FEMS Microbiology Letters, 365, 2018, fny098

doi: 10.1093/femsle/fny098
Advance Access Publication Date: 17 April 2018
Research Letter

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

RESEARCH LETTER – Environmental Microbiology

Characterization of biosurfactant from yeast using residual soybean oil under acidic conditions and their use in metal removal processes

Franciele Pereira Camargo[1,*], Aparecido Júnior de Menezes[2], Paulo Sérgio Tonello[3], André Cordeiro Alves Dos Santos[4] and Iolanda Cristina Silveira Duarte[1,4]

[1]Graduate Program in Biotechnology and Environmental Monitoring, Federal University of São Carlos- UFSCar, João Leme dos Santos Highway, Km 101, 18052-780, Sorocaba, São Paulo, Brazil, [2]Graduate Program in Material Sciences, Universidade Federal de São Carlos UFSCar, Rod. João Leme dos Santos, Km 101, 18052-780, Sorocaba, São Paulo, Brazil, [3]Graduate Program in Environmental Sciences, São Paulo State University (UNESP), Avenue Three March 511, Alto da Boa Vista, 18087-180, Sorocaba, São Paulo, Brazil and [4]Department of Biology, Federal University of São Carlos UFSCar, João Leme dos Santos Highway, Km 101, 18052-780, Sorocaba, São Paulo, Brazil

*Corresponding author: Graduate Program in Biotechnology and Environmental Monitoring, Federal University of São Carlos UFSCar, João Leme dos Santos Highway, Km 101, 18052-780, Sorocaba, São Paulo, Brazil. Tel: +55 (15) 997561146; E-mail: francielep.camargo@hotmail.com
One sentence summary: Acid metal removal by yeast biosurfactant.
Editor: Skorn Mongkolsuk

ABSTRACT

This study aimed at the production of biosurfactants from yeasts under acidic conditions using residual soybean oil as a carbon source, as well as the biosurfactant produced in the solubilization of metals in sewage sludge. The yeast *Meyerozyma guilliermondii* was considered the best producer in both pH 4.0 and 2.0; therefore, the product obtained by this yeast was characterized by Fourier transform infrared (FT-IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Moreover, it was applied in metal removal assays in anaerobic sewage sludge. The spectra obtained in FT-IR suggested that *M. guilliermondii*'s biosurfactant had a similar structure to glycolipids from the sophorolipid class, and it was confirmed by ^{1}H NMR spectroscopy. In the bioleaching assays, the application of biosurfactant (2%) produced by *M. guilliermondii* with pH adjusted to 2.0 was able to solubilize 15.9% of cadmium from the sewage sludge.

Keywords: anionic biosurfactant; glycolipid; low-cost substrate; sophorolipid; bioremediation; bioleaching

INTRODUCTION

Amphiphilic substances known for their ability to reduce surface tension and also interfacial tension between fluids with low miscibility are described as tensioactive or, more commonly known as surfactants. In addition to commercial surfactants, which are generally obtained from petroleum, these substances may also be produced extracellularly by microorganisms, and are known as biosurfactants (Franzetti *et al.* 2011; Saharan, Sahu and Sharma 2011; Singh 2012).

Received: 12 November 2017; **Accepted:** 13 April 2018
© FEMS 2018. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Biosurfactants can be anionic, cationic or nonionic in their hydrophilic portions, while their hydrophobic portions are commonly composed of linear or branched hydrocarbons that contain from 8 to 18 carbon atoms. Anionic and nonionic biosurfactants are common, while cationic biosurfactants are rare and often toxic (Franzetti *et al.* 2011). There are several types of biosurfactants, including glycolipids, phospholipids, fatty acids and peptides. Their main method of producing organisms is through the solubilization of insoluble substrates in water, facilitating their assimilation (Singh 2012).

Biosurfactants have been widely studied as they are considered to have less of an impact on the environment and have the same desirable properties for commercial and industrial applications. They are also effective in terms of solubilization and emulsification and are biodegradable and less toxic (Lawniczak, Marecik and Chrzanowski 2013).

Microorganisms producing biosurfactants, such as bacteria and fungi (especially yeasts), are considered as an alternative to the use of chemical surfactants, as among their other applications, toxic metals can be solubilized in soils (Lawniczak, Marecik and Chrzanowski 2013; Karwowska *et al.* 2014).

Although there are several methods of achieving solubilization for the subsequent removal of metals from aqueous environments, soils, sediments and sewage sludges, biological methods are considered the most environmentally and economically advantageous because of their low risk of secondary pollution (Franzetti *et al.* 2011). One of the methods is the addition of biosurfactants, which tend to interact with contaminants with poor solubility and transfer them to the aqueous phase, enabling their subsequent removal. These substances frequently have heteroatoms in their structure, so allowing the formation of complexes between potentially toxic metal ions and functional groups (hydroxyls, carbonyls, amines, etc.) and facilitating the process (Lawniczak, Marecik and Chrzanowski 2013).

Despite their known advantages, there are little data in the literature regarding the use of biosurfactants for the removal of toxic metals from environments contaminated with these analytes. Studies such as that by Maier *et al.* (2001) demonstrated the feasibility of this method for sewage sludges contaminated with copper (Cu), obtaining a metal recovery rate of 59.4% in a treatment with rhamnolipids (50 mM) for an exposure of 24 h. In soils, Yang *et al.* (2016) used glycolipids produced by *Burkholderia* sp. to remove tin (Zn), lead (Pb), manganese (Mn), cadmium (Cd) and copper (Cu), removing 44.0%, 32.5%, 52.2%, 37.7% and 24.1%, respectively.

