



GENETIC ADAPTATION OF *PSEUDOMONAS STUTZERI* KOS6 TO HIGH TOXIC HABITATS AND EVOLUTION OF SYSTEMS FOR DIAZOTROPHIC DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS

Alexander Laikov¹, Tatiana Grigoryeva¹, Rudolf Müller², Alexander Manolov³, Andrey Larin³, Irina Karpova³, Tatiana Semashko³, Dmitry Alexeev³, Elena Kostryukova³, Vadim Govorun³
(a.v.laikov@gmail.com)

¹Kazan Federal University, Institute of Fundamental Medicine and Biology, Russia; ²Technical University of Hamburg-Harburg, Institute of Technical Biocatalysis, Germany; ³Scientific Research Institute of Physical-Chemical Medicine, Russia

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Introduction. *Pseudomonas stutzeri* is a Gram-negative bacterium widely distributed in the environment with an extremely broad phenotypic and genotypic diversity. It can rarely be found as an opportunistic pathogen in humans [1]. These bacteria are known to be nutritionally versatile and especially interesting because of specific metabolic abilities (denitrification, degradation of aromatic compounds, nitrogen fixation) which were found in some of the strains [2].

Genome assemblies for ten strains of *P. stutzeri*, including naphthalene-degrading, nitrogen-fixing, carbazole-degrading, arsenite-oxidizing, natural transformation, lactate utilization are already available at GenBank. Most of them were isolated from environmental samples, but insufficiently known about strains inhabiting anthropogenic econiche, like high toxic hydrocarbon sludge.

Methods. *P. stutzeri* strain KOS6 was isolated from industrial hydrocarbon sludge of a chemical enterprise in Kazan, Russia. The waste was stored about ten years in a special repository and can impact to inhabiting microorganisms. We used whole genome data of ten referred strains for comparison with the genome of strain KOS6. First of all we are interested in features which can be related with surviving under chemical waste conditions and can be useful for bioremediation technology of the oily sludge. As it was shown early oily sludge has a specific chemical composition, high toxicity and contains a number of microorganisms with a broad stress resistance, hydrocarbon-degrading and nitrogen fixing abilities [3].

Results. The genome of strain KOS6 was sequenced using the PGM sequencer system (Life Technologies). The 79 contigs was formed. The draft genome sequence of strain KOS6 consists of 5,014,616 bp with a GC content of 62.55%. The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAAP). This Whole Genome Shotgun project has been deposited at GenBank under the accession AMZ00000000. Multiple-genome comparisons were performed with BLAST. Strain KOS6 was most related to strain CCUG 29243 (blast total score: 4.560e+06, 70% query coverage), followed by strain DSM 4166 (4.270e+06, 68%), strain ATCC 17588 (4.272e+06, 67%) and strain A1501 (4.127e+06, 66%). Along with housekeeping genes and a typical

denitrification system the genome of KOS6 contains a number of genes, which are important for phytoremediation technology. These genes are involved in plant growth promotion, in resistance to toxicants and in aromatic and polycyclic aromatic hydrocarbon degradation. In contrast to genomes of nitrogen-fixing *P. stutzeri* (A1501, DSM 4166) there are genes for Type VI secretion systems, for transport systems and for additional pathways of aromatic compounds degradation in our strain. Moreover, strain KOS6 contains 37 unknown and 38 annotated genes homological to *Tolomonas auensis* DSM 9187 which are presumably related with survival under extreme conditions in the waste. Future investigations in the relationships between the newly discovered genes will help to understand evolution and adaptation of *P. stutzeri* as well as broaden our knowledge of the ecology and physiology of nitrogen-fixing bacteria.

Table 1. Comparative analysis of some genetic systems by whole genome sequences

Strain of <i>P. stutzeri</i>	Isolation source	Nitrogen fixation	VI type secretion system	Naphthalene degradation
KOS6	chemical sludge	+	+	+
A1501	rhizosphere	+		
ATCC14405	sea water			
ATCC17588	clinical specimen			
CCUG29243	polluted marine sediments		+	+
DSM10701	soil			
DSM4166	rhizosphere	+		
SDM-LAC	ND			
T13	activated sludge			
TS44	arsenic-contaminated soil		+	
XLDN-R	soil			

ND – no data

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