Molecular Characterization of Biosurfactant Producing *Lactobacillus* strains and their Physicochemical Properties

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Abstract: Biosurfactants or surface-active compounds are biodegradable, non-toxic and ecofriendly compounds released by microorganisms. Biosurfactants are amphiphilic compounds cause surface tension reduction both aqueous solutions and hydrocarbon mixtures. The main purpose of this work was to characterize biosurfactant produced by *Lactobacillus* strains. Identification using 16s rDNA identified the isolates as *L. acidophilus* Effect different parameters (temperature, PH and Salinity) were studied to evaluate the stability of biosurfactant after treatment. In addition, critical micelle concentration of biosurfactant, emulsification index and viscosity reduction of palm and engine oils have been studied. The results revealed that, the biosurfactant from *L. acidophilus* and *L. pentosus* maintains its emulsifications activities unaffected in the wide range of parameter's study except slightly decreasing in emulsification index values at salinity 15%. The maximum reduction in surface tension was 18.05 mN/m with minimum concentration of critical micelle concentration of 7.5 mg/ml and high decrease of palm and engine oil viscosity of 110.1 and 165.3% respectively. This study concluded that, the emulsification activity, the surface activity and the stability to heat treatment, different PH and salinity of biosurfactant of *Lactobacillus* strains revealed the application of the biosurfactant in food, pharmaceutical, cosmetics industries and oilrecovery.

Keyword: Biosurfactant, Emulsification index, Surface tension, critical micelle concentration, *L. acidophilus*, *L. pentosus*

Introduction

The biosurfactants have several advantages over chemical surfactants including lower toxicity and higher biodegradability, better environmental compatibility, high selectivity and effectiveness at extreme temperatures, salinities or pH [1]. The disadvantages of the microbial surfactant or biosurfactant, comparing with synthetic surfactant are low yield and high production cost [2]. So, the method to improve the biosurfactant production with low cost is of interest.

Conventional methods include acid precipitation, solvent extraction, centrifugation and

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ammonium sulfate precipitation, recovery. In recent years, a few unconventional recovery methods have also been described, such as foam fractionation, ultrafiltration and ion exchanged chromatography. Often, a single technique is not enough for biosurfactants recovery and purification. For instance, extraction of low molecular weight biosurfactants normally involves an initial precipitation step, followed by extraction with different organic solvents according to the hydrophobicity and hydrophilic-lipophilic balance value of the compounds. On the other hand, high molecular weight biosurfactants, normally they are extracted by ammonium sulfate precipitation and then purified by dialysis[3].

The stability of biosurfactants to extreme conditions of pH, temperature and salinity make them desirable molecules for applications where these conditions prevail. Several studies showed that many biosurfactants are not affected by extreme environmental conditions. The lichenysin produced by *B. licheniformis* JF-2 is an example of a biosurfactant with good stability, not being affected by temperature up to 50C, pH 4.5 – 9.0, and by NaCl concentrations up to 50 g/l [4]. Therefore, the purpose of this study was to isolate the biosurfactant produced by *L. acidophilus* and *L. pentosus*and to investigate their physical properties.

Materials and Methods

Identification of LAB isolates

The LAB isolates that show biosurfactant activity in all the tests were identified by API 50 CH (API system, BioMérieux, France). The result was analyzed with API WEB (BioMerieux)[4].

Promising LAB isolates were further identified by 16 s rDNA using primer 16S forward: (5-AGAGTTTGATCCTGGCTC-3) and 16S reverse: (5-CGGGAACGTATTCAC-CG-3) [4].

Biosurfactant stability

In order to evaluate the stability of biosurfactant, the effects of temperature, pH and NaCl on the activity of the biosurfactant in the optimized conditions were assessed[4].

Physical properties of biosurfactant produced by *L. acidophilus*

The biosurfactant was extracted from *L. acidophilus* culture to evaluate their Physical properties. The acid precipitation for biosurfactants which become insoluble at low pH values were used to extract the biosurfactants from the culture medium[5].

Critical micelle concentration (CMC)

The CMC was evaluated as described by [5].

Determination of viscosity reduction

Viscosity reduction ability of two oils (palm oil and engine oil) by biosurfactant was tested. Viscosity was recorded by Ostwald's standard Viscometer at room temperature (28°C)[5].

Statistical Analysis

Results are presented as the mean ± standard deviation and all measurements were done in triplicate. Statistically significant differences of the conditions tested in the different assays were evaluated by a two-way ANOVA ($P < 0.05$) applying the Tukey test. Statistical

analyses were performed using SPSS software and the significant difference was considered if P <0.05.