Bioleaching is another biological process for the removal of metals from contaminated environments, taking advantage of the catalytic effect of the metabolic activity of microorganisms, which obtain energy through redox reactions, using iron or sulfur as an energy source (Pathak, Dastidar and Sreekrishnan 2009). This process has been studied for the removal of metals from sewage sludges and dredged sediments from rivers and soils (Fang *et al.* 2011). High levels of organic matter can inhibit the growth of these chemolithotrophic microorganisms, however. An alternative is the reconciliation of the bioleaching method and the use of heterotrophic biosurfactant producing microorganisms, which can lower the concentrations of organic acids in the medium (Fournier, Lemieux and Couillard 1998). In a previous study, Camargo *et al.* (2018) evaluated the influence of co-inoculation of *Acidithiobacillus* bacteria and the biosurfactant-producing yeast *Meyerozyma guilliermondii* in bioleaching processes, finding that after 10 days of incubation, 76.5% of Zn, 59.8% of Ni, 22.0% of Cu, 9.8% of Cd, 9.8% Cr and 7.1% of Pb were solubilized and the presence of yeast accelerated the time required for Cd solubilization from 240 to 96 h.

The co-inoculation of *Galactomyces* and *Acidithiobacillus* positively affected the bioleaching of metals in sewage sludge, as the yeast metabolized the organic acids present in the medium, making the performance of the *Acidithiobacillus* species more efficient, with 85% solubilization of Cu and 94% of Zn in the co-inoculation system compared to only 68% of Cu and 84% of Zn solubilization in the experiment without yeast in the same time (4 days). This genus of yeast is able to produce biosurfactants, which accelerated the rate of sulfur oxidation by *A. thiooxidans*, increasing their solubility (Zhou *et al.* 2013). Likewise, it is known that toxic metals may be solubilized by the application of biosurfactants (Banat *et al.* 2010).

Although the use of biosurfactants in bioremediation processes is both possible and recommended, its large-scale production is still impractical due to its high cost in comparison with the production of commercial surfactants (Accorsini *et al.* 2012). The use of raw waste material such as low-cost carbon sources, therefore, becomes desirable. It can also be considered to have a dual-purpose benefit, as in addition to promoting economically viable biosurfactant production, it can treat waste that could negatively impact the environment if disposed of improperly. Alternative carbon sources such as waste vegetable oils and industrial residues have been evaluated as a substrate for the production of biosurfactants, such as coffee processing waste, fruit, sugarcane, cassava, corn, soybean, etc. (Pandey, Soccol and Mitchell 2000; Saharan, Sahu and Sharma 2011).

Thus, the major purpose of this research was to evaluate the capacity of potentially biosurfactant producing yeasts under acidic conditions using soybean oil frying waste as the main source of carbon and to verify its application in bioremediation and metal removal processes in anaerobic sewage sludge. In addition, one of the purposes of this research was to characterize the biosurfactant produced by the yeast that could be considered as the best producer of this substance in acid conditions.

MATERIAL AND METHODS

Microorganisms

The performance of *Meyerozyma guilliermondii* (accession number KX455858) isolated from soil contaminated with diesel oil and seven previously isolated strains (Camargo *et al.* 2016) was evaluated. These were *Candida orthopsilosis* (accession number KR136231), *Cryptococcus luteolus* (accession number KR136232), *Rhodotorula glutinis* (accession number KR136236), *Rhodotorula mucilaginosa* (accession number KR136233), *Hannaela sinensis* (accession number KR136235), *Dipodascus australiensis* (accession number KR136229) and *Metschnikowia koreensis* (accession number KR136230).

Biosurfactant production

The strains were kept in tubes containing Sabouraud agar and refrigerated (4°C) until being used. The cells were transferred to Falcon tubes containing 10 ml of Ringer's solution sterilized with 0.22 μm of membrane filtration. Afterwards, 5 ml of this solution was transferred to erlenmeyer flasks containing 100 ml of an enrichment medium (KH_2PO_4 3 g·L^{-1}; $NaNO_3$ 30 g·L^{-1}; $MgSO_4$ 3 g·L^{-1}; yeast extract 10 g·L^{-1} and glucose 4%) with pH 4.0, adjusted with HCl and NaOH. The mixture was incubated and shaken at 2.64 g at 30°C for 48 h.

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

Biosurfactant production tests were carried out at an agitation rate of 2.64 g and 30°C for a period of seven days. Each erlenmeyer flask contained 100 ml of the production medium, in accordance with Kitamoto *et al.* (2001). A quantity of 5% of the enrichment medium after the incubation period, standardized in a spectrophotometer with absorbance between 0.400 and 0.500 at a wavelength of 660 nm, was used as an inoculum. In order to produce cheaper biosurfactants, soybean oil used for home or commercial frying was evaluated as a carbon source.

Surfactant activity determination

The emulsification index test (E_{24}) evaluates the ability of the biosurfactant to emulsify hydrocarbons (Bodour and Miller-Maier 1998) such as toluene. In order to estimate the emulsification index, 3.5 ml of the supernatant was added to 2 ml of toluene and vortexed for 2 min. The tubes were left to stand for a period of 24 h and the emulsification index was calculated from Eq. (1):

$$E_{24} = \frac{\text{height of emulsified layer (cm)}}{\text{total height (cm)}} \times 100 \quad (1)$$

A negative control using the production medium without fermentation was carried out. All the tests were done in triplicate and data presented were the mean and standard deviation of three independent samples.

