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PRODUCTION OF ANTI –ADHESIVE COATING AGENT FOR TABLE EGGS BY *LACTOBACILLUS ACIDOPHILUS* MTCC 447

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Abstract

The current study described the production of biosurfactant by *Lactobacillus acidophilus* MTCC 447 and its application in food storage and protection. The obtained biosurfactant gave a high emulsifying index (85%) at room temperature and low reduction in surface tension at 35mN/m with CMC 7.5 mg/ml. FTIR analysis showed that the biosurfactant is highly pretentious in its nature with small ratio of phosphate and polysaccharide groups. Our results showed a remarkable reduction in total viable count of *E. coli, Staphylococcus aureus, Staphylococcus epidermdis, and Salmonella enteritidis*. The average total viable count for all the tested bacterial species was reduced from $65x10^5$ to 2x10 CFU/cm^2 on table eggs surface by preventing the adhesion and biofilm formation. The biosurfactant was effective against *E. coli, Staphylococcus epidermdis, Salmonella enteritidis* even at low to moderate concentrations; however, the biosurfactant showed a low activity against *Staphylococcus aureus*. Biosurfactant has the ability to reduce the bacterial adhesion to eggs and prevent their spoilage. Reduction food spoilage will decrease the life threatening diseases and reduce the economic losses by using of this simple and cost-effective technique.

***Keyword*:** Biosurfactant, Anti-adhesive, table eggs, *Lactobacillus acidophilus*, PBS.

Introduction

Food safety is an important worldwide issue due to foodborne diseases that occur through chemical or microbial contamination at any stage of food production "farm to plate "(Allard, 2002). Food spoilage can cause dangerous life threatening diseases and make economic risks (Seema, 2015). Many research have been conducted to improve food safety and quality, including storage techniques. The bad environmental storage conditions can enhance the growth of pathogenic microorganisms especially in tropical and subtropical areas where the humidity and temperature are very important environmental factors (Theron *et al*., 2003; Martins *et al*., 1998; Kamel *et al*., 1980). Among the important types of food is table eggs, which can be contaminated by pathogenic microbes such as *E. coli, Salmonella, Staphylococcus spp.* and *Bacillus spp.* due to dirty handling and/or from the soil. Biofilms of these microbes can cause spoilage to eggs especially to cracked ones (Davies and Breslin, 2003; Board and Tranter, 1995). Pathogenic microbes have the ability to penetrate the shells of table eggs leading to their spoilage (Abdullahi, 2010).

Biosurfactants are chemical compounds that are produced by living microorganisms and have well defined hydrophilic and hydrophobic moieties with ability of reducing surface tension (Mostafa *et al*., 2019). Biosurfactants have different physicochemical properties according to their chemical structure (glycoproteins, polymeric types, phospholipid and lipopeptide) (Santos *et al*., 2018; Gudiña *et al*., 2016). Due to their anti-adhesive, antibacterial and antifungal activities, biosurfactants have been efficiently used in many applications in medicine and food industry (Muthusamy *et al*., 2008; Gao, 2018).

Biosurfactant that is produced by *Lactobacillus spp*. is surlactin, which is rich in protein (Reid *et al*., 1999). High yield of surlactin is produced during the stationary phase of bacterila growth and is stable at pH 6-7 and high temperature (Fouad, 2010). Surlactin has a number of beneficial characteristics, which made it a favorable agent in food production and storage. Biosurfactants prevent table eggs spoilage by disrupting the formation of microbial biofilms and prevent its adhesion to eggs surface. These characteristics include inhibition the adhesion of pathogenic microbes; cost-effectiveness of its production and a lower toxicity as compared with chemical surface active agents (Rodrigues *et al*., 2004). The objectives of this study were producing natural biosurfactants by a strain of probiotic bacteria (*L. acidophilus* MTCC 447) and testing their anti-adhesive activity against known pathogenic bacterial species that cause table eggs spoilage.

