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Effect of yeast extract on fluoranthene degradation and aromatic ring dioxygenase expressing bacterial community structure of a fluoranthene degrading bacterial consortium

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ABSTRACT

Fluoranthene (Fla) is a high molecular weight polycyclic aromatic hydrocarbon that exerts hazardous effects on living organisms. An efficient Fla degrading bacterial consortium LP was enriched from an oil contaminated soil sample, with and without yeast extract as a supplement. Objective of the present study was to see if there was any differential effect of yeast extract addition on Fla degradation potential and aromatic ring dioxygenase expressing bacteria (ARDB) of the enrichments. Primary enrichment of the soil sample was carried out in minimal salt medium (MSM) added with 500 mg l^{-1} Fla and 0.05% yeast extract (YMSM). Secondary, tertiary and subsequent enrichments were prepared in YMSM and MSM after every sixteen days of incubation. Fla was efficiently degraded by YMSM enriched culture than MSM enriched culture. However, when MSM enrichment was incubated longer instead of further sub-culturings, it also degraded Fla efficiently. All three enrichments exhibited growth of bacterial colonies on Fla sprayed minimal agar plates however only YMSM enrichment showed clear zone forming bacterial colonies. A positive effect was observed of yeast extract on ARDB population of LP consortium. To our limited knowledge this is first time that effect of yeast extract on ARDB population was studied.

1. Introduction

Extensive changes due to human activity in different ecosystems of our world have raised the issue of environmental pollution. One of the different sorts of environmental pollution is contamination of hydrocarbons in the form of crude oil, its refined products and different components, especially polycyclic aromatic hydrocarbons (PAHs). Numerous microorganisms with the potential to degrade low molecular weight (LMW) PAHs have been isolated however, only few isolated microorganisms can break down high molecular weight (HMW) PAHs consisting of four or more aromatic rings (Dean-Ross and Cerniglia, 1996; Pagnout et al., 2006; Sun et al., 2010). Fla, a four ring compound, is one of the 16 PAHs which have been identified as priority pollutants by US Environmental Protection Agency. Fla has been reported to be a recalcitrant genotoxic, cytotoxic, weakly mutagenic and potentially carcinogenic. Degradation of Fla as a sole carbon and energy source by pure bacterial strains was first reported in 1990 and since then several pure bacterial strains have been isolated with the potential to degrade Fla (Mueller et al., 1990; Weissenfels et al., 1990; Walter et al., 1991; Sepic et al., 1998; Herwijnen et al., 2003; Xu et al., 2011). However, recalcitrant compounds are generally better degraded by microbial consortia than any single microbial organism (Perry, 1979) and different microbial consortia, with the potential to degrade Fla efficiently, have been reported (Mueller et al., 1989; Trzesicka-Mlynarz and Ward, 1995; Abalos et al., 2004; Luan et al., 2006; Lin and Cai, 2008).

A variety of Gram-negative and Gram-positive bacteria possess aromatic-ring-hydroxylating dioxygenases (ARHDs) which catalyse the initial reaction in bacterial degradation of PAHs and a range of other compounds including monocyclic aromatic hydrocarbons, aromatic acids, chlorinated aromatics, heterocyclic aromatics as well as a wide range of other environmental pollutants (Gibson and Parales, 2000; Piper et al., 2004; Singh and Ward, 2004; Parales and Resnick, 2006; Xu-Xiang et al., 2006). Bacteria capable to produce these aromatic hydrocarbon dioxygenases are known as aromatic ring dioxygenase-expressing bacteria (ARDB). Abundance of ARDB with diverse range of PAHs catabolic oxygenase enzymes is desired

Abbreviations: ARDB, Aromatic ring dioxygenase expressing bacteria; ARHDs, Aromatic-ring-hydroxylating dioxygenases; DGGE, Denaturing gradient gel electrophoresis; Fla, Fluoranthene; HMW, High molecular weight; LMW, Low molecular weight; MSM, Minimal salt medium; MPN, Most probable number; PAHs, Polycyclic aromatic hydrocarbons; YMSM, Yeast extract added minimal salt medium.

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for effective biodegradation process. A well establish method of detecting ARDB is the use of colorimetric indicator indole (Singh and Ward, 2004). Indole in minimal salt agar is oxidized into a blue colour compound indigo by dioxygenase enzymes of ARDB and bacterial colonies on agar become blue in colour.

Nutrient supplements, inorganic or organic, are reported to stimulate the growth of PAHs degrading microorganisms and enhance their ability to degrade PAHs and oil contaminants (Rolling et al., 2002; Hamme et al., 2003; Zhou and Hua, 2004). Several inorganic and organic nutrients have been reported to influence the rate of hydrocarbons degradation (Walworth and Reynolds, 1995; Braddock et al., 1997; Wong et al., 2002; Ueno et al., 2006, 2007; Zhong et al., 2007). Different studies have reported enhancing effect of yeast extract on degradation of different hydrocarbons (Kim et al., 2003; Chang et al., 2008; Huang et al., 2008).

This research was carried out to (a) obtain an efficient Fla degrading bacterial consortium and (b) observe the effect of yeast extract addition during enrichment and in subsequent subculturings on ARDB community structure and Fla degradation potential of the obtained consortium.