Ionic character

The determination of the ionic charge of the biosurfactant was performed according to the double diffusion in the agar method described by Meylheuc, Van Oss and Bellon-Fontaine (2001), using $BaCl_2$ (50 mmol·L^{-1}) and SDS (20 mmol·L^{-1}) as well known cationic and anionic substances, respectively. The results were interpreted as positive when the formation of a precipitate line between the agar wells (1%) after 48 h occurred.

Dry biomass and pH

Cell growth was assessed using the pellet formed after centrifugation, transferred to the pre-weighed Petri dish and placed in a drying oven at 105°C until a constant weight was achieved. The dry weight was calculated from the difference between the two weights.

The pH was determined in a potentiometer by direct immersion of the electrode in the supernatant obtained after centrifugation.

Biosurfactant extraction and composition analysis

The composition of the biosurfactant produced by the yeast with the highest performance under acidic conditions (pH 4.0 and 2.0) was evaluated after purification by precipitation with acetone (Pruthi and Cameotra 1995). To identify functional groups, the FT-IR method was adopted at a range between 4000 and 400 cm^{-1} and 32 scans, using potassium bromide (KBr) pellets at a concentration of 1%.

The ^{1}H NMR spectra of the biosurfactant in dimethyl sulfoxide-d_6 were obtained on a Bruker 500 MHz NMR spectrometer (São Carlos, São Paulo, Brazil) at 25°C with 5000–5200 accumulations, pulse duration 5.9 micros, acquisition time 1.2 μs and a relaxation delay of 6 μs.

Sampling and characterization of sludge

Anaerobic sludge was collected from a wastewater treatment plant in Porto Feliz (São Paulo, Brazil), of the biological treatment step of the wastewater, directly from the upflow anaerobic sludge blanket reactor (UASB), and stored at 4°C until used. The collection and the storage were carried out following the 1060 method (APHA/AWWA/WEF 2012). The content of toxic metals was analyzed in the solid phase according to method 3050B (USEPA 1996) and in the liquid phase according to method 3030E (APHA/AWWA/WEF 2012) after the sludge centrifugation (11 739 g, 4°C, 10 min). The metals Cd, Cr, Cu, Ni and Zn were quantified by Inductively Coupled Plasma—Optic Emission Spectrometry (ICP-OES) before and after the batch sewage sludge washing studies.

Effect of the purified biosurfactant on the batch sewage sludge washing studies

Sewage sludge bioleaching experiments using the purified biosurfactant were carried out as the modified method described in Mulligan, Yong and Gibbs (2001). The product obtained after purification was suspended in ultrapure water (03.1 MΩ·cm) in the final concentration of 2%. The experiments were conducted in regular conditions (pH 6-5, without adjustment) and also in acid conditions (pH 2.0, adjusted with HCl 1 mol·L^{-1} and NaOH 1 mol·L^{-1}). The control experiments were conducted with ultrapure water in the same conditions.

The solution was added to the dried sludge (oven at 105°C for 24 h) in a proportion of 10:1 (10 g of solution in 1 g of dried sludge) and all Falcon tubes were incubated in a gyratory shaker at 30°C and 2.64 g for 24 h. After incubation, the samples were centrifuged (2934.75 g, 4°C, 10 min) and then the liquid phase was acidified with HNO_3 (pH < 2.0) and stored at 4°C until used. The metals and the physicochemical analysis were carried out as described in Sampling and characterization of sludge section.

Data analysis

The metal solubilization (%) in the solid phase of the sewage sludge at the end of each test was calculated by the ratio between the concentration of metal solubilized and the total concentration of the metal in the sludge.

The assay results were compared using the parametric analysis of variance (ANOVA) and Tukey analysis, evaluating their relevance using Graph Pad Instat software with a 95% confidence interval.

RESULTS AND DISCUSSION

Surfactant activity determination

The results of the emulsification index (E_{24}) showed that the *R. glutinis* (52.5%) and *M. guilliermondii* (42.8%) yeasts performed best (Fig. 1). The *C. ortholopsilosis*, *Cr. luteolus* and *H. sinensis* yeasts exhibited no emulsion, while all other strains showed percentages above 35%.

Despite an abrupt reduction in the production of dry biomass for both yeasts tested with an initial pH of 2.0, it was observed that the bio-emulsification potential (E_{24}) did not change much, since the E_{24} for *R. glutinis* was 45.8%, or in other words, this yeast was capable of maintaining 87.3% of its original E_{24}, while *M. guilliermondii* (E_{24} 39.2%) maintained 91.38% of its original E_{24}.