Material and Methods

Lactobacillus acidophilus strain (MTCC 447) was purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), India. Frozen bacteria were activated by streaking it on de Man, Rogosa and Sharpe (MRS) agar and incubated overnight at 37°C with 5% CO_2 (Sneath *et al*., 1986). The sub-culture was prepared by inoculating the colony in MRS broth and incubated overnight at the same conditions mentioned above. Table eggs were purchased from local markets to identify the eggs surface microbes. The target strains were isolated from the surface of 36 eggs by using moistened cotton swab that immersed with 0.1% peptone water. After that, the smear was transferred into nutrient broth and incubated at 37 °C for 24 hrs. to prepare it for culturing in MacConkey agar and Blood agar. The culturing activity and total microbial count were tested before and after anti-adhesive activity test. Biochemical tests , including citrate, methyl red, indole, nitrate, urease, hydrogen sulfide, sugar tests (glucose, sucrose, lactose)

and coagulase test were implemented to differentiate the isolated bacterial species (Jimenez-Lopez, 2012).

Biosurfactant production

Lactobacillus acidophilus MTCC 447 was cultured at 37°C for 72 hrs. in 1 L flask containing 500 mL of sterile MRS broth on a rotary shaker at 160 rpm. Five mL of overnight culture was used for inoculation. The optical density of bacterial growth at specific time interval was measured at 600 nm. Cells were collected by centrifugation at 10000 rpm under 4°C for 30 minutes, the cell free supernatant (CFS) was discarded. The precipitate was washed twice by deionized water for 2 hrs. at 25 °C with gentle shaking (Velraeds *et al*., 1996). The cells were suspended in phosphate buffered solution (PBS) to adjust pH at 6.5 for biosurfactant extraction. The cells were removed by centrifugation at 10000 rpm under 10°C for 5 minutes. The CFS was filtered through MF-Millipore Membrane Filter, 0.22 µm pore size, dialyzed against demineralized water, and the product was freeze-dried.

Oil spreading Method

Fifteen microliters of olive oil was added to a petri dish, which contained 30 mL of distilled water, then15 µl of aqueous solution of CFS was added to the center of olive oil film. The observation of a clear zone is an indication of the presence of biosurfactant in CFS (Cornea *et al*., 2016).

Emulsification index (EI24)

Two milliliters of CFS, 2 ml of sunflower oil and 4ml of distilled water were added to the same test tube. Then the mixture was vortexed for 2 min and left overnight to determine emulsification index. EI24 was calculated by dividing the height of the emulsified layer by the total height of the mixture and multiplied by 100 (Luna *et al*., 2013).

Surface tension and critical micelle concentration

The surface tension of distilled water, which contains CFS at different concentrations (0.0 - 13.0 mg/mL) was measured, and 15 ml of aqueous biosurfactant solution was used. These samples were measured and compared with surface tension of distilled water as a blank by using Sigma 703D Du-Nouy-Ring tensiometer. The reduction in surface tension detects the presence and activity of the produced biosurfactant. All the measurements were done in triplicates and the average values were taken for CMC determination.

Fourier transforms infrared spectroscopy (FTIR)

To determine the chemical compositions of the obtained biosurfactant, FTIR analysis was implemented. Two mg of freeze-dried biosurfactant were added to 100 mg of potassium bromide powder. The mixture was pressed under 8 bars for 60s to form pellets, which were placed in FTIR. Infrared radiation spectra were collected over a wave number range of 600 - 4000 cm^{-1} using a Sell FT-IR Spectrometer Model FTIR-600.

Anti-adhesive activity of the produced biosurfactant

To determine the activity of biosurfactant, 6 sets of treatments were prepared in triplicates in polyethylene containers, which were 1) untreated control containing 100 mL PBS only, 2) treatment 1 containing 100 mL PBS + 20 mg/mL of produced biosurfactant, 3) treatment 2, containing 100 mL PBS + 40 mg/mL of biosurfactant, 4) treatment 3, containing100 mL PBS + 60 mg/mL of biosurfactant, 5) treatment 4, containing 100 mL PBS + 80 mg/mL of biosurfactant, and 6) treatment 5, containing 100 mL PBS + 100 mg/mL of biosurfactant. In each treatment, 2 eggs were immersed in each replicate of the treatments for 15 minutes, removed from the treatment containers and left to dry. Then, all eggs were exposed to handling purpose and left at room temperature for 5 days. The inhibition of adhesion and growth of isolated strains (*E. coli, Staphylococcus aureus , Staphylococcus epidermdis, and Salmonella enteritidis*) was measured by culturing and counting the total viable count of these microbes after immersion according to the mentioned method for identification of microorganisms (Jimenez-Lopez, 2012).

