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Efficiency in hydrocarbon degradation and biosurfactant production by *Joostella* sp. A8 when grown in pure culture and consortia☆

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ABSTRACT

Joostella strains are emerging candidates for biosurfactant production. Here such ability was analyzed for *Joostella* strain A8 in comparison with *Alcanivorax* strain A53 and *Pseudomonas* strain A6, all previously isolated from hydrocarbon enrichment cultures made of polychaete homogenates. In pure cultures *Joostella* sp. A8 showed the highest stable emulsion percentage (78.33%), hydrophobicity rate (62.67%), and an optimal surface tension reduction during growth in mineral medium supplemented with diesel oil (reduction of about 12 mN/m), thus proving to be highly competitive with *Alcanivorax* and *Pseudomonas* strains. During growth in pure culture different level of biodegradation were detected for *Alcanivorax* strain A53 (52.7%), *Pseudomonas* strain A6 (38.2%) and *Joostella* strain A8 (26.8%). When growing in consortia, isolates achieved similar abundance values, with the best efficiency that was observed for the *Joostella-Pseudomonas* co-culture. Gas-chromatographic analysis revealed an increase in the biodegradation efficiency in co-cultures (about 90%), suggesting that the contemporary action of different bacterial species could improve the process. Results were useful to compare the efficiencies of well-known biosurfactant producers (*i.e. Pseudomonas* and *Alcanivorax* representatives) with a still unknown biosurfactant producer, *i.e. Joostella*, and to confirm them as optimal biosurfactant-producing candidates.

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Introduction

The genus *Joostella* (family *Flavobacteriaceae*) was firstly described by Quan et al. (2008) who isolated *J. marina* strain En5^T gen. nov., sp. nov., from coastal seawater (Quan et al., 2008; Hameed et al., 2014). A summary classification and a set of features for *J. marina*, in addition to the description of the complete genomic sequencing and annotation, was then presented by

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Stackebrandt et al. (2013). A second species, *J. atrarenae*, was described by Kim et al. (2011), but not yet appeared on a validation list. To date, information on *Joostella* spp. remain scarce and fragmentary. Only recently, members in the genus *Joostella* have been reported as biosurfactant (BS) producers by Rizzo et al. (2013) who isolated *Joostella* strain A8 from crude oil enrichment cultures, which were set up with homogenates of the polychaete *Megalomma claparedei* (Gravier, 1906). Further in depth investigation were carried out with the attempt to enhance BS production by *Joostella* strain A8 by varying culture conditions, including the carbon source (Rizzo et al., 2014), and link the influence of heavy metals on BS activity (Rizzo et al., 2015). Overall, gained data on *Joostella* strain A8 (Rizzo et al., 2013, 2014, 2015) were encouraging about its strong potentiality as BS producers and its possible application in microbial-mediated bioremediation process. Surface active agents, such as biosurfactants, facilitate the cellular uptake of insoluble substrates, by reducing the surface and interfacial tension, so increasing the solubility and emulsification of them, and resulted able to overcome the toxicity of synthetic compounds (Edwards et al., 2003). As it is well known, a limited range of petroleum substrates are metabolized by individual microorganisms, while mixed populations could exploit a variety of enzymatic abilities. This improve the biodegradation efficiency of complex hydrocarbon substrates, thanks to the complementary action of more than a single species with substrate specificity (Patil et al., 2012; Varjani and Upasani, 2013; Thomas et al., 2014). According to this, bacterial consortia should be regulated by a wide range of metabolic mechanisms for the enhancement of oil components transformation (Antoniou et al., 2015; Thomas et al., 2014). To date, bacterial consortia have been used to investigate microbial degradation efficiency, which is generally higher if compared to mono-cultures (Kadali et al., 2012), probably thanks to synergistic interactions among members of the association (Sampath et al., 2012). In contrast, the majority of studies on BS-mediated biodegradation were carried out with the use of mono-culture, and in rare occasions, mixed cultures (or co-cultures) were used (Ławniczak et al., 2013).