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

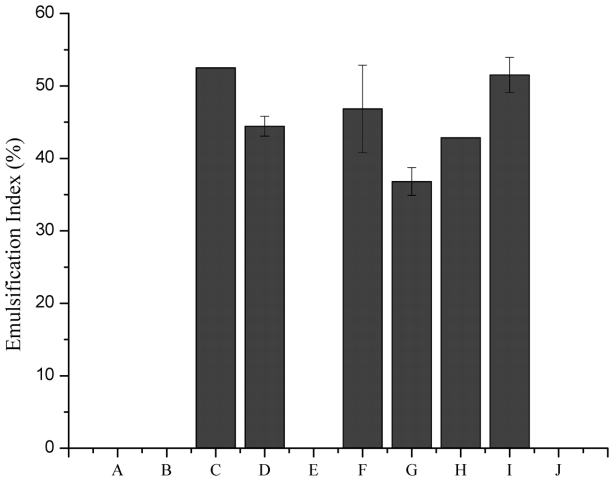

Figure 1. Emulsification index (E_{24}) at pH 4.0. (**A**) *C. orthopsilosis*, (**B**) *Cr. luteolus*, (**C**) *R. glutinis*, (**D**) *R. mucilaginosa*, (**E**) *H. sinensis*, (**F**) *D. australiensis*, (**G**) *Met. koreensis*, (**H**) *M. guilliermondii*, (**I**) SDS 50 mg·L^{-1} (+), (**J**) Production medium (–) (n = 3).

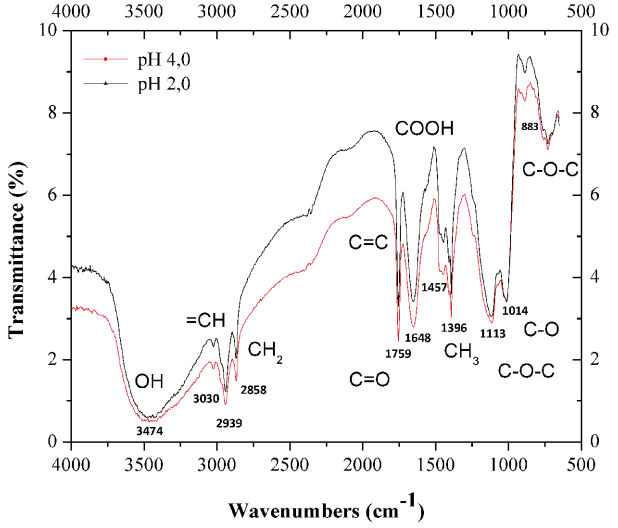

Figure 2. FTIR spectra of the *M. guilliermondii* supernatant. Spectrogram generated by OriginPro8.

Based on the data obtained from the analytical tests at pH 2.0, it was found that compared with other yeasts analyzed, *M. guilliermondii* had the most positive results and this yeast maintained the final pH of the culture medium closest to the initial value of 2.0 after fermentation.

Ionic character

The results obtained in the test for determining the ionic charge showed that the biosurfactant produced by *M. guilliermondii* is anionic, and may be the most suitable type of surfactant for bioremediation processes in environments contaminated by metals, usually cationic (Ahn *et al.* 2009).

Dry biomass and final pH

The results obtained for the final pH and dry matter biomass are available in Table 1. It can be seen that all the evaluated yeasts raised the pH of the medium at the end of the tests.

According to Bednarski *et al.* (2004), the acidity of the culture medium is directly related to the production of glycolipids by yeasts. Although alkalization of the medium by *M. guilliermondii* occurred after the test at pH 4.0, it was observed that at pH 2.0, maintenance of the acidity is possible, as its pH remained about 80.3%, while *R. glutinis* showed a higher pH both in the initial pH 4.0 and pH 2.0.

The biosurfactant production capacity of the *R. glutinis* yeast is already well known, particularly with regard to the production of polyol lipids (Saharan, Sahu and Sharma 2011), as is the biosurfactant production capacity of *M. guilliermondii* (*Candida guilliermondii*) in culture media with soybean oil as a carbon source, for which Coimbra *et al.* (2009) obtained values similar to those in this study (E_{24} between 40% and 50%).

Biosurfactant extraction and composition analysis

The purified supernatant of *M. guilliermondii* was analyzed by FT-IR (Fig. 2). The supernatants obtained for the biosurfactant production tests at pH 2.0 and at pH 4.0 were analyzed, but did not exhibit any differences. The results are presented at a frequency of 4000 to 400 cm^{-1}.

At approximately 3500 cm^{-1}, there are bands that are characteristic of the stretches of hydroxyl groups (–OH). The stretches between 1275 and 1020 cm^{-1} suggest the presence of C=O aliphatic ethers, while the angular deformation in the range between 1390 and 1370 cm^{-1} indicates the presence of CH_3. At 3030 cm^{-1}, there is a characteristic hydrogen band attached to unsaturated carbons. At 1648 cm^{-1}, there is a characteristic band for non-conjugated olefins (C=C) and at 1759 cm^{-1}, there is a stretch that can be assigned to carbonyl esters, carbonyl lactone or carboxylic acid carbonyls. These bands are

Table 1. Average cell growth (g·L^{-1}) and final pH of the biosurfactant production medium (n = 3).

	pH 4.0		pH 2.0	
Yeast	Dry weight (g · L^{-1})	pH	Dry weight (g · L^{-1})	pH
C. orthopsilosis	8.0 ± 0.0	7.1 ± 0.1	–	–
Cr. luteolus	5.0 ± 0.0	7.4 ± 0.0	–	–
R. glutinis	5.0 ± 0.0	7.6 ± 0.1	3.0 ± 0.0	6.3 ± 0.1
R. mucilaginosa	8.0 ± 0.0	6.9 ± 0.1	–	–
H. sinensis	7.0 ± 0.0	7.2 ± 0.0	–	–
D. australiensis	14.0 ± 0.0	4.8 ± 0.4	–	–
Met. koreensis	8.0 ± 0.0	6.3 ± 0.1	–	–
M. guilliermondii	18.0 ± 0.0	6.4 ± 0.2	2.0 ± 0.0	2.5 ± 0.1

(–) not evaluated in these conditions.