2. Materials and methods

2.1. Media composition, chemicals and stock solutions

Media used throughout the study were minimal salt medium (MSM) and MSM supplemented with 0.05% yeast extract (YMSM) at pH 7 (Wongsa et al., 2004). Stock solution of Fla (1%) was prepared by dissolving Fla crystals in hexane and acetone to be used in degradation test and spraying the minimal agar plates, respectively.

2.2. Sample collection, enrichment and subculturing

An oil contaminated soil sample was collected from an abandon oil field in Ishikari city, Hokkaido prefecture, Japan and enriched on Fla to isolate Fla degrading bacterial consortium. For primary enrichment, 1 g soil sample was directly inoculated in 30 ml YMSM added with Fla crystals (500 mg l^{-1}) in 100 ml screw cap flask and incubated at 30 °C and 180rpm for two weeks. First subculturing of enriched culture was carried out in MSM and YMSM in 1:10 ratio (secondary enrichment) and thereafter it was performed in 1:50 ratio (tertiary enrichment). Enriched culture was maintained by subculturing in MSM and YMSM on same parameters in 1:50 ratio, after every sixteen days of incubation (Mueller et al., 1989).

2.3. Preliminary screening for Fla degradation

Consortium, enriched and subcultured on 500 mg l^{-1} Fla in MSM and YMSM, was screened for degradation of 100 mg l^{-1} Fla in respective media. Stock solution of Fla was dispensed in 50 ml capacity teflon lined screw cap vials to give final concentration of Fla 100 mg l^{-1}. After hexane evaporation on clean bench under sterile condition, 10 ml MSM or YMSM was dispensed in vials and sonicated for 3 min to dissolve PAHs crystals. 10% V/V inoculum from MSM enrichment was inoculated in MSM containing vials whereas 10% and 1% V/V inoculum from YMSM enrichment was inoculated in YMSM containing vials. Control was set as well to see any autolytic growth. Culture and control, in triplicate, were incubated at 30 °C and 180rpm. Entire content of the flasks were extracted on 20th day of incubation and analysed by GC-FID.

2.4. Fla degradation potential of YMSM enrichment

Culture from YMSM enrichment was washed twice and suspended in YMSM or MSM. Cell suspension, at OD_{600nm} of 0.02, from YMSM and MSM enrichments was inoculated in YMSM and MSM, respectively with Fla 100 mg l^{-1}. Cultures and control, in triplicate, were incubated at 30 °C and 180rpm. Entire content of the flasks were extracted on 1st, 3rd, 5th and 8th day of incubation and analysed by GC-FID to observe gradual degradation of Fla. YMSM enriched culture was also grown the same way along with ampicillin 50 μg ml^{-1}, chloramphenicol 30 μg ml^{-1}, tetracycline 30 μg ml^{-1} or kanamycin 50 μg ml^{-1} to distinguish between prokaryotic and eukaryotic degradation of Fla (Ito et al., 2008). Cultures, in triplicate, were incubated at 30 °C and 180rpm and analysed on 8th day of incubation by GC-FID.

2.5. Fla degradation potential of longer incubated MSM enrichment

It was observed that if MSM enrichment was incubated longer instead of subsequent routine transfers in fresh medium, it attained the turbidity and medium colour changed to brown within five weeks. Experiment was set to scrutinize the effect of longer incubation on Fla degradation potential of the culture. 0.02 inoculum at OD_{600nm} from this enrichment was inoculated in MSM with Fla 100 mg l^{-1}. Culture and control, in triplicate, were incubated at 30 °C and 180rpm. Entire content of the flasks were analysed on 5th and 8th day of incubation by GC-FID.

2.6. Extraction and analysis of Fla

Samples were extracted in 1:1 ratio with chloroform/methanol solvent system. Residual Fla was extracted in 6 ml chloroform/methanol (2:1) followed by 2 ml (2X) of chloroform after addition of n-dodecane as an internal standard. 2 μl aliquots of extracted samples were analysed by GC-FID in split mode equipped with HR-17 column (25 m long, 0.25 mm i.d.). Initial oven temperature was set at 100 °C that gradually increased to 300 °C at the rate of 10 °C min^{-1}. Injector and detector temperatures were set at 300 °C. Carrier gas was nitrogen.

2.7. ARDB and clear zone forming population of enrichments

ARDB and clear zone forming bacterial population of MSM, longer incubated MSM and YMSM enrichments were compared during the subculturings. Dilutions from MSM, YMSM and longer incubated MSM enrichments were made in 0.89% saline. Diluted cultures were spread on minimal agar plates. Minimal agar plates were supplemented with 2 mM indole and 500 mg l^{-1} YE for total count and types of ARDB. For the growth of clear zone forming bacterial colonies, minimal agar plates were sprayed with Fla. Plates were incubated at 30 °C for two weeks.

3. Results and discussion

A bacterial consortium LP was isolated from an oil contaminated soil sample by enrichment on Fla. When LP consortium was enriched and subcultured in YMSM and MSM, clear differences were observed in turbidity, colour of media and amount of added Fla crystals. YMSM enrichment exhibited increase in turbidity within two weeks of incubation and medium colour changed from transparent to brown along with decrease in Fla crystals. MSM enrichment did not increase in turbidity and medium colour remained same in two weeks of incubation. When MSM enrichment was incubated longer, without further routine transfers in