FunTomP (2032)

FunTomP

FunctionalizedTomatoProducts

FunTomP General Assembly 23'
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Zied Zarai

Bioemulsifier derived from microorganisms with potential application in the food industry

FunTomP

University of Sfax
TUNISIA

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PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

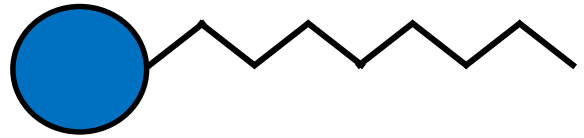
FunTomP

What are biosurfactants ?

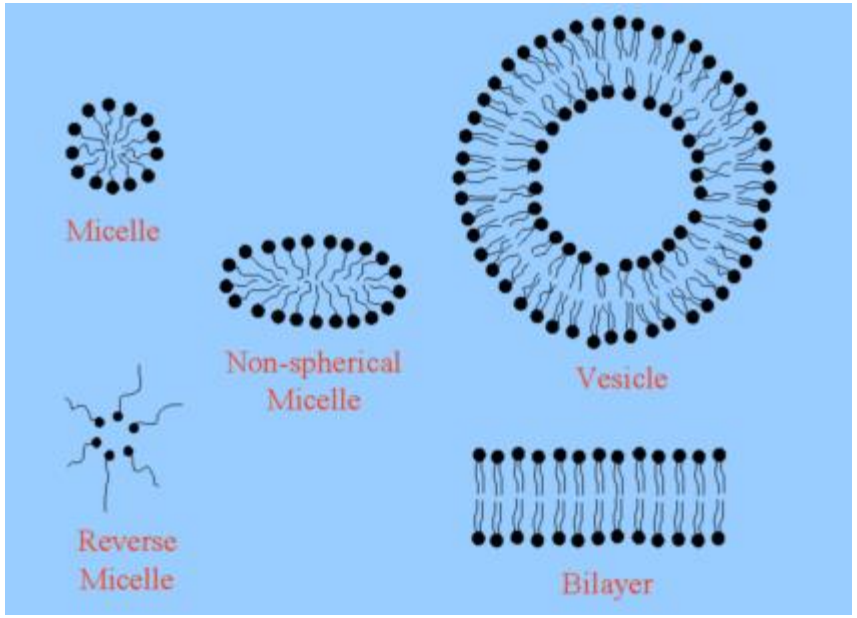
Biosurfactants are surface-active biomolecules produced by microorganisms.

Hydrophilic Moiety	Hydrophobic Moiety
• Acid • Peptide cations or anions • Mono-, di- or polysaccharides	• Unsaturated or saturated hydrocarbon chains • Fatty acids

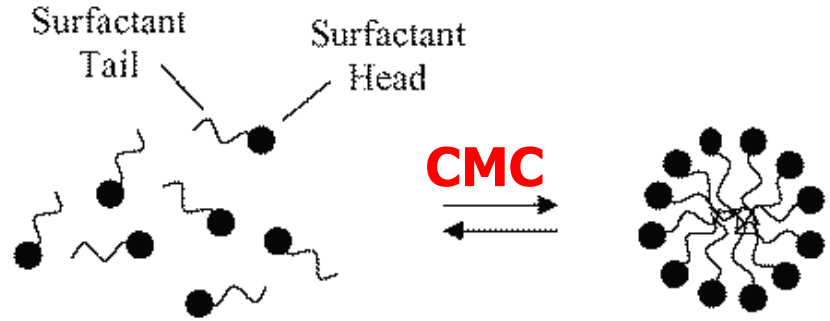
Biosurfactant



Hydrophilic Moiety (Surfactant head) **Hydrophobic Moiety** (Surfactant tail)



