Production of biosurfactants using Egyptian soil fungi

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Abstract

Soil fungi have unique physiological and metabolic features, and are considered as an important source of biomolecules. The present research aims to study the ability of local soil fungal isolates for the production of biosurfactants and to study the stability of these biosurfactants under different temperatures (50, 70, 90, 110, and 121 °C), different pH values (2, 3, 6, 10, 12, and 15), and different salinity concentrations (5, 10, 15, 20, and 25% NaCl) depending on the formation of clear zone using oil displacement area (ODA) test. The research also aims to study the enhancement effect of four vegetable oils including sunflower oil, waste frying oil, olive oil, and corn oil on the activity of the produced biosurfactants. The obtained results showed that *Aspergillus flavus*, *Aspergillus wentii* and *Fusarium sp.* had the maximum biosurfactants production. The stability of biosurfactants occurred at alkaline pH, wide range of temperatures, and under high salinity (15%). Sunflower oil had the maximum enhancement effect on the activity of the produced biosurfactants, while corn oil showed lower enhancement effect. Depending on the obtained results, it can be concluded that local soil fungal isolates are considered as a promising source for production of biosurfactants.

Key Points

- The article proved that soil fungi are a promising source for the production of different types of biosurfactants.
- According to available published articles, the current study is the first study to prove the ability of the soil fungus *Aspergillus wentii* to produce biosurfactants.
- The produced biosurfactants showed good stability at alkaline pH values and high salinity making it suitable for biodegradation of sea water polluted with oil.

Introduction

Biosurfactants are defined as unique amphipathic molecules containing polar and non-polar components with properties that are valuable for bioremediation and applications in some industries such as oil recovery, food processing, and pharmaceuticals (Desai and Banat 1997; Makkar and Cameotra 1998; Bodour et al. 2003). Kuyukina et al. (2005) and Batista et al. (2006) reported that most synthetic surfactants are hardly biodegradable and their residues are harmful to the soil. Microorganisms including bacteria, yeast, algae, and fungi can produce biosurfactants as metabolic byproducts. These biosurfactants have wide range and variety of chemical structures such as polysaccharide protein complexes, glycolipids, fatty acids, lipopeptides, neutral lipids, and phospholipids (Gudiña et al. 2016). The usage of biosurfactants is preferable to synthetic surfactants due to their biodegradability, foaming property, low toxicity, biocompatibility, environmentally safe, versatility, and tolerance to extreme pH, temperature and salinity (Georgiou et al. 1992). Biosurfactants are used widely to enhance oil recovery because are effective under a broad range of oil and reservoir conditions, very selective, and are used in small quantities (Lai et al. 2009; Pei et al. 2009). Biosurfactants are used in a wide range of food

industries such as lecithin, bakery, emulsifiers, food additives, meat products, and ethylene glycol (Besson and Michel 1992; Fiechter 1992). In addition, biosurfactants are used widely in pharmaceutical industries as immunological adjuvants, respiratory failure agent, gene delivery agent, adhesion inhibitor of pathogenic organisms to solid surfaces, anti-adhesive agent in surgical implants. Furthermore, they are used in the recovery of intracellular products, and as antimicrobial, anticancer, antiviral, as well as skin fibroblast metabolism stimulation agents (Balan et al. 2017). Mulligan and Gibbs (2004) reported that biosurfactants have the ability to improve the bioremediation process of hydrocarbons through two mechanisms. The first mechanism is through increasing the substrate bioavailability for microorganisms, and the second mechanism is through increasing the hydrophobicity via the interaction with the cell surface which allowing the association between microbial cells and hydrophobic substrates. A diverse group of microorganisms (bacteria, yeasts and fungi) can synthesis different kinds of biosurfactants using water-soluble compounds as substrates (glycerol, ethanol, sucrose and glucose) (Desai and Banat 1997). The quantity of biosurfactant produced by microorganisms is dependent on types and sources as well as their growth conditions such as pH, temperature, salinity, and pressure (Aliya et al. 2016). Bacteria, especially the genus *Pseudomonas*, play a major role in production of biosurfactants (Nadella et al. 2018). Moreover, many studies have reported the ability of different types of fungal species to produce biosurfactants such as sophorolipids produced by *Candida bombicola*, lipid carbohydrate protein based bioemulsifiers produced by *Yarrowia lipolytica* (Casas and Garcia-Ochoa 1999), monoketide and polyketide derivatives produced by *Penicillium chrysogenum* (Sanches et al. 2021), spiculisporic acid produced by *Penicillium spiculisporum* (Ban and Sato 1993), cellobiose lipids produced by *Ustilago maydis* (Teichmann et al. 2007), and chromone derivative produced by *Aspergillus versicolor* (Lin et al. 2003). The main aim of the present study is to investigate the production of biosurfactants from soil fungal isolates and to study the effect of sunflower oil, olive oil, corn oil, and waste frying oil on the production of these biosurfactants.

