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Induced Biosurfactant Production and Degradation of Lindane by Soil Basidiomycetes Yeast, Rhodotorula sp. VITJzN03

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ABSTRACT

The process of degradation in organochlorine insecticide lindane is greatly hindered by its high degree of hydrophobicity. In this work, the lindane-degrading yeast strain, Rhodotorula sp. VITJzN03 was found to show extracellular emulsification activity and reduction in surface tension while growing in mineral medium. The production of glycolipid biosurfactant was induced by addition of olive oil into the cultures of the yeast. Addition of olive oil increased the yield of biosurfactant to 7g/L. The biosurfactant produced was identified as a diacetyl sophorolipid by FTIR, NMR and GC-MS analysis. The purified sophorolipid had a critical micelle concentration of 110 mg/L. Induced production of sophorolipids by Rhodotorula VITJzN03, showed a positive effect on lindane degradation which was detected by the mineralization of lindane and release of chloride ion. Under the optimized growth conditions, 600 mg/L of lindane was completely mineralized with a release of 6mM of chloride ion by the end of the 5th day. The yeast cultures without olive oil showed the same result by the end of the 8th day. Therefore, it can be concluded that increased biosurfactant production could enhance lindane degradation by means of co-metabolism of olive oil.

Keywords: Biodegradation; Lindane; Olive oil; Sophorolipid; Rhodotorula sp.

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INTRODUCTION

Chlorinated organic pesticides are one of the major groups of chemicals which are responsible for environmental pollution. Lindane (gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane), a persistent, highly toxic, and bioaccumulative organochlorine insecticide, was used in agriculture and as a topical treatment for human head lice and scabies beginning in the 1940s [1]. The residues of lindane have been shown to accumulate in the environment and gain entry into the human body through the food chain [2]. Acute and chronic exposure of lindane has been shown to produce marked neurological changes including proconvulsive effects in humans and experimental animals [3-6]. Several soil microorganisms capable of degrading and utilizing lindane as a carbon source have been reported over last two decades. In selected bacterial strains, the genes encoding the enzymes involved in the initial degradation of lindane have been cloned, sequenced, expressed and the gene products were characterized. Most studies report the degradation of relatively low (<500 mg/kg) concentrations of lindane in soil [7]. In our previous study, we isolated soil basidiomycetes yeast, Rhodotorula sp. VITJzN03 which could grow and degrade lindane at a concentration of 600 mg/L during a period of 8 days with a half-life of 1.66 days [8].

A promising method that can improve the effectiveness of lindane bioremediation process is the use of biosurfactants. The addition of biosurfactants is expected to enhance lindane degradation by mobilization, solubilization or emulsification. Manickam et al. demonstrated enhanced degradation of HCH isomers by Sphingomonas sp. NM05 in the presence of three biosurfactants which were individually added into the culture of the degrading bacteria [9]. In our recent study, the ability of glycolipid MEL produced by the yeast strain Pseudozyma VITJzN01 was reported to form bio-microemulsions for enhanced degradation of lindane from aqueous cultures as well as soil slurry [10]. In the present study we have explored the possibilities to induce biosurfactant production in aqueous culture of Rhodotorula sp. VITJzN03 by the incorporation of olive oil as an additional carbon source. Effect of olive oil in the production of glycolipids was studied and the degradation of lindane in the presence of olive oil induced glycolipids was studied.

MATERIALS AND METHODS

Materials

Lindane was purchased from Sigma-Aldrich (USA). All the solvents used were HPLC grade and were procured from SRL Chemicals India Ltd. All the media components were obtained from Hi-media chemicals (Mumbai, India).

Microorganism and culture maintenance

Rhodotorula sp. VITJzN03 (GenBank: JX310560), a strain isolated from sorghum cultivation fields, Vellore, Tamil Nadu, India was screened for lindane degradation and biosurfactant production . The pure culture obtained stored at -80 ºC as master stock. The working culture was preserved at 4 ºC on yeast extract peptone dextrose (YEPD) agar slants and subcultured every two weeks.

Detection of biosurfactant production during lindane degradation

The degradation studies on lindane were performed in 100 mL Erlenmeyer flask containing 25 mL mineral medium with lindane at a concentration of 600 mg/L [8]. The formation of biosurfactant in mineral medium during yeast growth was monitored by the reduction in the surface tension of the medium as well as the emulsification activity of the medium. The biosurfactant produced into the culture medium was extracted from the cell free supernatant as described by Salam and Das [10].

Induction of yeast strain for biosurfactant production

As it was found that lindane was not a good substrate for biosurfactant production for the yeast Rhodotorula sp. VITJzN03, olive oil (2%) was added as an inducer to increase the hydrophobicity of the mineral medium along with lindane. The surface tension of the culture medium was monitored at regular intervals.

