

# Purification and Characterization of Surlactin Produced by *Lactobacillus Acidophilus*

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## *Abstract*

**Objectives:** The aim of research is purification and characterization of surlactin produced by *Lactobacillus acidophilus* isolated from vaginal swabs of healthy women.

**Materials and Methods:** The stationary growth phase for *Lactobacillus acidophilus* isolated from vaginal swabs of healthy women from (Kamal Al-Samaraei and Al-Alweia Maternity Hospitals) in Baghdad was adopted on production of surlactin, after separation of bacterial cells from the growth media (Man-Rogosa Sharpe, MRS), incubated in phosphate buffer (0.1 molar) for four hours at temperature of 25 °C to produce Surlactin. It was purified by using paper filtration, dialyses and concentration using sucrose. Purified Surlactin is characterized by the best pH, incubation temperature and time, inoculation volume per 10 milliliter of the media, storage condition and total molecular weight obtained by gel filtration. Also it studies the importance of magnesium and manganese sulfate for the bacterial growth and for surlactin production.

**Results:** Results of purified and characterized surlactin showed that the best pH was at 6.0, while the incubation temperature and time were 25°C for four hours with inoculation volume of 600 µ liter (6×10<sup>7</sup> cell / milliliter) per 10 milliliter of the media. It is active at a temperature up to 75°C for an hour and it keeps its activity for six months when storage it at -20 °C , while at 4 °C it remains active for only one month.

Total molecular weight is obtained by gel filtration and found to be 60-80 K Dalton. Magnesium and manganese sulfate are important for the bacterial growth and for surlactin production consequently at concentration of 0.04 and 0.01%, respectively for isolates.

**Conclusion:** *Lactobacillus acidophilus* was isolated from healthy women, who were capable of producing crude surlactin in stationary phase of growth, then purified and characterized. Some physical and chemical factors affecting the ability of the bacteria in the production of surlactin had been studied, later identified optimal conditions for growth and increase production of surlactin.

**Keywords:** *Lactobacillus acidophilus*, Surlactin, Biosurfactants, Purification.

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## *Introduction*

The biosurfactants agents in general are articles of emulsifier, solvent, wetted, producing foam and adapted to be used in many industries, especially manufacturing detergents, cosmetics, as a material impact on the surface tension of the surfaces and to reduce infections and to maintain the vitality of some of the material <sup>(1-3)</sup>. These materials vary in terms of chemical composition; biosurfactants has to be discipline glycolipids, lipopeptids, Fatty acids or glycoprotein. The biosurfactants agents were produced either chemically or as bio product, as a result of many microorganisms <sup>(4)</sup>.

Biosurfactants produced by *Lactobacillus spp.* is one of the most important bioactive and effective materials of these bacteria. It is particular importance compared with biosurfactants produced by other types of living microorganisms, that's because of low toxicity and the ability to biodegradation of many materials. Therefore it is not used for treatment of the heavy-water, in addition to its important in the medical applications by reduction of bacterial contamination and bioactive treatments <sup>(5-7)</sup>. Biosurface agents produced by bacteria *Lactobacillus spp* are known as surlactin or what is known as surface lactin. It is believed that the reason for this name is due to the chemical nature (Mucoproteins) in addition to their impact on the surfaces <sup>(8)</sup>.

In present time their is an increased aspects of attention to treatment of lactobacillus products because it is based on the Glycosylation in addition to other attributes, so it can take advantage of its bioactivity in treatment <sup>(7)</sup>.

Also it was found that this type of microbiology product had antitumer effectiveness of cancer of the gastrointestinal tract, bladder, and cervix <sup>(8, 10, 11, 12, 13)</sup>.

Many researchers found that the possible use of surlactin produced by *Lactobacillus spp.* was in the medical field, as many of the materials have some events for the growth of antimicrobial, including effective antitumer agent and some of them ant-mutagenic.

Also they found it possible to use immuno-modulators and its ability to conserve cholesterol levels and reduce the concentration of sugar lactose in humans <sup>(14,15)</sup>.

## *Materials and Methods*

### A- Biosurfactants production:

For select off a number of *Lactobacillus acidophilus* strain, 100-ml cultures in MRS broth were grown overnight (for 18 hours) to invest the stationary phase of bacterial growth. The cells were harvested by centrifugation at 6000 rpm/minutes for 30 minutes at 5°C, washed twice in demineralized water, and resuspended in 10 ml of sterile phosphate buffer saline, PBS (pH 7). The lactobacilli were incubated at room temperature for 2 hrs. with gentle stirring for biosurfactants production. Subsequently, the bacteria were removed by centrifugation, and the remaining supernatant liquid was filtered through a 0.22-mm-pore-size filter (Millipore). Filtered product was dialyzed against demineralized water at 5°C overnight membrane tube (molecular weight cutoff, 6,000 to 8,000) and subsequently against sucrose to be concentrated <sup>(16)</sup>.

### B-Isolation and purification of surlactin:

Velraeds,et<sup>(17)</sup> method had been used in isolation and purification of surlactin for *L.acidophilus*

#### C- Characterization of surlactin:

1- Determination of total molecular weight using sephadex G-200 filtration and composite protein molecular weight by electrophoresis using polyacryl amide in presence of sodium dodecyle sulphate<sup>(18,19)</sup>.

2- Effect of high temperature on surlactin activity:-

Surlactin samples of 497-514 microgram milliliter concentration were incubated, respectively at temperature 50, 75, 100 °C for a period of one hour and then the effectiveness of these models was tested. The membrane on the susceptibility of *S.epidermidis* was removed by a way of test tube and its ability to inhabit the adhesion tested bacteria to epithelial cells<sup>(19)</sup>.

