Science of the Total Environment 511 (2015) 767–776

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Development of eukaryotic zoospores within polycyclic aromatic hydrocarbon (PAH)-polluted environments: A set of behaviors that are relevant for bioremediation

Rungroch Sungthong [a], Pieter van West [b], Manuel Cantos [a], Jose Julio Ortega-Calvo [a,*]

[a] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Apartado 1052, 41080 Seville, Spain
[b] Aberdeen Oomycete Laboratory, University of Aberdeen, Institute of Medical Sciences, Foresterhill, AB25 2ZD Aberdeen UK

HIGHLIGHTS

- Zoospore development shows possible enhancement of PAH bioavailability.
- Toxicity of PAHs is highly dependent on their bioavailability.
- PAH-degrading bacteria suppress toxic influence of PAHs on zoospore development.
- Development of zoospores is applicable for improving bioremediation.

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 26 December 2014
Accepted 26 December 2014
Available online 22 January 2015

Editor: D. Barcelo

Keywords:
Eukaryotic zoospore
Oomycetes
PAH-polluted scenario
PAH-degrading bacteria
Zoospore development
Bioremediation

ABSTRACT

In this study, we assessed the development (formation, taxis and settlement) of eukaryotic zoospores under different regimes of exposure to polycyclic aromatic hydrocarbons (PAHs), which imitated environmental scenarios of pollution and bioremediation. With this aim, we used an oomycete, *Pythium aphanidermatum*, as a source of zoospores and two PAH-degrading bacteria (*Mycobacterium gilvum* VM552 and *Pseudomonas putida* G7). The oomycete and both bacteria were not antagonistic, and zoospore formation was diminished only in the presence of the highest bacterial cell density (10^8–10^{10} colony-forming units mL^{-1}). A negative influence of PAHs on zoospore formation and taxis was observed when PAHs were exposed in combination with organic solutions and polar solvents. Co-exposure of PAHs with non-polar solvents [hexadecane (HD) and 2,2,4,4,6,8,8-heptamethylnonane (HMN)] did not affect zoospore settlement at the interfaces of the organic solvents and water. However, zoospores settled and created mycelial networks only at HD–water interfaces. Both bacteria diminished the toxic influence of PAHs on zoospore formation and taxis, and they did not interrupt zoospore settlement. The results suggest that zoospore development could be applicable for toxicity assessment of PAHs and enhancement of their bioavailability. Microbial interactions during both swimming modes and community formation at pollutant interfaces were revealed as major factors that have potential relevance to bioremediation.

1. Introduction

In nature, free-swimming zoospores are produced through asexual reproduction by various eukaryotic organisms, e.g., algae, protists, fungi and oomycetes. Although these eukaryotic zoospores are produced from very distant phylogenetic taxa, they share a typical behavioral sequence of development, including swimming, settlement, encystment, germination and orientation of the germ-tube (Walker and van West, 2007; Gleason and Lilje, 2009). The development of zoospores is highly dependent on their chemoresponses to different chemical effectors that are found in the environment, which may cause an incomplete development sequence (Jones et al., 1991; Donaldson and Deacon, 1993; Walker and van West, 2007). Zoospore producers have been found in a wide variety of ecological niches, e.g., cattle's rumen, freshwater lakes, mangrove forests and oceans (James et al., 2006; Walker and van West, 2007; Gleason and Lilje, 2009). Within such ecological niches, they may play diverse roles as symbionts, phototrophs, saprophytes and parasites.