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

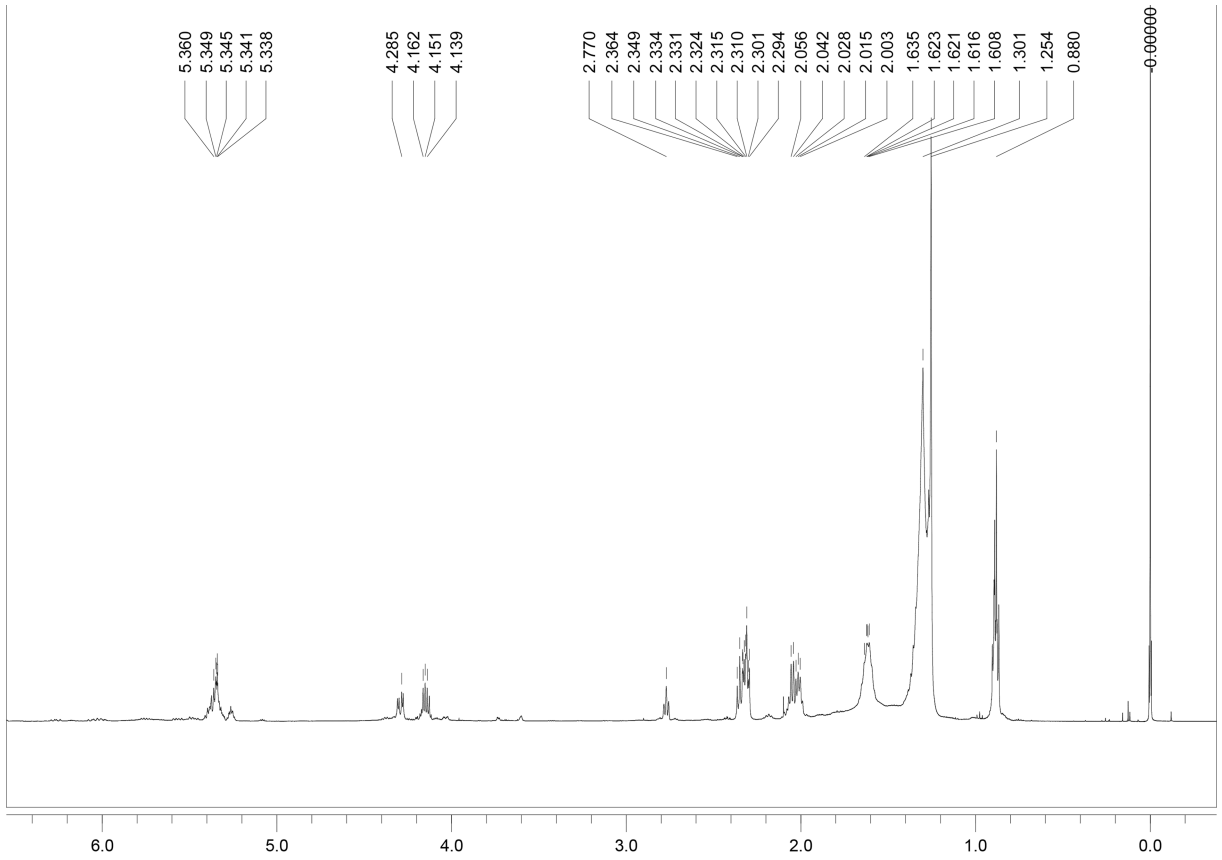

Figure 3. ^{1}H NMR spectrum of the purified biosurfactant produced by *M. guilliermondii*. Analysis performed on ACD Spectrus Processor software.

characteristic of sophorolipid spectra, while the bands at 1113 cm^{-1} (C^1–O–C^5) and 883 cm^{-1} (C^1–O–C^2) can be related to ether groups in the structure of the sophorolipids. The band at 1457 cm^{-1} corresponded to the plane bending of the C–O–H group of carboxylic acid (COOH). In the portion between 2960 and 2850 cm^{-1}, there is confirmation of the lipid portion as band stretches in this range are characteristic of aliphatic CH. Similar bands were found in the sophorolipid spectra analyzed by Parekh, Patravale and Pandit (2012) and Mousavi, Beheshti-Maal and Massah (2015).

The spectra of the purified supernatants are compatible with the groups found in glycolipids from the sophorolipid class, which are non-ionic biosurfactants, containing lactone or are anionic, containing sophoroside acid, usually produced by non-pathogenic yeasts, especially *Candida bombicola* (Hirata *et al.* 2009) and other *Candida* species (Van Bogaert *et al.* 2007).

To confirm the data obtained by FT-IR, the sample was submitted to the quantitative analysis of ^{1}H protons in NMR (Fig. 3). The signal between 2.0 and 2.08 ppm confirms the presence of the –$COCH_3$ group, and the signal between 5.33 and 5.37 ppm indicates the presence of –CH=CH. The aliphatic portion is confirmed by a strong signal in the region between 1.20 and 1.40 ppm, while signals between 4.16 and 4.20 ppm indicate the presence of the –OH function. Signals in similar regions were observed by Daverey and Pakshirajan (2009) in the characterization of sophorolipids produced by *Candida bombicola*.