Results

Identification of LAB Isolates

The two LABs isolates that showed biosurfactant activity were identified by phenotypic and genotypic identification

Phenotypic Identification

Results from API 50 CH test kits and API web identified the two LAB isolates (Fm1 and Y1) as

*L. acidophilus and L. pentousus*with similarity 99.2 and 82.9%, respectively.

Genotypic Identification

Genotype identification of DNA using universal primer showed clear bands of isolates (Figure 1) with approximate molecular weight 1500 bp and similarity 99.9% for (LAB-Fm1) *L. acidophilus* and 100% for (LAB-Y1) *L. pentosus*. The sequences of these isolates were determined and deposited in the Gene Bank database under accession number GU138532.1 and GU451063.1, respectively.

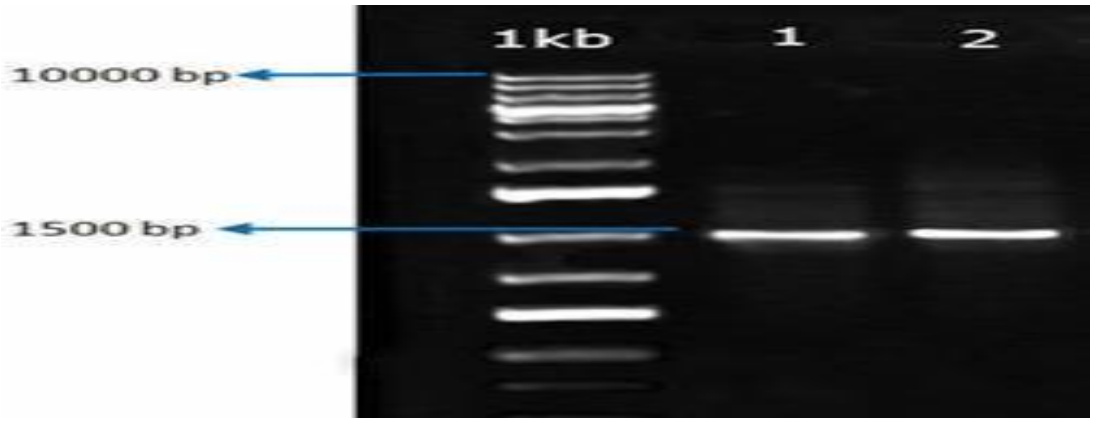

Figure 1: The DNA bands of LABs on the 1.5 % agarose gel using primers 16S.S, Lane 1: Fm1 and 2. Y

BiosurfactantStability Effect of Temperature

The stability of the biosurfactant was tested over a wide range of temperature from 25Cº to 100 Cº comparison with 1 % SDS which showed a significant increase (P<0.05) in the surface tension and a significant loss (P<0.05) of EI24. The biosurfactant produced by L. acidophilus was shown to be thermo stable. The *EI 24* was stable at the temperature used (*EI 24* = 100 %) in comparison with the synthetic surfactant 1 % SDS, which exhibited a significant loss (P<0.05) of *EI 24* beginning at 70 Cº (*EI 24*= 43.2 %) (Figure 2, 3).