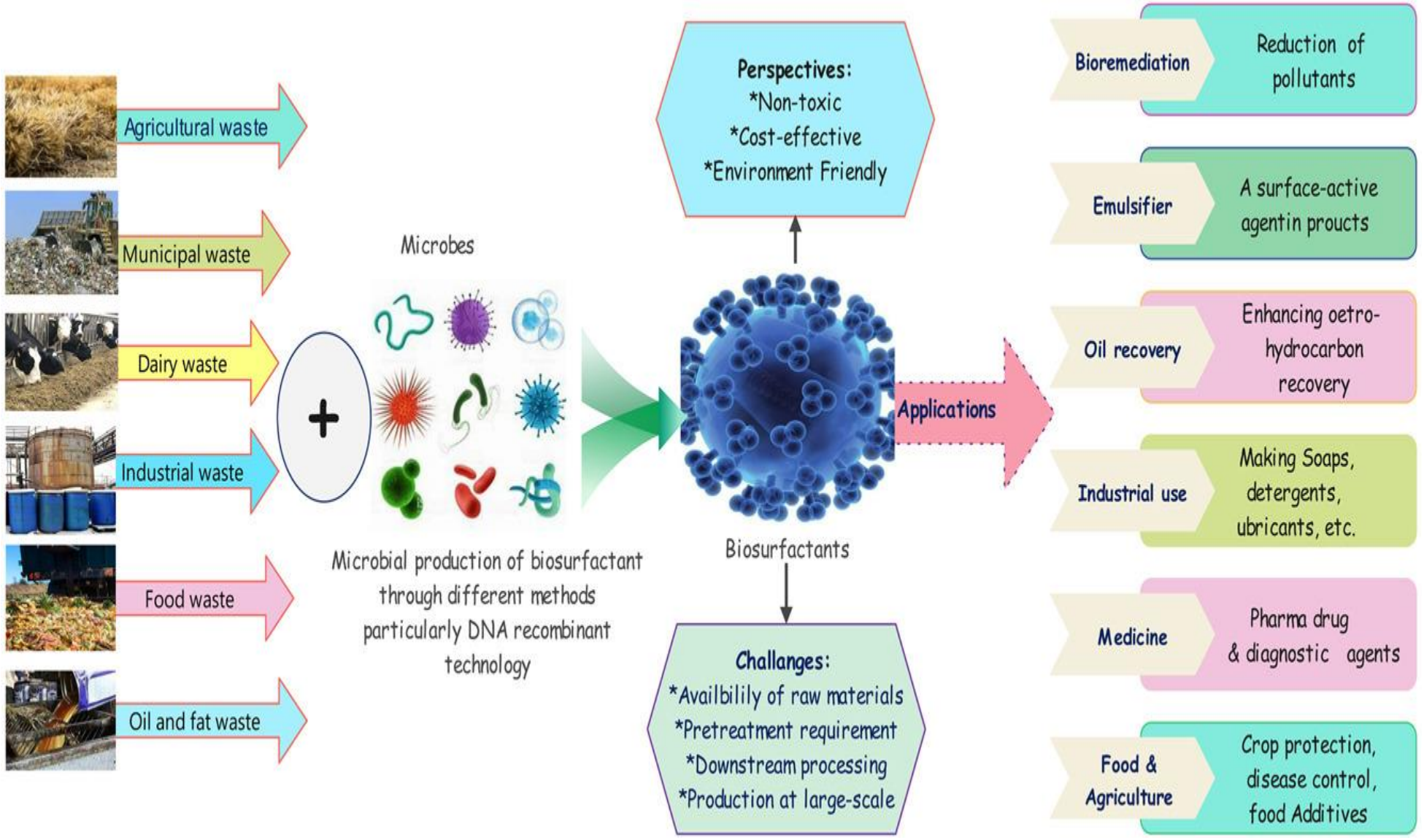
(http://people.umass.edu/mcclemen/Group.html)



(http://www.ualberta.ca/~csps/JPPS8(2)/C.Rangel-Yagui/solubilization.htm)

CMC = Critical Micelle Concentration

Applications of biosurfactants in various industries.



Teklit et al., 2021

The important properties of biosurfactants , explaining their advantages over the common chemical surfactants.

Excellent surface and interface activity

Biodegradability: Biosurfactants are environmental friendly compounds

Thermophilic: The temperature and pH tolerance capacity

Low toxicity

Emulsifying agents

The important properties of biosurfactants , explaining their advantages over the common chemical surfactants.

Biosurfactants are widely used as drug delivery and therapeutic agents in pharmaceutical industries.

In preparation of micro-emulsion systems and nano particles

As antioxidants

Biofilm disruption

As anti-adhesives

The Emulsion stability can be improved by using biosurfactants

Emulsification imparts texture, consistency and dispersion patterns to food products.

In food industries

An emulsifier stabilizes the emulsion by managing the mixture by aggregation of globules

Low molecular weight amphiphiles are used to provide stability to various food products as beverages, dressings, sauces etc.

The majority of biosurfactants made by bacteria can function in a variety of pH and temperature conditions, hence they are employed in place of traditional emulsifiers.

Microbial surfactants are also used to provide texture, to improve shelf life and to modify rheological properties of dough. They are also used to enhance texture, delay staling and stabilize fats in ice creams

Microbial biosurfactants are also utilized for making cookie formulations.

Application of biosurfactants in juice industry:

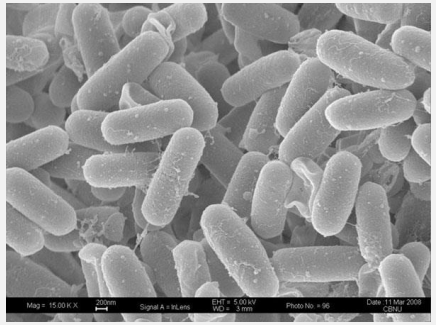
- As emulsifier/de-emulsifiers
- As antibacterial
- As preserver and stabilizer

Some biosurfactants show antimicrobial or antiviral properties making them potential candidates for future use in therapeutics substitute as emulsifier and active ingredient at the same time.

Objectives

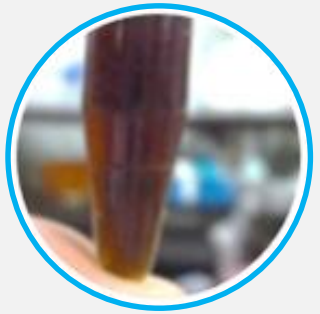
Low cost substrates???