The values of the integrals obtained when analyzing the peaks were considered coherent with the number of atoms in the sophorolipid molecule elucidated in previous studies (Daverey and Pakshirajan, 2009; Hirata *et al.* 2009). In addition,

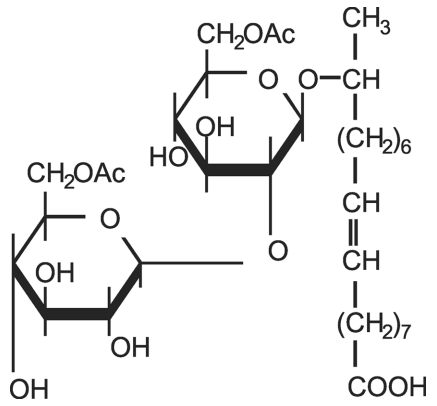

Figure 4. Chemical structure of the glycolipid of the sophorolipid class (anionic type), containing sophoroside acid. Source: adapted from Fig. 1 in Hirata *et al.* (2009).

the attribution tool of probable structures available in the ACD Spectrus Processor software considered the similarity between the structure of this molecule and the spectrum obtained as 0.85, while the structure of the sophorose showed a similarity of 0.74 (Fig. 4).

Mulligan, Yong and Gibbs (2001) reported that the application of sophorolipids in concentrations of 4% in sediments contaminated with Cu and Zn could solubilize up to 15% and 60% of these metals, respectively, because acid functional groups can sequester metal ions by adsorption (Ahn *et al.* 2009).

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

Effect of the purified biosurfactant on the batch sewage sludge washing studies

The results shown in Fig. 5 compare the efficacy of different treatments for the removal of metals in sewage sludge. In the control assays, it was observed that the solubilization of metals was higher in the assay with the pH adjusted to 2.0 compared to the assays without pH adjustment, and the same was observed in the assays with an addition of biosurfactants, except for Cd.

All treatments presented a statistically significant difference in relation to the control assay ($P < 0.001$), except for Cr in the co-inoculation assay ($P > 0.05$). There was also a significant statistical difference between bioleaching and co-inoculation assays, except for Cu ($P > 0.05$) and Ni ($P > 0.05$) (Table 2).

The best results for metal removal were observed for Cd, where the solubilization was 15.9% for the assay with pH adjustment to 2.0% and 7.6% in the assay without pH adjustment. The Cu, Pb, Ni, Zn and Cr metals presented 5.7%, 5.6%, 4.9%, 4.0% and 3.1% of removal, respectively, for the biosurfactant addition test with a pH adjustment to 2.0, and 4.3%, 4.6%, 1.9%, 3.4% and 3.4% of removal for the assays without pH adjustment, respectively. It can be observed that the addition of the biosurfactant increased the solubilization of metals in all assays (except for zinc).

The difference of solubilization between the different metals can be explained by their sorptive affinity, which depends on their properties, surface type and also the experimental conditions used. The higher solubilization in the treatments where the solution had its pH adjusted to 2.0 is due to the fact that the binding sites found in the sewage sludge are mostly pH dependent; therefore, the more acidic the solution, the higher the solubilization of cationic metals should be (McLean and Bledsoe 1992).

Compared with Mulligan,Yong and Gibbs (2001), where the authors described results of the application of sophorolipids for the removal of Cd and Zn, it can be observed that the results obtained in this study were lower for the assays with an addition of biosurfactants and an adjustment of pH, since, as stated above, there was 5.7% solubilization of copper, while Mulligan,Yong and Gibbs (2001) obtained 15% solubilization of this metal. However,

