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Characterization of Two Hydrocarbon-degrading Bacteria and Determination of Their Biodegradation Potential

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Backround

Oil contaminated soil is a widespread problem that requires clean-up of the contaminated site. Biodegradation by naturally occurring microorganisms could be a major mechanism for the removal of petroleum from the environment. Microbial strains needs to be examined in laboratory conditions in order to use them in full-scale bioremediation procedure.

Aim

The aim of this study was to characterize two hydrocarbon-degrading bacteria isolated from oil polluted site and examine their biodegradation potential.

Material and Methods

Bacteria were isolated from Oil rafinery Pancevo (Serbia) and identified based on 16S RNA sequences.Bacterial strains were characterized using API system (API 20NE, API Coryne; bioMerieux, France). Biodegradation potential of isolated bacteria has been tested for degradation diesel oil (2000ppm), at 28°C and rotation of 120 rpm for one month. TPH content lost was followed using GC-MS and gravimetry measurements.

Results

Isolated bacteria were identified as *Rhodococcus sp* and *Planomicrobium sp*, based on 16S RNA sequence. Both bacterial strains gave positive results in following test: Nitrate reduction, mannitol, glucinate and malate assimilation. In addition, *Planomicrobium sp* was positive in glucose fermentation test and has cytochrome oxidase. *Rhodococcus sp* is positive in citrate i phenyl-acetate assimilation, and doesn't have cytochrome oxidase. Biodegradation test showed both strains are extremely efficient in diesel oil degradation, with 98.75 % and 99.45 % of biodegraded TPH after 30 days, for *Planomicrobuim sp* and *Rhodococcus sp*, respectively.

Conslusion

On the basis of results it can be concluded that hydrocarbon-degrading bacteria isolated from petroleum polluted site is extremely effective in biodegradation process. Furthermore, obtained results have shown occurrence of natural selection among microorganisms in petroleum-containing sites which supports thesis that is enough to stimulate these strains during bioremediation.

Keywords: Petroleum biodegradation, hydrocarbon-degrading bacteria

CHARACTERIZATION OF TWO HYDROCARBON-DEGRADING BACTERIA AND DETERMINATION OF THEIR BIODEGRADATION POTENTIAL

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BACKROUND

Oil contaminated soil is a widespread problem that often requires clean-up of the contaminated site. It has been suggested that the addition of hydrocarbon-degrading microbes to polluted site is less effective in hydrocarbons removal than stimulating the growth of the indigenous microorganisms with potential in biodegradation. That's why biodegradation by naturally occurring microorganisms could be a major mechanism for the removal of petroleum from the environment. First of all, microbial strains needs to be examined in laboratory conditions in order to use them in full-scale bioremediation procedure.

The aim of this study was to characterize two hydrocarbon-degrading bacteria isolated from oil polluted site and examine their biodegradation potential.

METHODS

Bacteria were isolated from Oil refinery Pancevo (Serbia) and identified based on 16S RNA sequences. Bacterial strains were characterized using API system (API 20NE, API Coryne; bioMerieux sa, France). Biodegradation potential of isolated bacteria has been tested for degradation diesel oil (2000ppm), at 28°C and rotation of 120 rpm for one month. TPH content lost was followed using GC-MS and gravimetry measurements.

RESULTS

Isolated bacteria were identified as *Planomicrobium sp.* RNP01 and *Rhodococcus sp.* RNP05, based on 16S RNA sequence. Figure 1. shows phylogenetic distance between these two isolates.

Fig 1. Phylogenetic tree showing the relationship between two ispolates

Both bacterial strains gave positive results in following API system tests: nitrate reduction, mannitol, glucinate and malate assimilation. In addition, *Planomicrobium sp* RNP01 was positive in glucose fermentation test and has cytochrome oxidase. *Rhodococcus sp* RNP05 is positive in citrate and phenyl-acetate assimilation, and doesn't have cytochrome oxidase.

Biodegradation test showed both strains are extremely efficient in diesel oil degradation, with 98,75 % and 99,45 % of biodegraded TPH after 30 days, for *Planomicrobuim sp.* RNP01 and *Rhodococcus sp.* RNP05, respectively.

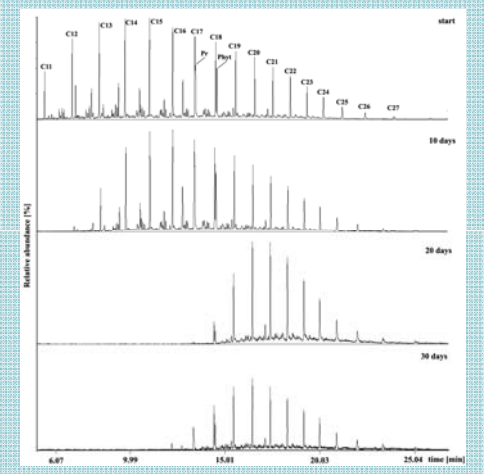

Fig 2. GC-MS analysis of diesel oil biodegradation by *Planomicrobium sp.* RNP01

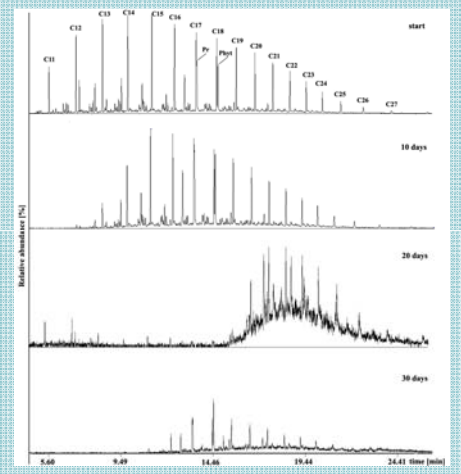

Fig 3. GC-MS analysis of diesel oil biodegradation by *Rhodococcus sp.* RNP05

Figures 2. and 3. shows fragmentograms of *n*-alkane fractions changes (*m/z* 57) during biodegradation. Both bacteria in the first twenty days degraded alkanes with lower molecular weight. At the end of experiment *n*-alkanes were almost completely degraded in both experiments. GC-MS analysis after 30 days of experiment showed that *Rhodococcus sp.* RNP05 is more efficient in diesel oil degradation than *Planomicrobium sp.* RNP01, which is in correlation with gravimetric analysis.

CONCLUSIONS

On the basis of results it can be concluded that hydrocarbon-degrading bacteria isolated from petroleum polluted site is extremly effective in biodegradation process. Furthermore, obtained results have shown occurence of natural selection among microorganisms in petroleum-containing sites which supports thesis that is enough to stimulate these strains during bioremediation.

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