Results and Discussion

Oil spreading method

It is a simple and cheap test to detect the production of biosurfactant and its activity through the reduction of water-oil interfacial tension (Cornea *et al*., 2016). Clear zone was appeared due the presence of effective biosurfactant (Fig. 1).

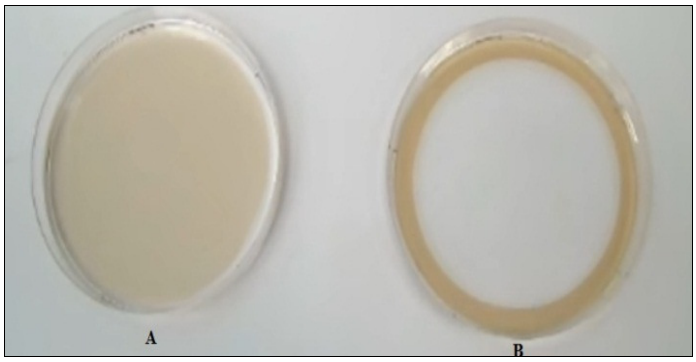

Fig. 1 : Oil spreading method (A) control; (B) sample containing biosurfactant solution.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectroscopy results (Fig. 2) showed that the produced biosurfactant has a pretentious structure and has a relatively high and distinctive absorption bands at 1654 and 1535 cm-1, which due to presence of amide I and amide II, respectively. Other distinctive bands were at 1200 and 1050 cm-1, which due to presence of phosphate and polysaccharide, respectively. It did not show an absorption bands at 2000 to 3000 cm^{-1} because it's obtained during stationary phase. The produced biosurfactant in the current study was not pure, but it is a mixture of proteins, phosphate and polysaccharide. The crude form of biosurfactant has an ability to inhibit the formation of pathogenic bacteria biofilm even at low concentrations.

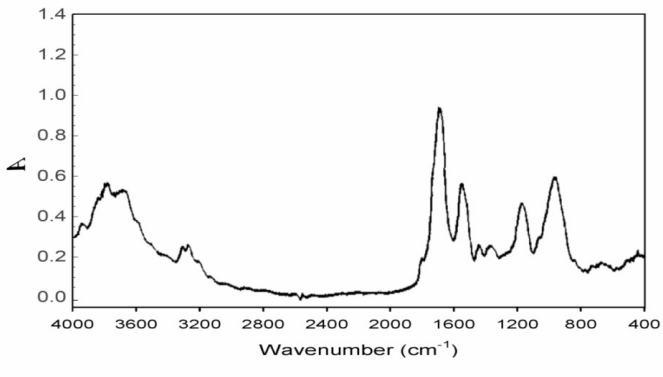

Fig. 2 : FTIR spectrum of biosurfactant produced by *L. acidophilus* MTCC 447

Emulsification index (EI_{24})

Emulsification index is known as the ability of biosurfactant to maintain at least 50% of original emulsion volume after 24 hrs. from the time of its formation. The emulsification index of the produced biosurfactant showed that the EI_{24} was 85%, which indicates presence a high emulsification activity; therefore, the bacterial strain *Lactobacillus acidophilus* MTCC 447 was considered as a promising strain for biosurfactant production (LUNA *et al*., 2013).

Critical micelle concentration (CMC)

CMC is defined as the concentration of biosurfactant at which the formation of micelles begins, and at this point, there will be no more reduction in surface tension even with additional amount of biosurfactant (Ayed *et al*., 2015).