3- Effect of low temperature store on surlactin activity:-

The effect of keeping at low temperatures (4, -20) °C on effective of surlactin in the removal of the membrane of *S.epidermidis* is by method test tube and inhibition of bacterial adhesive to the epithelial cells. It has been tested weekly know the effectiveness of the surlactin, which is preserved at 4°C for a period of one month, and monthly for the models to be preserved at temperatures -20 °C for a period of six months<sup>(20)</sup>.

4- Typical pH for surlactin activity:

Surlactin which is concentrated 497 and 514 microgram/ ml, respectively, was assessed to its effectiveness to inhabit bacterial cells to the epithelial cells and remove the bioactive membrane of *S.epidermidis* using test tube method by measuring the optical density at a wave length 550 nm, and pH values ranged between (2-12)<sup>(21)</sup>.

Determination of optical condition for growth and production of surlactin

#### A-Effect of pH and temperature:

Method of Christensen<sup>(16)</sup> was adopted to study the effect of the pH<sup>(12, 10, 8, 6, 4,2)</sup> and different degrees of heat (40, 30, 25, 4) °C.

B - Effect of bacterial inoculation volume in production of surlactin

Inoculated tubes containing the productive media, 10 ml PBS with different sizes of homogeneous *L.acidophilus* suspension (bacterial number of  $1 \times 10^8$  cell / ml) ( 200, 400, 600, 1000 micro liter) which is equivalent to (  $2 \times 10^7$ ,  $4 \times 10^7$ ,  $6 \times 10^7$ ,  $8 \times 10^7$ ,  $1 \times 10^8$  cell), respectively. Incubated tubes at room temperature for two hours, then quickly centrifuge the cultivated at 6000 cycles/minute at temperatures 4 °C for half hour and take supernatant containing surlactin. Used Millipore filter with a pore size of 0.22 micrometer. Protein and carbohydrates concentration was measured. The effectiveness of the product bioactivity was also measured.

C-Effect of primary inoculation media composition:-

Prepare tubes containing 10 ml of primary liquid MRS with different additions which include the addition of glycerin 2% to each tube, the amino acid alalnin 0.01% to the second tube, and 3% glucose to third tube, MgSO4 0.04% to the fourth tube and 0.01 % MnSO4 to the fifth tube( 22) .

D- Statically analysis:

Make the appropriate statistical analysis to demonstrate the variation in the production of isolates surlactin and the factors affecting the production, using the SPSS Statistical graphics system stat graph, where Achtbarthalil used variation of the one-way multiple comparisons adopt Duncan method to determine if there are differences among different factors, and determine optimal conditions for production.

## *Results and Discussion*

Reference to the results of studies on surlactin produced by *L.acidophilus* and other types of bacteria, in addition to results of current study, which confirmed that the surlactin produced in the stationary stage. Therefore adopted this stage in the production of surlactin<sup>(18, 23)</sup>.

Once, *L.acidophilus* was isolated from vaginal swabs of healthy women, which is located normally<sup>(24)</sup>. After separation of the cells from the growth media (MRS), surlactin was prepared as a crude from the isolate by incubation of bacteria in phosphate buffer saline for two hours at 25°C<sup>(25)</sup>. Purification was achieved by using Millipore filter, dialysis in concentrated sucrose solution. It was observed that the activity of the obtained surlactin was increased upon purification, while the best activity was obtained at pH 6.0 but did not lose its activity at extreme pH of 2.0 and 12.0, but decreased its activity which may be the result of damage (Denaturation) in composition. The results of this study agreed with the results of Stefan Roos and Hans Jonsson (2002) in their studies on the lactic acid bacteria<sup>(26)</sup>.

The surlactin stands active at a temperature up to 75°C for an hour, while it completely loss activity at 100 °C for the same period. May be due to the impact on the protein in surlactin at 100 °C, therefore the temperature range (50-75)°C concenter the best rang to testing of this product at high temperature treatment .

In storage at -20 °C, surlactin keeps its activity for six months, while at 4 °C it remains active for only one month<sup>(27)</sup>.

Total molecular weight was obtained by gel filtration for isolates found to be 60 - 80 K Dalton. The molecular weight of the protein component was measured by polyacryl amide gel electrophoresis in the presence sodium dodecyle sulfate.

Two bands of protein were obtained for surlactin for isolates with molecular weight of 100 and 50 K Dalton for isolate, and 40 and 32 K Dalton for other isolate. Thus, differentiate as higher molecular weight component (glycoprotein) compose of protein and sugars<sup>(7, 17)</sup>.

The convenient conditions to promote good yield of surlactin production were also studied. These conditions include; chemical composition of the first growth media, pH, time and temperature of incubation, inoculate volume...etc.

It was found that the presence of magnesium and manganese sulfate is important for the bacterial growth and for surlactin production consequently at concentration of 0.04 and 0.01 % respectively for isolates. The fact that the elements are working as cofactor<sup>(24)</sup> and when add magnesium to the bacterial production media leads to deposition of surface active agents, which re-producing bacteria, to production of a new. The purpose of this process is an increase in extra cellular surlactin production.

Result showed that the best pH was at 6.0, while the incubation temperature and time were 25°C for four hour with inoculation volume of 600 µliter (6×10<sup>7</sup>cell / milliter) per 10 milliter of the media. But when you increase the volume of vaccine to more than 600 micro liter found that the concentration of the produced surlactin starts to decline.

The decline may be due to a decline in cell efficiency due to change in the content of the media and accumulations of metabolic products, which make the production conditions not suitable for high concentration ( 24 ).

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