Bioinformatics characterization of PCBs and benzoate metabolism genes from Pseudomonas pseudoalcaligenes KF707

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Motivation

PCBs (polychlorinated biphenyls) had been widely used for a variety of industrial purposes. Due to their classification as persistent organic pollutants, their production was banned in 1979. However, PCBs-polluted sites are still present worldwide, thus leading to the need for environmental remediation strategies for PCB compounds. Several PCB-degrading bacteria had been previously isolated from anthropogenic polluted zones and had been screened to select those useful in bioremediation [1]. In this context, the γ-proteobacterium Pseudomonas pseudoalcaligenes KF707 is considered as one of the best candidate, due to its high heavy metal-resistance and its capability to grow either in planktonic or biofilm state [2]. Our aim was to investigate, within P. pseudoalcaligenes KF707 genome, bph and ben gene operon features, involved in the PCBs and the benzoate degradation pathways respectively. Since benzoate catabolism play a key role in aerobic bacterial PCBs degradation, we particularly focused on ben gene operons. Comparative genomic analyses extended to other classes out of γ-proteobacteria including order Pseudomonadales and phylogenetic analyses of protein encoding in ben operon were performed, in order to trace their ancestry.

Methods

P. pseudoalcaligenes KF707 genome sequence and the related annotations were previously described [3] and are available at NCBI BioProject Database (AC: PRJNA83639). As of March 2013, the KF707 Genome Project includes 233 contigs and the related annotated CDS total 6111. P. pseudoalcaligenes KF707 genes involved in the PCBs and benzoate degradation were identified by combining the inspection of genome annotations and the blast results of KF707 proteome against the protein RefSeq bacterial database (including the KF707 proteome itself) [4]. Virtual Footprint software [5] was used for promoter sequence search. Sequence Feature Scan tools, which combines different protein recognition methods within Swiss-model Workspace [6], was used to improve Ben and Bph protein predictions, to identify conserved domains and to predict elements of secondary structure. MAFFT [7] and the phylogeny tool herein implemented (Neighbour-Joining method) were used to carry out protein multi-alignments and the related phylogenetic trees. With the purpose to evaluate the whole KF707 metabolic pathway set, proteome and operons organization, the KFCyc based on the Pathway Tools software, was built.

Results

We found a putative bph operon with an arrangement that confirmed the evidences reported in Furukawa [1]. Thirty-five ben genes putatively involved in benzoate metabolism were recognized within P. pseudoalcaligenes KF707 genome and BlastP results suggested functional redundancy for 26% of them. The ben genes were apparently arranged in five operons (hereafter named ben operons) with different gene content. Out of the five ben operons, four contained the gene encoding for AraC family transcriptional regulator BenR, and in two of them a BenR binding dimeric motif (TGCA6GGNTA) was recognized. This evidence suggested that the regulation of the ben operons could be similar to the one previously described in Pseudomonas putida [8], one of the phylogenetic closest bacteria. Promoter search analysis showed two promoter classes among the five ben operons; AlgU(-10) and RhlR promoter elements were recognized upstream to ben operons. Phylogenetic analyses of Ben proteins from KF707 and the most similar homologues identified with BlastP
suggested that multiple copies of bene genes could derive from either intra-genomic duplications or events of horizontal transfer. One possible cause implies horizontal transfer or internal chromosomal rearrangements. Overall, our analyses could suggest that P. pseudoalcaligenes KF707 probably combined benzoate degradation capabilities from different bacterial species belonging to the same ecological niche. With the aim to broaden our knowledge on ben genes and to deeply investigate the whole KF707 proteome and interactome, the next step will deal with the Pathway/Genome Database KFCyc finishing and tuning thus allowing also a merging between dry and wet data.

References