At a concentration lower than CMC, the surface tension will decrease with additional amount of biosurfactant, and the surface becomes saturated and formation of micelles will initiate. According to Fig. 3, the biosurfactant gave the lowest surface tension at 35 mN/m with CMC 7.5 mg/ml and above this value there was no reduction in surface tension.

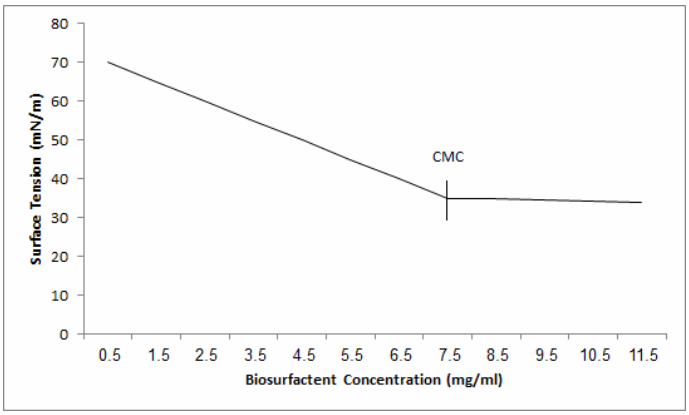

Fig. 3 : Surface tension (mN/m) versus concentrations of biosurfactant (mg/ ml) obtained by *L. acidophilus* MTCC 447. CMC value was determined from the intersection between the regression lines that better described the two parts of the curve.

Anti-adhesive activity of biosurfactant

Anti-adhesive activity of the produced biosurfactant was observed by detection of bacterial growth after immersion of table eggs in different concentrations of biosurfactant as compared with the control. The results (Tab. 1) showed that at 20 mg/ml of biosurfactant the colonies growth of all target strains were detected with little inhibition to *E. coli*. At 40 mg/ml and 60 mg/ml the anti-adhesive activity was comparable, the growth of *Salmonella enteritidis* and *E. coli* wasn't detected. However, there wasn't any inhibition to growth of *S. aureus* because many colonies were detected. *S. epidermidis* was not detected at 60 mg/ml, but there was a growth of few colonies at 40mg/ml. This indicates that as the concentration of biosurfactant increases its inhibitory activity will increase. The growth of all target strains was inhibited at 80 mg/ml and 100mg/ml, whereas a few colonies of *S.aureus* were detected, this species is resistant to the anti-bacterial activity of biosurfactant even at high concentrations. A study reported that biosurfactants exert little activity against *S. aureus* (Bento *et al*., 2005). Total viable count of microorganism declines after coating table eggs with biosurfactant at a concentration of 100mg/ml from $65x10^5$ to 2x10 CFU/cm^2. Biosurfactant inhibits the growth of microorganisms by inhibition the formation of biofilm of microbes.

Briefly, as the concentration of biosurfactant increases the inhibition activity will also increase.

Table 1 : Antimicrobial activities of surlactin from *L. acidophilus* MTCC 447 at different concentrations against several pathogenic bacteria.

Microorganism	Biofilm inhibition at different concentration (mg/ml) of surlactin					
	Control (PBS)	20	40	60	80	100
Escherichia coli	+	±	–	–	–	–
Salmonella enteritidis	+	+	–	–	–	–
Staphylococcus aureus	+	+	+	+	±	±
Staphylococcus epidermidis	+	+	±	–	–	–

+many colonies detected; ±few colonies detected; –no colonies detected.

Conclusion

Lactobacillus acidophilus MTCC 447 produces safe and cost effective biosurfactants that can be beneficially applied in coating eggs and other food products to reduce thie risk of microbial contamination. The process of coating table eggs and food products with anti-adhesive biosurfactant can increase food storage time and reduce food spoilage and consequently lower economic losses.

References

Abdullahi, I.N. (2010). Isolation and identification of some bacterial isolates from table eggs. Alanbar Journal of Veterinary Sciences, 3(2): 59-67.

Allard, D.G. (2002). The 'farm to plate' approach to food safety–everyone's business. Canadian Journal of Infectious Diseases and Medical Microbiology, 13: 185–190.

