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Synchronization of lipid-based biofuel production with waste treatment using oleaginous bacteria: A biorefinery concept

Samia Qadeer[a], Shahid Mahmood[a], Muzammil Anjum[e], Noshin Ilyas[c], Zulfiqar Ali[d], Azeem Khalid[a,b,*]

[a] Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan
[b] Trees and Timber Institute – National Research Council of Italy, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy
[c] Department of Botany, PMAS-Arid Agriculture University, Rawalpindi, Pakistan
[d] Department of Chemical Engineering, COMSATS Institute of Information Technology, Lahore, Pakistan
[e] Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia

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ABSTRACT

The use of oleaginous bacteria to produce lipid-based biofuels from organic waste is an emerging approach. The present study was designed to isolate oleaginous bacteria capable of growing on food-processing waste to produce bio-fuels on a sustainable basis. About 26 oleaginous bacteria were isolated from natural crude oils using Luria-Bertani (LB) medium under nitrogen-deficient conditions. The GC–MS analysis confirmed the ability of 10 isolates to produce free fatty acids, where oleic acid appeared to be the most recurring compound among the identified fatty acids. The results of aerobic wet digestion (batch mode) showed the potential of the strain KM15 for simultaneous lipid accumulation and waste treatment. Among different types of waste, removal of volatile solids (VS) up to 38.5% and oxidizable organic matter removal (COD-based) up to 48.9% was achieved by strain KM15, while simultaneously showing an accumulation of lipids up to 41.5% in 96 h. The degradation efficiency of organic matter was 30.9% and 31% for apple and orange waste after 96 h with a lipid accumulation of 21% and 25% respectively. Overall, *Bacillus cerus* strain KM15 was the most effective strain in the degradation of mango waste and, correspondingly, the production of biolipids from waste. This study illustrates the concept of biorefinery for sustainable waste management and simultaneous production of lipid-based biofuels. The use of waste as the sole source of nutrition could be a key factor in reducing the total production cost of lipid-based bacteriological biorefineries.

1. Introduction

The search for alternative sources of energy to reduce dependence on fossil fuels and emissions from fossil fuels has led to the production of biofuels (Cortes and de Carvalho, 2015). Significant work has been done on the production of plant-based biofuels, but the use of food crops for fuel production can cause food security problems and has also been proven by the increased cost (per bushels) of corn in recent years (Thompson, 2012). Alternatively, oleaginous microbes have been identified as one of the competent sources to produce bio-fuels based

* *Corresponding author at*: Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan.
E-mail address: azeem@uaar.edu.pk (A. Khalid).
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on biolipids using an organic material as a substrate (Kumar et al., 2015; Ratledge and Wynn, 2002; Soccol et al., 2017; Vicente et al., 2009). In addition, the fuel composition can be modified by modifying the substrate. Studies have reported the potential of microalgae (Chisti, 2007; Cortes and de Carvalho, 2015; Mata-Sandoval et al., 2001), fungi (Thiru et al., 2011) and some bacterial species (Alvarez et al., 2000) for lipid accumulation. The oleaginous microbes are characterized by their capacity to accumulate lipids more than 20% during the stationary phase of their growth (Muniraj et al., 2013), whereas this character is limited to their exposure to stressful conditions with an excess of carbon and limited nitrogen (Meng et al., 2009). The production of lipid-based biofuels is limited due to the high cost of production associated with lipid accumulation using glucose as a substrate. Therefore, finding a promising raw material to produce lipids is a growing concern.

Fortunately, oleaginous microbes can also efficiently use high-carbon waste for lipid production (Kot et al., 2017; Kumar et al., 2015; Muniraj et al., 2013). In this regard, food processing waste (FPW) can be considered as a potential substrate. The FPW encompasses large volumes of different substances, including peels, pulps, juices, process water and many other things that are not of consumer demand. FPW is not specifically limited to consumer consumption behavior, but also occurs throughout the supply chain, from production to post-harvest and processing stages (Manzocco et al., 2016). According to FAO (2011), one-third of the produced food ends up into waste, accounting for nearly 1.3 billion tons per year. Among the various types of food waste, fruit and vegetable waste (FVW) makes the highest proportion of waste, ending into approximately 45% loss in total production. Therefore, producing environmental nuisance and adding to the additional management costs of the food processing industries (Plazzotta et al., 2017). This waste is mainly generated in solid or liquid form and has a characteristic high organic fraction (Yasin et al., 2013). In contrast, the organic fraction of food waste is considered as a competent resource for the production of value-added products in recent years (Van Dyk et al., 2013). The organic fraction of food waste could also be a potent substrate for oleaginous microbes to produce biofuels.