The improvement of microbial hydrocarbon degradation and BS production represents a promising approach in the control and remediation of this kind of pollution, so that various hydrocarbon-degrading bacteria have been isolated during last decades. The present study had a dual aim. Firstly, BS production and hydrocarbon degradation by *Joostella* sp. A8 were compared with those by isolates of the same origin (Rizzo et al., 2013) belonging to well-known genera in this field, i.e. *Alcanivorax* strain A53 and *Pseudomonas* strain A6. The genus *Alcanivorax* is an alkane degrader and producer of an efficient glucose–lipid surfactant (Fernàndez-Martìnez et al., 2003). The genus *Pseudomonas* is the best known rhamnolipid producer, able to use different substrates such as fructose, glycerol, mannitol, glucose, n-paraffin and vegetable oils (Desai and Banat, 1997), and most promising candidate for BS-production on large scale. Secondly, BS production and hydrocarbon degradation capacity was evaluated in co-cultures of *Joostella* strain A8 grown together with *Alcanivorax* strain A53 or *Pseudomonas* strain A6 in order to establish if they might have reciprocal advantage in substrate degradation thanks to the involvement of different metabolic abilities.

1. Material and methods

1.1. Bacterial strains

BS-producing bacterial strains used in this study were previously isolated from crude oil enrichment cultures which were set up with homogenates of the Polychaete annelids *Megalomma claparedei* (Gravier, 1906) (i.e. *Joostella* strain A8, **J**, Accession number **JX298555**; *Pseudomonas* strain A6, **P;** Accession number **JX298544**) and *Branchiomma luctuosum* (i.e. *Alcanivorax* strain A53, **A,** Accession number **JX298541**) from the brackish Lake Faro, Messina, Italy (Rizzo et al., 2013). Main features of the three BS-producing isolates are summarized in Table 1. Isolates were grown in both pure cultures and co-cultures (consortia) to monitor cell abundances, hydrocarbon degradation and BS production over time, as described in the following sections.

1.2. Culture set-up

1.2.1. Pure cultures

Experiments were carried out in 500 mL Erlenmeyer flasks containing 150 mL of the mineral salt medium Bushnell Haas Broth (BH) supplemented with NaCl (3%, W/V) and diesel oil (DO; 2%, V/V). Culture broth was inoculated with 10% (V/V) of an overnight pre-culture ($OD_{580} \approx 0.6$), and incubated at 25°C in a shaker (160 r/min) for 480 hr. Biodegradation assays were carried out under three parallel culture sets, as follows: Set I, Bacterial cells plus BH plus DO was used for chemical analyses; Set II, Bacterial cells plus BH plus DO was used for monitoring BS production and bacterial abundance; Set III, Bacterial cells plus BH plus DO plus sodium dodecyl sulfate (SDS) was used to investigate the effect of a synthetic surfactant on hydrocarbon degradation. Uninoculated control experiments were simultaneously carried out, with 150 mL of BH supplemented with DO (2%, V/V) to monitor abiotic losses of the substrate.

1.2.2. Co-cultures

Equal volumes of bacterial cultures ($OD_{580} \approx 0.700$) (for a total inoculum of 10%) were combined to inoculate 150 mL of BH *plus* DO (2%, V/V). Consortia were incubated at 25°C under shaking (160 r/min) for 480 hr. In detail, *Joostella* strain A8 was grown together with *Pseudomonas* strain A6 (consortium J-P) or *Alcanivorax* strain A53 (consortium J-A). Uninoculated control experiments were simultaneously carried out, with 150 mL of BH broth supplemented with DO (2%, V/V) to monitor abiotic losses of the substrate.

1.3. Estimation of bacterial abundances

1.3.1. Estimation of bacterial abundances in pure cultures

Aliquots from the pure cultures were collected at regular intervals (48 hr) to monitor bacterial abundance by optical density measurement at 580 nm (OD_{580}) using a spectrophotometer (UV-mini-1240, Shimadzu, Japan). Additionally, sub-samples were collected at 0, 240 and 480 hr of incubation (T_0, T_{240} and T_{480}, respectively) and fixed with formaldehyde (final concentration 2%, V/V) for the subsequent total counts using epifluorescence microscope and flow cytometry, as follows. For microscope

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