Materials & Methods

Biosurfactant-producing fungi isolation and purification

In order to isolate the biosurfactant producing fungi from the Egyptian soil, the dilution-plate technique was used (Johnson et al. 1959). The inorganic salt medium (ISM) was modified and used for screening of biosurfactant-producing fungal strains with the following composition, MgSO_4 (0.5 g/L), KH_2PO_4 (1 g/L), NaNO_3 (2 g/L), K_2HPO_4 (2 g/L), CaCl_2 (0.1 g/L), KCl (0.1 g/L), trace salt solution (1 ml/L). pH of ISM was modified and adjusted to 7 for filamentous fungi (Rapp and Backhaus 1992). Soybean oil (1.0 % v/v) was used as a carbon source in ISM for biosurfactant production since soybean oil characterized by its simple carbon chains (less complex). 125-mL Erlenmeyer flasks were used to keep the different fungal cultures at 30 °C. Erlenmeyer flasks were placed inside an orbital incubator (New Brunswick Scientific) at 120 rpm under continuous stirring for 7 days. In parallel, some Erlenmeyer flasks containing 50 mL of culture medium and 1 % soybean oil (v/v) without inoculation of any fungi were used for control purpose. The culturing of each fungal strain was carried out in triplicate and at the end of the incubation period,

membrane filters with 0.45 mm porosity (TPP, Europe/Switzerland) coupled with sterilized syringe (20-mL) were used for filtration of the culture media. The purified fungal isolates were given the code NRCB as abbreviation for National Research Centre Biosurfactant.

Biosurfactant-producing fungi identification

The isolated fungi were subjected to morphological identification depending on some characteristics such as diameter of colonies, conidia's color, reverse mycelium's color and pigmentation, and extracellular exudates. The isolated fungi were also subjected to microscopic examination using optical light microscope (10 x 90) Olympus CH40 and depending on some features including heads of conidia, sporulation degree, fruting bodies, and the homogeneity characters of conidiogenous cells (Barron 1968; Booth 1971; Booth 1977; Ainsworth et al. 1971; Ellis 1971 & 1976; Pitt 1985). Malt extract agar (MA) was used for culturing of the isolated fungi at 28°C for 7-10 days, then the fungal cultures were kept at 4 °C for further experiments.

Biosurfactant production determination

1. Oil displacement activity (ODA).

The oil displacement activity (ODA) test (Datta et al. 2018) was used with minor modifications for the determination of biosurfactant production by fungal isolates. The used engine oil (100 µL) was placed on the surface of the Petri dishes containing distilled water (40 mL), then cell-free broth (10 µL) was dropped on oil-coated thin film, and the diameter of the circle clear zone of displaced oil was measured.

2. Emulsification index (EI 24%).

Emulsification index (EI 24%) is another technique that used for screening of biosurfactant-producing fungi. 2 mL of the supernatant (cell-free) with 2 mL of kerosene were mixed together for two minutes using vortex. After mixing, the mixture was left for 24 h at room temperature. The results were observed and calculated using the following equation (Cooper and Goldenberg 1987):

$$\text{EI 24\%} = (\text{emulsified layer height after 24 h} / \text{total height of liquid}) \times 100$$

3. CTAB Agar Test

This method was developed by Siegmund and Wanger (1991), and used for the detection of anionic surfactants. ISM media with supplemented was 0.5 mg/mL cetyltrimethyl ammonium bromide (CTAB) + 0.2 mg/mL methylene blue (Satpute et al. 2008). With a sterile cork borer (10 mm) wells were made in agar plate. Each well was filled with the supernatant of the culture. The plates were incubated at 30 °C for 48 hrs and left in the room temperature for 12 hrs. The plates were observed for the development of bluish or bluish green color around the walls.

4. Foam formation

A known volume of each supernatant was introduced into a graduated capped cylinder, tightly closed and was vigorously shaken for 1 min the formation of foam was observed and reported.

Stability of the biosurfactants

1. Effect of temperature

Ten mL of supernatant were exposed to various temperatures (50–121 °C) for 30 min and then allowed to cool at room temperature. The activity of the biosurfactant was measured by ODA.

2. Effect of pH value

The supernatant of each culture was adjusted at different pH values (2-15). The activity of each was tested using ODA.

3. Effect of salinity

Different concentrations of NaCl (5-25 %, w/v) were prepared using the supernatant of each culture and left for 20 min, after which the activity of each was tested using ODA.

Production of biosurfactants using vegetable oils

Fifty mL of ISM medium (without 1 % soybean oil) supplemented separately by the following vegetables oils (corn oil- olive oil- sunflower oil- waste frying oil) as carbon sources, inoculated with tested fungal strains and incubated at 30 °C under shaking condition (135 rpm) for 7 days. At the end of the incubation period, the production of biosurfactants was examined using ODA test.

Results

Isolation and purification of biosurfactant producing fungi

Seventeen different isolates of filamentous fungi belonging to five genera and twelve species were selected depending on the growth on the modified ISM (containing oil) and identified morphologically. These isolates were isolated from soil contaminated with oil. Scientific names of local soil fungal isolates were summarized in Table 1. All isolated fungi were identified as biosurfactant producing fungi depending on the ability of fungi to grow in the media containing soybean oil.

