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Bacillus thuringiensis a promising bacterium for degrading emerging pollutants

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ABSTRACT

In the last decades, a wide range of organic pollutants has been identified as emerging pollutants in the aquatic environment. However, limited work has been done examining the ability of microorganisms to degrade emerging pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and pesticides.

In this work, a novel PAH and pesticide degrading bacterium was isolated from polluted marine sediment. After morphological and genetic characterization, the novel strain showed the highest similarity to *Bacillus thuringiensis*. The ability of the isolated bacterium to degrade the target pollutants was evaluated in shake flasks and bioreactor assays, reaching high levels of degradation for the model pollutants studied, phenanthrene and imidacloprid. Furthermore, the plausible degradation pathways of both pollutants were established. Based on the reported results, it can be concluded that *B. thuringiensis* has an enormous potential to mineralize a wide spectrum of emerging pollutants, such as PAHs and pesticides.

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1. Introduction

In the last decades, more and more pollutants have been released into the environment as a result of anthropogenic activities. A wide range of organic pollutants have been found in aquatic environments, including PAHs, organochlorine pesticides, polychlorinated biphenyls, organophosphates pesticides and carbamates (Sánchez-Avila et al., 2011). Water consumption in urban areas has been increasing, due to population growth, industrial development and the expansion of irrigated agricultural land. Water sources may be considered as one of the most essential parts of the environment, since they are a limited natural resource (Cobas et al., 2013).

In the last years, several studies have surveyed aquatic environment management (Zhang et al., 2012; Ma et al., 2013a). Some studies focus on developing and applying model tools to simulate aquatic environment policy, such as applying Markov model (Ma et al., 2012c) or system dynamic model (Ma et al., 2012a) to simulate coastal dynamics; developing modes for aquatic environment restoration (Ma et al., 2012b). Some studies focus on assessing aquatic environment policy and management, such as aquatic environment management assessment, aquatic environment ecological requirement assessment health status evaluation (Ma et al., 2013b), and assessment of economic development and aquatic environment conservation (Ma et al., 2013a).

In addition, some organic pollutants that have been identified in groundwater or surface waters, such as PAHs, halogenated pesticides and phenols, are highly toxic, carcinogenic and mutagenic (Xu et al., 2008; Payne et al., 2013). In

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recent years, the European Union and the Environmental Protection Agency of the United States have established lists of priority pollutants. The current list includes 126 different pollutants, which include PAHs and pesticides.

PAHs are may be present naturally as well as anthropogenically but they are normally associated with the release of petroleum hydrocarbons (Chagger and Jones, 2000). The detection of these compounds in the environment may indicate the presence of petroleum pollution. It is estimated that between 1.7 and 1.8 million metric tons of oil are release into the aquatic and soil environments every year. Furthermore, approximately 30% of the oil that is spilled reaches freshwater ecosystems (Dadrasnia and Agamuthu, 2013).

Pesticides are used to control, repel, prevent or eradicate pests. They not only include a wide range of chemical compounds but also antimicrobial or disinfectant agents. Pesticides are usually classified according to their active chemical group, one of which (neonicotinoids) is the focus of this work. Neonicotinoid pesticides are the fastest growing family of insecticides. The *in vivo* action of this type of compound is similar to that of epibatidine and nicotine, with the common target being the nicotinic acetylcholine receptor (Zhao et al., 2009).

In order to address the challenges of the negative impact of these emerging pollutants, research and development of innovative environmental biotechnology solutions is being undertaken. The role of microorganisms in the removal of toxic organic compounds to a large extent involves biodegradation. Bioremediation can be regarded as an attractive technology that results in the complete transformation of organic compounds to harmless end products such as CO_2 and H_2O. Bioremediation is also considered as a cost-effective and environmentally friendly means for the treatment of organic compounds in comparison to the physical and chemical methods of cleaning up pollutants (Farhadian et al., 2008). Microorganisms that are able to degrade organic pollutants can be isolated from many different environments, including both contaminated and uncontaminated sites and even marine sediments (Harayama et al., 2004). In recent years, the ability of microorganisms to degrade these pollutants has been found to be associated with specific species of *Burkholderia* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Coriolus sp. Serratia* sp., *Enterobacter* spp., *Flavobacterium* sp., *Brevundimonas diminuta*, *Mycobacterium* sp., *Novosphingobium* sp., and *Rhizobium tropici* (Jariyal et al., 2014; Kim et al., 2014). However, the ability of these strains to degrade different emerging pollutants, as PAHs and pesticides, is limited.

