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Short Communication

Diversity of Alkane-1-Monooxygenase Genes in Actinobacteria of the Genus *Rhodococcus*

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Abstract

By analyzing genetic similarity of *rhodococci* actinobacteria seven phylogenetic groups are allocated on the base on alkane-1-monooxygenases sequences. *R. qingshengii* F2-2 genome have been shown to have alkane-1-monooxygenase genes affiliated to five different types. We assume the use of alkane-1-monooxygenase genes diversity as a molecular marker to identify *Rhodococcus* species.

Keywords: *Rhodococcus*, Alkane degradation, Alkane-1-monooxygenase genes, classification

Short Communication

Rhodococci actinobacteria can degrade hydrocarbon compounds representative of various chemical structures and are used in bioremediation technologies. *Alk*ane-1-monooxygenase is the first enzyme catalyzing *alk*ane cleavage, transforming *alk*anes into primary alcohol. We analyzed protein sequences that include functional domains distinctive for the known *rhodococcal alk*ane-1-monooxygenases: Hist1 (HELGHK), Hist2 (EHNXGHH), Hist3 (LQRHSDHHA) and HYG (NYLEHYGI). Search for homologous *alk*ane-1-monooxygenases of *rhodococci* (threshold E value below 1e-156) was performed in an NCBI database using psi-blast utility. Using USEARCH software (version 11.0.667_i86linux32) [1] proteins were excluded whose homology to an *alk*ane-1-monooxygenase did not exceed 60%. Amino acid sequences of homologous *alk*ane-1-monooxygenases were aligned with a help of Clusta lX, version 2.1 [2] and then used to search for functional domains using MEME software, version 5.1.1 [3]. Search for regulatory sequences was performed using Sigmo ID software [4]. We have revealed that the quantity of *alkB* genes and their genetic organization depend on taxonomic affiliation of bacteria under study (Table 1). Phylogeny of *alk*ane-1-monooxygenase corresponds to phylogeny of *Rhodococcus* bacteria offered based on comparing genomes, vital genes, conservative proteins, DNA-DNA hybridization, and physiologo-biochemical traits [5]. By analyzing genetic similarity of *rhodococci* bacteria seven phylogenetic groups are allocated: A, B, C, D, E, F and G, with B (B1 and B2) group and E (E1 and E2) one having the highest polymorphism. Due to Table 1, most typical representative of A group is *R. hoagie/R. equi*; B1- *R. aetherivorans, R. ruber*; B2 - *R. coprophilus, R. pyridinivorans, R. rhodochrous*; C - *R. opacus, R. jostii, R. wratislaviensis, R. koreensis*; D - *R. erythropolis*, *R. qingshengii*; E - *R. fascians*; G - *R. triatomae.*

Table 1: Diversity of *rhodococcal* alkane-1-monooxygenases.

Group	Representative	*alk*B Gene Type in an Operon	Type of a Free Localized *alk*B gene	*alk*B Genes Quantity
A	*R. hoagie, equi*	*alk*B2	no	2
B1	*R. aetherivorans*	*alk*B1	*alk*B6	2
	R. rubber	*alk*B1		
B2	*R. coprophilus*	*alk*B2		
	R. pyridinivorans	*alk*B2		
	R. rhodochrous	*alk*B2		
C	*R. opacus*	*alk*B1	no	1
	R. jostii	*alk*B1		
	R. wratislaviensis	*alk*B1		
	R. koreensis	*alk*B1		
D	*R. erythropolis*	*alk*B1, *alk*B2	*alk*B3, *alk*B4	4
	R. qingshengii	*alk*B1, *alk*B2	*alk*B3, *alk*B4, *alk*B5	5
E	*R. fascians*	*alk*B1	*alk*B7, *alk*B8	3
F*	*R. corynebacterioides*	no	*alk*B8, *alk*B9	2
	R. kroppenstedtii		*alk*B8, *alk*B10	
G	*R. triatomae*	*alk*B1	no	1

Note: For bacteria *R. corynebacterioides* and *R. kroppenstedtii* one each genome as non-assembled nucleotide sequences set is in open access, and this does not allow to have final conclusions.