Morphological identification of biosurfactant producing fungi

As mentioned previously, seventeen different filamentous fungi isolates belonging to five genera and twelve species were isolated. Macroscopic and microscopic features of isolated microorganisms were examined and observed as shown in the Figure 1. The initial growth of *Aspergillus niger* is white in color but after few days, it converts into black with production of conidial spores. Microscopically, *A. niger* is characterized by smooth colored conidia (with radial heads and biseriate) and conidiophores (hyaline

and septate). *Aspergillus flavus* have a thick mycelial mat that have a size of 3 to 6 µm. the conidiophores are colorless and rough-textured. *Aspergillus wentii* colonies appear dense, fluffy to cottony, and are white in color and reverse is reddish, the growth rate is slow to moderate. Due to cleistothecial production, colonies of *A. glaucus* color ranged from grayish-turquoise to deep green with yellow central areas with a reverse color range from pale yellow to pale brown. Colonies of *A. ornatus* growing very sparingly, conidial heads radiate and borne on conidiophores and no cleistothecia produced. Colonies of *A. terreus* growing rapidly, marked by shallow radial furrows and velvety. *Penicillium cyaneum* is a species of the genus of *Penicillium* which was isolated from an oil-field. Mycelium consists of highly branched networks of multinucleated, usually colorless hyphae. *Penicillium frequentans* showed rapid growth, green color with yellowish border and valvate appearance and the back side of colony was reddish in color with yellowish border on PDA media. The fast-growing colonies of *Trichoderma* sp. appear white and downy at first then develop in small areas (or in concentric ring-like zones) yellowish-green to deep green compact tufts on the surface of the agar. Colonies of *Fusarium* sp. are rapidly grown with white aerial mycelium then changes to purple with discrete orange sporodochia, *Gibberella* sp. is a teleomorph of *Fusarium moniliforme*.

Biosurfactant production determination

A survey was conducted to determine the extent of studied fungal isolates to investigate their abilities to produce biosurfactants using different parameters (ODA, CTAB agar test, foam formation, and EI 24%) as shown in Table 2. It was found that all examined fungal isolates were positive for ODA test but with different clear zone diameter values. Isolate NRCB11 (*Fusarium* sp.) showed a remarkable oil displacement clear area (8 cm diameter) and the same isolate showed high EI 24% value, with two other isolates NRCB10 (*Aspergillus wentii*) and NRCB8 (*Aspergillus flavus*) of 57.14 %, 60.0 % and 61.76 %, respectively.

As the ability of tested isolates to form bluish color (CTAB agar test), the results fluctuated between very faint bluish color as in case of isolates (NRCB1, NRCB6, NRCB8 and NRCB16) and very bluish color as in case of isolates (NRCB3, NRCB10 and NRCB11). Regarding to the ability of tested isolates to form foam, it was found that some isolates showed good ability of foam formation as in case of isolates (NRCB1, NRCB2, NRCB4, NRCB5, NRCB10, NRCB12, NRCB15 and NRCB17) while the other isolates showed no ability of foam formation. Depending on the obtained results in the current study, it was clear that there was no correlation between results of foam formation test and results of other tests (ODA, CTAB agar test, and EI 24%).

Stability of the produced biosurfactants

In order to apply biosurfactants in several fields, it depends on their stability at different pH values, wide range of temperatures as well as high salinity. The stability of produced biosurfactants were analyzed using oil displacement area (ODA) test. The stability of produced crude biosurfactants by studied fungal isolates were examined at different temperature degrees (50 °C, 70 °C, 90 °C, 110 °C, and 121 °C),

different pH ranges (2, 3, 6, 10, 12 and 15) and at different saline (NaCl conc. (w/v)) concentrations (5 %, 10 %, 15 %, 20 %, and 25 %).

1. pH

Figure 2 shows that maximum ODA (8 cm) was observed using the produced biosurfactants by fungal isolates NRCB9 (*Aspergillus niger*) and NRCB13 (*Aspergillus glaucus*) at pH 12, while the ODA results retreated at other pH values (2, 6, and 10) and the lowest ODA result was observed at pH 15. These results indicated that alkaline medium (pH 12) had a positive effect on emulsion stability and emulsification activity of the produced biosurfactants.

2. Temperature

The temperature stability of the produced biosurfactants using ODA test is represented in Figure 3. The results showed that some tested biosurfactants were stable at different temperatures such as NRCB3 (*Trichoderma sp.*), NRCB6 (*Penicillium frequentans*) NRCB8 (*Aspergillus flavus*), NRCB9 (*Aspergillus niger*), NRCB10 (*Aspergillus wentii*) and NRCB15 (*Aspergillus glaucus*), while other biosurfactants stability affected by the change in temperature. The results showed also the stability of some biosurfactants at sole temperature degree such as biosurfactants NRCB12 (*Aspergillus versicolor*) and NRCB13 (*Aspergillus glaucus*) at 110 °C, and NRCB16 (*Aspergillus glaucus*) at 90 °C.

Moreover, biosurfactant NRCB17 (*Aspergillus terreus*) showed no stability at all examined temperatures.

3. Salt concentration

Figure 4 shows the stability of produced biosurfactants at different salt concentrations (5 %, 10 %, 15 %, 20 %, and 25 % NaCl) depending on ODA test results. It was found that the maximum activity of all biosurfactants occurred at salt concentration of 15 % NaCl, followed by salt concentrations of 10 % and 20 %, while both of lower salt concentration (5 %) and higher salt concentration (25 %) showed the lowest activity.

