

Original Research Article

Antimicrobial Activity and Toxicity of Sophorolipids Produced by *Candida haemulonis* and *Saccharomyces cerevisiae* Against Some Selected Microorganisms

Abstract

Sophorolipid have been identified to possess varying antagonism relationship to a number microbiota, although having been identified as being ecologically friendly. The biosafety of biologically synthesized materials has been identified as a major challenge to commercialization and scale-up. Sophorolipid was produced by *Candida haemulonis* and *Saccharomyces cerevisiae* in Palm oil mill effluent impacted soil in Emohua, Rivers State. Submerged fermentation was employed in the production of the sophorolipid while the Well-in-agar approach was employed in the antimicrobial susceptibility was conducted using 20%, 40%, 60%, 80% and 100% on both beneficial and pathogenic bacteria and fungi namely *Staphylococcus* sp, *Nitrobacter* sp, *Klebsiella* sp, *Bacillus* sp while the fungal flora were *Aspergillus* sp, *Penicillium* sp, *Rhizopus* sp, *Candida* sp, *Mucor* sp and *Saccharomyces* sp. Acute toxicity was conducted using *Nitrobacter* sp, *Nitrosomonas* sp and *Thiobacillus* sp. Probit based determination of acute toxicity after a 48hr and 96hr exposure to the test organisms. Antagonistic nature of the sophorolipids showed there was little or no antagonistic activity on the bacterial isolates than the fungal isolates. The sophorolipid formulation synthesized from both isolates were observed to have a zone of inhibition between 10 mm to 24 mm for *Nitrosomonas* sp. while to *Aspergillus* sp it had a range between 4.0 mm to 13.0 mm. Acute toxicity of sophorolipid produced by *Candida haemulonis* against *Nitrosomonas* sp 0.054 mg/L while to *Thiobacillus* sp it had an acute toxicity index of 0.107 mg/L. This study identified that the sophorolipid produced by the yeast isolate had the potential of being employed in a number of sectors. This study has pioneered the untapped benefits in the POME-induced flora and how they can be veritable tools in the biotechnology industry.

Keyword: Sophorolipids, Biosafety, Probit, Antagonism, Acute toxicity, Microbiota

INTRODUCTION AND BACKGROUND

Surfactants have been described in certain reports as being an undisputable tool in man's everyday life. Applications reaching far beyond our hygienic needs, ranging from asphalt, concrete and food to fuel additives Delvelter and Laurysen (2010). They are broadly classified as glycolipids, lipoproteins or lipopeptides, phospholipids, fatty acids or natural lipids, polymeric surfactants and particulate surfactants (Moya *et al.*, 2015; Hoskova *et al.*, 2013). Biosurfactants are amphiphilic compounds synthesized by a wide variety of microorganisms, which either adhere to cellular membrane or are excreted extracellularly into the culture medium (Liu *et al.*, 2016). Sophorolipids are members of the glycolipids that can be produced by bioconversion of native and renewable feedstocks. They are biologically surface-active agents commonly synthesized by *Candida* spp, *Wickerhamiella* sp and *Rhodotorula* sp (Li *et al.* 2020). They may exhibit some variations in their morphology on the basis chemistry. Sophorolipids such as cyclic lipopeptide (CLP) are stable over a wide pH range (7.0-12.0) and heating them at high temperature does not result in any loss of their surface-active property (Mukherjee, 2007).

Sophorolipids are considered secondary metabolites whose synthesis are believed to be associated with the end of the exponential phase. A number of substrates have been associated with production ranging from oleophilic and hydrophilic materials (Celligio *et al.*, 2020).

Antimicrobial susceptibility indices of biologically synthesized materials have been identified as a critical and defining process in the definition of biosafety and ecosystem partitions. Although, the number of previous investigations has applied the area of biotechnology is yet to show *Candida haemulonis* sourced sophorolipid as being biosafe for environmental. Gharaei-Fathabad (2011) reported that several biosurfactants have strong antibacterial, antifungal and antiviral activity; these surfactants play the role of anti-adhesive agents to pathogens making them useful for treating many diseases as well as its use as therapeutic and probiotic agent. Sophorolipids exerts its toxicity on the cell membrane permeability bearing the similitude of a detergent like effect (Zhao *et al.*, 2010).