Rhodococcus qinqshengii F2-2 strains with high petroleum-utilizing activity was isolated from soils sampled from oil deposit Festivalnoe in Western Siberia, Russia [6]. The strain grows in media with crude oil and diesel fuel, produces tregalolipid biosurfactants and can use *alk*anes and benzene as a sole source of carbon and energy. In the study the genome of *Rhodococcus qinqshengii* F2-2 was sequenced using technologies of Oxford Nanopore and Illumina MiSeq and assembled. Genome includes a chromosome of 6.3 mb [7], two linear [8-9] plasmids of 156 (pLP156) and 337 kb (pLP337), and a circular one, pCP209, of 209 kb [10,11]. Surprisingly, it has managed to find a gene cluster encoding for phenazine synthesis on pLP156, that includes five genes: phzF, trans-2,3-dihydro-3-hydroxyanthranilate isomerase (EC:5.3.3.17), phzE, 2-amino-4-deoxychorismate synthase (EC:2.6.1.86), phzA_B, phenazine biosynthesis protein, phzG, dihydrophenazinedicarboxylate synthase (EC:1.10.3.16), and phzS, 5-methylphenazine-1-carboxylate 1-monooxygenase (EC:1.14.13.218). Genome has 6741 coding sequences (CDS), three rRNA clusters (5S, 16S and 23S) and 59 tRNAs. 5234 CDS can be affiliated to 25 different clusters of orthologous genes. *R. qingshengii* F2-2 genome has five *alk*ane-1-monooxygenase genes (Table 2).

Table 2: Alkane-1-monooxygenases of a strain *Rhodococcus sp*. TMP2 revealed in *R. qingshengii* F2-2.

Strain	*Alk*B1	*Alk*B2	*Alk*B3	*Alk*B4	*Alk*B5
Rhodococcus sp. TMP2 [11]	BAG06232.1 (partial)	BAG06233.1 (partial)	BAG06234.1 (partial)	BAG06235.1 (partial)	BAG06236.1 (partial)
R. qingshengii F2-2	ULD44392.1	ULD40270.1	ULD39415.1	ULD40441.1	ULD43115.1

The gene *alkB1* has a classic organization, namely, is in an operon presented by five genes (*alkB1, rubA1, rubA2, rubB* and *alkU1*). Three genes of the operon being electron carriers (rubredoxins encoded by *rubA1* and *rubA2* and a rubredoxin reductase encoded by *rub*B) provide an *alk*ane-1-monooxygenases functional activity. The gene *alkB2* is part of operons (*alkB2*, *rubA3*, *rubA4* and *alkU2*) with the last one's non-possessing determinants encoding for rubredoxin reductases. Thus, in genomes of *rhodococci* two type of *alkB* genes have managed to distinguish that are part of differently arranged operons, encoding *alk*ane-1-monooxygenases of two types-*AlkB1* и *AlkB2*, correspondingly. Herewith *AlkB1* type enzymes are typical for non-pathogenic degrader bacteria affiliated to B1 phylogenetic group (*R. aetherivorans, R. ruber*), C (*R. opacus, R. jostii, R. wratislaviensis, R. koreensis*) and G (*R. triatomae*) and

plant pathogens of E group (*R. fascians*). Enzymes of *AlkB2* type are synthesized in cells of B2 group-affiliated degrader bacteria (*R. coprophilus, R. pyridinivorans, R. rhodochrous*) and A group animal pathogens (*R. hoagie/equi*). D group is on a special place that includes degrader bacteria and best studied biosurfactant producers (*R. erythropolis*, *R. qingshengii*) that synthesize two types of phylogenetically distant *alk*ane-1-monooxygenases. *AlkB1* type is the closest to the same one in G group bacteria (*R. triatomae*), and *AlkB2* type demonstrates the greatest similarity to the same one of A group pathogenic bacteria (*R. hoagie/equi*). The highest quantity of distinct *alkB* genes (*alkB3*, *alkB4*, *alkB5*) is detected in bacterial genomes belonging to D phylogenetic group (*R. erythropolis*, *R. qingshengii*). Herewith, genes *alkB3* and *alkB4* are present in all the representatives of this group, whereas *alkB5* genes are detected in *R. qingshengii* strains only [12].

Based on the data analysis performed it may be concluded that phylogenetically most diverse *alk*ane-1-monooxygenases are produced by *R. erythropolis*, *R. qingshengii* belonging to group D (from four to five types of the enzymes). Separately localized *alkB* determinants would seem to emerge owing to duplication of *alkB* genes occurring in the corresponding operons, and further divergence process of gene copies proceeds. However, their conservative localization contradicts the last assumption: once appeared, *alkB* genes keep their localization, only their nucleotide sequences are changing. This situation is possible only because of horizontal gene transfer, much common process in prokaryotes. Understanding the genome formation of bacteria important for human is essential since cognate proteins must realize similar functions. *Alk*ane-1-monooxygenase genes appear to be a molecular marker to identify *Rhodococcus* species. The patterns revealed in this study complement the available to date data and can be useful to determine taxonomy and phylogeny of closely related *rhodococci*.

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Conflicts of Interest

The authors declare no conflict of interest.

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