IS THE ABILITY OF MICROORGANISMS TO DEGRADE POLYCYCLIC AROMATIC HYDROCARBONS PLASMID MEDIATED?

Isolation and Growth Characteristics of a Marine Vibrio from the Great Sippewissett Salt Marsh

Asa Jouper

INTRODUCTION

Microorganisms in soil and waters convert many synthetic organic chemicals to inorganic products. Other compounds are transformed by cometabolism. Very little is known about these processes, although the fate of these compounds in the environment is of concern. Catabolism of polycyclic aromatic hydrocarbons (PAH) has been shown to be plasmid mediated in some cases (e.g. Furukawa et al., 1983). It was of interest to see if we could find a PAH-degrader in the great Sippewissett salt marsh, and if the degrading ability was encoded by a plasmid. We also wanted to know if the microorganisms made the PAH water soluble by hydroxylation or by conjugation.

MATERIALS AND METHODS

Isolation of the organism.

Samples were taken from the control area and the highly fertilized area at the Great Sippewissett salt marsh. One gram of soil from each area was put in E-flasks containing artificial seawater with 4 mg phenanthrene for enrichment of phenanthrene utilizing organisms. After 6 days, samples were taken from the enrichment cultures, diluted and spread on agar plates. Two different plates were used, neo-plates containing enopeptone 0.1g, yeast extract 0.1g, glycerol 0.5 ml per 1000 ml artificial seawater (ASW) and ASW-plates (Shiaris and Cooney, 1983).

The plates were covered with a thin overlay of phenanthrene (phe). The overlay was an emulsion of phe, 2 mg in 0.4 ml acetone and ASW-agar. Plates were dried overnight at 30°C to allow the acetone to evaporate. Neo-plates without the overlay were used as controls.

Zones of clearing on the plates were used to detect the degradation of phe, which fluoresce in long-wave UV-light. Colonies with clearing zones were picked and put in neo-broth with phe and ASW with phe. The tubes that showed a slight turbidity in the ASW plus phe broth were screened for plasmids

Plasmid DNA isolation and agarose gel electrophoresis.

The method used for plasmid DNA isolation and gel electrophoresis was developed by C.I. Kado and S-T. Liu (1981).

Extraction of the metabolic products.

The cells were grown in neo plus PAH broth and in ASW plus PAH broth. Neo plus PAH grown cells were harvested after 24 hr and the ASW+PAH grown cells were harvested after five days. The cells were centrifuged and the

supernatant was extracted with three equal volumes of ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate and the solvent was removed in vacuo.

Deconjugation experiments.

The aqueous layer was divided into three 10-ml portions and diluted 1:1 with 0.2 M sodium acetate buffer (pH 4.5). The first sample was incubated with β-glucuronidase, the second with arylsulfatase and the third sample was incubated without enzyme and served as a control. Each mixture was incubated for 20 hr at 37°C on a shaker. Samples were then extracted as described above.

Analysis of metabolic products.

Separation was achieved by thin-layer chromatography (TLC). TLC was performed on silica gel plastic plates with fluorescent indicator, developed in toluene=acetic acid 10:1. When the silica gel plates were dry they were sprayed with Gibb's reagent (2% in methanol) and put in heat (100°C) for 15 min. Glucuronide and sulfate conjugated aromatic hydrocarbons were detected under long-wave UV-light. The hydroxylated aromatic intermediates were identified after color and position in the chromatogram.

RESULTS

Organisms were found growing in presence of phe. Clearning zones were detected after about one to two weeks. These isolates were transferred to neo plus phe broth and screened for plasmids.

In the first screening experiment isolate CEP-9 (Control area-Enrichment culture-Primary metabolizer, no.9) was shown to harbour a plasmid. To determine whether the ability to degrade a PAH was plasmid mediated or not, this organism was chosen. The other isolates could also have harbored a plasmid but the method used here could have been inappropriate for their identification.

Strain CEP-9 is a gram negative, motil, polar flagellated, curved rod. The strain is catalase positive, fermentative and requires sodium chloride for growth. CEP-9 clumps easily, forms clusters when it is grown anaerobically. These are indicates that CEP-9 is a Vibrio.