Given the limited substrate range of the microorganisms already isolated and the wide range of emerging pollutants, there is an urgent need to find microorganisms that are able to degrade those compounds. Therefore, the aim of this study was to isolate microorganisms with this ability. Marine sediments were chosen as the source for this work since they may contain these emerging pollutants and therefore microorganisms which have the ability to degrade them.

2. Materials and methods

2.1. Polluted marine sediment

Samples of marine sediment from the Ria de Vigo (NW Spain) were collected at a depth of 20 cm as used as the source of microorganisms. The samples were collected (using a stainless steel spoon), placed in glass bottles and preserved at 4 °C. This location was chosen since it has been polluted by a series of crude oil spills and contains organic pollutants such a PAHs (Viñas et al., 2009).

2.2. Pollutants

Phenanthrene (PHE) and imidacloprid (IMI) (Table S1) were used as model PAH, and pesticide, respectively. All chemicals were supplied by Sigma Aldrich and their properties and characteristics are showed in Table S1

2.3. Isolation of microorganisms from polluted marine sediment

Erlenmeyer flasks (250 mL) were inoculated with 2 g of polluted sediment and 50 mL of growth medium (GM). GM was composed of 10 g L^{-1} bacteriological peptone, 2 g L^{-1} yeast extract, 2 g L^{-1} casein peptone, 6 g L^{-1} NaCl and 10 g L^{-1} glucose (Hou et al., 2005). The flasks were placed on an orbital shaker (Thermo Scientific MaxQ800) at 37 °C and 100 rpm and cellulose stoppers were employed to achieve a passive aeration. After 24 h, 200 μL of liquid media were removed from the flask and spread on a Petri plate which contained GM and 1.5% agar. The Petri plate was maintained for 24 h at 37 °C. The colonies grown on the plates were picked and streaked onto a new Petri plate for the isolation of pure cultures. This procedure was repeated several times in order to obtain a pure culture.

2.4. Isolation of PAH-degrading microorganisms

A Petri plate which contained the isolated microorganism was washed with sterile 0.9% NaCl solution (1.5 mL). This solution was then used to inoculate an Erlenmeyer flask (250 mL) that contained 50 mL of minimal medium (MM) and 17.8 mg/L of phenanthrene (PHE; the selected model PAH) with 1% Tween 80 as solubilizing agent (Moscoso et al., 2012). The amount of PHE used was 17.8 ppm based on the work of Moscoso et al. (2012). The Erlenmeyer flasks were incubated for approximately one week in an orbital shaker at 37 °C and 100 rpm. Samples were periodically removed in order to analyse microbial growth and PHE degradation. All experiments were carried out in duplicate and the reported results were the mean values.

2.5. Identification

2.5.1. Gram staining

Gram staining was carried out using the 77730 Gram Staining Kit (Fluka Analytical, Sigma-Aldrich) and one drop of pure culture. For this purpose, one drop of pure culture was placed on a microscope slide and fixed to the surface by passing the slide quickly through a flame. Afterwards, the slide smear was flooded with Gram's crystal violet Solution, than Gram's Iodine Solution and Gram's Decolorizer Solution, at the end the smears were counter stained with Gram's Safranin Solution. After all, samples were examined under microscope lens 1000× magnification (Olympus System Microscope Model BX41).

2.5.2. DNA extraction and molecular identification

Bacteria isolates were inoculated five times into GM and maintained at 37 °C for 24 h. Five samples of 2 mL each were collected and centrifuged at 13,400 rpm for 10 min (Eppendorf MiniSpin 9056, F-45-12-11). DNA extraction was performed

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