MATERIALS AND METHODS

Sophorolipid production

Eighteen-hour (18hrs) yeast culture was grown on the Glucose-Yeast Peptone media at room temperature and inoculum standardization was done using Macfarland standard. About 0.1% inoculum was dispensed aseptically into the mineral-salt-broth glycerol 3%, Urea, 4.0%, KH_2PO_4 4.03 mg.L^{-1} , MgSO_4 0.4 mg.L^{-1} , NaCl 1.0 g.L^{-1} , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g.L^{-1} , Urea, 4.46 g.L^{-1} , 0.1L trace metal solution containing $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 1.5 g.L^{-1} , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g.L^{-1} , $\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$ 0.1 g.L^{-1} , $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ 1.5 g.L^{-1} , and H_3BO_3 0.3 g.L^{-1}). The growth media was placed in orbital shaker at 120 r.p.m (Effiong *et al.*, 2019; Peekate and Abu, 2017).

Antagonistic activity of sophorolipids on microbial isolates

The antagonistic activity of the sophorolipids synthesized by the yeast isolates was assessed using the well-in-agar method on microbial isolates that was obtained crude oil impacted and unimpacted environments. Microbes in their log-phase of growth were attained from subculturing (Agwa *et al.*, 2012). The cell density and concentration were determined from Macfarland scale. Turbidity was extrapolated from its optical density and plate counts respectively. A sterile swab stick was used to spread the organism on the plates while a micropipette was used to introduce 5 μl of the sophorolipid into the well, zones of inhibition was read-off from the plates after incubation (Sobrinho *et al.*, 2008).

Assessment of Toxicity of sophorolipids

Acute toxicity studies were carried out on the sophorolipids produced by the yeast cells (Ajuzieogu and Odokuma, 2018). The concentrations ranging from 0.001, 0.01, 0.1, 1.0 and 10.0, mg.mL^{-1} of sophorolipids was used for the study and water and detergents was used as control. The study was observed and monitored for toxicity indices and growth performance proxies during the exposure. Biototoxicity was determined from probit analysis

Results and Discussion

Biosafety and Antimicrobial studies of the sophorolipids produced by yeast isolates

Tables 1- 4 shows the antimicrobial interaction of the sophorolipids produced against some select microbes ranging from pathogenic to beneficial bacterial and fungal flora. The study showed that the sophorolipid produced by the *Candida haemulonis* did not have any form of antagonistic activity against *Bacillus* sp, *Klebsiella* sp. and *Staphylococcus* sp. There was a varying effect on *Nitrobacter* and *Nitrosomonas* sp as the zone of inhibition increased from 6.0 mm to 16.0 mm for *Nitrobacter* sp while that the *Nitrosomonas* ranged from 10.0 mm to 24.0 mm. Table 2 shows the antimicrobial studies of the sophorolipid produced by *Saccharomyces cerevisiae*. The study shows that the sophorolipid did not have any form of antagonistic activity on *Bacillus* sp, *Klebsiella* sp and *Nitrobacter* sp. Although there was a slight antagonistic activity on *Staphylococcus* sp at high concentration between 60-100% which had a zone of inhibition of 8.0 mm to 10.0 mm. Although there was a slightly antagonistic activity on *Nitrosomonas* sp. with zone of inhibition ranging from 10.0 mm to 22.0 mm for 20 and 100% concentrations of the *Saccharomyces* sp. based sophorolipids. Table 3 shows antagonistic performance of sophorolipid sourced from *Candida haemulonis* on the fungal isolates. The study observed that the antimicrobial activity of the sophorolipid to *Saccharomyces* sp. and *Candida* sp. was not observed while the study underscored the lower concentrations between 20% to 60% did not have a significant antagonistic activity with *Penicillium* sp, *Mucor* sp and *Rhizopus* sp. There was a slight antimicrobial activity between 80% to 100%. This was similar to the performance of the *Saccharomyces* sp. based sophorolipids.