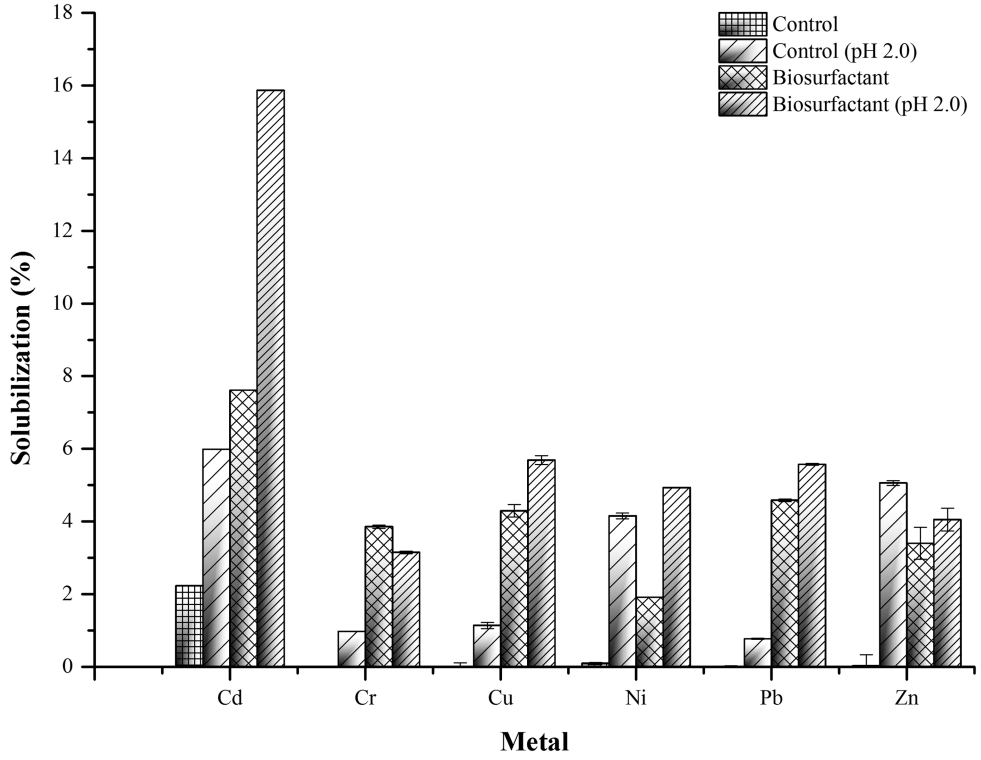

Figure 5. Solubilization (%) of metals from sewage sludge by adding biosurfactant (2%) with both pH adjustment to 2.0 and without pH adjustment (n = 2).

Table 2. Correlation coefficient and p value for the tests performed. Data obtained by Graph Pad Instat software for parametric ANOVA test with 95% confidence interval.

	Metals					
	Cd	Cr	Cu	Ni	Pb	Zn
Control vs. Control (pH 2.0)	0.1610	0.9798	0.9476	0.02218	0.9493	0.5204
Control vs. Biosurfactant	0.03006	0.5259	0.8876	0.02921	0.2712	0.7780
Control vs. Biosurfactant (pH 2.0)	0.002204	0.2466	0.5625	0.4704	0.5612	0.4977
Control (pH 2.0) vs. Biosurfactant	0.3124	0.7096	0.9975	0.09754	0.4462	0.9999
Control (pH 2.0) vs. Biosurfactant (pH 2.0)	0.007053	0.3537	0.8230	0.07361	0.8187	0.9490
Biosurfactant vs. Biosurfactant (pH 2.0)	0.02293	0.8502	0.8992	0.07361	0.8601	0.9349

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

it is worth mentioning that in this study, the concentration of biosurfactants used was 2%, while in the cited work the concentration was 4%. It can be claimed that the biosurfactant produced by *M. guilliermondii* has potential for to be used in metal removal processes.

CONCLUSIONS

Using raw waste material as a carbon source can be considered to have a dual-purpose benefit. Not only does it promote economically viable biosurfactant production, it can also treat waste that could have a negative impact on the environment. The application of biosurfactants produced in acidic conditions using residual oil as a carbon source can be considered inexpensive and an environmental friendly method to remove metals, and therefore using them in bioleaching processes is desirable. In this study, we characterized the biosurfactant produced by *Meyerozyma guilliermondii* as glycolipids and found that it could be used for removing cadmium from sludges.

After comparing the spectra obtained in FT-IR in NMR and in literature data, it can be suggested that the biosurfactant obtained after fermentation of the culture medium containing soybean oil frying waste as a carbon source has a similar structure to glycolipids of the sophorolipid class. It is known that sophorolipids can assist in the solubilization of metals, and so its production in bioleaching reactors in co-inoculation with acidophilic bacteria is desirable. The best results for metal removal were obtained for cadmium, where the application of a solution of 2% biosurfactant produced by *M. guilliermondii* with pH adjusted to 2.0 was able to solubilize 15.9% of this element.

ACKNOWLEDGEMENTS

The authors would like to thank André Henrique Rosa (PhD) and Cláudia Watanabe (MSc) for their help with the metal analysis, the *Laboratório de Hidrometalurgia from the Universidade Estadual Paulista* for the concession of *Aciditiobacillus* strains and the *Centro de Análises Químicas* (CAQI) from *Universidade de São Paulo* (USP) for the NMR analysis, and to the Journal of Bioscience and Bioengineering for granting us the use of Fig. 1B published by Hirata *et al.* (2009) (https://doi.org/10.1016/j.jbiosc.2009.03.012). This work was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Council for Scientific and Technological Development) (CNPq) (process numbers 442833/ 2014-8) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Development Committee for Higher Education Personnel) (CAPES).

Conflict of interest. None declared.

REFERENCES

Accorsini FR, Mutton MJR, Lemos EGM *et al.* Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. *Braz J Microbiol* 2012;**43**:116–25.

Ahn CK, Park D, Woo SH *et al.* Removal of cationic heavy metal from aqueous solution by activated carbon impregnated with anionic surfactants. *J Hazard Mater* 2009;**164**:1130–6.

APHA/AWWA/WEF. *Standard Methods for the Examination of Water and Wastewater*. 22nd ed. Washington, DC: American Public Health Association, 2012.

Banat IM, Franzetti A, Gandolfi I *et al.* Microbial biosurfactants production, applications and future potential. *Appl Microbiol Biotechnol* 2010;**87**:427–44.

Bednarski W, Adamczak M, Tomasik J *et al.* Application of oil refinery waste in the biosynthesis of glycolipids by yeast. *Bioresour Technol* 2004;**95**:15–8.

Bodour AA, Miller-Maier RM. Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J Microbiol Methods* 1998;**32**:273–80.

Camargo FP, Araujo ACV, Moraes EM de *et al.* A comparison between cactophilic yeast communities isolated from *Cereus hildmannianus* and *Praecereus euchlorus* necrotic clados. *Fungal Biol* 2016;**120**:1175–83.

