Supplementary File 1.

Multiple and overlapping functions of quorum sensing proteins for cell specialization in *Bacillus* species.

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Methods for phylogenetic reconstruction of Rap- and NprR- Like proteins and homologs search.

Phylogenetic reconstruction

Amino acid sequences of NprR- and Rap- like proteins were obtained from a previous work (1). From this dataset, we excluded sequences from eukaryotic organisms and then classified the remaining sequences in the following taxas: Actinobacteria, Aquificae, Bacteroidetes, Cyanobacteria, Clostridia, Eryspelotrichia, Lactobacillales, Other Bacillales, *Bacillus cereus* group, *Bacillus subtilis* group and other *Bacillus.* The *B. cereus* group includes all sequences from *B. anthracis, B. cereus, B. thuringiensis, B. mycoides, B. pseudomycoides, B. cytotoxicus* and *B. weihenstephanensis.* The *B. subtilis* group comprises all sequences from *B. subtilis, B. amyloliquefaciens, B. licheniformis, B. atrophaeus, B. mojavensis, B. sonorensis, B. tequilensis* and *B. vallismortis.* Sequences with insufficient data to be classified into any of these categories were excluded. For each tree, sequences where aligned using the Auto strategy of MAFFT v. 7 (2). Both phylogenies were then constructed in PhyML 3.0 (3) using the automatic model selection SMS (4), with the Akaike Information Criterion and the Approximate Likelihood-Ratio Test for Branches (aLRT) to support branches (5). For better visualization, individual branches were collapsed into the categories mentioned above.

NprR and Rap homologs search

We conducted BlastP searches for identifying homologs of NprR and Raps. For NprR, we queried the NprR sequence of *B. thuringiensis* 407 (AFV16330.1) against the dataset used for the phylogenetic reconstructions using the Blast2 online tool (6). This sequence was also queried against the NCBI Nucleotide collection (nr/nt) database (as of January 2020). NprR homolog sequences were identified as hits above ≥70% of identity. For Raps, we queried the RapH sequence of *B. subtilis* (WP_003243267.1) against the dataset used for the phylogenetic reconstructions. Rap homolog sequences were recognized as such when the BlastP results showed ≥ 60% of query cover with the query sequence and ≥ 20% of identity for Rap.

Supplementary File 2 Description.

Sheet 1-2. Delta-BLAST results for NprR (sheet 1) and Rap (sheet 2) queries from (1), used as dataset for phylogenetic reconstructions.

Sheet 3-4. NprR (sheet 3) and Rap (sheet 4) homolog sequence search in the dataset used for phylogenetic reconstructions. # Hit column represents the sequence hit number from sheets 1-2 (1). Identifier column: Actino, Actinobacteria; Aqui, Aquificae; Bact, Bacteroidetes; Cyano, Cyanobacteria; Proteo, Proteobacteria; Clos, Clostridia; Ery, Erysipelotrichia; LB, Lactobacillales; NBG (Non-Bacillus Genus), Other Bacillales; Bc, *B. cereus* group; Bs, *B. subtilis* group; Ob, Other *Bacillus*.

Sheet 5. tBlastn results for NprR query against the NCBI Nucleotide collection (nr/nt) database of the *B. cereus* group.

Sheet 6. tBlastn results for NprR query against the NCBI Nucleotide collection (nr/nt) database excluding the *B. cereus* group.

References

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