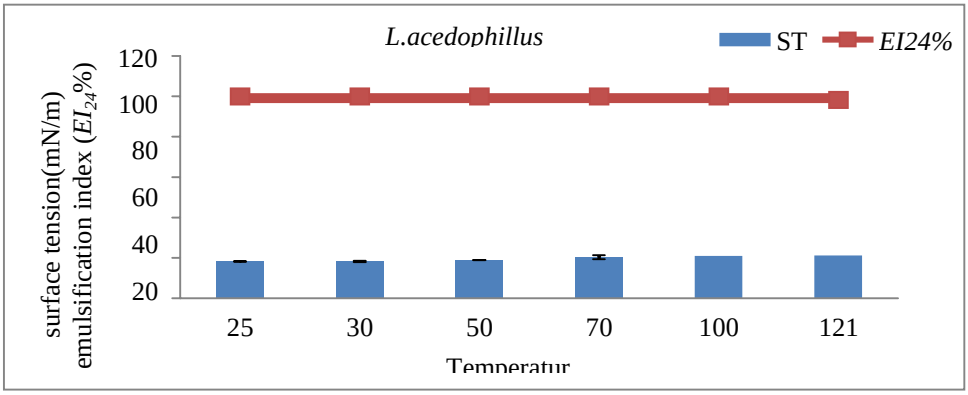

Figure 2: Effect of temperature on *L. acidophilus* biosurfactant stability

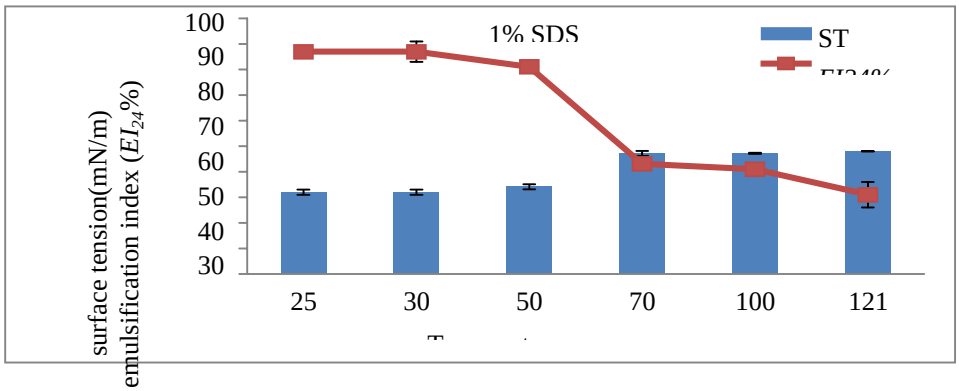

Figure 3: Effect of Temperature on 1 % SDS

Effect of pH

The maximum reduction in surface tension (18.6 mN/m) and the higher *EI24* (100 %) were obtained at pH 7. In comparison with the synthetic surfactant 1 % SDS, there was quite increase in the surface tension from 31 to 48 mN/m and a significant loss ($P<0.05$) of *EI24* started from pH 7(87%) Figure 4, 5).

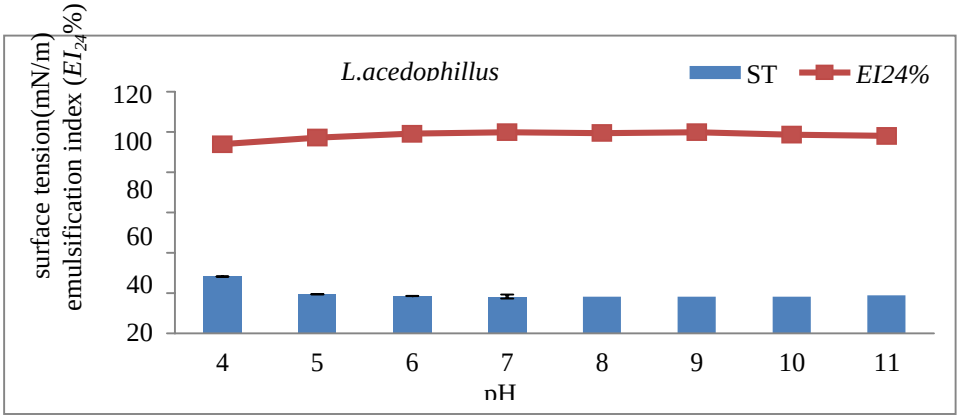

Figure 4: Effect of pH on *L. acidophilus* biosurfactant stability

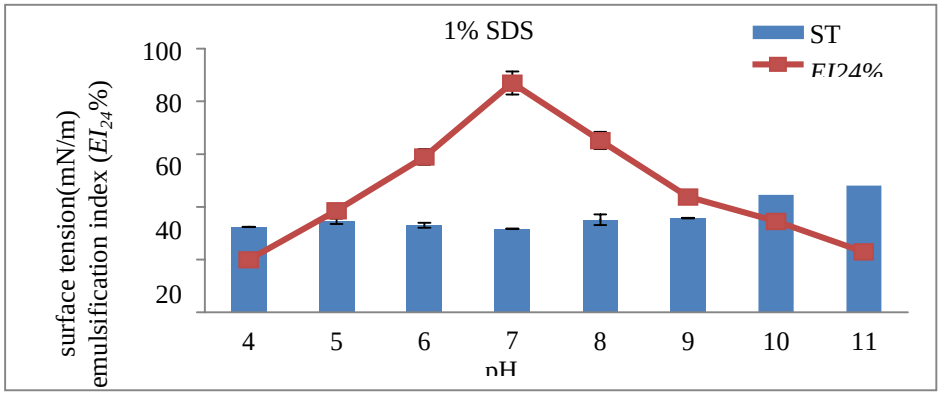

Figure 5: Effect of pH on 1 % SDS Stability

Effect of Salinity

The lowest surface tension about 18 mN/m and the highest *EI24* about100 % at 1-8 % NaCl, by *L. acidophilus*. In compare with 1 % SDS, there was a significant increase ($P<0.05$) in the surface tension at concentration 1 to 10 % from 32 to 70 mN/m and a significant loss ($P<0.05$) of *EI24* from 86.7 to 7 % at the same pH range (Figure 6, 7).