Chemical characterization of the biosurfactant

The Critical Micelle Concentration CMC value of the purified biosurfactant was determined by measuring the surface tension of water at various concentrations of the biosurfactant. The primary characterization of the biosurfactant was done by TLC. Compositional analysis was done by determining the total carbohydrates, proteins and lipids. Further characterization was performed with the help of FTIR, NMR spectroscopy and GC-MS analysis.

Biodegradation assays in aqueous culture

The degradation of lindane by Rhodotorula sp. VITJzN03 in mineral medium was tested in the presence of glucose and olive oil as inducers of biosurfactant production. Experiments were conducted in 250 mL Erlenmeyer flask, containing 100 mL mineral medium with lindane alone or along with the inducers and were incubated at 30 ºC for 8 days. The residual lindane in the culture medium was analyzed at regular intervals by means of gas chromatography (GC) [8]. The free chloride released into the medium was determined by mercuric thiocyanate method [11]. The biosurfactant production was monitored by means of emulsification activity in the culture medium.

RESULTS AND DISCUSSION

Production of biosurfactant during lindane degradation

As an indication of production of surfactant by Rhodotorula sp. VITJzN03 during lindane degradation, a slight decrease in surface tension together with an increase in emulsification activity was observed (Fig.1A). The biosurfactant production continued in the lindane degrading cultures till the yeast approached stationary phase, by the end of 7^{th} day of incubation. The surface tension of the culture was reduced to a final value of 60 mN/m by this time. The reduction of the surface tension indicated the ability lindane to induce biosurfactant. The amount of biosurfactant was very low (< 2 g/L) indicating that lindane is

not a good substrate (Fig. 1B). Increase in the amount of biosurfactant in the culture could enhance the degradation of lindane.

Manickam et al demonstrated that addition of surfactants into the cultures of HCH isomers degrading Sphingomonas sp. NM05 [9]. To increase the yield of biosurfactant by the yeast, olive oil was added into the culture. The surfactant produced was extracted by centrifugation and ice cold ethanol precipitation. The yield of surfactant was raised to 7 g/L by induction (Fig. 1B). The biosurfactant was purified by column chromatography and lyophilized.

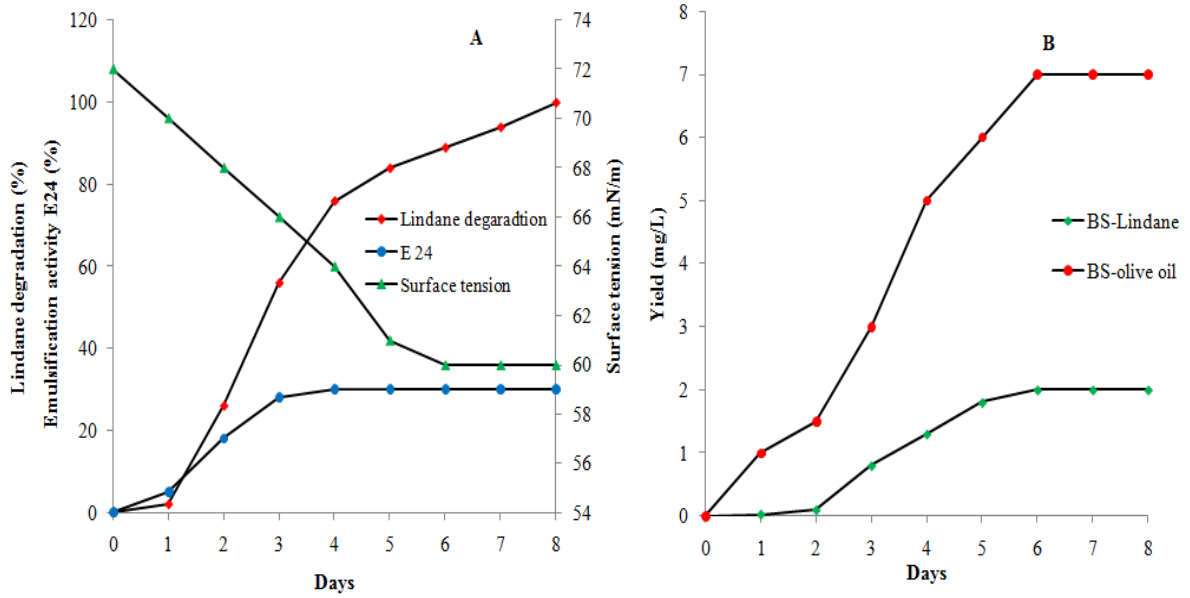

Figure 1. A) Lindane degradation in mineral medium along with reduction of surface tension and increase in emulsification activity. B) Biosurfactant production yield in the mineral medium with lindane and with olive oil.

