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Burkholderia thailandensis as a microbial cell factory for the bioconversion of used cooking oil to polyhydroxyalkanoates and rhamnolipids

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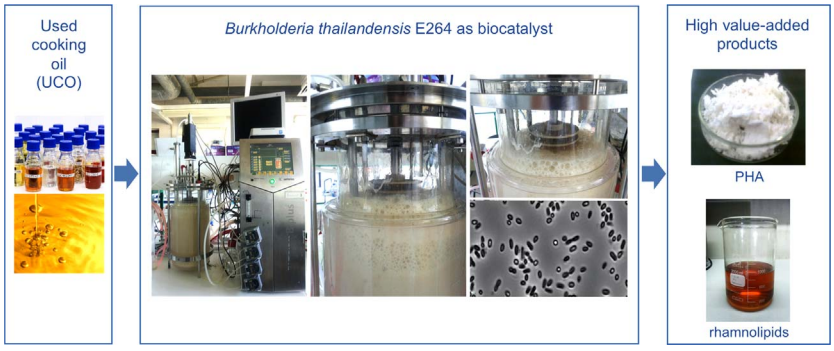
GRAPHICAL ABSTRACT



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ABSTRACT

The present work assessed the feasibility of used cooking oil as a low cost carbon source for rhamnolipid biosurfactant production employing the strain *Burkholderia thailandensis*. According to the results, *B. thailandensis* was able to produce rhamnolipids up to 2.2 g/L, with the dominant congener being the di-rhamnolipid Rha-Rha-C_{14}-C_{14}. Rhamnolipids had the ability to reduce the surface tension to 37.7 mN/m and the interfacial tension against benzene and oleic acid to 4.2 and 1.5 mN/m, while emulsification index against kerosene reached up to 64%. The ability of *B. thailandensis* to accumulate intracellular biopolymers, in the form of polyhydroxyalkanoates (PHA), was also monitored. Polyhydroxybutyrate (PHB) was accumulated simultaneously and consisted of up to 60% of the cell dry weight. PHB was further characterized in terms of its molecular weight and thermal properties. This is the first study reporting the simultaneous production of polyhydroxyalkanoates and rhamnolipids by the non-pathogen rhamnolipid producer *B. thailandensis*.

1. Introduction

Burkholderia thailandensis E264 was isolated from a rice field soil sample in Central Thailand. It is a saprophyte gram-negative motile strain, due to the presence of a polar tuft of 2–4 flagella, with diverse nutritional requirements. It can grow in a wide range of temperatures, between 25 and 42°C, but most importantly there is no correlation of human disease to this organism (Brett et al., 1998; Tseng et al., 2016). A previous study revealed that *Burkholderia thailandensis* E264 is capable of producing rhamnolipids, as it contains gene orthologs *rhlA*, *rhlB* and *rhlC,* which are responsible for their biosynthesis (Dubeau et al., 2009).

Rhamnolipids (RLs) are classified as low molecular weight

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glycolipid biosurfactants. These amphiphilic molecules comprise of a hydrophilic part, consisting of one (mono-rhamnolipid) or two (di-rhamnolipid) rhamnose sugars, and a hydrophobic region, consisting of one or two β-hydroxy fatty acids (ranging of eight to sixteen carbon atoms), linked through a glycosidic bond (Abdel-Mawgoud et al., 2010). Their bacterial production occurs in the form of mixtures of rhamnolipid homologues, including mono- and di-rhamnolipids, with predominant and minor components. To date, around 60 different rhamnolipid homologues have been identified and reported, mainly produced by *Pseudomonas*, *Burkholderia*, *Acinetobacter* and *Enterobacter* species (Costa et al., 2011; Dubeau et al., 2009; Hošková et al., 2015).

Due to their unique physicochemical properties they may be used for emulsification and demulsification, wetting and spreading, foaming, solubilization purposes and as detergents. In addition, their biological properties allow them to act in numerous ways, i.e. to protect certain bacteria and inhibit growth of others. Due to their features their applications are quite diverse including enhanced oil and petroleum recovery, formulation of cosmetics and pharmaceuticals, water treatment and environmental cleanup, toiletries and household cleaners, food processing, pesticides and agrochemicals, environmental control and management (Kourmentza et al., 2017a).

Secretion of rhamnolipids primarily occurs from the end of the exponential or the onset of the stationary growth phase, and are therefore characterized as secondary metabolites (Abdel-Mawgoud et al., 2011). Their production is strongly associated with the opportunistic pathogen *Pseudomonas aeruginosa,* a model organism extensively studied for rhamnolipid synthesis and regulation (Toribio et al., 2010). As an alternative, heterologous rhamnolipid production has been proposed, which is considered beneficial in non-pathogenic hosts since the native and complex quorum sensing regulation in *P. aeruginosa* is avoided (Beuker et al., 2016). However, rhamnolipid production rates and space-time yields are still significantly lower compared to the ones obtained from *P. aeruginosa* (Wittgens et al., 2011). An alternative is the production of rhamnolipids by non-pathogenic bacteria, that has only started being explored, and is considered advantageous in terms of large-scale production as it increases user safety and minimizes security measures and control during the fermentation process.