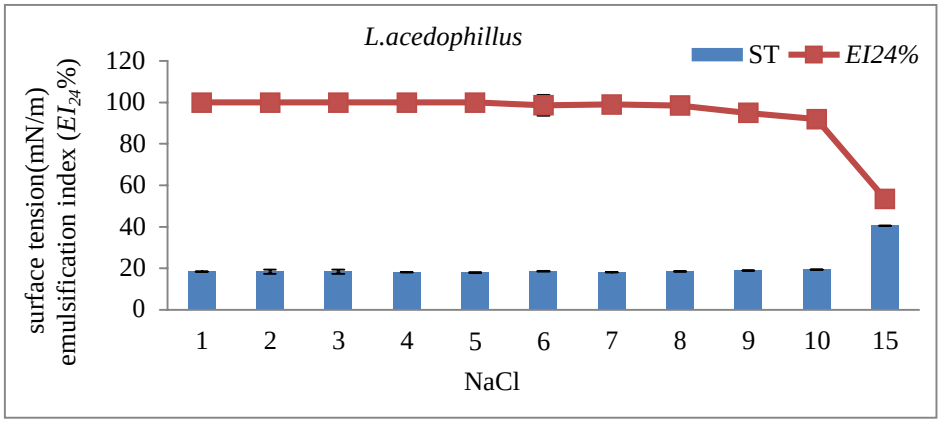

Figure 6: Effect of salinity on *L. acidophilus* biosurfactant stability

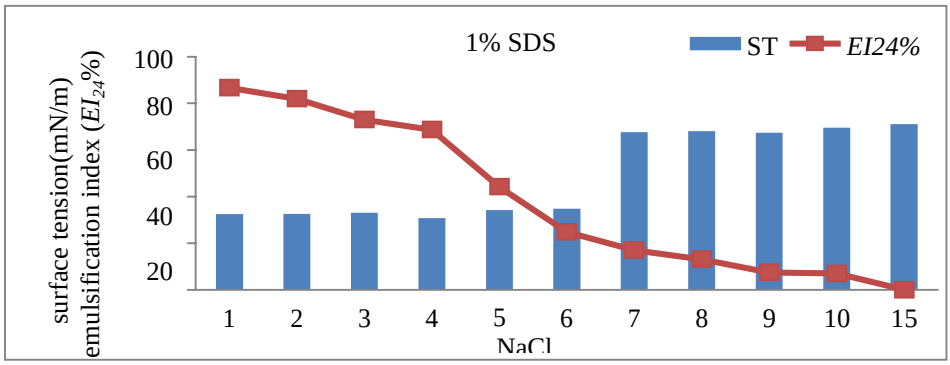

Figure 7: Effect of Salinity on 1 % SDS Stability

Physical properties of biosurfactant produced by *l. acidophilus*

In order to determine the activity of biosurfactant produced by *L. acidophilus* the biosurfactant was extracted by acid precipitation.

Extraction of the Crude Biosurfactant

Result of acid precipitation showed the masses of biosurfactants produced by the *Lactobacillus* strains were 1.93 g/L.

CMC of Crude Biosurfactant

The biosurfactant showed the lowest surface tension at 18.05 mN/m with CMC approximately 7.5mg/ml by *L. acidophilus*.

Table1: CMC of *Lactobacillus* biosurfactant

Isolates	ST (mN/m)	CMC (mg/ml)
L. acidophilus	18.0 ± 0.0^{a}	7.5 ± 0.2^{a}

Different letters in the same column represents significant differences at $p<0.05$

Emulsification Index of Crude biosurfactant(*EI24*)

It was observed that the biosurfactant from *L. acidophilus* were able to maintain the emulsion stability after 24 h with$\%EI_{24}$ of 100%for palm oil and 83% for engine oil respectively (Figure 8).