Vegetable oils for production of biosurfactants

The utilization of some vegetable oils (corn, olive, sunflower, and waste frying) was investigated as carbon sources instead of soybean oil to study the ability of these oils to enhance the production of biosurfactants using fungal isolates. Figure 5 shows the influence of four types of vegetable oils on the activity of produced biosurfactants by tested fungal isolates using ODA test. It was clear that sunflower oil has the highest enhancement effect on the activity of produced biosurfactants by all fungal isolates, followed by waste frying oil. The maximum ODA (8.5 cm) occurred in case of using sunflower oil as a carbon source for the production of biosurfactant by fungal isolate number NRCB10.

Discussion

Isolation and purification of biosurfactant producing fungi

Seventeen different isolates of filamentous fungi belonging to five genera and twelve species were selected depending on the growth on the modified ISM (containing oil) and identified morphologically. These isolates were isolated from soil contaminated with oil. Scientific names of local soil fungal isolates were summarized in Table 1. All isolated fungi were identified as biosurfactant producing fungi depending on the ability of fungi to grow in the media containing soybean oil. The results in Table 1 showed that all studied soil fungal isolates had the ability to produce biosurfactants. There are some species of the genus *Aspergillus* which have been reported as biosurfactant producers using different sources such as *Aspergillus flavus* using citrus fruits (Ishaq et al. 2015), *Aspergillus niger* using banana stalks powder during solid state fermentation (Asgher et al. 2020), and *Aspergillus terreus* using oil spills (Spina et al. 2018; Pitocchi et al. 2020). All previously mentioned *Aspergillus* sp. were isolated from different sources but not from soil source as we made in this study. However, some other species of the genus *Aspergillus* were not proven yet as biosurfactant producers such as *Aspergillus glaucus*, *Aspergillus wentii*, and *Aspergillus ornatus*. *Fusarium* sp. has been classified as biosurfactant producers which isolated from *Eichhornia crassipes* collected from oil contaminated water (Lima et al. 2016). In contrast, *Gibberella* sp. (telomorph of *Fusarium*) has not yet recorded as biosurfactant producer. Additionally, different species of *Penicillium* genus and *Trichoderma* genus have been reported as biosurfactant producers (Sena et al 2018; Spina et al. 2018; Pitocchi et al. 2020). According to our results, it was found that each of the following fungal isolates: *Aspergillus glaucus*, *Aspergillus wentii*, *Aspergillus versicolor*, *Aspergillus ornatus* and *Gibberella* sp. were recorded for the first time as biosurfactant producers from soil source.

Biosurfactant production determination

According to the results of the present study (Table 2), *Aspergillus flavus* (NRCB8), *Aspergillus wentii* (NRCB10) and *Fusarium* sp. (NRCB11) isolates showed the best results for biosurfactant production. These results were in comparable with previous studies which reported that main genera of ascomycetes such as *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. have the ability to produce biosurfactants with wide range variety and spectrum (Santhappan and Pandian 2017; Reis et al. 2018; Sanches et al. 2021). According to available published articles, the current study is the first study to prove the ability of *Aspergillus wentii* to produce biosurfactant.

Stability of the produced biosurfactants

The stability results of produced biosurfactants at different pH, temperatures, and saline concentrations were summarized in Figures 2, 3, and 4, respectively.

1. pH.

The obtained results in Figure 2 indicated that alkaline medium (pH 12) had a positive effect on emulsion stability and emulsification activity of the produced biosurfactants. This could be attributed to the

superior stability of fatty acid surfactant micelles in the presence of NaOH as well as the secondary metabolites' precipitation at alkaline pH values (Khopade et al. 2012). These results were in agreement with the results reported by Sarubbo et al. (2007) and Andrade Silva et al. (2014) since the produced biosurfactants by *Cunninghamella echinulata* and *Candida lipolytica*, respectively showed noticeable stability at pH 12. Factors such as temperature, pH and salinity concentration are well known to influence the activity and stability of produced biosurfactants (Andrade Silva et al. 2014).

2. Temperature.

The observed variation in temperature stability of produced biosurfactants (Figure 3) depends on the chemical structure of biosurfactants and may be related to the strength of hydrogen bonds between the hydroxyl groups present which allows or prevents any significant dehydration relative to high temperatures (Stubenrauch et al. 2001; Udoh and Vinogradov 2019). Ahmad et al. (2021) reported that higher temperatures up to 100 °C had no significant adverse effect on activity of surface tension for biosurfactant produced from *Klebsiella* sp. There are many studies have showed the positive effect of different temperature degrees ranged from 30 °C to 120 °C (Vaz et al. 2012; Qazi et al. 2014; Mouafi et al. 2016) on the stability of biosurfactants produced by different microorganisms such as *Candida lipolytica* (Rufino et al. 2007), *Candida glabrata* (Luna et al. 2009) and *Bacillus subtilis* C9 (Haba et al. 2000).

3. Salt concentration.

Depending on the obtained results (Figure 4), the salt concentration ranged from 10 % to 20 % (NaCl) had the maximum activity of produced biosurfactants with maximum activity at salt concentration of 15 %. Similar results were observed for biosurfactant produced by *Candida tropicalis* (Muthusamy et al. 2008), *Fusarium* sp. BS-8 (Qazi et al. 2013) and *Candida sphaerica* (Luna et al. 2011). Most of the produced biosurfactants showed stability at high salinity and alkaline pH values; such biosurfactants may be useful for biodegradation of oil spills in marine environment because of its stability in alkaline condition and in the presence of high salt concentrations (Khopade et al. 2012).