The ecological impact of zoospores and their development have been studied primarily in natural habitats or under axenic conditions (Gleason and Lilje, 2009; Savory et al., 2014), which are rarely observed within polluted environments. Some authors recently reported that mycelial networks formed by a zoospore producer, *Pythium ultimum*, facilitated the transport of either polycyclic aromatic hydrocarbons (PAHs) or PAH-degrading bacteria, thus enhancing the bioavailability of PAHs

* Corresponding author.
E-mail address: jjortega@irnase.csic.es (J.J. Ortega-Calvo).

http://dx.doi.org/10.1016/j.scitotenv.2014.12.089
0048-9697/

(Wick et al., 2007; Furuno et al., 2010, 2012). This zoospore producer is a rhizosphere oomycete that belongs to the pseudofungi that live at the interface of biphasic habitats (solid–liquid, solid–air or liquid–air). Although mycelial networks of the oomycete are applicable for enhancement of pollutant bioavailability, the development of its zoospores in contact with pollutants and pollutant-degrading bacteria still remains relatively unexplored.

Among hazardous chemicals, PAHs are ubiquitous and considered important pollutants that are of critical concern for animal and human health (Keith and Telliard, 1979; Ortega-Calvo et al., 2013). Emissions of PAHs into the environment occur through anthropogenic activities and natural incidents such as forest fires and volcanic eruptions. These pollutants can be transferred spontaneously by wind and/or rain into various ecosystems (Martínez-Lladó et al., 2007; Vergnoux et al., 2011). Persistence of PAHs in nature is often caused by their association with organic matter and nonaqueous-phase liquids (NAPLs), such as light or crude oil, creosote, coal tar and soot-like materials (Tejeda-Agredano et al., 2011, 2014; Ortega-Calvo et al., 2013). Different exposure regimes can be caused by exchange between these solid and liquid phases. This exchange relies on the physicochemical properties of PAHs, such as low water solubility and high hydrophobicity, and can have a profound impact on their bioavailability (Schwarzenbach et al., 2003; Mackay et al., 2006). The persistence of PAHs through association to organic matter and NAPLs is therefore the major cause of reduced bioavailability for microbial degradation. Because bioavailability limits the effectiveness of PAH-degrading activity in bioremediation, some applications to enhance bioavailability, with a focus on their environmental risks, have been proposed (Ortega-Calvo et al., 2013). Among all applications, the mycelial networks of oomycete and fungi are an ecological application that could enhance the bioavailability of PAHs. However, mycelial networks are applicable only for increasing the bioavailability of flagellated PAH-degrading bacteria, e.g., *Achromobacter* sp., *Sphingomonas* sp. and *Pseudomonas* sp. (Kohlmeier et al., 2005; Wick et al., 2007; Furuno et al., 2010). Non-flagellated PAH-degrading bacteria, such as *Mycobacterium* sp., have not been included in these ecological applications (Kohlmeier et al., 2005), which is problematic because they are found abundantly within PAH-polluted sites (Uyttebroek et al., 2006).

As the development and subsistence of zoospores within polluted environments and bioremediation scenarios are poorly understood, the ecological impacts of zoospores may provide additional knowledge for further improvements to innovative bioremediation technology. With this hypothesis, we assessed zoospore development of a rhizosphere oomycete, *Pythium aphanidermatum*, in different PAH-polluted environments. Two common PAH-degrading bacteria (*Mycobacterium gilvum* VM552 and *Pseudomonas putida* G7) were also used; these bacteria exhibit differences in their physiology and PAH-degrading capabilities. The development of zoospores was evaluated at three general stages of their life cycle: formation, taxis and settlement. The PAH-polluted environments were constructed with different levels of bioavailability, as determined by the different exposure regimes of PAHs in combination with chemical effectors. The term 'chemical effectors' refers to a set of environmental chemicals that may have a profound influence on zoospore development and/or environmental relevance in PAH pollution and bioremediation scenarios. The possible applications of zoospores at each stage of their development to the improvement of bioremediation technology are discussed in this article.