Substrates



Microorganisms

Production & processes



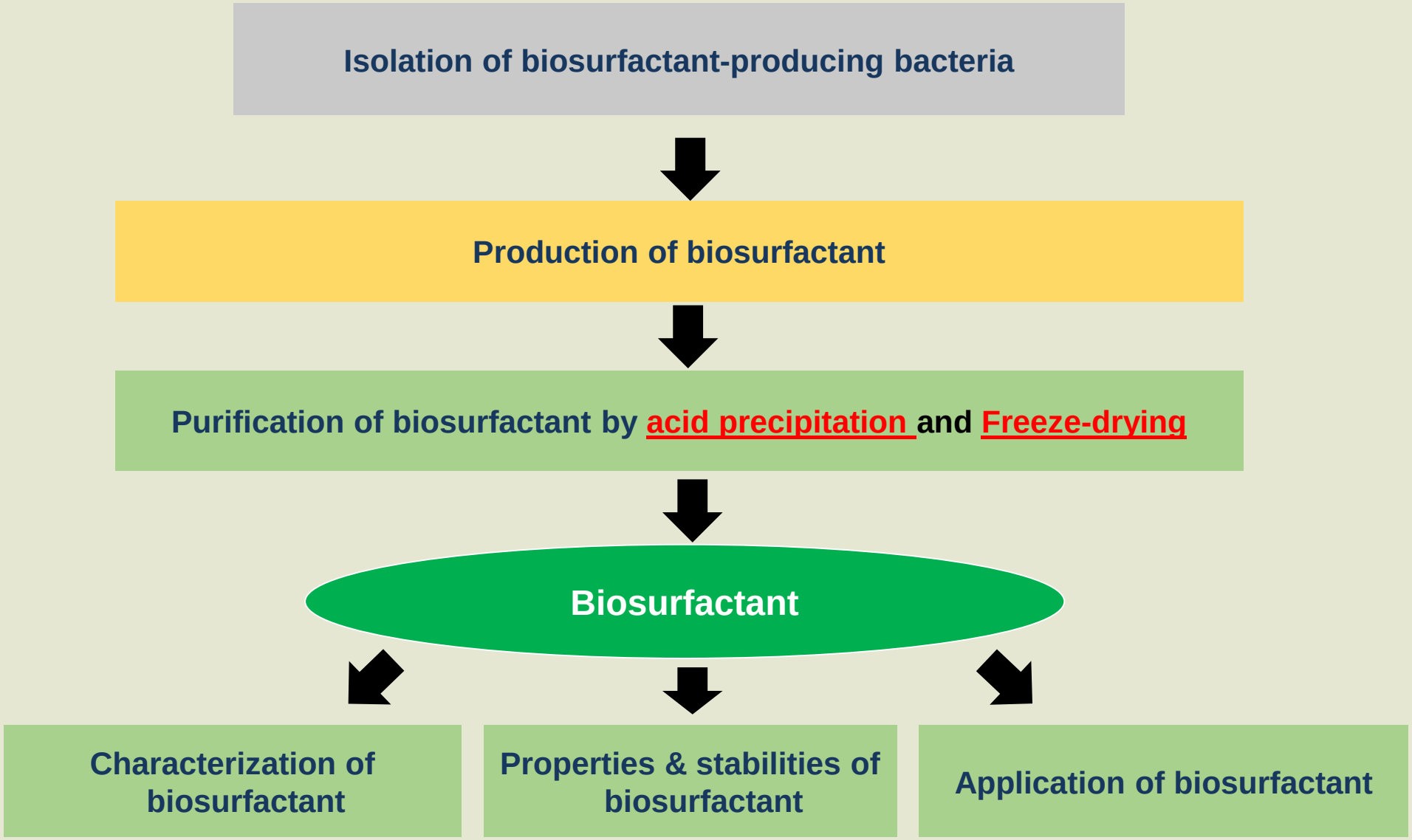
Biosurfactants

Biosurfactant producing Microorganisms???

Production & Processes ???

application???

Scope of Experiment



Biosurfactant producing microorganisms & Substrate selection

Isolation of microorganisms

Microbial screening

40 strains

Surface tension < 30 mN/m

10 strains

Bacillus sp.

Highest yield

culture conditions

5 cultures in liquid media were carried out in the presence of one of the **5 carbon sources (**starch, fructose, glucose, saccharose and lactose): 0.5; 1; 1.5 and 2% (w/v).

chemical additives

T° (25, 30, 35, 40 and 45 °C)

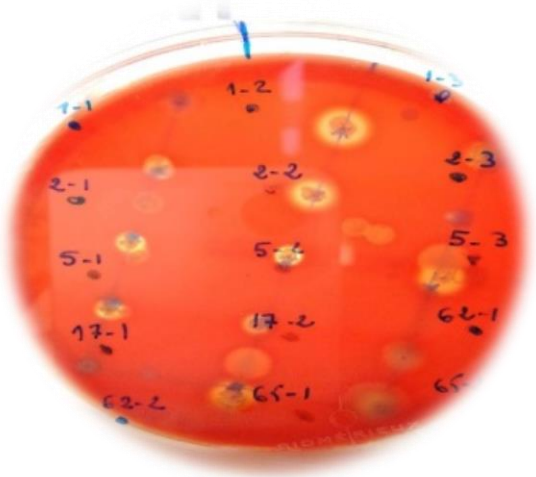
pH (5, 6, 7, 8 and 9)