Table 1: Antimicrobial Activity of Isolate *Candida haemulonis* (Sophorolipid) to Beneficial and Pathogenic Bacterial Isolates

Test Isolates	Concentration (%)					Control
	20	40	60	80	100	
Zone of Inhibition (mm)						
<i>Staphylococcus</i> sp	0	0	0	2.0	4.0	0
<i>Nitrobacter</i> sp	6.0	8.0	14.0	16.0	16.0	0
<i>Klebsiella</i> sp	0	0	0	0	0	0
<i>Bacillus</i> sp	0	0	0	0	0	0
<i>Nitrosomonas</i> sp	10.0	16.0	20.0	22.0	24.0	0

Table 2: Antimicrobial Activity of Isolate *Saccharomyces cerevisiae* (Sophorolipid) to Beneficial and Pathogenic Bacterial Isolates

Test Isolates	Concentration (%)					Control
	20	40	60	80	100	
Zone of Inhibition (mm)						
<i>Staphylococcus</i> sp	0	0	0	8	10	0
<i>Nitrobacter</i> sp	0	0	0	0	0	0
<i>Klebsiella</i> sp	0	0	0	0	0	0
<i>Bacillus</i> sp	0	0	0	0	0	0
<i>Nitrosomonas</i> sp	10.0	14.0	17.0	19.0	22.0	0

Table 3: Antimicrobial activity of isolate *Candida haemulonis* (Sophorolipid) to beneficial and pathogenic fungal isolates

Test Isolates	Concentration (%)					Control
	20	40	60	80	100	
<i>Aspergillus</i> sp	2.0	4	7.0	10.0	13.0	1.0
<i>Penicillium</i> sp	0	0	0	8.0	11.0	0
<i>Rhizopus</i> sp	0	0	2.0	5.0	8.0	0
<i>Candida</i> sp	0	0	0	0	0	0
<i>Mucor</i> sp	0	0	0	4.0	6.0	0
<i>Saccharomyces</i> sp	0	0	0	0	0	0

Table 4: Antimicrobial activity of isolate *Saccharomyces cerevisiae* (Sophorolipid) to beneficial and pathogenic fungal Isolates

Test Isolates	Concentration (%)					Control
	20	40	60	80	100	
<i>Aspergillus</i> sp	1.0	3.0	5	7	8	1.0
<i>Penicillium</i> sp	0	1.0	4.0	6.0	7.0	0
<i>Rhizopus</i> sp	0	0	2.0	4.0	6.0	0
<i>Candida</i> sp	0	0	0	0	0	0
<i>Mucor</i> sp	0	0	2.0	5.0	7.0	1.0
<i>Saccharomyces</i> sp	0	0	0	0	0	0

Toxicity studies of sophorolipid produced by yeast isolates

Figure 1-6 shows the acute toxicity of the crude sophorolipid on *Nitrosomonas* sp, *Nitrobacter* sp and *Thiobacillus* sp. The acute toxicity of the sophorolipid produced by *Candida haemulonis* to *Nitrosomonas* sp was determined to be 0.0542mg.L^{-1} while that of the sophorolipid from *Saccharomyces cerevisiae* was 0.0891mg.L^{-1} . Similarly, the LC_{50} of the sophorolipid produced by *Candida haemulonis* to *Nitrobacter* sp was 0.309mg.L^{-1} while that of the sophorolipid from *Saccharomyces cerevisiae* was 0.269mg.L^{-1} . The acute toxicity of the sophorolipid produced by *Candida haemulonis* to *Thiobacillus* sp was determined to be 0.776mg.L^{-1} while that of the sophorolipid from *Saccharomyces cerevisiae* was 0.138mg.L^{-1} , this can be seen in Fig 5-6 below. Table 5 show acute toxicity indices from probit analys.