Vegetable oils for production of biosurfactants

In order to enhance the production of biosurfactants using soil fungal isolates, four different types of vegetable oils (corn, olive, sunflower, and waste frying) were used instead of soybean oil as carbon sources (Figure 5). It was clear that sunflower oil had the maximum enhancement effect. Ferraz et al. (2002) reported that sunflower oil contains about 60 % linoleic acid which was responsible for the enhancement of biosurfactant production by *Serratia marcescens*. Also, Zadeh et al. (2017) reported that waste frying oil showed the maximum biosurfactant production (11.7 cm oil spreading) when used as a carbon source by *Mucor circinelloides*. Olive oil showed a moderate enhancement effect on the activity of produced biosurfactants with a maximum ODA of 5 cm in case of fungal isolate NRCB9. Olive oil showed no increase in the biosurfactant production from *Yarrowia lipolytica* which was due to the adhesion of surfactant to the cell wall of *Yarrowia lipolytica* (Fontes et al. 2010; Qazi et al. 2013). However, corn oil showed the lowest enhancement effect on the activity of produced biosurfactants by all fungal isolates.

Finally, the present study concluded that local soil fungal isolates have the ability to produce biosurfactants. Depending on oil displacement area (ODA) test results, most of the produced biosurfactants showed stability under wide range of temperatures (50 °C–121 °C), different pH values (2–15), and different salt concentrations (NaCl; 5–25 %). Sunflower oil had the maximum enhancement effect on the activity of the produced biosurfactants followed by waste frying oil. However, olive oil and corn oil showed less enhancements. Local soil fungal isolates are considered as a promising source for production of biosurfactants. According to available published articles, the current study is the first study to prove the ability of *Aspergillus wentii* to produce biosurfactant. Further study will be conducted in order to determine the chemical structure of the promising produced biosurfactant from the local soil fungal isolate NRCB10 (*Aspergillus wentii*).

Declarations

Author Contributions: SRH and RSA conceived and designed research. SRH, RSA, and SR conducted experiments. SRH and SR analyzed data. SRH and RSA wrote the initial manuscript. SRH, RSA, and SR wrote the final manuscript. All authors read and approved the manuscript.

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Compliance with Ethical Standards

Conflict of interest: Shimaa R. Hamed declares that she has no conflict of interest. Raed S. Al-Wasify declares that he has no conflict of interest. Samar Ragab declares that she has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

Ahmad Z, Zhang X, Imran M, Zhong H, Andleeb S, Zulekha R, Guansheng L, Iftikhar A, Frederic C (2021) Production, functional stability, and effect of rhamnolipid biosurfactant from *Klebsiella* sp. on phenanthrene degradation in various medium systems. *Ecotoxicol Environ Safety* 207:111514. <https://doi.org/10.1016/j.ecoenv.2020.111514>.

Ainsworth GC, James PW, Hawksworth DL (1971) Ainsworth & Bisby's Dictionary of the Fungi. 6th edn Kew: Commonwealth Mycological Institute.

- Aliya Y, Gulzhan K, Almagul B, Azhar Z (2016) Microbial Enhanced Oil Recovery. In book: Chemical Enhanced Oil Recovery (cEOR) - a Practical Overview. P:147-167. <http://dx.doi.org/10.5772/64805>.
- Andrade Silva NR, Luna MA, Santiago AL, Franco LO, Silva GKB, de Souza PM, Okada K, Albuquerque CD, da Silva CA, Campos-Takaki GM (2014) Biosurfactant-and-bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from Caatinga soil in the northeast of Brazil. Int J Mol Sci 15(9):15377-15395. doi: [10.3390/ijms150915377](https://doi.org/10.3390/ijms150915377) .
- Asgher M, Arshad S, Qamar SA, Khalid N (2020) Improved biosurfactant production from *Aspergillus niger* through chemical mutagenesis: characterization and RSM optimization. SN Appl Sci 2:966. <https://doi.org/10.1007/s42452-020-2783-3>.
- Balan SS, Kumar CG, Jayalakshmi S (2017) Aneurinifactin, a new lipopeptide biosurfactant produced by a marine *Aneurinibacillus aneurinilyticus* SBP-11 isolated from Gulf of Mannar: Purification, characterization and its biological evaluation. Microbiol Res 194:1-9. <https://doi.org/10.1016/j.micres.2016.10.005>.
- Ban T, Sato T (1993) Aqueous microbial biosurfactant solution exhibiting ultra-low tension at oil-water interfaces. In book: Microbial enhancement of oil recovery-recent advances. Develop Petrol Sci 39:115–125.
- Barron GL (1968) The Genera of Hyphomycetes from soil. Williams and Wilkins Co., Baltimore, Maryland.
- Batista SB, Mouteer AH, Amorim FR, Tótola MR (2006) Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresour Technol 97(6):868–875. <https://doi.org/10.1016/j.biortech.2005.04.020>.
- Besson F, Michel G (1992) Biosynthesis of iturin and surfactin by *Bacillus subtilis* evidence for amino acid activating enzymes. Biotechnol Lett 14:1013–1018. <https://doi.org/10.1007/BF01021050>.
- Bodour AA, Drees KP, Maier RM (2003) Distribution of biosurfactant producing bacteria in undisturbed and contaminated arid southwestern soils. Appl Environ Microbiol 69(6):3280–3287. doi: 10.1128/AEM.69.6.3280-3287.2003.
- Booth C (1971) The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England, pp 237.
- Booth C (1977) *Fusarium* laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew, Surrey, England, pp 58.
- Cooper DG, Goldenberg BG (1987) Surface-active agents from two *Bacillus* species. Appl Environ Microbiol 53(2):224-229. <https://doi.org/10.1128/aem.53.2.224-229.1987>.