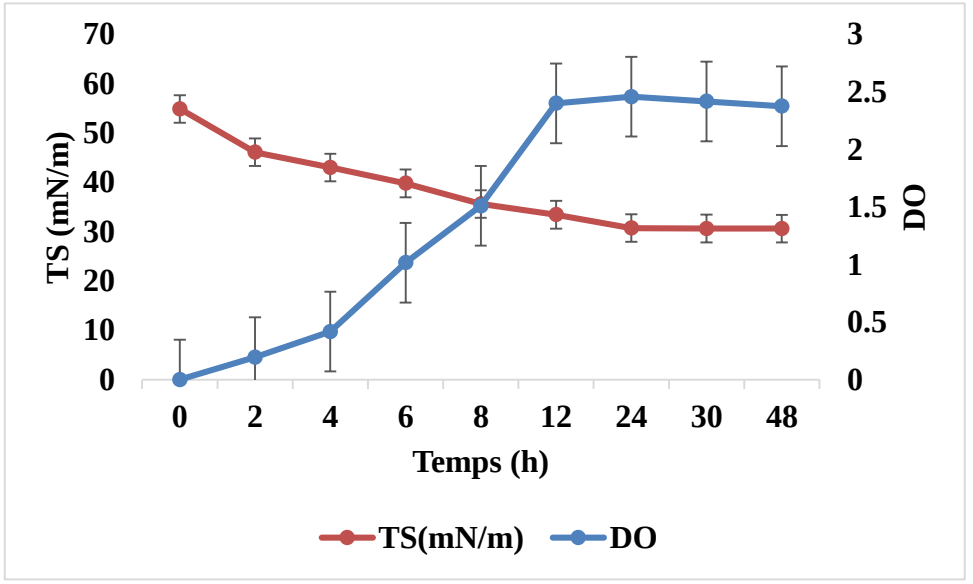
Time (24, 48, 72, 96 et 120 hours).

- **High yield**
- **Low cost**

Bacterial growth kinetics and surface tension monitoring

Bacillus thuringiensis
Bacillus thuringiensis
Bacillus thuringiensis
Bacillus cereus
Bacillus cereus
Bacillus cereus
Bacillus subtilis
Bacillus subtilis
Bacillus subtilis
Bacillus subtilis
Bacillus subtilis
Bacillus halotolerans
Bacillus halotolerans
Bacillus halotolerans
Bacillus halotolerans
Staphylococcus saprophyticus
Staphylococcus saprophyticus
Staphylococcus aureus
Bacillus pumilus
Bacillus velezensis
Bacillus megaterium





Effect of different conditions on growth and biosurfactant production

Production of biosurfactant

Peptone: 2%
Yeast extract 0.45%
Saccharose 2.5%,
K2HPO4 0.20%
MgSO4 0.06%
MnSO4 0.0006%
pH 7

Culture (48-72/30°C/100 rpm

↓

Acid precipitation of Biosurfacatant at pH 2, HCl (6N), 24h

↓

centrifuged at 10,000g for 20 min

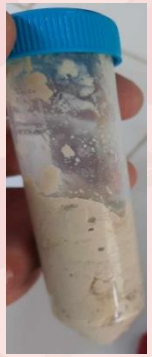
↓

Freeze-drying

↓

Biosurfactant

Biosurfactant extraction

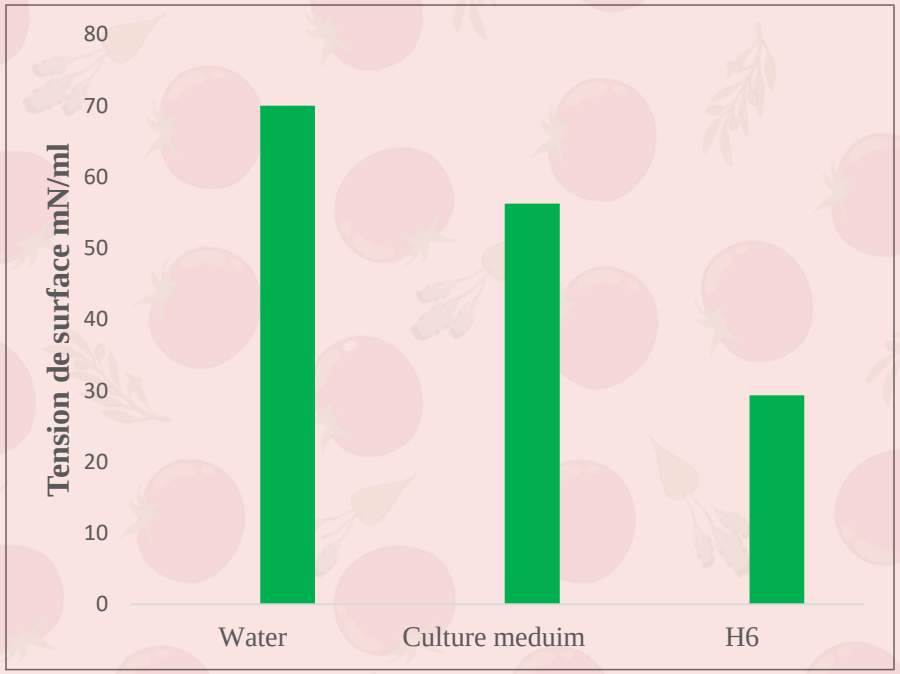


Powdered biosurfactant

Surface tension measurement (ST)