Chemical and structural characterization of the biosurfactant

The CMC value of the purified biosurfactant was calculated as 110 mg/L, by taking the intercept of graph surface tension Vs concentration of biosurfactant (Fig. 2A). Compositional analyses revealed that the biosurfactant produced from olive oil by Rhodotorula sp. VITJzN03 might be a glycolipid primarily consisting of 67% lipid and 31% carbohydrates**.** There were no protein fractions in the biosurfactant. The same was confirmed in the FTIR spectrum of the purified biosurfactant (Fig. 2B). There were broad absorbance at 3232.70, 1236.37, 1139.93 and 1116.76 cm^{-1} which indicated hydroxyl group or OH stretch in the carboxylic acid stretch. Vibrational changes at wavenumber 3005.10 implied presence of C=C-H and terminal C-H stretches. Strong absorbance in the range 2850-2930 cm^{-1} as well as 1460 and 730 indicated the presence of long linear aliphatic chain in the lipid moiety of the glycolipid. There were no evidences of –NH or C-N stretches in primary, secondary or tertiary amines which confirmed that protein moieties were absent in the biosurfactant.

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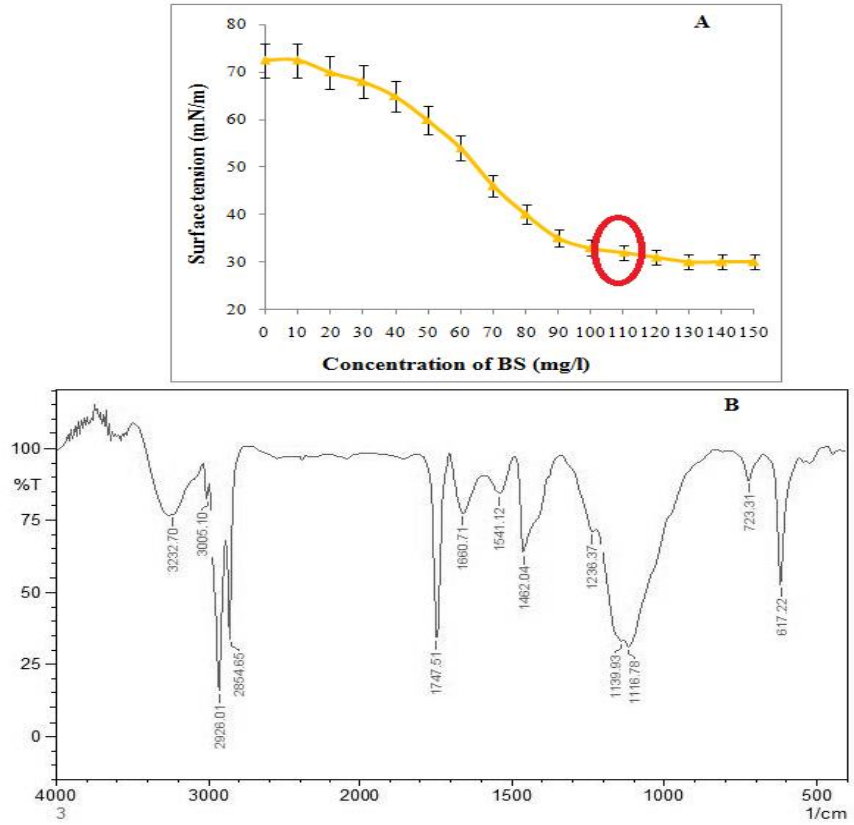

Figure 2. A) Critical micelle concentration of the biosurfactant (BS). B) FTIR spectrum of the purified glycolipid.

The ^{1}H NMR data interpreted that acyl group is attached to the C-6' carbon of the hexose sugar. The number of carbons was determined by ^{13}C NMR and determined as 30. The hydrophobic lipid chain was analysed by GC-MS of the methyl esters of the surfactant. The major fatty acids were C18:0 and C16:1. The structure of the glycolipid produced by Rhodotorula sp. VITJzN03 was elucidated based on the FTIR, NMR and GC-MS analyses and is given in Fig. 3.

Yeasts have been reported as copious producers of glycolipid biosurfactants from substrates such as soybean oil and other hydrocarbons [12-17].

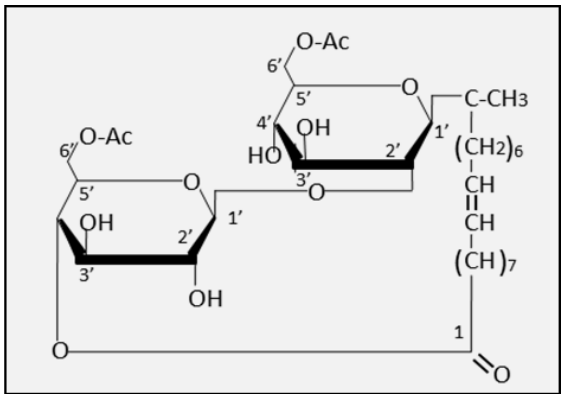

Figure 3. Diacetyl sophorolipid produced by Rhodototrula sp. VITJzN03.