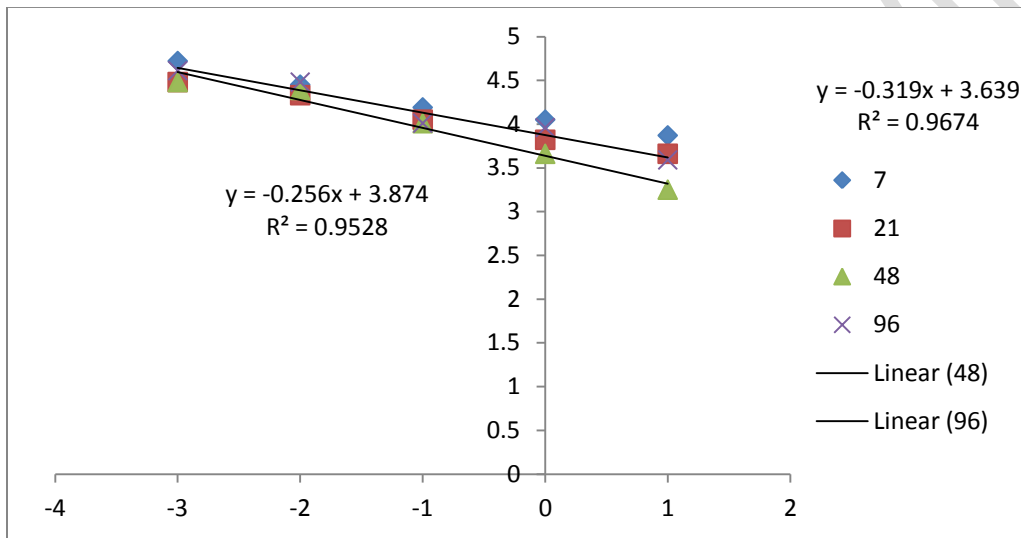


Figure 1: Toxicity of *Candida haemulonis* (Sophorolipid) against *Nitrosomonas* sp

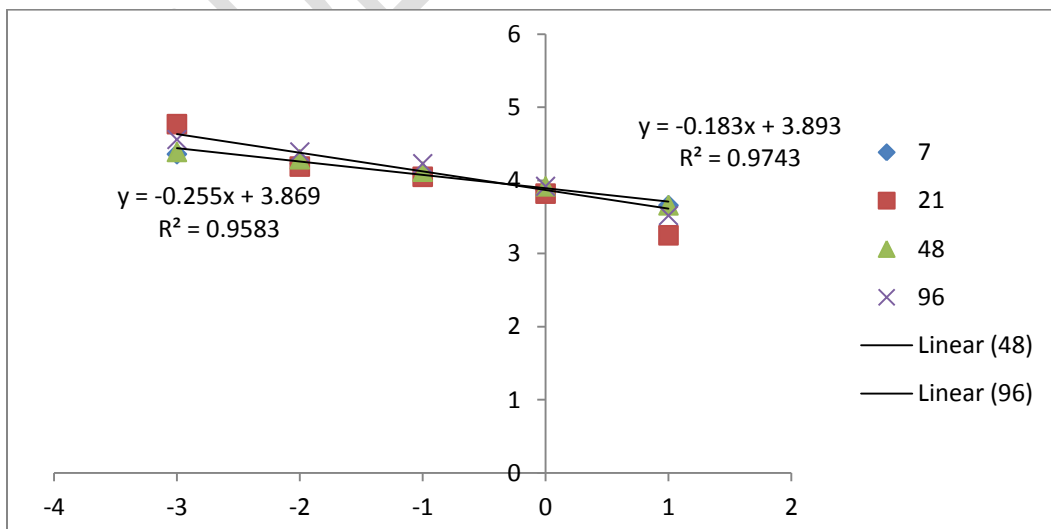


Figure 2: Toxicity of *Saccharomyces cerevisiae* (Sophorolipid) against *Nitrosomonas* sp.

