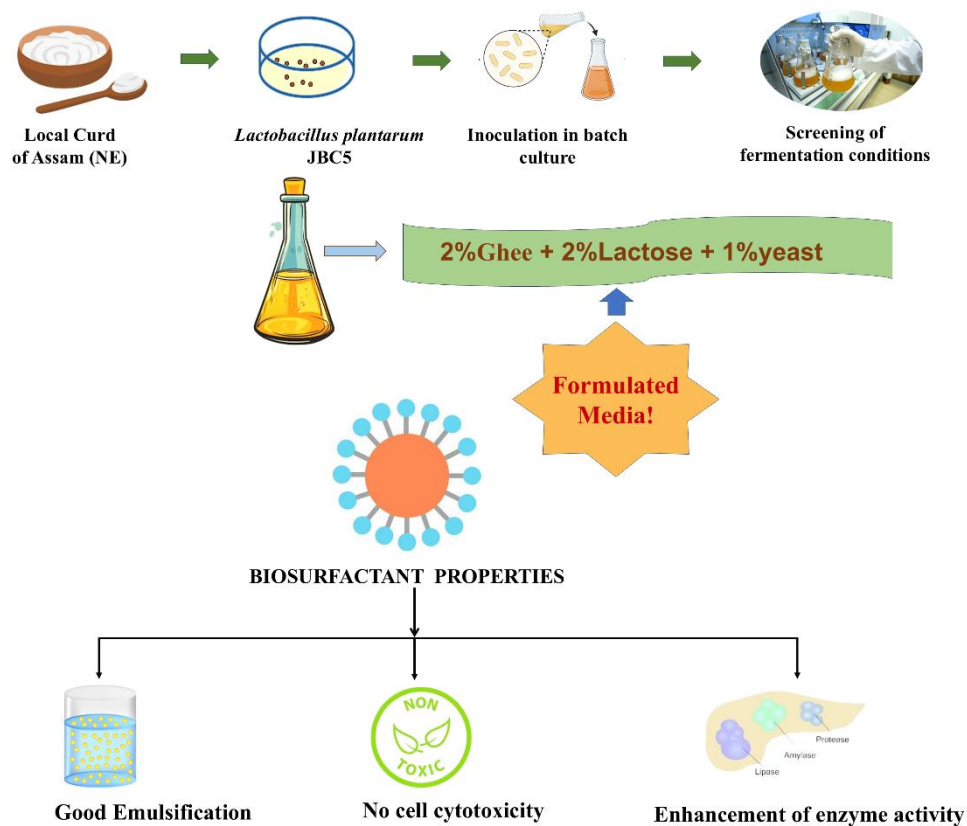


A Novel Media Composition for the Efficient Production of Biosurfactant with Superior Properties from *Lactobacillus Plantarum* JBC5 and Use Thereof



Brief description of the technology

The present invention is to provide a biosurfactant from a novel strain identified as *Lactobacillus plantarum* JBC5 that can utilize Ghee as a medium for biosurfactant production. The present invention is to provide a method for efficient production of biosurfactants with superior properties such as good emulsification index and enhancement of enzymatic activity of amylase, pepsin and lipase.

Details about the technology

Objective 1: To evaluate the effect of lactose, yeast extract, and Ghee to produce biosurfactant by LPJBC5

For this, the following media compositions were prepared:

- (i) M1, 2% ghee without any external components,
- (ii) M2, 2% Lactose without any external components,
- (iii) M3, 1% yeast without any external components,
- (iv) M4, 2% Ghee, 2% lactose, and 1% yeast extract.

Results:

Table 1: Effect of different media on growth and biosurfactant production by LPJBC5 after 48 hours of inoculation. The surface tension reduction in M4 was statistically significant, with a $**P < 0.05$. Values are mean $\pm$ SD of triplicate determinations.

