XVII. INTERNATIONAL SYMPOSIUM ON THE BIOLOGY OF ACTINOMYCETES (ISBA'17)
&
APPLICATIONS AND BIOTECHNOLOGY OF ACTINOMYCETES

BOOK OF ABSTRACTS

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Meta Basım Matbaacılık Hizmetleri
87 Sok. No. 4 / A Bornova
☎ (0.232) 343 64 54 ✉ metabasim@gmail.com
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Dear Fellow Actinomycetologists,

Welcome to the Aegean Shores of Turkey where the ***XVII. International Symposium on the Biology of Actinomycetes (ISBA'17)***, and a satellite meeting ***Applications and Biotechnology of Actinomycetes***, will take place.

ISBA'17 again will provide a successful platform for discussions related to new advances in the biology, molecular aspects and natural product chemistry of actinomycetes. The two meetings will be held jointly to gather researchers from multidisciplinary fields to encourage the exchange of new information, support interchange of opinions and establish effective communication between them.

We wish you a very pleasant stay in Kusadasi and thank you for your contributions towards the success of another ISBA.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF FIVE ACTINOBACTERIA STRAINS ISOLATED FROM HYDROCARBON-CONTAMINATED SOIL SAMPLES

Marija Ljesevic[1], Gordana Gojgic-Cvijovic[2], Jelena Milic[2], Vladimir Beskoski[1], Miroslav M. Vrvic[1]

[1]Faculty of Chemistry, University of Belgrade, Studentski Trg 16, 11158 Belgrade, P. O. Box 51, Serbia
[2]ICHTM- Department of Chemistry, University of Belgrade, Njegoševa 12, 11158 Belgrade, P. O. Box 2 473, Serbia

Hydrocarbon contaminated soil has a great number of substrates suitable for the growth of complex microbial community. Isolation and characterization of bacteria involved in transformation of these substrates helps optimize the process of bioremediation.
In this study chemotaxonomic and biochemical methods were used to compare five Gram positive bacterial strains labeled RNP05, CHP-ZH25, CHP-NR31, CHP-315 and NS094. The strains were isolated from contaminated soil samples taken near oil refineries in Pancevo and Novi Sad (Serbia).

The strains were identified by 16S rRNA gene sequencing. Fatty acid composition was determined by GC/MS after derivatization in methanol: toluene: sulphuric acid mixture. Utilization of different carbon sources (phenanthrene, phenol, 4-hydroxybenzoic acid, 3,4-hydroxybenzoic acid, sodium benzoate, diesel fuel, motor oil) was examined on mineral medium. Tolerance to heavy metals was studied on Mueller-Hinton agar with increasing concentrations of $CuSO_4x5H_2O$, $Cd(CH_3COO)_2$, $NiCl_2$, $Zn(CH_3COO)_2$ and $K_2Cr_2O_7$. Specific enzyme activities were detected using API ZYM test.

The strains RNP05 and CHP-NR31 were identified as members of *Rhodococcus* genus, while strains CHP-ZH25, CHP-315 and NS094 represent *Oerskovia*, *Gordonia* and *Micromonospora spp.* respectively. *Rhodococcus sp.* CHP-NR31 is rich in palmitic, myristic, oleic and tuberculostearic (10-methyloctadecanoic) acid. It shows the highest tolerance to nickel (Ni^{2+}) and tested positive for esterase C4, esterase lipase C8, lipase C14, leucine and cysteine arylamidase, acid phosphatase, α-glucosidase, α-galactosidase and β-galactosidase. *Rhodococcus sp.* RNP05 contains more than 30% of 10-methyloctadecanoic acid. It has the highest tolerance to zinc (Zn^{2+}). It tested positive for alkaline and acid phosphatase, esterase C4, esterase lipase C8, leucine, valine and cysteine arylamidase, trypsine, α-chymotrypsine, naphtol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase. *Oerskovia sp.* CHP-ZH25 has a high amount of branched chain fatty acids (12-methyltetradecanoic, 13-methyltetradecanoic and 15-methylhexadecanoic acid). It has the highest tolerance to nickel (Ni^{2+}). *Micromonospora sp.* NS094 is rich in palmitic, octadecenoic and 13-methyltetradecanoic acid. It shows the highest tolerance to chromium (Cr^{3+}). It tested positive for esterase lipase C8, leucine and valine arylamidase, and β-galactosidase. *Gordonia sp.* CHP-315 is rich in 14-methylpentadecanoic, 10-methyloctadecanoic and octadecenoic acid. It has the highest tolerance to copper (Cu^{2+}) and positive reaction for alkaline and acid phosphatase, esterase lipase C8, lipase C14, leucine and valine arylamidase and α-glucosidase. All the strains were capable of using phenol, 4-hydroxybenzoic acid, diesel fuel and motor oil as a sole source of carbon. *Rhodococcus sp.* RNP05 could use 3,4-hydroxybenzoic acid. Phenanthrene was used by all the strains except *Gordonia sp.* CHP-315 and sodium benzoate by *Rhodococcus sp.* CHP-NR31, *Rhodococcus sp.* RNP05 and *Micromonospora sp.* NS094.