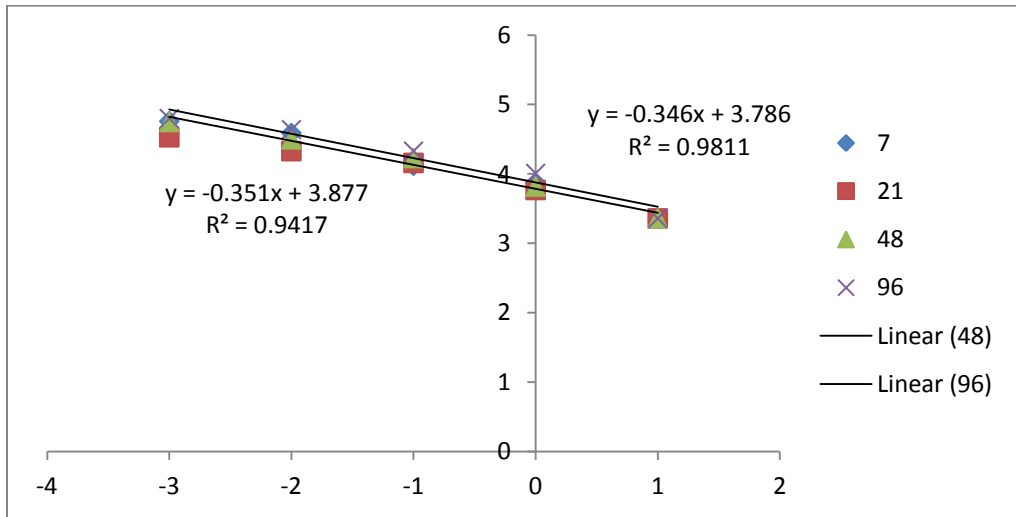


Figure 3: Toxicity of *Candida haemulonis* (Sophorolipid) against *Nitrobacter* sp.

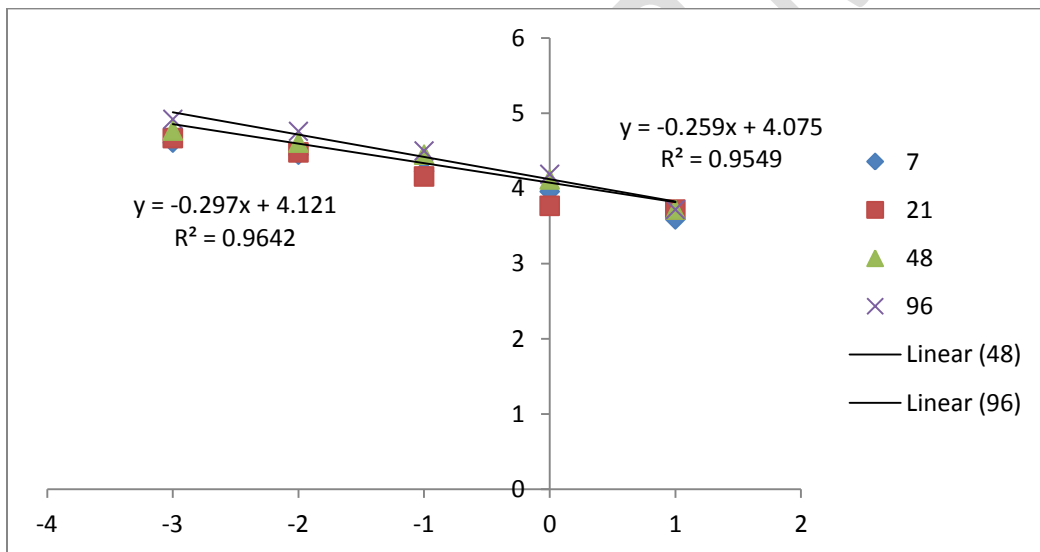


Figure 4: Toxicity of *Saccharomyces cerevisiae* (Sophorolipid) against *Nitrobacter* sp.