2. Materials and methods

2.1. Zoospore-producing organism and formation of zoospores

The primary stock of the oomycete *Py. aphanidermatum* originated from the culture collection of Aberdeen Oomycete Laboratory, University of Aberdeen, United Kingdom. It was grown in diluted V8 juice (DV8) agar [4% (v/v) filtered Campbell's V8 juice; 20 g agar powder (Panreac, Barcelona, Spain); 1 L distilled water] at 25 °C. To form zoospores, 10 pieces (1 cm^2) of a 4-day-old hyphal mat growing on the agar were soaked with 10 mL of sterilized lake water (Embalse Torre del Águila, Seville, Spain) in a 50-mL Erlenmeyer flask. Zoospores were released after incubation at 25 °C for 5–6 h. The zoospore density obtained by this process was 10^4 zoospores mL^{-1} (quantified in BLAUBRAND® counting chambers, BRAND GMBH + CO KG, Wertheim, Germany).

2.2. PAH-degrading bacteria

The bacterial strains used in this study were *M. gilvum* VM552, which was isolated from PAH-polluted soil and is able to use phenanthrene, naphthalene, fluoranthene, pyrene and anthracene as sources of carbon and energy, and the motile naphthalene-degrading *P. putida* G7. *M. gilvum* VM552 was supplied by D. Springael (Catholic University of Leuven, Leuven, Belgium), whereas *P. putida* G7 was supplied by C.S. Harwood (University of Washington, USA). These bacteria were maintained in mineral salt media supplemented with phenanthrene for *M. gilvum* VM552 (Tejeda-Agredano et al., 2011) and naphthalene for *P. putida* G7 (Jimenez-Sanchez et al., 2012); the two PAHs served as the sole carbon and energy sources. For the purposes of tests with zoospores, the bacteria were routinely grown in tryptic soy broth (TSB) (Sigma-Aldrich, Germany) through shaking incubation at 150 rpm and 30 °C for 4 days. Bacterial cells were harvested by centrifugation at 4303 $\times g$ for 5 min and then washed twice and re-suspended with sterilized lake water. The initial cell density of bacteria was adjusted to an optical density ($OD_{600\ nm}$) of 1.5. This OD corresponded to 10^{10} and 10^8 colony-forming units (CFU) mL^{-1} for *P. putida* G7 and *M. gilvum* VM552, respectively.

2.3. Antagonism tests and the influence of bacteria on zoospore formation

The possible antagonistic effects between *Py. aphanidermatum* and PAH-degrading bacteria were studied within two different habitats, including a solid surface of agar media and a liquid phase of sterilized lake water. To determine the interaction during surface growth on solid media, an antagonism test was performed using a dual culture technique (Fokkema, 1978). Two different media [DV8 agar and tryptic soy agar (TSA) (Sigma-Aldrich, Germany)] were used. These two media vary in terms of their compositions and the concentrations of total carbon that are available for growth. The estimated total carbon in TSA was 2% (w/v) and 0.2% (w/v) in DV8 agar; the composition and nutritional information about each medium were used to perform the estimation. First, the bacterial inoculum was streaked at ~2 cm from the edge of the agar plate, which was then incubated at 30 °C until visible bacterial biomass developed. Then, an agar plug (Ø = 0.5 cm) of the oomycete (previously grown on DV8 agar at 25 °C for 4 days) was placed opposite to the bacterial growth. The dual culture plates were incubated at 25 °C and observed every day until the oomycete mycelia reached over the bacterial growth (~1 week). All tests were performed in triplicate.

To determine the interaction within an aqueous habitat, we performed a test for zoospore formation and used the number of zoospores produced as an indicator of the influence of the bacteria. A set of different bacterial cell densities was prepared and introduced into the zoospore formation system. The bacteria were grown for 4 days under the conditions described previously. Their initial cell density was adjusted to an $OD_{600\ nm}$ of 1.5, which was serially diluted 10-fold using sterilized lake water. The number of zoospores produced with dilutions of bacterial cells was counted and compared with the control without bacterial cells. The count was performed twice after 4 and 6 h of incubation. All tests were performed at least in triplicate. The highest cell density of each bacterium that did not exhibit a negative influence on zoospore formation was selected as the optimal cell density for further co-existence experiments.