Surface tension

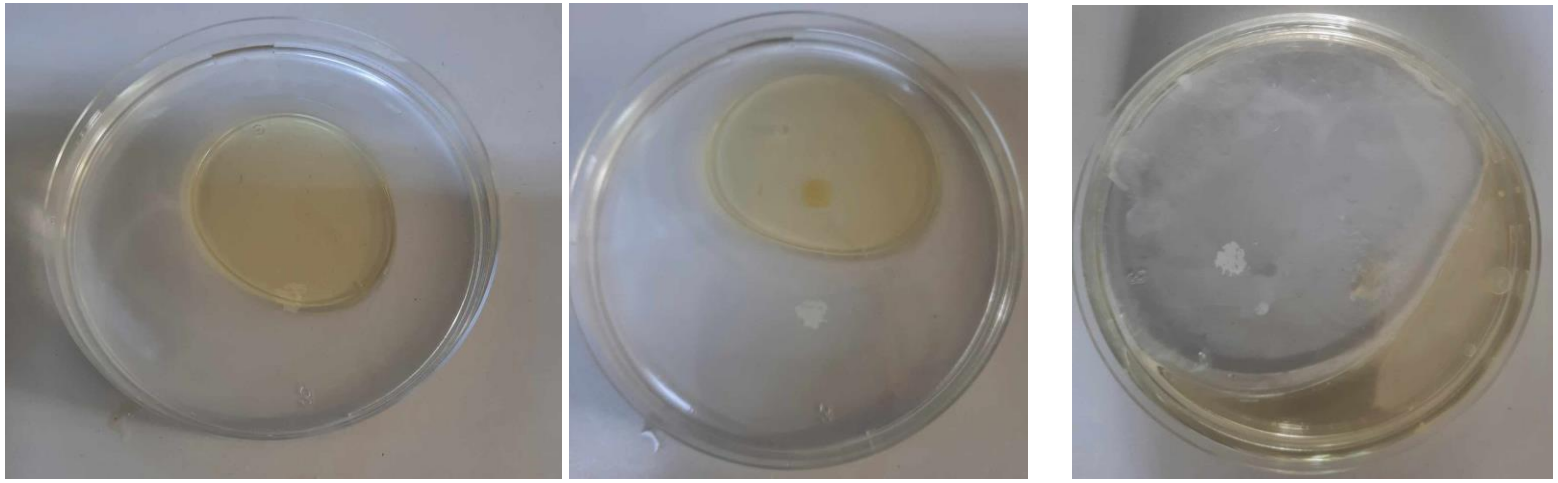
To determine the decrease in the surface tension due to biosurfactant production, uninoculated LB should be used as a control.



A significant decrease in surface tension is observed as we move from distilled water to non-inoculated culture medium, and finally to the supernatant of the inoculated H6 strain (29.37mN/m)

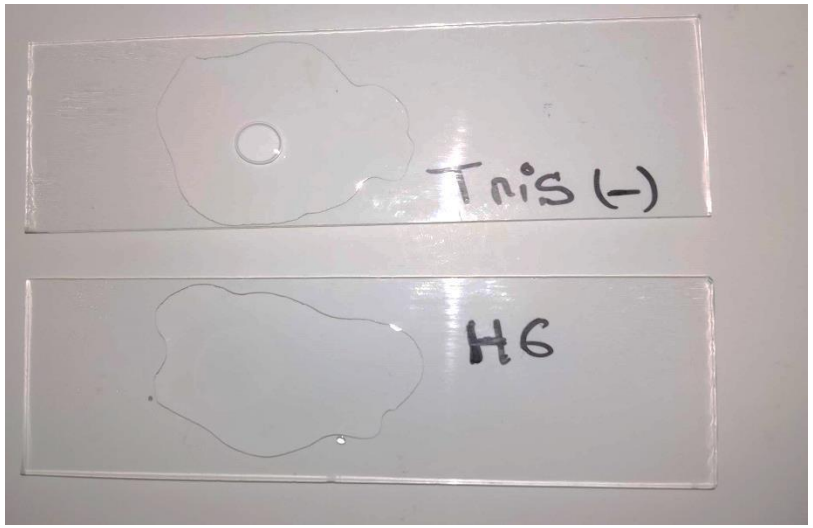
Physiochemical properties of biosurfactants

Oil spreading assay



A clearing zone is formed because of oil displacement, the sample positive for the presence of the biosurfactant.

