ISOLATION AND CHARACTERIZATION OF THERMOTOLERANT HYDROCARBON-UTILIZING BACTERIA FROM MARINE, SHALLOW HYDROTHERMAL VENTS

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Abstract

In order to isolate hydrocarbon-utilizing microorganisms water samples were collected from shallow vents offshore the coasts of the Aeolian Islands (Southern Italy) and inoculated onto modified natural sea water (NSW) agar supplemented with emulsified crude oil (Arabian light, 5 gl^{-1}). Temperature and pH values recorded during sample collection and ranged from 30 to 95°C and from 5.2 to 6.4 respectively. Bacterial isolates were characterized by morphological, physiological and biochemical properties. Facultative anaerobic, spore-forming bacteria were identified at genus level as Bacillus spp. Strains able to grow on crude oil as sole source of carbon and energy were lipolytic on Tween 20 and 80 and exopolysaccharide producers.

Key-words: bacteria, thermal vents, petroleum, Tyrrhenian Sea

Introduction

The marine, shallow hydrothermal vents around the Aeolian Islands represent an easier accessible field than the deep-sea oceanic sites to studying unusual environments. Microbiological investigations in these sites have been shown the presence of mesophilic phototrophic, chemotrophic and heterotrophic bacteria [1, 2, 3, 4]. These sites allow the growth of thermophilic and thermotolerant microorganisms. Their enzymes display high activity and thermal stability.

A number of reports on the distribution of marine hydrocarbon-utilizing bacteria from temperate and tropical zones demonstrated the widespread presence of this activity in the marine environment [5, 6, 7, 8]. Moreover, Mills *et al*, (1978) [6] reported that all strains of oil-degrading bacteria were lipolytic, but the converse was not always true.

The hydrophobic nature of the outermost bacterial surface seems to be an important factor in the growth of microorganisms on insoluble substances, such as hydrocarbons, as well as in the adherence of bacteria to non-wettable plastic surface [9]. Many hydrocarbon-degrading microorganisms produce emulsifier, which enhance growth by increasing the available surface area and provide a mechanism for desorption [10].

The aim of this study is to investigate the ability of isolates from marine, shallow hydrothermal vents to degrade crude oil. The selection of strains was based on the presuntive correlation between the lipolytic and hydrocarbon clastic activity. Moreover, the hydrocarbon utilization was related to the exopolysaccharides production.

Materials and Methods

Study sites - The Aeolian Islands represent an arc of vulcanic origin where submarine hot waters and gases flow from sea-floor at various depths. The sites of the sampling stations in correspondence of the hydrothermal vents off the Aeolian Islands are depicted in Figure 1.

Samples collection - During an oceanographic cruise around the Aeolian Islands, venting water samples were collected by SCUBA-divers using sterile samplers. Temperature and pH values were immediately recorded by a multiparameter probe during samples collection.

Isolation of hydrocarbon utilizing bacteria - To isolate hydrocarbon-utilizing bacteria was used a mineral natural sea water (NSW) agarized [8] consisting of ammonium nitrate 1g, dipotassium hydrogen phosphate 0.2 g, ferric citrate 0.02 g, pH 7.8, dissolved in 800 ml sea water and 200 distilled water, agar 30 g, added with crude oil (Arabian light, 5 $g.l^{-1}$). Inoculated plates were incubated at 55°C for three days.

After isolation and purification 51 strains were checked for the following characteristics: growth at different temperatures (37, 55, 60, 65, 70 and 75°C), pH values (5.5, 6, 7, 8, 9) and NaCl contents (0, 2, 3, 5, 7, 10%). All strains were tested for Gram stain, spore-production, cellular morphology, and for the tests contained in the standardized and miniaturized API 20E and API 20NE systems. The strains were characterized at genus level according to the Bergey's Manual.

The lipolytic activity on Tween 20 and 80 was studied on Degryse *et al.* (1978) [11] medium (peptone 0.5%, NaCl 0.5%, $CaCl_2.H_2O$ 0.01%, agar 3%, pH 7). Incubation was carried out at optimal temperature of each isolate for three days.

Lipolytic strains were successively tested for their ability to grow into NSW medium supplemented with gasoline or kerosene at final concentration of 2%. Emulsion at the interface culture-hydrocarbons was visually observed and the bacterial growth was spectrophotometrically monitored. A pre-screening on exopolysaccharides production was based on i) the ability of each isolate to grow in the 162 mineral basal medium [11] with glucose or saccharose at a final concentration of 0.6%; ii) the ability to produce extracellular carbohydrate, demonstrated by staining technique proposed by Allison and Sutherland [12].

Results and discussion

Temperature and pH values recorded during sample collection and ranged from 30 to 95°C and from 5.2 to 6.4 respectively.

All strains were Gram-positive, spore-forming rods, moderately halophilic, facultative anaerobic and were distinct in thermophilic or thermotolerant according to their optimal values of growth. Mostly of the isolates were positive for gelatinase, esculine hydrolase, amilase, nitrato-reductase, oxidase and catalase tests; utilized glucose and saccharose. According to the Bergey's Manual, they were ascribed to the genus Bacillus.

Most (50) of the 51 isolates were lipolytic on Tween 20 and 80 media. Only 12 strains were able to utilize gasoline and kerosene as sole source of carbon and energy.

All of the hydrocarbon-utilizing isolates were positive for the pre-screening of exopolysaccharides production. Since many microbially derived surface-active compounds are known to be involved in the hydrocarbons degradation [13], the exopolysaccharides could mediate the attack to crude oil.

The recovery of thermotolerant hydrocarbon-utilizing microorganisms from shallow hydrothermal vents should allow to develop strategies for marine bioremediation. From an ecological point of view, the thermotolerance may be an useful tool to survive at the environmental variations that the hydrothermal habitat may offer.

References

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