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

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

ISBN 978 – 605 – 65121 – 0 – 0

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İzmir, Eylül – 2014
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.

On behalf of the Organizing committee for ISBA'17,

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

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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 CuSO4x5H2O, Cd(CH3COO)2, NiCl2, Zn(CH3COO)2 and K2Cr2O7. Specific enzyme activities were tested 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-methylotadecanoic) acid. It shows the highest tolerance to nickel (Ni2+) 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-methylotadecanoic acid. It has the highest tolerance to zinc (Zn2+). It tested positive for alkyllic and acid phosphatase, esterase C4, esterase lipase C8, leucine and cysteine arylamidase, trypsin, α-chymotrypsin, naphthol-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 (Ni2+). Micromonospora sp. NS094 is rich in palmitic, octadecenoic and 13-methyltetradecanoic acid. It shows the highest tolerance to chromium (Cr3+). It tested positive for esterase lipase C8, leucine and valine arylamidase, and β-galactosidase. Gordonia sp. CHP-315 is rich in 14-methylpentadecanoic, 10-methylotadecanoic and octadecenoic acid. It has the highest tolerance to copper (Cu2+) and positive reaction for alkyllic 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 biochemical characterization of five Actinobacteria strains isolated from hydrocarbon-contaminated soil samples

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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 isolated as RNP05, CHP-ZH25, CHP-NR31, CHP-315 and NS094. The strains were isolated from contaminated soil samples taken near oil refineries in Pančevo 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 CuSO4·5H2O, CdCl2·H2O·2H2O, NiCl2, ZnCl2·H2O, K2Cr2O7. Specific enzyme activities were detected using API ZYM test.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>CdCl2·H2O·2H2O</th>
<th>NiCl2</th>
<th>K2Cr2O7</th>
<th>ZnCl2·H2O</th>
<th>CuSO4·5H2O</th>
<th>Pb</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodococcus sp. RNP05</td>
<td>25</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Micromonospora sp. NS094</td>
<td>1</td>
<td>2.5</td>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Oerskovia sp. CHP-ZH25</td>
<td>2.5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Rhodococcus sp. CHP-NR31</td>
<td>&lt;1</td>
<td>&gt;50</td>
<td>5</td>
<td>2.5</td>
<td>2.5</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Gordonia sp. CHP-315</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>2.5</td>
<td>2.5</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenol</th>
<th>Phanenche</th>
<th>3,4-hydroxybenzoic acid</th>
<th>Sodium benzoate</th>
<th>Motor oil</th>
<th>Diesel fuel</th>
<th>4-hydroxybenzoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oerskovia sp. CHP-ZH25</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Micromonospora sp. NS094</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Rhodococcus sp. RNP05</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gordonia sp. CHP-315</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rhodococcus sp. CHP-NR31</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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.

Acknowledgement

Serbian Ministry of Education, Science and Technological Development (Project no. III 43004)

References