Myristic acid derived sophorolipid: efficient synthesis and enhanced anti-bacterial activity

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Running Head: Synthesis of short chain derived sophorolipid

Supporting information

Materials and methods:

Thin Layer Chromatography:

Preliminary characterization of different components was analyzed by performing thin layer chromatography on silica gel plates (Merck DC Keiselgel 60 F254). The solvent system used was Chloroform/methanol/water in the ratio 65:15:2 v/v. Sample spots were detected using anisaldehyde method. Briefly, the silica plate is immersed into the charring solution containing...
[absolute ethanol (135ml), conc. H$_2$SO$_4$(5ml), glacial acetic acid(1.5ml), p-anisaldehyde(3.7ml)]
and then the plate is heated for visualization of the components (Dubey, 2014).

Oil Displacement:

Oil displacement assay was performed to access the surfactant property of the synthesized MASL. For the assay, a 55mm petri plate with 20 ml of distilled water was taken. On to it a thin film of oil was formed by adding 1 ml of rapeseed oil. Onto this equilibrated film, 10 µl of MASL (0.1mg/ml) was added. A clear halo upon addition of sample determines the surfactant ability of the MASL.

HPLC

The different ratios of acidic to latonic components present in MASL were assessed using HPLC (Water 515 System, C18 Column, UV detector-2489). The solvent system consisted of ACN/Water (70:30). The injection volume was 50 µL and the retention time was 35 minutes. Flow rate of the sample was maintained to 0.7 ml min$^{-1}$.

High-Resolution Mass Spectrometry:

The mass spectrum of crude MASL along with mass spectra of individual congeners post purification are represented in the following images.

Anti-bacterial Activity:

Anti bacterial activity was assessed against gram positive and gram negative bacteria using contact method. After 24 hours of incubation the number of colonies formed onto the plates were counted. From this the % survival graph is plotted. The cumulative images for each organism are presented.

**Result and discussions:**
Thin Layer Chromatography:

The TLC of crude MASL (S1) exhibited 5 bands depending upon the respective polarity. MASL was spotted with only oil (Myristic acid). MASL showed no band in accordance to the oil band indicating complete conversion of the substrate into sophorolipid production.

Figure S1: TLC of crude MASL

Oil Displacement assay:

Oil displacement of MASL(S2) was done to determine the surfactant property. The clear halo in the test images verifies the surfactant ability of the synthesized MASL.

Figure S2: Oil Displacement assay of crude MASL using rapeseed oil. Control: Without addition of MASL; Test: On addition of MASL.

High Performance Liquid Chromatography (HPLC)
Crude MASL is a mixture with ratio of 80:20 (acidic:lactonic). Acidic components (R.T- 4.0- 6.5) are eluted first while the lactonic (R.T- 6.9- 28.17) are eluted later due to their higher hydrophobicity.

Figure S3: HPLC of crude MASL displaying the acidic and lactonic ratio

High-Resolution Mass Spectrometry:

The following figures depict the mass spectra of crude MASL: to identify the presence of different modifications. Figure S4a reveals the mass spectrum of native MASL with molecular formula C_{26}H_{48}O_{13}. The spectrum shows presence of protonated molecular ion peak at m/z 569.3170. The lactonic part (Figure S4b) (C_{26}H_{46}O_{12}) is confirmed by the presence of sodium adduct at m/z 573.28871.
Figure S4 (a): Mass spectrum of component a (non-acetylated acidic form) S4 (b): Mass spectrum of non-acetylated lactonic form

Figure S5a reveals the mass spectrum of monoacetylated MASL with molecular formula C_{28}H_{50}O_{14}. The mass spectrum shows presence of protonated molecular ion peak at m/z 611.3285.
The lactonic part (Figure S5b) \((\text{C}_{28}\text{H}_{48}\text{O}_{13})\) is also confirmed by presence of protonated molecular ion peak at \(m/z\) 593.3169.

Figure S5 (a): Mass spectrum of monoacetylated acidic form, S5(b): Mass spectrum of monoacetylated lactonic form
The presence of diacetylated MASL form is revealed through Figure S6a. The diacetylated form has molecular formula $C_{30}H_{52}O_{15}$, is depicted by its sodium adduct at m/z 675.3203. The lactonic component are depicted in Figure S6b. The lactonic component ($C_{30}H_{50}O_{14}$) is depicted by its sodium adduct at m/z 657.3109.

Figure S6 (a): Mass spectrum of diacetylated acidic form, S6 (b): Mass spectrum of diacetylated lactonic form
High-Resolution Mass Spectrometry:

The below graphs depict the chromatogram and corresponding mass spectrum of individual congeners post purification (Fractions collected from dry column chromatography).

For fraction I a peak was obtained at 1.23 RT (Figure S7) which corresponds to acidic sophorolipid protonated ion, with molecular formula $\text{C}_{26}\text{H}_{49}\text{O}_{13}$ and $m/z$ 569.3163.

Figure S7: Extracted ion chromatogram and mass spectrum of fraction I
For fraction II a peak was obtained at 1.24 RT (Figure S7) which corresponds to lactonic monoacylated sophorolipid sodium adduct ion, with molecular formula $C_{28}H_{48}O_{13}$ and m/z 615.2985. The small peak at 0.91 is attributed to the solvent system used.

![Figure S8: Extracted ion chromatogram and mass spectrum of fraction II](image)

For fraction III a peak was obtained at 1.39 R.T (Figure S9), which corresponds to acidic monoacylated sophorolipid sodium adduct ion, with molecular formula $C_{28}H_{50}O_{14}Na$ and m/z 633.3107.
A sharp peak was obtained at 1.80 R.T for fraction IV (Figure S10) which corresponds to acidic diacetylated sophorolipid sodium adduct at m/z 675.3190, with molecular formula C$_{30}$H$_{52}$O$_{15}$Na.
For fraction V a sharp peak was obtained at 1.39 R.T (Figure S11), which corresponds to lactonic di-acetylated sophorolipid protonated ion with molecular formula C$_{30}$H$_{50}$O$_{14}$ and m/z 657.3086.
Anti Bacterial activity:

The figure S12 represents the colony count of *S. aureus*. From the images it is evident that as the concentration of MASL increases, the colony count decreases. The similar trend is seen in case of time interval.
Figure S12: Reduction in the colony count of *S.aureus* at different concentrations of MASL and at different time intervals

Figure S13 represent colony count of *P.arenuginosa*. With increase in concentration the colony count decreases. As compared to gram positive, higher concentration of MASL is needed to inhibit the gram negative organisms.
Figure S13: Reduction in the colony count of *P. aeruginosa* at different concentrations of MASL and at different time intervals

The following table summarizes the MIC (IC$_{80}$) value of MASL against both organisms in terms of concentration and time interval.

| Organism         | Concentration (µg/ml) | Time Interval |
|------------------|-----------------------|---------------|
| Gram Positive    | *S. aureus*           | 150           |
| Gram Negative    | *P. aeruginosa*       | 350           |

Table S1: Summary of anti bacterial

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