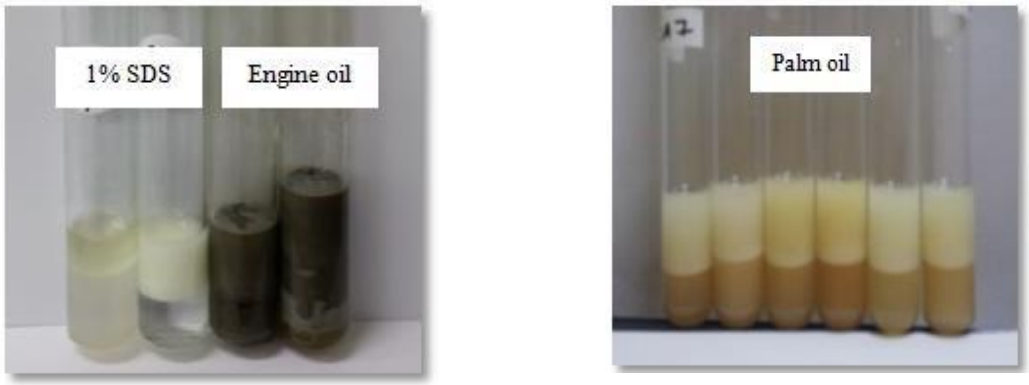

Figure 8: The emulsion layer of biosurfactant mixed with oil at24h

Determination of Viscosity Reduction

The biosurfactantfrom*L. acedophillu*caused reduction in viscosity of palm oil and engine oil from 402 to 110.1cP and from 736.0 to 165.3 cPespectively (Table 2). In comparison with the viscosity of the control (402.0 and 736.0 cP), there was a significant reduction ($P<0.05$) in the viscosity of palm and engine oil respectively caused by biosurfactant.

Table 2: Reduction of oil viscosity caused by biosurfactant

Viscosity	*L. acidophilus*
palm oil (cP)	402.0 ± 0.5^{bA}
reduction (cP)	110.1 ± 0.0^{aA}
engine oil (cP)	$736.0\ \pm0.7^{bA}$
reduction (cP)	$165.3\ \pm0.2^{aA}$

Different letters in the same column (lower case) and in the same row (upper case) represents significant differences at $p<0.05$

Discussion

The biosurfactant produced by L. acidophilus was shown to be thermo stable. The results obtainedby[12] concerning heat treatments at 25, 50, 75 and 100ºC for 15 min, show that temperature didn't have any significant effect ($p<0.05$) on the emulsifying and surface activities of biosurfactants extracted from *Lactobacillus* stains.

The biosurfactant produced by *L. acidophilus* showed a high stability not being affected by pH up to 11, except slightly decrease in the surface activity at pH 4. This could be due to the better stability of fatty acid surfactant micelles in the presence of NaOH and the precipitation of secondary metabolites at higher pH values [15].

This work showed that there was no significant effect ($P<0.05$) of NaCl at all concentrations tested on the surface tension reduction and the EI_{24} of biosurfactants except the increase in surface tension from 19 to 41 mN/m and the decrease in the EI_{24} from 92 to 53 % with an increased concentration of NaCl up to 15 % (w/v). These results were in agreement with those reported by [16]*)* which showed that the stability of biosurfactants at NaCl concentration below 15 % could be due to the presence of phosphates groups in the biosurfactants which can prevent the relegate of proteins.

The amount of biosurfactant in the this study was higher than those obtained by [12] which showed the masses of biosurfactants produced by the *Lactobacillus* strains were varying from

0.710 to 1.20 g/L. This difference could be explained by the fact that in the present study MRS was used and supplemented with yeast extract and peptone which according to [14] were essential component for bacterial growth and biosurfactant production by *Lactobacillus* spp.

The CMC defined as the concentration of an amphiphilic compound in solution at which the formation of micelles is initiated. Results of CMC were higher than those reported by [14] with *L. paracasei:* CMC at 2.5 mg/mL.

Most data published in the literature reported that bacteria with high potential of emulsifying activity of 50 to 60% are promising microbial candidates for biosurfactant production [26]. The biosurfactant from *L. acidophilus* were able to maintain the emulsion stability after 24 h with $\%EI_{24}$ of 100% for palm oil and 83% for engine oil respectively (Figure 11).

The biosurfactantfrom*L. acedophillu*caused reduction in viscosity of palm oil and engine oil which was higher than observed by [29]. They reported that two mechanisms that increase and decrease the viscosity using hydrophobic substrates and the new biosurfactant is a candidate for mediated enhanced oil recovery[30].

This study reported that biosurfactant are stable and not affected by extreme environmental conditions and it has the ability to reduce the viscosity of oils (palm oil and engine oil) which facilitates the mobility of oils and ease of transportation.

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