In this study, fruit waste was used as a substrate because of its high sugar content and high production volumes. The high sugar content and readily available biomass for fermentation could serve as a proportionate raw material to produce waste-based lipids. Bacterial strains were isolated from crude oils which can potentially store lipids in their cells. This approach also offers dual benefits of waste reduction and the production of lipid-based biofuels, leading to a biorefinery concept.

2. Materials and methods

The study was conducted to isolate lipid-accumulating bacteria, and then exploit potent strains for lipid accumulation using food processing waste as sole substrate. All the reagents used in the study were of analytical quality and purchased from Sigma–Aldrich (USA).

2.1. Sampling of crude oil

Samples of crude oil in the form of thick black liquid were collected from five different sources, namely Karak MOL, Karak MPCL, Kohat MOL, Attock oil fields and Pindi oil fields in Pakistan. These samples were collected in amber colored bottles and transported to the laboratory in airtight bottles at 4 °C.

2.2. Isolation and screening of oleaginous bacteria

The oleaginous bacteria were isolated from crude oil using Luria-Bertani (LB) medium under aerobic conditions. Briefly, 5 ml of crude oil was introduced into 500 ml sterilized LB broth medium containing yeast extract (2.5 g), peptone (5 g) and NaCl (5 g). The pH of the medium was maintained at 7. The culture medium was kept under shaking for 48 h and then the isolation was carried out on LB agar plates using the dilution plate technique. Plates were incubated at 32 ± 1 °C for 48 h. The pure cultures were obtained by repeated streaking on an agar medium (Gao et al., 2012).

2.3. Primary screening of oleaginous strains (lipid accumulating bacteria)

The initial screening of the oleaginous bacteria was carried out as a function of their ability to grow on a nitrogen deficient medium. The medium (1 l) contains 10 g of K_2HPO_4, 5 g of NaH_2PO_4, 5 g of glucose, 1 g of yeast extract, 0.2 g of $MgSO_4$, 2.5 mg of $CaCl_2$ and 1.5 mg of $FeSO_4$. Isolates capable of growing on a nitrogen-limited medium were further screened on the basis of their capacity to store lipids in CDW (cell dry weight). For the estimation of lipid content, the selected isolates were grown for 72 h at 32 ± 1 °C. Subsequently, at the stationary phase growth, the cells were harvested by centrifugation for 15 min at 5000 rpm. The cell biomass was washed twice with deionized distilled water to remove excess nutrients (Zhang and Hu, 2011). The cell pellets were dried for 24 h at 60 °C to constant dry weight. Finally, the CDW was measured and further analyzed for lipid accumulation capacity by gravimetric method (Ongmali et al., 2014).

2.4. Secondary screening of oleaginous bacteria using food processing waste as a substrate

Three types of FPW such as orange, mango, and apple were used as the sole substrate for bacterial growth. Waste samples were mechanically shredded to a uniform particle size of 2–5 mm. The waste slurry was prepared by adding 5 g (wet weight) of fruit waste containing pulp and peels to 95 ml of distilled water to achieve a final volume of 100 ml for each waste. To ensure the growth of the selected isolates, the slurry was autoclaved at 120° C for 20 min (Nanolytik Nanoclave 1, Germany). The 24 selected isolates were subjected to a secondary screening process, where each isolate was tested for its potential to use FPW as a primary substrate. The potential of each isolate was estimated by measuring optical density (OD_{600}) of the culture at 600 nm using Photoelectric Colorimeter (ERMA Inc., Tokyo, Japan).

2.5. Experimental setup for waste treatment and simultaneous lipid production

Five isolates with the highest growth (OD_{600}) on FPW were further evaluated for their simultaneous potential of waste treatment and lipid accumulation. To carry out a waste treatment experiment, slurries were prepared by mixing each waste with water at a ratio of 1:19 (w/v). In the preliminary analysis, each waste slurry (apple, mango, and orange waste) was characterized for physicochemical characteristics such as pH, electrical conductivity (EC), total dissolved solids (TDS), total solids (TS) and volatile solids (VS). Thereafter, the strain KM15 showing best results was further evaluated for lipid accumulation and waste degradation (COD removal). For this, the slurry was divided into two equal proportions (250 ml each). After autoclaving the slurry, each flask containing a single type of waste was inoculated with strain KM15. The flasks were incubated at 30 °C with shaking at 150 rpm and periodic sampling was carried out at 48, 72, 96 and 168 h. The collected samples were analyzed for lipids, COD, VS, TDS, EC,

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