- Datta P, Tiwari P, Pandey LM (2018) Isolation and characterization of biosurfactant producing and oil degrading *Bacillus subtilis* MG495086 from formation water of Assam oil reservoir and its suitability for enhanced oil recovery. *Biores Technol* 270:439-448. <https://doi.org/10.1016/j.biortech.2018.09.047>.
- Desai JD, Banat IM (1997) Microbial production of surfactants and their commercial potential. *Microbiol Mol Biol* 61(1):47-64. doi: 10.1128/mmbr.61.1.47-64.1997.
- Reis CBLD, Morandini LMB, Bevilacqua CB, Bublitz F, Ugalde G, Mazutti MA, Jacques RJS (2018) First report of the production of a potent biosurfactant with α , β -trehalose by *Fusarium fujikuroi* under optimized conditions of submerged fermentation. *Braz J Microbiol* 49(1):185-192. doi: [10.1016/j.bjm.2018.04.004](https://doi.org/10.1016/j.bjm.2018.04.004).
- Ellis MB (1971) Dematiaceous Hyphomycetes. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England.
- Ellis MB (1976) More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England, pp 507.
- Ferraz C, de Araújo AA, Pastore GM (2002) The Influence of vegetable oils on biosurfactant production by *Serratia marcescens*. *Appl Biochem Biotechnol* 98–100:841-847. doi: 10.1385/abab:98-100:1-9:841.
- Fiechter A (1992) Biosurfactants: Moving towards industrial application. *Trends Biotechnol* 10:208–217. [https://doi.org/10.1016/0167-7799\(92\)90215-H](https://doi.org/10.1016/0167-7799(92)90215-H).
- Fontes GC, Fonseca-Amaral PF, Nele M, Coelho MA (2010) Factorial design to optimize biosurfactant production by *Yarrowia lipolytica*. *J Biomed Biotechnol* 2010:821306-821306. doi: 10.1155/2010/821306.
- Georgiou G, Lin S, Sharma MM (1992) Surface active compounds from microorganisms. *Biotechnol (NY)* 10(1):60-65. doi: 10.1038/nbt0192-60.
- Gudiña EJ, Teixeira JA, Rodrigues LR (2016) Biosurfactants produced by marine microorganisms with therapeutic applications. *Marine Drugs* 14(2):38. doi: 10.3390/md14020038.
- Haba E, Espuny MJ, Busquets M, Manresa A (2000) Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. *J Appl Microbiol* 88:379-387. doi: [10.1046/j.1365-2672.2000.00961.x](https://doi.org/10.1046/j.1365-2672.2000.00961.x).
- Ishaq U, Akram MS, Iqbal Z, Rafiq M, Akrem A, Nadeem M, Shafi F, Shafiq Z, Mahmood S, Baig MA (2015) Production and characterization of novel self-assembling biosurfactants from *Aspergillus flavus*. *J Appl Microbiol* 119(4), 1035-1045. doi: 10.1111/jam.12929.
- Johnson IF, Curl EA, Bond JH, Fribourg HA (1959) Methods for studying soil microflora-plant disease relationships. Burgess publishing Co., Minneapolis. USA, pp 15.

- Khopade A, Biao R, Liu X, Mahadik K, Zhang L, Kokare C (2012) Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4. *Desalination* 285:198-204. <https://doi.org/10.1016/j.desal.2011.10.002>.
- Kuyukina MS, Ivshina IB, Makarov SO, Litvinenko LV, Cunningham CJ, Philp JC (2005) Effect of biosurfactants on crude oil desorption and mobilization in a soil system. *Environ Int* 31(2):155-161. doi: 10.1016/j.envint.2004.09.009.
- Lai CC, Huang YC, Wei YH, Chang J (2009) Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. *J Hazard Mater* 167(1-3):609-614. <https://doi.org/10.1016/j.jhazmat.2009.01.017>.
- Lima JMS, Pereira JO, Batista IH, Neto PC, dos Santos JC, de Araújo SP, Pantoja MC, da Mota AJ, de Azevedo JL (2016) Potential biosurfactant producing endophytic and epiphytic fungi, isolated from macrophytes in the Negro River in Manaus, Amazonas, Brazil. *Afri J Biotechnol* 15(24):1217-1223. doi: 10.5897/AJB2015.15131.
- Lin W, Brauers G, Ebel R, Wray V, Berg A, Sudarsono, Proksch P (2003) Novel chromone derivatives from the fungus *Aspergillus versicolor* isolated from the marine sponge *Xestospongia exigua*. *J Nat Prod* 66(1):57-61. <https://doi.org/10.1021/np020196b>.
- Luna JM, Rufino RD, Albuquerque CDC, Sarubbo LA, Campos-Takaki GM (2011) Economic optimized medium for tensio-active agent production by *Candida sphaerica* UCP0995 and application in the removal of hydrophobic contaminant from sand. *Int J Mol Sci* 12(4):2463-2476. doi: 10.3390/ijms12042463.
- Luna JM, Sarubbo LA, Campos-Takaki GM (2009) A new biosurfactant produced by *Candida glabrata* UCP1002: characteristics of stability and application in oil recovery. *Braz Arch Biol Technol* 52:785-793.
- Makkar RS, Cameotra SS (1998) Production of biosurfactant at mesophilic and thermophilic conditions by a strain of *Bacillus subtilis*. *J Ind Microbiol Biotechnol* 20:48-52. <https://doi.org/10.1038/sj.jim.2900474>.
- Mouafi FE, Elsoud MMA, Moharam ME (2016) Optimization of biosurfactant production by *Bacillus brevis* using response surface methodology. *Biotechnol Rep* 9:31-37. <https://doi.org/10.1016/j.btre.2015.12.003>.
- Mulligan CN, Gibbs BF (2004) Types, production and applications of biosurfactants. *Proc Indian Nat Sci Acad* 70:31-55.
- Muthusamy K, Gopalakrishnan S, Ravi K, Sivachidambaram, P (2008) Biosurfactants: properties, commercial production and application. *Current Science* 94:736-