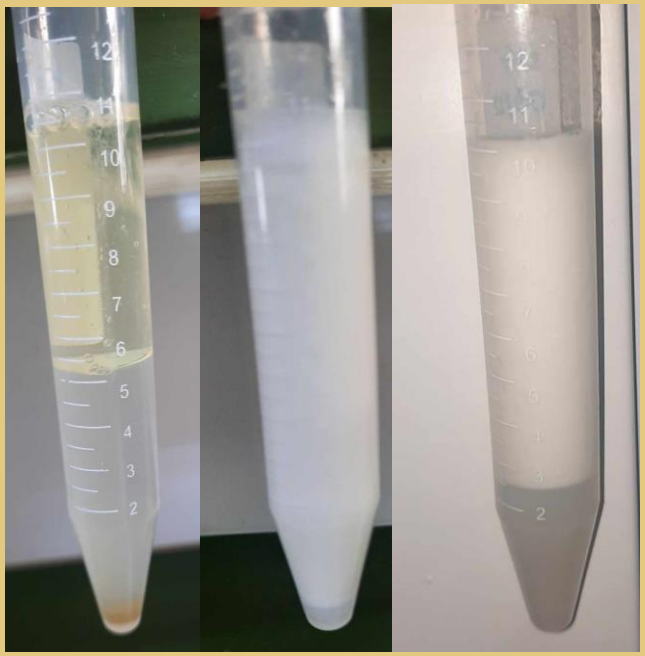
Drop collapse assay



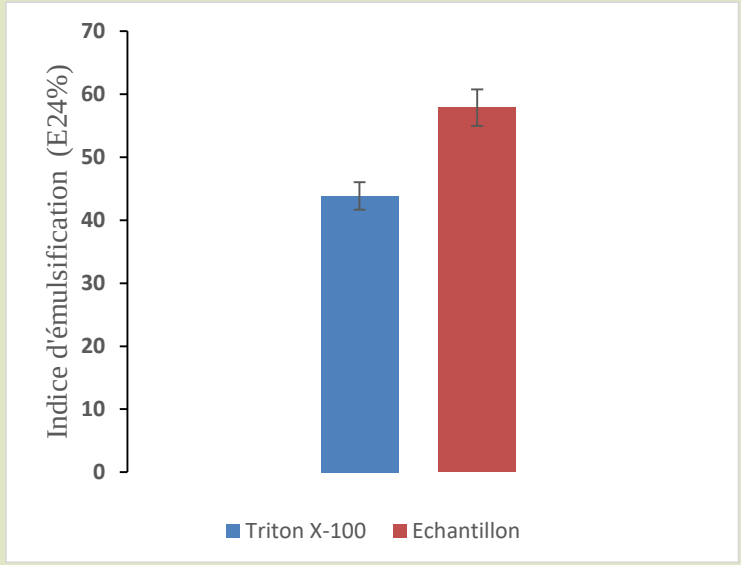
The drop collapses, the sample positive for the presence of biosurfactant.

Emulsification properties

Emulsion index assay (E24 assay)



After 24 h



An emulsion (oil in water) is observed after 24 h, the sample is likely to contain the biosurfactant.

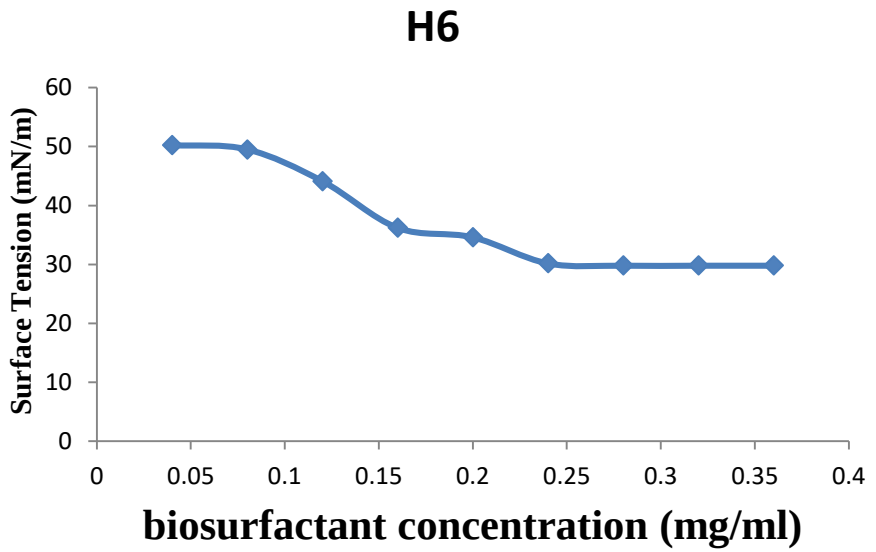
E24 = 66 %
C= 2 mg/ml

The biosurfactant from Bacillus subtilus H6 promoted intense foam formation, indicating considerable emulsifying capacity.