The rhamnolipid biosynthetic pathway in *P. aeruginosa* has been reported to show metabolic links with a variety of microbial products such as alginate, lipopolysaccharide (LPS), polyhydroxyalkanoates (PHAs) and 4-hydroxy-2-alkylquinolines (HAQs) (Bredenbruch et al., 2005; Choi et al., 2011; Gutierrez et al., 2013; Pham et al., 2004). Similarly, the non-pathogenic strain *B. thailandensis* has been reported for the production of LPS (Novem et al., 2009) and HAQs (Vial et al., 2008). Moreover, strains such as *B. cepacia* and *B. sacchari* have been studied regarding their PHA production potential (Mendonça et al., 2014; Pan et al., 2012).

Rhamnolipids, LPS and HAQs are compounds that are secreted in the medium. On the other hand, PHAs are linear polyesters of high molecular weight that are accumulated in the form of intracellular granules by a wide variety of bacteria. Their physicochemical characteristics resemble the ones of conventional polymers, such as polypropylene (PP) and low-density polyethylene (LDPE), while their most important benefits is that they are fully biodegradable and biocompatible (Kourmentza et al., 2009). Therefore, they are considered an attractive alternative as environmentally friendly replacements of their synthetic counterparts, with a wide range of applications in various spheres like packaging, biomedical engineering, pharmacology, cosmetics, food, agriculture and others.

It is a fact that petrochemical products have long life-cycles thus leading to their accumulation in natural environments and contamination (Singh and Sharma, 2016). On the other hand, bio-based and biologically derived products present huge environmental and societal advantages compared to their chemically synthesized counterparts due to their biodegradability, low toxicity and renewable nature. Nowadays, the main factor that restricts the widespread production and use of PHAs and rhamnolipids is their high production cost, mainly due to high raw materials cost, downstream processing required for their recovery and purification, and in several cases low manufacturing output (Banat et al., 2014; Kourmentza and Kornaros, 2016).

Ongoing research and development is targeted towards integrated biorefinery schemes, for the biotransformation of waste and biomass by-products to high value-added ones, in order to develop a circular economy model balanced in terms of economic and ecological benefits (Kourmentza et al., 2017b). PHA market is expected to grow from an estimated USD 73.6 million in 2016 to USD 93.5 million by 2021 characterized by a CAGR (Compound Annual Growth Rate) of 4.88% (Markets and Markets, 2017) while global biosurfactants market is set to be worth around USD 2.69 billion by 2023 (Global Market Insights, 2016).

Simultaneous production of PHAs and rhamnolipids is feasible and has been reported in the past by *Pseudomonas aeruginosa* (Hori et al., 2011, 2002; Marsudi et al., 2008). Since PHAs are accumulated inside the cells and rhamnolipids are secreted in the medium broth, their separation can be easily performed by centrifugation without interference in each product's downstream processing.

After preliminary screening, performed in our laboratory for various bacterial strains, it was shown that *B. thailandensis* E264 was also capable of PHAs production. The scope of the present study was to evaluate the production of PHAs and rhamnolipids from *B. thailandensis* E264, using as carbon source used cooking oil deriving from sunflower (UCO). This is the first study that describes the simultaneous production of PHAs and rhamnolipids by the non-pathogenic strain *B. thailandensis* E264 and the first report on its PHA production potential.

The recycling cooking oil industry has expanded within the past years. UCO instead of being viewed as a waste product it has become a valuable commodity, particularly for biodiesel production. UCO is characterized by its low market value and high availability while several studies have evaluated its potential biotransformation to PHAs (Cruz et al., 2016a, b; Martino et al., 2014; Obruca et al., 2014a). In addition, selection of the carbon source was based on the fact that hydrophobic substrates induce the production of rhamnolipids. This is based on the fact that bacteria may develop mechanisms in order to enhance the bioavailability of, and gain access to, hydrophobic compounds, referred to as 'micelle solubilization' or 'pseudosolubilization' (Smyth et al., 2010). Moreover, in the present study, an alternative scenario of UCO being converted to high value-added materials, instead of biofuels, is presented.

2. Materials and methods

2.1. Bacterial strain and cultivation conditions

Burkholderia thailandensis E264 was obtained by Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures). Upon revitalization, cultures were grown on agar plates and stored at 4 °C (short-term storage for 4–6 weeks), or cryopreserved at −80 °C by supplementation of 20% v/v glycerol (long-term storage). Nutrient broth (NB, or else Medium 1 as described by DSMZ) was used for bacterial growth, consisting of peptone (5 g L^{-1}) and meat extract (3 g L^{-1}), whereas pH was adjusted to 7. For the preparation of solid cultures agar (15 g L^{-1}) was also added in the medium.

In order to initiate bioreactor fermentation a bacterial inoculum was prepared as followed: a loopful of *B. thailandensis* colonies was suspended in fresh NB medium and left to grow overnight at 37 °C and 200 rpm. Re-inoculation with 5% of the formed cell suspension was performed in Erlenmeyer flasks containing NB medium supplemented with 4% w/v of UCO and bacterial cultures were incubated for 48 h at 37 °C and 200 rpm.

PHA and rhamnolipids production was performed in a 10 L reactor, with an active volume of 8L, while a 5% (v/v) inoculum was used under batch mode. NB medium was supplemented with UCO (4% w/v),