774. <http://www.jstor.org/stable/24100627>.

Nadella RK, Murugadas V, Basha KA, Joseph TC, Lalitha KV, Prasad MM (2018) Biosurfactant production by *Pseudomonas aeruginosa* isolated from aquaculture farm soil and its optimisation. Indian J Fish 65(4):127-134.

Casas JA, Garcia-Ochoa F (1999) Sophorolipid production by *Candida bombicola* medium composition and culture methods. J Biosci Bioeng 88(5):488-494. [https://doi.org/10.1016/S1389-1723\(00\)87664-1](https://doi.org/10.1016/S1389-1723(00)87664-1).

Pei X, Zhan X, Zhou Z (2009) Effect of biosurfactant on the sorption of phenanthrene onto original and H₂O₂-treated soils. J Environ Sci 21(10):1378-1385. doi: 10.1016/s1001-0742(08)62429-8.

Pitocchi R, Cicatiello P, Birolo L, Piscitelli A, Bovio E, Varese GC, Giardina P (2020) Cerato-Platanins from marine fungi as effective protein biosurfactants and bioemulsifiers. Int J Mol Sci 21(8):2913. doi: [10.3390/ijms21082913](https://doi.org/10.3390/ijms21082913).

Pitt JI (1985) A laboratory guide to common *Penicillium* species. Academic Press, Australia, pp 182.

Qazi MA, Kanwal T, Jadoon M, Ahmed S, Fatima N (2014) Isolation and characterization of a biosurfactant-producing *Fusarium* sp. BS-8 from oil contaminated soil. Biotechnol Prog 30:1065-1075. doi: 10.1002/btpr.1933.

Qazi MA, Subhan M, Fatima N, Ali MI, Ahmed S (2013) Role of biosurfactant produced by *Fusarium* sp. BS-8 in enhanced oil recovery (EOR) through sand pack column. Inter J Biosci Biochem Bioinfo 3(6):598-604.

Rapp P, Backhaus S (1992) Formation of extracellular lipases by filamentous fungi, yeast, and bacteria. Enzyme Microb Technol 14(11):938-943. [https://doi.org/10.1016/0141-0229\(92\)90059-W](https://doi.org/10.1016/0141-0229(92)90059-W).

Rufino R, Sarubbo L, Campos-Takaki G (2007) Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. World J Microbiol Biotechnol 23:729-734. doi: 10.1007/s11274-006-9278-2.

Sanches MA, Luzeiro IG, Cortez AA, de Souza ÉS, Albuquerque PM, Chopra HK, de Souza JVB (2021) Production of biosurfactants by Ascomycetes. Inter J Microbiol 2021:11 pages. <https://doi.org/10.1155/2021/6669263>.

Sanches MA, Luzeiro IG, Cortez ACA, de Souza ES, Albuquerque PM, Chopra HK, de Souza JVB (2021) Production of biosurfactants by Ascomycetes. Inter J Microbiol, Article ID 6669263, 11 pages (2021). <https://doi.org/10.1155/2021/6669263>.

Santhappan R, Pandian MR (2017) Characterization of novel biosurfactants produced by the strain *Fusarium oxysporum*. Bioremediat Biodegrad 8(6):416. doi: [10.4172/2155-6199.1000416](https://doi.org/10.4172/2155-6199.1000416).