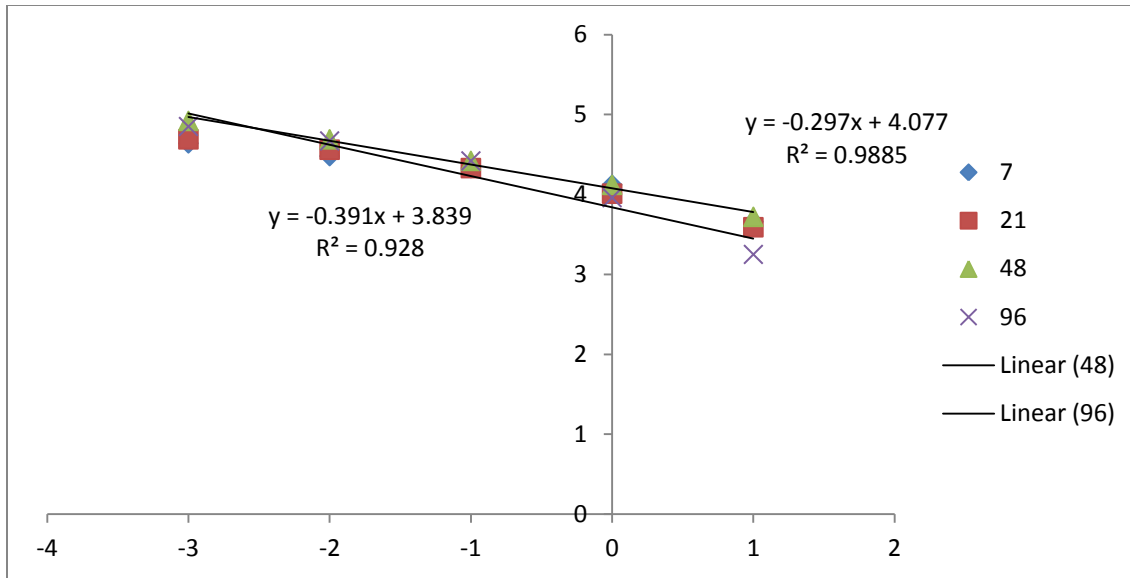


Figure 5: Toxicity of *Candida haemulonis* (Sophorolipid) against *Thiobacillus* sp.