On the basis of hydrocarbon utilization and metal tolerance tests, *Rhodococcus* strains have the highest biodegradation potential. The cellular fatty acid profiles of all tested strains are in accordance with data previously reported in the literature.

Keywords: Hydrocarbon contamination, Bacterial characterization

Molecular and bioochemical characterization of five Actinobacteria strains isolated from hydrocarbon-contaminated soil samples

M. Lješević[1,*], G. Gojgić-Cvijović[2], J. Milić[2], V. Beškoski[1], M.M. Vrvić[1]

[1] *Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11158 Belgrade, P. O. Box 51, Serbia*
[2] *IChTM- Department of Chemistry, University of Belgrade, Njegoševa 12, 11158 Belgrade, P. O. Box 473, Serbia*
*For correspondence: marijaljesevic@gmail.com

Introduction

Hydrocarbon contaminated soil has a great number of substrates suitable for the growth of complex microbial community. Microbial strains isolated from contaminated environment have attracted much attention not only as a rich source of novel pathways and metabolites, but also as potential bioremediation agents. In selection and evaluation of environmental isolates for future implementation the different methods have been employed.

In the present study chemotaxonomic and biochemical methods were used in order to compare five Gram positive bacterial strains labeled as RNP05, CHP-ZH25, CHP-NR31, CHP-315 and NS094. The strains were isolated from contaminated soil samples taken near oil refineries in Pancevo and Novi Sad, Serbia [1,2].

Material and methods

The bacterial strains were identified by 16S rRNA gene sequencing. Composition of fatty acids was determined by GC/MS after derivatization in methanol : toluene : sulphuric acid mixture. Utilization of different carbon sources (phenanthrene, phenol, 4-hydroxybenzoic acid, 3,4-hydroxybenzoic acid, sodium benzoate, diesel fuel, motor oil) was examined on mineral medium. Tolerance to heavy metals was studied on Mueller-Hinton agar with increasing concentrations of $CuSO_4x5H_2O$, $Cd(CH_3COO)_2$, $NiCl_2$, $Zn(CH_3COO)_2$ and $K_2Cr_2O_7$. Specific enzyme activities were detected using API ZYM test.

Table 1. Tolerance to metal ions, minimum inhibitory concentration MIC (mmol/L)

Strain	Cadmium $Cd(CH_3COO)_2$	Nickel, $NiCl_2$	Copper, $CuSO_4x5H_2O$	Zinc, $Zn(CH_3COO)_2$	Chromium, $K_2Cr_2O_7$	Pb	Fe
Rhodococcus sp. RNP05	50	25	10	>50	50	>50	50
Micromonospora sp. NS094	1	2.5	1	/	2.5	/	/
Oerskovia sp. CHP-ZH25	2.5	50	10	10	10	/	/
Rhodococcus sp. CHP-NR31	<1	>50	5	2.5	2.5	/	/
Gordonia sp. CHP-315	10	10	25	10	2.5	/	/

Table 3. Cellular fatty acid composition of isolated strains, % of total detected

Fatty acid	CHP-NR31	CHP-ZH25	CHP-315	RNP05	NS094
i12:0	nd	nd	nd	nd	0.55
12:0	0.74	0.07	0.09	nd	0.4
i13:0	nd	nd	nd	nd	5.7
ai13:0	nd	nd	nd	nd	2.15
13:0	0.21	0.30	0.04	nd	nd
i14:0	nd	nd	nd	nd	1.82
14:0	9.93	13.31	0.19	5.8	2.63
i 15:0	nd	19.20	nd	nd	11.79
ai 15:0	1.98	34.43	nd	nd	4.44
15:0	4.79	5.03	1.80	6.53	0.84
i 16:0	0.05	9.69	46.68	nd	5.62
16:1	0.04	0.13	0.51	1.96	1.54
16:0	57.72	0.18	nd	44.08	28.07
i 17:0	0.07	16.19	nd	3.33	8.51
ai17:0	nd	nd	nd	nd	6.83
cy 17:0	0.01	nd	0.04	nd	nd
17:0	1.68	0.34	4.67	1.22	1.77
i18:0	nd	nd	nd	2.58	nd
18:1	11.19	nd	3.32	3.66	13.14
18:1	0.33	nd	10.88	nd	nd
18:0	1.92	1.14	3.61	0.70	3.97
i19:0	nd	nd	nd	30.15	nd
cy 19:0	nd	nd	0.07	nd	nd
19:0	9.34	nd	27.91	nd	nd
20:0	nd	nd	0.20	nd	nd

References

1. G.D. Gojgic-Cvijovic et al., (2012) Biodegradation of petroleum sludge and petroleum polluted soil by a bacterial consortium: a laboratory study, Biodegradation 23 :1-14.
2. J. S. Milic et al., (2009) Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium, J. Serb. Chem. Soc. 74 (4) : 455-460.