Media Compositions	Surface Tension at 48 hours	Δ Surface Tension (mN/m) (At 48 hours)	Yield (g/L)
M1	36.2	8.23	1.3
M2	56.7	7.71	0.36
M3	50.9	7.87	0.86
M4	22.7	10.87	2.3

Objective 2: To determine the properties of partially purified biosurfactant produced by LPJBC5

2.1 Critical micelle concentration (CMC)

The CMC value was then determined from the breaking point of the surface tension versus its log of bulk concentration curve. 200 mg/L of the partially purified biosurfactant was prepared in distilled water. It was then serially diluted from 10mg/L to 200mg/L, and the prepared dilutions' surface tension was measured with the tensiometer.

Results: The partially purified biosurfactant obtained from M4 displayed a critical micelle concentration of 100mg/L in water. Whereas M1, M2 and M3 demonstrated CMC values of 150mg/L, 170mg/L, and 170mg/L, respectively.

2.2 Emulsification Index (E_{24})

Emulsification of partially purified biosurfactant obtained from M4 media was evaluated against four oils: mustard, vegetable, olive, and coconut.

Results: The emulsification index of a partially purified biosurfactant obtained from M4 media against mustard, vegetable, olive, and coconut oil was 59.3%, 45.75%, 34.2%, and 43.4%, respectively. As the emulsification index was illustrated higher with mustard oil, the emulsification index of partially purified biosurfactant obtained from all the tested media was compared. E24 of M1, M2, and M3 biosurfactants was found to be 26.35%, 24.5%, and 53.85%, significantly lower than the biosurfactant obtained from M4.

2.3 *In-vitro* cell cytotoxicity

The partially purified biosurfactant was evaluated against mammalian cell line (L9-29 fibroblast) to confirm their toxicity or non-toxicity and proceed for further clinical studies.

Results: Treatment of the biosurfactant obtained from LPJBC5 grown in M4 media showed no toxic effect on the mouse fibroblast L9-29 cell line except for the 0.5mg/mL concentration incubated for 48 hours, where the cell viability slightly decreased. This result demonstrates the potency of biosurfactants in meeting human and animal safety regulations.

Objective 3: Elucidation of application of partially purified biosurfactant in enhancing the digestive enzymes activity

3.1 α -amylase activity

Amylolytic activity was determined by using DNSA method. Biosurfactant was added to the reaction mixture, in amount 1:5 and 1:10 ratio to the commercial amylase enzyme.

Results: Enzyme activity of commercial α - amylase enzyme towards starch were determined to be 1141.54 U/mL (mean $\pm$ S.D., n = 3). On incubating the reaction mixture with partially purified biosurfactant in 1:5 ratio and 1:10 ratio, the enzyme activity has significantly increased and was determined to be 1334.27 and 1579.22U/mL.

3.2 Protease (papain) activity

The protease activity was evaluated by 12.5% SDS-PAGE. The assay mixture contained 50 μ l of soluble and filtered egg albumin (1 %, w/v) and 0.25 μ g of purified commercial pepsin solution at pH 2 and 37°C for 5 mins.

Results: In SDS PAGE, by quantifying and comparing the intensity of bands, before and after hydrolysis of 1% egg albumin, it is revealed that the addition of partially purified biosurfactant to commercial pepsin enzyme has significantly hydrolysed more of the substrate as compared to that of only pepsin.

3.3 Lipase activity

100 μ g partially purified biosurfactant was added to 2.5 μ g of purified lipase enzyme to check the lipase activity by titrimetric method using olive oil as substrate.

Results: The enzyme activity of lipase on olive oil as substrate is determined to be 4737.18 U/mL and on addition of partially purified biosurfactant to it the activity has enhanced to 10,303.02 U/mL.

Applicability of the Technology

1. Utilisation of Ghee as a substrate by *Lactobacillus plantarum* JBC5 for efficient biosurfactant production with higher yield, improved surface tension reduction, and emulsification index against vegetable oils. Since, the media composition is majorly food based also the bacteria is promising probiotic bacterial strain, the biosurfactant can be used for many cosmetic, pharmaceutical and food-based applications.
2. The enhancement of various enzymatic activities aligns with their potential applications in pharmaceuticals.

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