Critical micelle concentration

Another important parameter for evaluating the efficiency of a biosurfactant is the CMC, which is the minimum concentration of biosurfactant necessary for the maximum reduction in surface tension. In the present study, the biosurfactants isolated from the *Bacillus* strains exhibited excellent surfactant properties, yields and CMCs

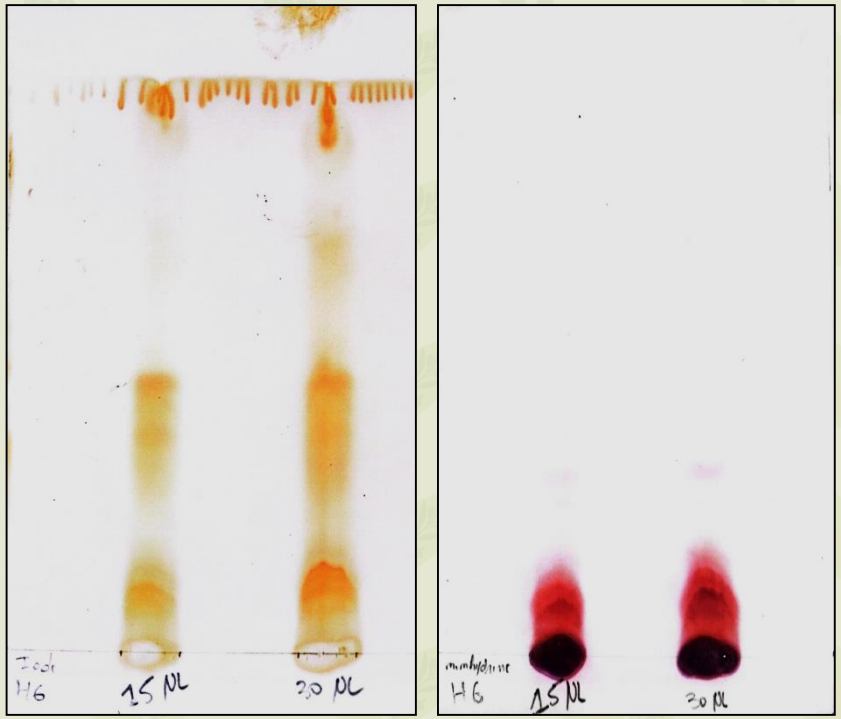
Microorganisms	Surface tension (mN/m)	CMC (mg/L)	Yield (g/L)
Bacillus Subtlis H6	29,79 ± 0.30	0,28	10.0 ± 0.5
Bacillus thirigensis 40	26.40 ± 0.23	0,20	2.0± 0.5



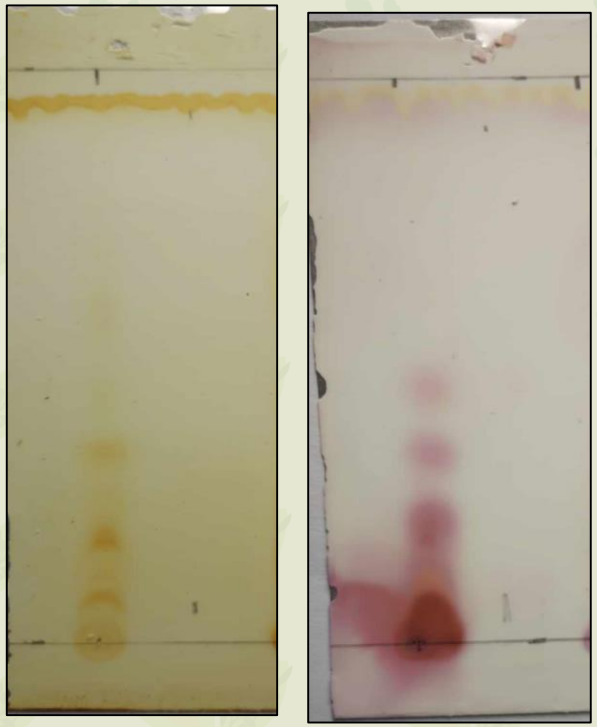
Detection of Biosurfactant by Thin Layer Chromatography

(TLC)

(a) (b)