Results

The strains RNP05 and CHP-NR31 were identified as members of *Rhodococcus* genus, while strains CHP-ZH25, CHP-315 and NS094 represent *Oerskovia*, *Gordonia* and *Micromonospora spp.* respectively. *Rhodococcus sp.* CHP-NR31 (GenBank JX965395) is rich in palmitic, myristil, oleic and tuberculostearic acid. It has highest tolerance to nickel (Ni^{2+}) and tested positive for esterase C4, esterase lipase C8, lipase C14, leucine and cysteine arylamidase, acid phosphatase, α-glucosidase, α-galactosidase and β-galactosidase. *Rhodococcus sp.* RNP05 (GenBank JQ065876) contains more than 30% of 17-methyiloctadecanoic acid. It has the highest tolerance to zinc (Zn^{2+}) and tested positive for alkaline and acid phosphatase, esterase C4, esterase lipase C8, leucine, valine and cysteine arylamidase, trypsine, α-chymotrypsine, naphtol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase. *Oerskovia sp.* CHP-ZH25 (GenBank JX430000) has the high amount of branched chain fatty acids (12-methyltetradecanoic, 12-methyltetradecanoic and 15-methylhexadecanoic acid). It has the highest tolerance to nickel (Ni^{2+}). *Micromonospora sp.* NS094 (GenBank JF826530) is rich in palmytic, octadecenoic and 13-methyltetradecanoic acid. It has the highest tolerance to chrome (Cr^{3+}) and nickel (Ni^{2+}). It has tested positive for esterase lipase C8, leucine and valine arylamidase, and β-galactosidase. *Gordonia sp.* CHP-315 (GenBank JX429999) is rich in 14-methylpentadecanoic, 10-methyloctadecanoic and octadecenoic acid. It has the highest tolerance to copper (Cu^{2+}) and positive reactions for alkaline and acid phosphatase, esterase C4, esterase lipase C8, lipase C14, leucine and valine arylamidase and α-glucosidase. All the strains were capable of using phenol, 4-hidroxybenzoic acid, diesel fuel and motor oil as a sole source of carbon. *Rhodococcus sp.* RNP05 could use 3,4-hydroxybenzoic acid. Phenanthrene was used by all the strains except *Gordonia sp.* CHP-315 and sodium benzoate by *Rhodococcus sp.* CHP-NR31, *Rhodococcus sp.* RNP05 and *Micromonospora sp.* NS094. The detailed results are shown in Tables 1-4.

Table 2. Microbial growth on diesel fuel and different aromatic compounds as the sole C source

Strain	Phenol	Phenanthrene	3,4-hydroxybenzoic acid	Sodium benzoate	Motor oil	Diesel fuel	4-hydroxybenzoic acid
Oerskovia sp. CHP-ZH25	+	+	-	-	+	+	+
Micromonospora sp. NS094	/	+	/	+	/	+	/
Rhodococcus sp. RNP05	+	+	+	+	+	+	+
Gordonia sp. CHP-315	+	-	-	-	/	+	+
Rhodococcus sp. CHP-NR31	+	+	-	+	+	+	+

Table 4. Api Zym

Reaction	CHP-315	RNP05	NS094	CHP-NR31	Reaction	CHP-315	RNP05	NS094	CHP-NR31
Alkaline phosphatase	+	+	-	-	Naphtol-AS-BI-phosphohydrolase	-	+	-	-/+
Esterase C4	+	+	-	+	α-galactosidase	-	-	-	+
Esterase lipase C8	+	+	+	+	β-galactosidase	-	-	+	+
Lipase C14	+	-	-	+	β -glucuronidase	-	-	-	-
Leucine arylamidase	+	+	+	+	α-glucosidase	+	+	-	+
Valine arylamidase	+	+	+	-	β-glucosidase	-	+	-	-
Cysteine arylamidase	-	+	-	+	N-acetyl-β-glucosaminidase	-	+	-	-
Trypsine	-	+	-	-	α-mannosidase	-	-	-	-
α-chymotrypsine	-	+	-	-	α-fucosidase	-	-	-	-
Acid phosphatase	+	+	-	+					

Conclusion

On the basis hydrocarbon utilization and metal tolerance tests the studied Rhodococcus strains have the highest biodegradation potential. The cellular fatty acid profiles of all tested strains are in accordance with data previously reported in the literature.

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