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Original Research Paper

Control-release of antimicrobial sophorolipid employing different biopolymer matrices

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ABSTRACT

Sophorolipid (SL) purified from fermentation broth of *Candida bombicola* grown on oleic acid and glucose substrates was embedded at 0%, 9%, 17%, and 29% (%-total weight of final product) in solvent-cast films of poly(L-lactic acid) (PLLA), poly(ε-caprolactone), and poly(hydroxybutyrate) (PHB). Growth-inhibition activity of the SL-biopolymers against *Propionibacterium acnes*, a causative agent of acne vulgaris skin condition, is dependent on the SL contents of the films; the degree of inhibition as determined from the width of the zone of inhibition in agar-plate assays follows the order of SL-PCL > SL-PLLA > SL-PHB. The release of SL from the films into aqueous medium after a 4-d shaking at 25 °C showed that SL-PLLA (30.1 ± 1.7 wt% SL released) most readily released the embedded SL, followed by SL-PHB (11.4 ± 4.3 wt%) and SL-PCL (4.3 ± 1.4 wt%). Thermal properties as determined by differential scanning calorimetry showed that SL decreases the heat of fusion (ΔH) and the melting temperature (T_m) of the biopolymers, indicating for the first time its usefulness as a plasticizer to prevent crystallization. In summary, the study shows the feasibility of controlling the release of antimicrobial SL by varying the type of biopolymer used, with the added advantage of SL functioning as a plasticizer to improve the physical properties of the film in term of lower crystallinity. Future research could benefit the agricultural sector via new developments as varied as antimicrobial food packaging and algal bloom mitigation.

1. Introduction

Sophorolipids (SLs) are a class of glycolipids produced and secreted by *Candida bombicola*, *Candida apicola, Candida stellata, Cataglyphis floricola, Candida riodocensis, Candida batistae, Pichia anomala, Rhodotorula bogoriensis*, and *Wickerhamiella domercqiae* (Van Bogaert et al., 2011). *C. bombicola* is the best known organism for high-yield production (up to 400 g SL/L culture) of SL (Kim et al., 2009; Pekin et al., 2005). The predominant molecular species of SL synthesized by *C. bombicola* grown on oleic acid and glucose are 17-L-[(2′-*O*-*β*-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-9-octadecenoic acid 1′,4′′-lactone 6′,6′′-diacetate (*SL-1*; Fig. 1A) and its free-acid form (*SL-1A*; Fig. 1A) (Asmer et al., 1988). Being an amphiphilic glycolipid, SL has excellent surface active properties suitable for use as surfactant, emulsifier, and wetting agent. Moreover, SL has been found to possess strong antimicrobial activity against many microorganisms (Kitamoto et al., 1993; Kim et al., 2002; Shah et al., 2007; Sleiman et al., 2009). Furthermore, the importance of SL in agriculture is underscored by its algicidal activity as demonstrated in the mitigation of harmful algal bloom (Lee et al., 2008; Sun et al., 2004). SL is therefore valuable for use not merely as a "green" biobased substitute for nonrenewable petrochemical surfactants, but also for dual-functional applications that take advantage of its additional value-added properties such as antimicrobial/algicidal activity and plasticizer functionality.

To date, the biological activities of SL have largely been studied in solution (Kitamoto et al., 1993; Kim et al., 2002; Shah et al., 2007; Sleiman et al., 2009; Baek et al., 2003; Borzeix Concaix, 2003). Study on the effects of embedding SL into a solid matrix or carrier on its antimicrobial activity is lacking in the literature (Ashby et al., 2011). In addition, even though surfactants have been widely studied as plasticizers in many formulations (Ghebremeskel et al., 2006, 2007; Collins et al., 2004; Vieira et al., 2011; Patra et al., 2013; Hayes, 2011), the effects of SL incorporation on the physical and mechanical properties of polymers have not been widely reported (Ashby and Solaiman, 2014). The aims of this study are to examine the effects of incorporating SL on the thermal and physical properties of the films of three well-known

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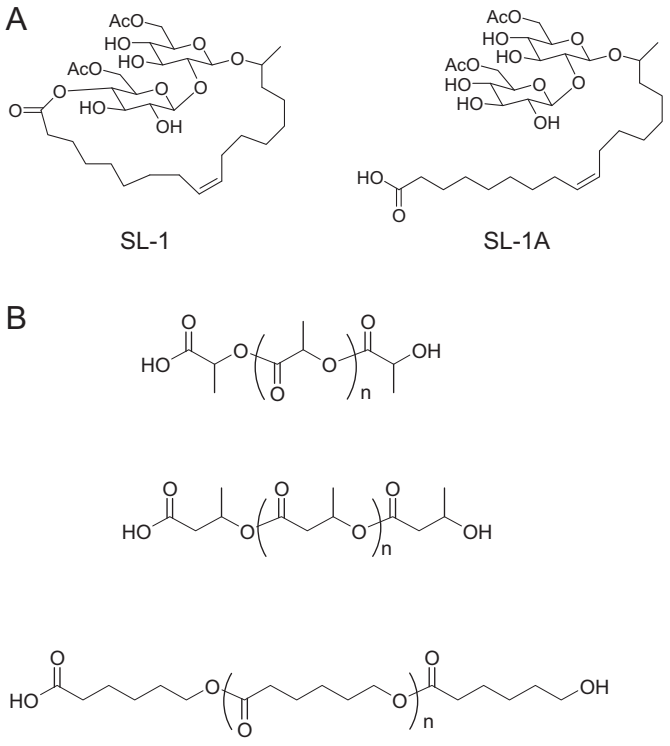

Fig.. 1 **A.** Structures of sophorolipids synthesized predominantly by *C. bombicola*. The 17-L-[(2′-*O*-*β*-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-9-octadecenoic acid 1′,4′′-lactone 6′,6′′-diacetate (SL-1) and free-acid (SL-1A) form. **B.** Chemical structures of PLLA (*top*), PHB (*middle*), and PCL (*bottom*).

biodegradable polymers, i.e., poly(L-lactic acid) (PLLA), poly(ε-caprolactone) (PCL), and poly(hydroxybutyrate) (PHB), and to characterize the antimicrobial activity of these films as the embedded SL is released. The results are expected to expand our understanding of SL-biopolymer interaction that is useful in the designing of tailored SL-delivery biopolymer films.