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Biodegradation assays

As seen in the Fig.4A, the biodegradation of lindane was enhanced when the production of biosurfactant (Fig. 4B) was induced by the addition of olive oil. The degradation of lindane was complete, with no apparent residues detected in GC at the end of 8 days of 30% only. The degradation of lindane was delayed when glucose was added as an inducer of biosurfactant production. But gradually, the yeast culture achieved complete degradation of lindane by the end of the 8th day. The delay in degradation takes place because the yeast prefers glucose rather than lindane as its sole carbon source. Once the glucose concentration is depleted in the medium, the yeasts utilize lindane for its growth. The degradation is efficient here due the increased biomass, which is produced from glucose. The culture reached equilibrium again by the end of 8th day. No change was noted in the emulsification activity. This implies that glucose is also not a good inducer of biosurfactant production. On the other hand, the use of olive oil as an inducer increased the degradation efficiency greatly. This was evident from the absence of any lindane residue after the 5th day of incubation. The addition of olive oil stimulated the release of glycolipids into the medium by Rhodotorula sp. The enriched glycolipids reduced the hydrophobicity of lindane helping in the solubilization of the compound which could be taken up by the yeast. When olive oil and lindane were present together in mineral medium, lindane degradation started earlier compared to other cases where lindane was alone or in combination with glucose (Fig 4A). This suggested the possibility of co-metabolic utilization of lindane and olive oil together by the yeast. The emulsification activity of the culture was 78% confirming the release of surfactants into the medium by the yeast as shown in Fig. 4B.

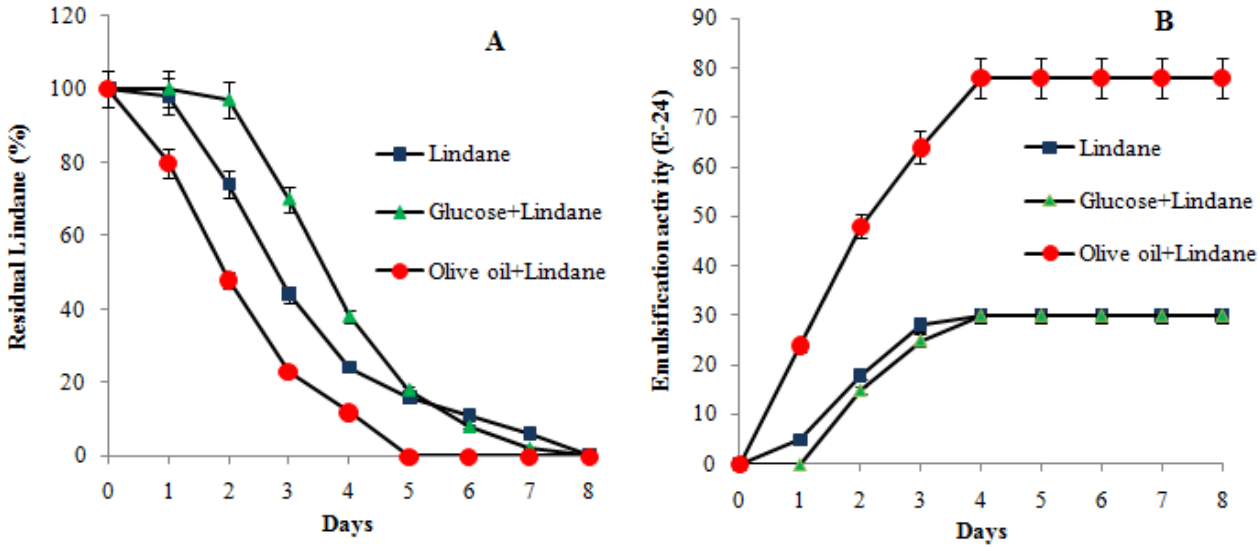

Figure 4. Residual lindane in mineral medium (A) and Emulsification activity (E24) of the yeast culture (B).

CONCLUSION

There are reports on enhancement of degradation efficiency using synthetic surfactants or biosurfactants for bioremediation of hydrophobic pollutants. But in the present study, we have induced the production of biosurfactant in the culture enhancing the degradation of lindane by the soil yeast Rhodotorula sp. VITJzN03. Olive oil was proved to be the better substrate for biosurfactant production compared to other substrates

studied like glucose and lindane. By the induction of biosurfactant production in the yeast culture, the degradation of lindane was increased by 20%, which was completed by the end of 5th day instead of 8 days under normal conditions. This approach can be implemented in the bioremediation of lindane contaminated sites.

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