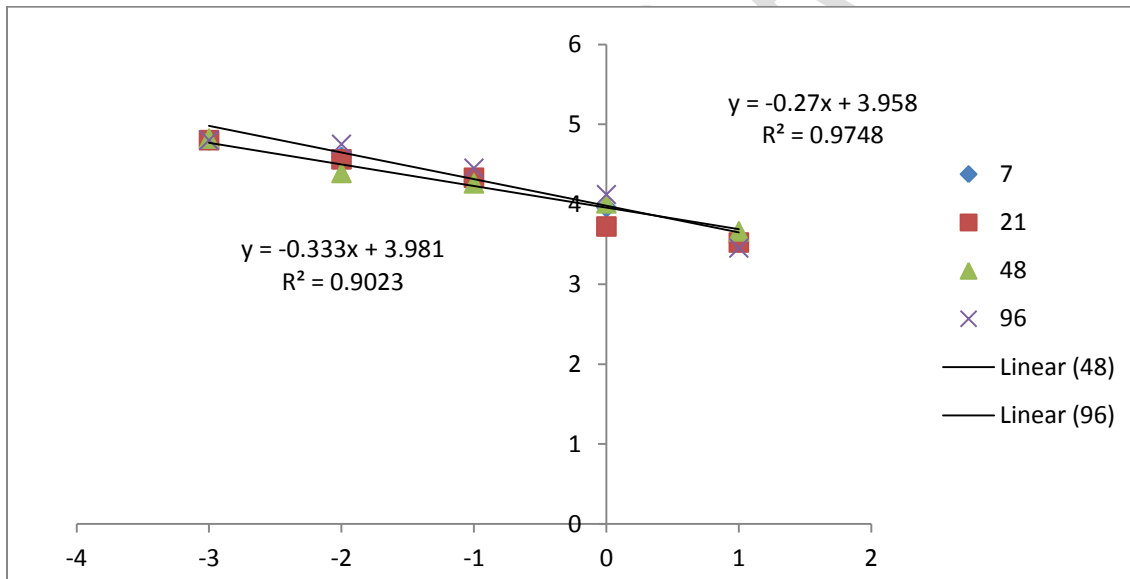


Figure 6: Toxicity of *Saccharomyces cerevisiae* (Sophorolipid) against *Thiobacillus* sp.

Table 5: Acute toxicity indices from probit analysis

Test Isolates	<i>Candida haemulonis</i>		<i>Saccharomyces cerevisiae</i>	
	(Sophorolipid)		(Sophorolipid)	
Time	48hrs	96hrs	48hrs	96hrs
<i>Nitrosomonas sp</i>	0.0542 mg.L ⁻¹	0.0398 mg.L ⁻¹	0.0891 mg.L ⁻¹	0.0363 mg.L ⁻¹
<i>Nitrobacter sp</i>	0.309 mg.L ⁻¹	0.631 mg.L ⁻¹	0.269 mg.L ⁻¹	0.110 mg.L ⁻¹
<i>Thiobacillus sp</i>	0.776 mg.L ⁻¹	0.107 mg.L ⁻¹	0.138 mg.L ⁻¹	0.871 mg.L ⁻¹

5.0 Discussion:

Antimicrobial studies have been identified as a key biosafety protocol for a number biologically synthesized materials for quite some time now. A number of previous investigations have applied the area of biotechnology. The study showed that the sophorolipid produced by the *Candida haemulonis*. Did not have any form of antagonistic activity against *Bacillus sp*, *Klebsiella sp*. and *Staphylococcus sp*. There was a varying effect on *Nitrobacter* and *Nitrosomonas sp* as the zone of inhibition increased from 6.0 mm to 16.0 mm for *Nitrobacter sp* while that the *Nitrosomonas* ranged from 10.0 mm to 24.0 mm. The study shows that the sophorolipid did not have any form of antagonistic activity on *Bacillus sp*, *Klebsiella sp* and *Nitrobacter sp*. Although there was a slight antagonistic activity on *Staphylococcus sp* at high concentration between 60-100% which had a zone of inhibition of 8.0 mm to 10.0 mm. Although there was a slightly antagonistic activity on *Nitrosomonas sp*. with zone of inhibition ranging from 10.0 mm to 22.0 mm for 20 and 100% concentrations of the *Saccharomyces sp*. based sophorolipids. The previous study conducted by Kim *et al.* (2002) have identified the qualities of sophorolipid as an antimicrobial agent. They were able to evaluate the antimicrobial quality of sophorolipid produced by *Candida bombicola* against a number of bacterial and fungal isolates, their study identified 50% of the biomaterial inhibited the activity of *Botrytis cineria* although they reported there was no significant effect on the *Escherichia coli* Their study on *Bacillus subtilis* strongly agreed with the report of the present study as it corroborates that the zero antagonism. In a related study Solaiman *et al.* (2016) tested the antagonistic relationship of sophorolipids produced by *Candida bombicola* to both Gram positive and negative bacterial. In the same vein, their study corroborated that the *Bacillus spp* had a very low antagonism compared to other bacterial isolates both as pure and mixed cultures. They further reported an

