree(s) for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with a superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 1.2410)]. The tree was drawn to scale with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were total of 315 positions in the final dataset. The branch support values which are less than 50% are not shown. The 26 PqqEs selected for multiple sequence alignment are marked in yellow.