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7th BALKAN CONGRESS OF MICROBIOLOGY
8th CONGRESS OF SERBIAN MICROBIOLOGISTS

PROCEEDINGS

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New Bacillus circulans strain isolated from oil shale

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New strain was obtained during experiments of oil shale demineralization, which was carried out in order to get "pure" organic matter (kerogen). The application of microbiological method for demineralization of oil shale by B. circulans resulted in higher demineralization efficiency.

Cells of isolated strain were Gram-positive, spore-forming regular rods, 0.5 x 4 µm, with rounded ends, often in pairs. Spores were ellipsoid with terminal position. On the modified Ashby’s medium, the isolates formed mucous, transparent convex colonies, with even edges and smooth surface. The strain was also catalase positive. After applying API 50CHB and 20E tests, the new strain showed a positive reaction in fermentation of ribose, glucose, fructose, N-acetil-glucosamine, arbutine, esculine, salicine, cellobiose, maltose, trehalose, glycogen, gluconate, glycerol and hydrolysis of gelatin and starch. Chemotaxonomically, strain is characterized by a guanine-to-cytosine DNA ratio of 43.2 mol%. The most abundant cellular fatty acids are C15:0 ai, C16:0, C16:0 ai and C17:0 ai. The BLAST sequence homology search revealed that 16S rDNA sequence of strain VD01 showed homology with 16S rRNA from B. circulans strains. B. circulans strain exhibits a unique chromosome profile as it was detected in PFGE analysis and results clearly indicated that both AcsI and NotI treatments are well adapted to PFGE of B. circulans, because they generated DNA fragments well resolved for visual comparison. The protein patterns of newly isolated strain exhibited clear bands detected within the range from 30 to 160 kDa.

On the bases of the intensive comparisons with known species of Bacillus, the newly isolated strain was identified as a new one of B. circulans VD01, which proved to be very efficient in the bacterial desilification of oil shale.
NEW BACILLUS CIRCULANS STRAIN ISOLATED FROM OIL SHALE

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INTRODUCTION

B. circulans is a typical chemomangaferritrophic bacterium using mono, di- and polysaccharides and polyhydroxylic alcohols as source of carbon, energy and electrons. The efficiency in disintegrating silicates and aluminosilicates is not equal in all strains of siliceous bacteria and it varies depending on the nature of silicates and the bacterial metabolic products, primarily organic acids and the mucous polysaccharide capsule. The activity of B. circulans is attributed to its metabolites and their specific reactions such as acidolysis, alkalization and complexolysis.

Kerogen is a mixture of organic compounds. It represents an insoluble component of oil shale, a compact sedimentary rock of homogenous fine-composition. Preparation of pure and unaltered kerogen concentrate was always of geochemical interest. Investigations of the kerogen chemical composition and structures generally requires preliminary isolation of unchanged organic material. The isolation of native kerogen is a difficult task due to its complex nature and insolubility and the fact that in sediments, kerogen is mixed intimately with large amount of diverse minerals, such as carbonates, silicates and pyrite. Siliceous bacterium B. circulans was also proposed for bacterial desilification of the oil shale.

RESULTS

iserite was mixed with petroleum. On the modified Ashby’s medium, the colonies formed were transparent, circular colonies, with even edges and smooth surface. The colonies of these microorganisms were catalase positive. It was clear that the newly isolated strain belonged to the genus Bacillus.

After applying API 50 CH and 20 E tests, the new strain showed a positive reaction in fermentation of ribose, glucose, fructose, N-acetyl-glucosamine, arbutin, esculine, salicine, celebicine, maltose, trehalose, glycerogen, glucosone, glyceral and hydrolysis of gelatin and starch. According to the biochemical features, newly isolated strain was very similar to B. circulans.

Chromatographically, strain is characterized by a guanine-to-cytosine DNA ratio of 43.2 mol%. Hence the range of mol % G + C in Bacillus strains is from about 33 to 65%, although strains of most species cluster between 40-50%, we have good correspondence here. Percentage calculated was somewhat higher than the reference value for B. circulans, but variations are possible and they show a wide range.