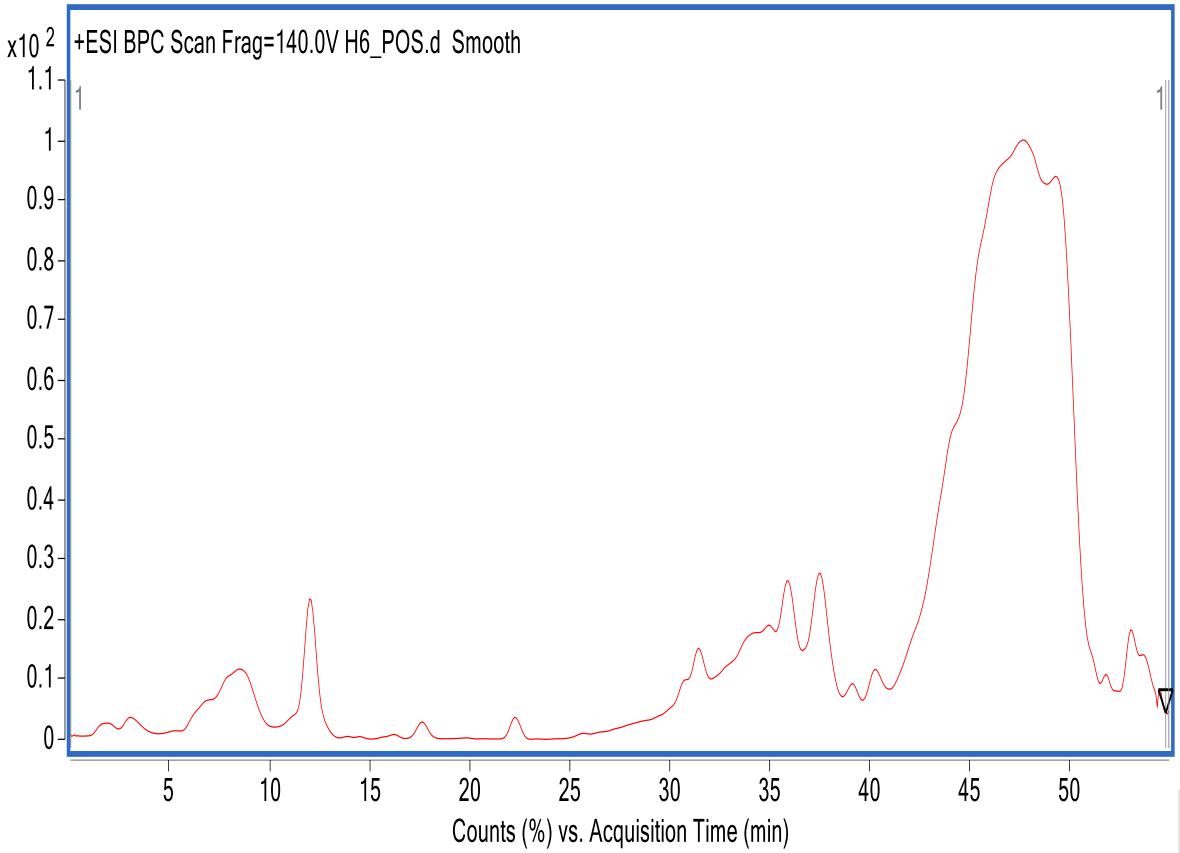


(a) (b)



(**a**) Plates stained with iodine vapor: Various spots developed on the TLC plate show that the extracted biosurfactants are a mixture of various compounds containing a lipid group.
(**b**) Plates stained with ninhydrin: A purple spots appeared when the plates were stained with ninhydrin. This represented presence of amino acids in the biosurfactant mixture and

LC–MS/MS analysis



Strain *Bacillus subtilis* H6

Pics	Tr (min)	Ion précurseur m/z	Poids moléculaire estimé (Da)	Identification
1	41.095	1506	1505	C17 Fengycin B
2	40.026	1492.8	1492.8	C18 Fenycin B
3	43.315	1491.8	1490.8	C16 Fenycin B
4	39.76	1478.8	1477.8	C17 Fengycin A
5	42.43	1477.8	1476.8	C15 Fengycin A
6	38.842	1464.8	1463.8	C14 FenycineB
7	38.57	1463.8	1462.8	C14 FengycineA
8	39.509	1093.5	1092.5	C16 Iturin A
9	37.35	1057.58	1056.5	C15 Iturin A
10	39.74	1022.7	1021.7	C14 Surfactin

10 compounds were identified using the mass spectrometry technique. The compounds identified belong to the 3 families of best-known lipopeptides which are **fengycin**, **iturin** and **surfactin**.

food utilization :

Biosurfactants show several properties which could be useful in many fields of food industry;

The combination of particular characteristics such as emulsifying and antimicrobial activities presented by biosurfactants suggests potential application as multipurpose ingredients or additives.

Scant information regarding toxicity, combined with high production costs seems to be the major cause for the limited uses of biosurfactants in food area.

Biosurfactants obtained from Generally Regarded As Safe microorganisms like lactobacilli and the toxicological aspects of new biosurfactants should be emphasized in order to certify the safe of these compounds for food utilization.

planned work

- The biosurfactants can be characterized using various spectroscopic techniques.
- The purification and chemical analysis of biosurfactants, by using reverse phase high performance liquid chromatography (RP-HPLC) and liquid chromatography-mass spectrometry (LC-MS) techniques.
- Ultra performance liquid chromatography-tandem mass spectrometer (UPLC-MS) method to identify the structures of novel biosurfactants produced.
- The molecular structure of biosurfactants will be further identified by gas chromatography-mass spectrometry (GC–MS) analysis
- Chemical nature of the biosurfactant
- Nuclear magnetic resonance of biosurfactant
- Evaluation of the cytotoxic activity of a biosurfactant derived from Bacillus strains, This test will be created by utilising cell culture (mouse macrophage, fibroblast cells, …)
- Antiradical activity of biosurfactant
- Antimicrobial activity of biosurfactant
- Cytotoxicity activity of biosurfactant: Effect of biosurfactant on cell proliferation

Thank you for your attention

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