- Sarubbo L, Farias C, Campos-Takaki GM (2007) Co-utilization of canola oil and glucose on the production of a surfactant by *Candida lipolytica*. *Curr Microbiol* 54(1):68-73. doi: 10.1007/s00284-006-0412-z.
- Satpute SK, Bhawsar BD, Dhakephalkar PK, Chopade BA (2008) Assessment of different screening methods for selecting biosurfactant producing marine bacteria. *Indian J Marine Sci* 37(3):243-250.
- Sena HH, Sanches MA, Rocha DFS, Filho Segundo WOP, de Souza ÉS, de Souza JVB (2018) Production of biosurfactants by soil fungi isolated from the amazon forest. *Inter J Microbiol* 8 pages. <https://doi.org/10.1155/2018/5684261>.
- Siegmund I, Wagner F (1991) New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. *Biotechnol Tech* 5:265-268. <https://doi.org/10.1007/BF02438660>.
- Spina F, Spini G, Poli A, Romagnolo A, Zanellati A, Bentivegna NG, El-Azhari N, Regnier T, Blieux A, Echairi A, Prigione V, Puglisi E, Varese GC (2018) Screening of anionic biosurfactants production among fungi and bacteria. *Chem Eng Trans* 64:493-498. <https://doi.org/10.3303/CET1864083>.
- Stubenrauch C (2001) Sugar surfactants—aggregation, interfacial, and adsorption phenomena. *Curr Opin Colloid Interface Sci* 6(2):160-170. [https://doi.org/10.1016/S1359-0294\(01\)00080-2](https://doi.org/10.1016/S1359-0294(01)00080-2).
- Teichmann B, Linne U, Hewald S, Marahiel MA, Bölker M (2007) A biosynthetic gene cluster for a secreted cellobiose lipid with antifungal activity from *Ustilago maydis*. *Mol Microbiol* 66(2):525-533. doi: 10.1111/j.1365-2958.2007.05941.x.
- Udoh T, Vinogradov J (2019) Experimental investigations of behavior of biosurfactants in brine solutions relevant to hydrocarbon reservoirs. *Colloids Interfaces* 3(1):24. <https://doi.org/10.3390/colloids3010024>.
- Vaz DA, Gudiña EJ, Alameda EJ, Rodrigues LR (2012) Performance of a biosurfactant produced by *Bacillus subtilis* strain isolated from crude oil samples as compared to commercial chemical surfactants. *Colloids Surf B: Biointerfaces* 89:167-174. doi: 10.1016/j.colsurfb.2011.09.009.
- Zadeh PH, Moghimi H, Hamed J (2017) Biosurfactant production by *Mucor circinelloides* on waste frying oil and possible uses in crude oil remediation. *Water Sci Technol* 76(7-8):1706-1714. doi: 10.2166/wst.2017.338.

Tables

Table 1. Scientific names and code numbers of the different fungal isolates.

Fungal isolate number*	Scientific name
NRCB1	<i>Aspergillus niger</i> Van Tieghem
NRCB2	<i>Penicillium cyaneum</i> (Bainier & Sartory) Biourge ex Thom
NRCB3	<i>Trichoderma sp.</i> (Persoon) Horz
NRCB4	<i>Aspergillus niger</i> Van Tieghem
NRCB5	<i>Gibberella sp.</i> Schwabe
NRCB6	<i>Penicillium frequentans</i> Westling
NRCB7	<i>Aspergillus niger</i> Van Tieghem
NRCB8	<i>Aspergillus flavus</i> Link
NRCB9	<i>Aspergillus niger</i> Van Tieghem
NRCB10	<i>Aspergillus wentii</i> Wehmer
NRCB11	<i>Fusarium sp.</i> Link
NRCB12	<i>Aspergillus versicolor</i> (Vuill.) Tiraboschi
NRCB13	<i>Aspergillus glaucus</i> Link
NRCB14	<i>Aspergillus ornatus</i> Raper, Fennell and Tresner
NRCB15	<i>Aspergillus glaucus</i> Link
NRCB16	<i>Aspergillus glaucus</i> Link
NRCB17	<i>Aspergillus terreus</i> Thom

*NRCB: National Research Centre Biosurfactant.

Table 2. Screening of tested fungi for biosurfactant production.

Fungal Isolate number*	ODA diameter (cm)	CTAB agar test	Foam formation	EI 24%
NRCB1	0.5	Very Faint bluish color	Good	54.28
NRCB2	2.5	Bluish color	Good	52.94
NRCB3	7.0	Very bluish color	Negative	37.5
NRCB4	3.5	Bluish color	Good	47.36
NRCB5	3.0	Bluish color	Good	44.11
NRCB6	1.0	Very faint bluish color	Negative	50.0
NRCB7	4.5	Bluish color	Negative	42.85
NRCB8	0.2	Very faint bluish color	Negative	61.76
NRCB9	4.5	Bluish color	Negative	45.45
NRCB10	2.5	Very bluish color	Good	60.0
NRCB11	8.0	Very bluish color	Negative	57.14
NRCB12	2.5	Bluish color	Good	51.11
NRCB13	5.5	Bluish color	Good	40.0
NRCB14	4.0	Bluish color	Excellent	52.63
NRCB15	2.5	Bluish color	Excellent	54.28
NRCB16	2.0	Very faint bluish color	Negative	50.0
NRCB17	1.0	Faint bluish color	Good	45.0

*NRCB: National Research Centre Biosurfactant.

Figures

Figure 1

Microscopic observations of isolated microorganisms.

Figure 2

Effect of different pH values on biosurfactants stability.

Figure 3

Effect of different temperature degrees on biosurfactants stability.

Figure 4

Effect of different salt concentrations on biosurfactants stability.

Figure 5

Effect of vegetable oils as carbon sources for production of biosurfactants.