The BLAST sequence homology search revealed that 16S rDNA sequence of strain VDO1 showed homology with 16S rDNA from B. circulans strains.

The most abundant cellular fatty acids are C15:0 al, C16:0, C16:0 ai and C17:0 ai and collectively account for up to 85% of the total fatty acid content. (Table 1).

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Fatty acid content (% of total fatty acids)</th>
<th>Fatty acid content (% of total fatty acids)</th>
</tr>
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<tbody>
<tr>
<td>C15:0</td>
<td>5.6</td>
<td>2</td>
</tr>
<tr>
<td>C16:0</td>
<td>4.6</td>
<td>2</td>
</tr>
<tr>
<td>C17:0</td>
<td>2.6</td>
<td>2</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.8</td>
<td>1</td>
</tr>
<tr>
<td>C20:0</td>
<td>1.3</td>
<td>1</td>
</tr>
</tbody>
</table>

The protein patterns of newly isolated strain exhibited clear bands detected within the range from 30 to 180 kDa.

CONCLUSION

On the bases of the intensive comparisons with known species of Bacillus, the newly isolated strain was identified as a new one of B. circulans VDO1, which proved to be very efficient in the bacterial desilification of oil shale.

MATERIAL AND METHODS

Biochemical characteristics

Bacilli, fulfilling the morphology and general biochemical characteristics of genus Bacillus, were assayed in commercial kits API 20E and 50CH as described by Berkeley et al. Strain was identified by analyzing the test results (scoring according to manufacturer’s protocol) with API Lib Plus PC version software.

Amplification of 16S rDNA by PCR and sequence determination

In order to obtain 16S rDNA sequences, polymerase chain reaction (PCR) was carried out using primers P1 and P2 and the PCR products were analyzed by agarose gel electrophoresis. The PCR products were cloned into plasmid pBR322, and subsequently into M13. The nucleotide sequence of the cloned inserts was determined using the dideoxy method. The sequences obtained were analyzed using the seqman software package.

PFGE of genomic DNA

The low melting point agarose (Sigma Chemical Co., St. Louis, USA) embedded chromosomal DNA of the newly isolated strain was prepared from 6 h cultures according to Giesa’s method. The DNA was restricted with HaeIII (Roche Biosciences, Mannheim, Germany), EcoRI, and SpeI. The restriction digest, was loaded onto a 0.8% agarose gel in 0.5X TBE buffer at 100 V for 3 h, and the gel was stained with ethidium bromide (1 µg/mL) and UV illuminated with a UV transilluminator. The gel was transferred to a nylon membrane, and the DNA hybridized to a probe using the alkaline elution method. The probe was labeled with [32P]dCTP (1000 Ci/mmol) and [3H]dCTP (100 Ci/mmol) by 32P- and 33P-labeling, respectively.

SDS-PAGE of whole cell proteins

The analysis was performed as described by Costas (2). Electrophoresis was performed at the constant current of 20 mA, using a 10% separating gel (pH 8.8) and a 5% stacking gel (pH 6.8). The mini gel electrophoresis equipment SE250 was purchased from Hoefer Scientific Instruments (San Francisco, CA, USA). Molar mass standard (15-250 kDa) was purchased from Amersham (Bioscience).

Determination of fatty-acid composition (FAME)

The holoell cell fatty acid composition of (100 mg in 5 mL of NaOH-CH3OH) was heated for 15 min at 300°C. The saponified material was cooled and acidified to pH 2. Boron trifluoride methanol (BF3-CH3OH) reagent was added in portion of saponified material (4 mL), and the mixture was re-heated for 5 min at 300°C. The content of the tube was added to 10 mL of saturated sodium chloride solution and the methyl esters were extracted twice with an equal volume of 1:1 hexane:isoamyl alcohol.

The determination of fatty-acid composition of bacterial biomass was performed on Agilent 6890 chromatograph with Agilent 5973 mass detector (MSD).

REFERENCES