2. Materials and methods

2.1. Chemicals, biologicals and microorganism

All reagent-grade chemicals and organic solvents were variously purchased from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Fair Lawn, NJ), Honeywell Burdick & Jackson (Muskegan, MI), and other commercial suppliers. PLLA and PCL were obtained from Sigma-Aldrich. *Pseudomonas oleovorans* NRRL B-14682 containing the genetic system needed for PHB biosynthesis (Solaiman and Ashby, 2005) was originally obtained from ARS Culture Collection (Peoria, IL), as was *Propionibacterium acnes* NRRL B-4224 strain used as the test organism in antimicrobial assay. *C. bombicola* ATCC 22214 for SL production was purchased from American Type Culture Collection (Manassas, VA).

2.2. Biosynthesis

PHB was obtained by fermentation using *P. oleovorans* NRRL B-14682 (Ashby et al., 2012). Production was performed with a Bioflo 3000 fermenter (New Brunswick Scientific, Edison, NJ) in 10-L medium E* (for media composition see Brandl et al. (1988)) containing 1% glycerin as carbon source. The medium was inoculated with bacterial cells collected by centrifugation (8000 × g, 4 °C, 15 min) from 1 L *P. oleovorans* NRRL B-14682 culture (previously grown for 24 h) (Ashby et al., 2012). Fermentation of the 10-L culture was carried out for 48 h at 30 °C, 250 rpm impeller rotation speed, and 3 L/min aeration. Cells were then harvested by centrifugation (8000 × g, 4 °C, 15 min) and dried by lyophilization. PHB was recovered from the lyophilized cells by chloroform extraction at 30 °C with shaking at 250 rpm overnight. Insoluble cellular material was removed by filtration through a Whatman #2 filter paper, and the chloroform of the clear filtrate was evaporated to concentrate the crude polymer. The crude polymer was precipitated a total of 3 times by dropwise addition to cold methanol. The polymer was recovered and dried *in vacuo* for 24 hours. The yield of polymer was obtained by weighing the dried materials.

Sophorolipid (SL) was isolated from *C. bombicola* ATCC 22214 grown on oleic acid and glucose (Ashby et al., 2008). As with PHB, SL synthesis was performed at a 10-L working volume of medium in a 12-L capacity bench-top fermenter (Bioflo 3000 Batch/Continuous Bioreactor, New Brunswick, NJ). The basal Candida Growth Media (CGM; glucose 10% w/v, yeast extract 1% w/v, urea 0.1% w/v) production medium was prepared, autoclaved, allowed to equilibrate to 26 °C, and then supplemented with oleic acid as the lipid co-substrate to a final concentration of 2% (w/v). A 50-mL frozen inoculum culture (previously stored in −80 °C freezer) was thawed and used to inoculate the 10-L CGM (2% oleic acid) medium. The fermentation was performed at 26 °C, an impeller speed of 700 rpm, 2 L/min aeration, and no pH control. On day 2 (∼48 h) after inoculation, an additional 7.5% (w/v) of dry glucose and 2% (w/v) oleic acid were added to the culture. On day 5 (∼ 120 h) after inoculation, an additional 1% (w/v) of oleic acid was added. The fermentation was continued for an additional 2 days, and the entire culture (cells and broth) was then collected and lyophilized to dryness (∼2 days). SL was extracted from the dried residues by shaking in Erlenmeyer flasks with excess ethyl acetate at room temperature for 2 days. The extract was filtered through Whatman No. 2 filter paper, and the remaining solids were washed two additional times with ethyl acetate to maximize recovery. The combined ethyl acetate fractions containing the SLs were concentrated by evaporation and precipitated into 1 L aliquots of hexane to obtain the pure SLs. The pure SLs (as indicated by an HPLC-ELSD analysis) were recovered on a Whatman No. 2 filter paper, and were then vacuum-dried to a fine white powder in a desiccator. The chemical structures of SLs purified as described here had been previously been determined by LC/MS analysis (Fig. 1A) (Nuñez et al. (2001)). The yield of SL was obtained by weighing the dried white powder.

2.3. Preparation of PLLA, PCL and PHB biopolymer films containing SL

Poly(L-lactic acid) (PLLA), poly(ε-caprolactone), and poly(hydroxybutyrate) (PHB) films containing various amounts of SL were prepared by solvent casting technique. Samples (250 mg) of PLLA, PCL and PHB and an appropriate amount of SL were dissolved in 20 mL of dichloromethane. Four concentrations (wt% of composite) of SL were investigated: 0%, 9%, 17% and 29%. The solution mixtures were heated (about 40 °C) and stirred about an hour until they were uniformly mixed. The mixture of biopolymer and SL was then poured on a glass plate. Upon cooling and solidification, the thin film was peeled off and dried in a vacuum oven for several days at room temperature before using in various analyses.

2.4. HPLC-ELSD analysis of SL

SL concentration was determined using an HPLC system (Model UFLC, Shimadzu) equipped with an Evaporative Light Scattering Detector (Model ELSD-LT II, Shimadzu). The temperature of the ELSD drift tube was maintained at 40 °C, and in-house N_2 set at 50 psi was used as nebulizing gas. A reverse-phase C_{18} column