Camargo FP, Prado PF do, Tonello PS *et al.* Bioleaching of toxic metals from sewage sludge by co-inoculation of Acidithiobacillus and the biosurfactant-producing yeast *Meyerozyma guilliermondii*. *J Environ Manage* 2018;**211**:28–35.

Coimbra CD, Rufino RD, Luna JM *et al.* Studies of the cell surface properties of candida species and relation to the production of biosurfactants for environmental applications. *Curr Microbiol* 2009;**58**:245–51.

Daverey A, Pakshirajan K. Production, characterization, and properties of sophorolipids from the yeast Candida bombicola using a low-cost fermentative medium. *Appl Biochem Biotechnol* 2009;**158**:663–74.

Fang D, Zhang R, Zhou L *et al.* A combination of bioleaching and bioprecipitation for deep removal of contaminating metals from dredged sediment. *J Hazard Mater* 2011;**192**:226–33.

Fournier D, Lemieux R, Couillard D. Essential interactions between *Thiobacillus ferrooxidans* and heterotrophic microorganisms during a wastewater sludge bioleaching process. *Environ Pollut* 1998;**101**:303–9.

Franzetti A, Gandolfi I, Fracchia L *et al.* Biosurfactant use in heavy metal removal from industrial effluents and contaminated sites. In: Kosaric N, Vardar-Sukan F (ed.). *Biosurfactants: Production and Utilization—Processes, Technologies, and Economics*. 2nd ed. New York: CRC Press, 2011, 361–70.

Hirata Y, Ryu M, Oda Y *et al.* Novel characteristics of sophorolipids, yeast glycolipid biosurfactants, as biodegradable low-foaming surfactants. *J Biosci Bioeng* 2009;**108**:142–6.

Karwowska E, Andrzejewska-Morzuch D, Łebkowska M *et al.* Bioleaching of metals from printed circuit boards supported with surfactant-producing bacteria. *J Hazard Mater* 2014;**264**:203–10.

Kitamoto D, Ikegami T, Suzuki GT *et al.* Microbial conversion of n-alkanes into glycolipid biosurfactants, mannosylerythritol lipids, by Pseudozyma (*Candida antarctica*). *Biotechnol Lett* 2001;**23**:1709–14.

Lawniczak L, Marecik R, Chrzanowski L. Contributions of biosurfactants to natural or induced bioremediation. *Appl Microbiol Biotechnol* 2013;**97**:2327–39.

Maier RM, Neilson JW, Artiola JF *et al.* Remediation of metal-contaminated soil and sludge using biosurfactant technology. *Int J Occup Med Environ Health* 2001;**14**:241–8.

McLean JE, Bledsoe BE. Behavior of Metals in Soils. Ground Water Issue. U.S. Environmental Protection Agency 1992; EPA/540/S-92/018:1-25.

Meylheuc T, Van Oss CJ, Bellon-Fontaine MN. Adsorption of biosurfactant on solid surfaces and consequences regarding the bioadhesion of *Listeria monocytogenes* LO28. *J Appl Microbiol* 2001;**91**:822–32.

Mousavi F, Beheshti-Maal K, Massah A. Production of sophorolipid from an identified current yeast, *Lachancea thermotolerans* BBMCZ7FA20, isolated from honey bee. *Curr Microbiol* 2015;**71**:303–10.

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019

Mulligan CN, Yong RN, Gibbs BF. Heavy metal removal from sediments by biosurfactants. *J Hazard Mater* 2001;**85**: 111–25.

Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I-bioprocesses and products. *Process Biochem* 2000;**35**:1153–69.

Parekh VJ, Patravale VB, Pandit AB. Mango kernel fat: a novel lipid source for the fermentative production of sophorolipid biosurfactant using *Starmerella bombicola* NRRL-Y 17069. *Ann Biol Res* 2012;**3**:1798–803.

Pathak A, Dastidar MG, Sreekrishnan TR. Bioleaching of heavy metals from sewage sludge: a review. *J Environ Manage* 2009;**90**:2343–53.

Pruthi V, Cameotra SS. Rapid method for monitoring maximum biosurfactant production obtained by acetone precipitation. *Biotechnol Tech* 1995;**9**:271–6.

Saharan BS, Sahu RK, Sharma D. A Review on biosurfactants: fermentation, applications, current developments and perspectives. *Genet Eng Biotechnol J* 2011;**GEBJ-29**:1–42.

Singh V. Biosurfactant – isolation, production, purification & significance. *Int J Sci Res Publ* 2012;**2**:2250–3153.

U.S. EPA. *Method 3050B: Acid Digestion of Sediments, Sludges, and Soils*. 1996, Washington, DC.

Van Bogaert INA, Saerens K, De Muynck C *et al*. Microbial production and application of sophorolipids. *Appl Microbiol Biotechnol* 2007;**76**:23–34.

Yang Z, Zhang Z, Chai L *et al*. Bioleaching remediation of heavy metal-contaminated soils using Burkholderia sp. Z-90. *J Hazard Mater* 2016;**301**:145–52.

Zhou J, Zheng G, Zhou L *et al*. The role of heterotrophic microorganism Galactomyces sp. Z3 in improving pig slurry bioleaching. *Environ Technol* 2013;**34**:35–43.

Downloaded from https://academic.oup.com/femsle/article-abstract/365/10/fny098/4975271 by Universidade Estadual Paulista Jï¿½lio de Mesquita Filho user on 21 August 2019