Neo plus PAH grown cells were shown to form sulfate and glucuronide conjugates of PAH. No other intermediates were detected except for cells grown in neo+biphenol. Deconjugation with β-glucuronidase showed a different intermediate compared to the phe and the napthalene grown cells. This intermediate could be a meta-phenophenol. The other tests, even the control, showed two metabolites that were not identified.

ASW plus PAH grown cells showed no sign of conjugation of the PAH. After deconjugation experiments the same metabolites that were found above were also identified here, in the cells grown in phe and naph.

Dry weight of the ASW plus PAH grown cells showed that ASW plus phe had four times higher dry weight than the other two cultures. PAH was not limiting for growth in the cultures.

As an attempt to cure CEP-9 from the plasmid the organism was grown on benzoate as a sole carbon source. To determine whether the plasmid was responsible for the synthesis of enzymes involved in the degradation of PAH or not, the isolate was spread on neo plus phe- and ASW plus phe-plates. No clearing zones were observed after three days.

These results clearly indicate that there are PAH-degraders in the Great Sippewissett salt marsh, and that at least one of these organisms can utilize phe as a carbon source.

Cometabolism seems to be more common and more rapid than primary metabolism. The isolate CEP-9 seemed to harbour a plasmid that is responsible for the ability to degrade PAH ad demonstrated by the poor growth of the "cured" strain on ASW plus phe plates.

SHEATH PIGMENT FORMATION IN A BLUE-GREEN ALGAE LYNGBYA AESTUARII AS AN ADAPTATION TO HIGH LIGHT

Lisa K. Muehlstein

INTRODUCTION

The Great Sippewissett salt marsh has extensive areas of intertidal microbial mats. The top layer of the mats is composed primarily of blue-green algae Lyngbya aestuarii and Microcoleus chthonoplastes. These mats are subjected to long exposures of high light intensity which can potentially cause photodynamic damage to the microorganisms. L. aestuarii with heavily pigmented sheaths is often found in these exposed areas. The conditions for sheath pigment formation are not yet understood. Iron concentrations in the environment, periodic desiccation and light intensity are possible factors which may influence sheath pigment formation. From the results of the present study, however, exposure to high light appears to be the primary requirement for sheath pigment formation.

The yellow-brown sheath pigment has yet to be fully characterized. The staining of the Lyngbya sheaths is due to the pigment scytonemine (as cited in Birke, 1974) and is very effective in reducting incident light. There has been speculation that the sheath pigment evolved in the Precambrian, as a primitive form of protection from high light intensity, including ultraviolet radiation (Rambler et al., 1977). No recent or detailed chemical analysis of this pigment has been reported.

MATERIALS AND METHODS

Axenic cultures of Lyngbya aestuarii, grown in modified artificial Von Stosch medium (Table 1) were used for this project. To obtain a somewhat homogeneous inoculum, Lyngbya was put in a Waring blender for approximately 10 sec and 125 ml Erhlenmeyer flasks were inoculated with 50 ml artificial Von Stosch medium.

Table 1. Modified Artificial Von Stosch Medium with Instant Ocean.

Stock (20X conc.)
per liter distilled water

0.1 g EDTA
4 ml Nitsch's trace element mixture
20 ml $FeCl_3$ solution (0.29 g/l)
0.84 g $NaNO_3$
1.00 g NH_4Cl
0.08 g $NaH_2PO_4\ H_2O$
20 ml Na_2SeO_4 (0.01 mM)
5 ml $NiSO_4(NH_4)_2\ 6H_2O$ (0.01 mM)
33.0 g NaCl

Dilute 50 ml with 950 ml Instant Ocean Seawater Mix*, add 1 ml vitamin mix (D.N.), check and adjust pH 8.0-8.3, and autoclave

*Instant Ocean 33 g/liter distilled water

Three different light intensities wre used for this study: (a) direct sunlight with intensity ranging from 400-850 watts/m^2, (b) a cardboard box partially covering the flasks to provide some shading with intensity ranging from 250-500 watts/m^2, and (c) in the 30°C incubator under coolwhite fluorescent light, with continuous lighting at 25 watts/m^2. Cultures from