MIC value of $4.88\text{mg}\cdot\text{mL}^{-1}$ for *Bacillus licheniformis* adding a number of both intrinsic and physiological properties as being pivotal to the efflux system employed for the positive interaction of the isolate to the biomaterial. The report conducted by Sleiman *et al.* (2009) suggested from his report that most of these sophorolipids may function as anti-inflammatory agents or modulators. Their findings were observed not have a significant antimicrobial activity on clinically relevant isolates. Abhyankar *et al.* (2021) reported that the fatty acid component of sophorolipids produced by *Starmerella bombicola* called the Myristic acid did not have effect on most Gram-negative bacteria and had a minor antagonism on Gram positive like the *Bacillus* sp. as observed in the present study. Their findings corroborate the findings of the present study that the presence of fatty acid ends could hinder the growth of microbes. This further corroborate with the report of Fontoura *et al.* (2020) reported that the sophorolipid obtained from *Candida bombicola* sourced sophorolipid which had a varying effect on Gram positive and enteric pathogens which was in tandem with the report of the present study.

Candida haemulonis sourced sophorolipids were determined on the fungal isolates. The study observed that the antimicrobial activity of the sophorolipid to *Saccharomyces* sp. and *Candida* sp. was not observed while the study underscored the lower concentrations between 20% to 60% did not have a significant antagonistic activity with *Penicillium* sp, *Mucor* sp and *Rhizopus* sp. There was a slight antimicrobial activity between 80% to 100%. This was similar to the performance of the *Saccharomyces* sp. based sophorolipids. The work of de Caretta *et al.* (2021) identified the antimicrobial activity of rot fungi of tomatoes as a solution to biodeterioration of agro-products. Their study conducted on molds such as *Botrytis cinerea*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium ultimum* in their observation the lactonic groups was able to inhibit necrosis of leaves of tomatoes caused by pathogenic fungi. The work of Silveira *et al.* (2019) supported the role of sphorolipids produced by *Starmerella bombicola* was able to inhibit the growth of fungi and enteric pathogens as they observed that an MIC of $5.0\text{mg}\cdot\text{L}^{-1}$ was able to partition with kanamycin. Their study posited their role as topical agents for agro-allied uses in plant yield promoters.

Acute toxicity (LC_{50}) of sophorolipid synthesized from both *Candida haemulonis* and *Saccharomyces cerevisiae* isolated from palm oil impacted soil. The LC_{50} of sophorolipid produced by *Candida haemulonis* was tested against *Nitrosomonas* sp was $0.0542\text{mg}\cdot\text{L}^{-1}$ while that of the sophorolipid from *Saccharomyces cerevisiae* was $0.0891\text{mg}\cdot\text{L}^{-1}$. Similarly, the toxicity of the sophorolipid produced by *Candida haemulonis* to *Nitrobacter* sp was $0.309\text{mg}\cdot\text{L}^{-1}$ while that of the sophorolipid from *Saccharomyces cerevisiae* was $0.269\text{mg}\cdot\text{L}^{-1}$. this finding agrees with Gudina *et al.* (2015) who reported a low toxicity effect for a bioemulsifier produced by *Paenibacillus* sp.

Conclusion

In this study, the antagonistic and toxicity profile of sophorolipids synthesized by *Candida haemulonis* and *Saccharomyces cerevisiae* niche-mined from palm oil mill effluent impacted soil in Emohua, Rivers State, Nigeria has shown huge level of biosafety in the soil microorganisms and exhibited a good environmental compatibility as it deduced from the antimicrobial activity and toxicity assay. The study identified that minimal antagonistic activity to bacterial isolates such as *Bacillus* sp., *Klebsiella* sp and *Nitrobacter* sp; at concentration between 20-60% v/v. The acute toxicity profile to *Nitrobacter* sp was $0.309\text{mg}\cdot\text{L}^{-1}$ while that of *Thiobacillus* sp had $0.78\text{mg}\cdot\text{L}^{-1}$. These finding further indicate that the biorefinery and industrial application of biomaterials such as sophorolipids isolated from indigenous microbiota has the potential to preserve and restore the ecological balance of impacted environmental matrices without creating harmful or detrimental interactions of the microflora. These ease to handle and non-toxic microbial products has the capacity to be used in a number of sectors to create wealth and preserve the ecosystem.

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