Ayed, H.B.; Jemil, N.; Maalej, H.; Bayoudh, A.; Hmidet, N. and Nasri, M. (2015). Enhancement of solubilization and biodegradation of diesel oil by biosurfactant from Bacillus amyloliquefaciens An6. International Biodeterioration & Biodegradation, 99: 8-14.

Bento, F.M.; De-oliveira Camargo, F.A.; Okeke, B.C.; Frankenberger, W.T. (2005). Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. Microbiological research, 160(3): 249-255.

Board, R.G. and Tranter, H.S. (1995). The microbiology of eggs. Egg science and technology, 4: 81-104.

Cornea, C.P.; Roming, F.I.; Sicuia, O.A.; Voaideș, C.A.; Zamfir, M.E. and Grosu-Tudor, S.S. (2016). Biosurfactant production by *Lactobacillus* spp. strains isolated from Romanian traditional fermented food products. Romanian Biotechnology Letter, 21(2): 11312-20.

Davies, R.H. and Breslin, M. (2003). Investigation of Salmonella contamination and disinfection in farm egg-packing plants. Journal of Applied Microbiology, 94: 191–196.

Fouad, H.K.; and Khanaqa, H.H. and Munira, C.I. (2010). Purification and characterization of surlactin produced by Lactobacillus acidophilus. Al-Anbar Medical Journal, 8(1): 34-39.

GAO, C. (2018). Experiences of microbial enhanced oil recovery in Chinese oil fields. Journal of Petroleum Science and Engineering, 166: 55-62.

Gudiña, E.; Teixeira, J. and Rodrigues, L. (2016). Biosurfactants produced by marine microorganisms with therapeutic applications. Marine drugs, 14(2): 38-53.

Jimenez-Lopez, J.C. (2012). Biochemical testing. In Tech Janeza Trdine. P 217. ISBN 978-953-51-0249-6.

Kamel, B.; Bond, C. and Diab, M. (1980). Egg quality as affected by storage and handling methods. Journal of Food Quality, 3: 261–273.

Luna, J.M.; Rufino, R.D.; Sarubbo, L.A.; Campos-Takaki, G.M. (2013). Characterisation, surface properties and biological activity of a biosurfactant produced from industrial waste by *Candida sphaerica* UCP0995 for application in the petroleum industry. Colloids and surfaces B: Biointerfaces, 102: 202-209.

Martins, H.M.; Bernatdo, F.M. and Martins, M.L. (1998). Effect of *Saccharomyces cerevisiae* ATCC 97631 on aflatoxins production., 4th World Cong. Foodborne Infect. and Intoxi.

Mostafa, N.A.; Tayeb, A.M.; Mohamed, O.A. and Farouq, R. (2019). Biodegradation of Petroleum Oil Effluents and Production of Biosurfactants: Effect of Initial Oil Concentration. Journal of Surfactants and Detergents, 22(2): 385-394.

Muthusamy, K.; Gopalakrishnan, S.; Ravi, T.K. and Sivachidambaram, P. (2008). Biosurfactants: properties, commercial production and application. Current Science (00113891), 94(6): 736-747.

Reid, G.; Heinemann, C.; Velraeds, M.; Van der Mei, H.C. and Busscher, H.J. (1999). Biosurfactants produced by Lactobacillus. In Methods in enzymology pp. 426-433. ISBN 9780121819613.

Rodrigues, L.; Van der Mei, H.C.; Teixeira, J. and Oliveira, R. (2004). Influence of biosurfactants from probiotic bacteria on formation of biofilms on voice prostheses. Applied Environmental Microbiology, 70(7): 4408-4410.

Santos, A.P.; Silva, P.; Costa, M.D.S.; Rufino, E.V.L.; Santos, R.D.; Ramos, V.A.; Sarubbo, C.S.; Porto, A.L.F. (2018). Production and characterization of a biosurfactant produced by *Streptomyces* sp. DPUA 1559 isolated from lichens of the Amazon region. Brazilian Journal of Medical and Biological Research, 51(2): e6657.

Seema, R. (2015). Food Spoilage: Microorganisms and their prevention. Pelagia Research Library. Asian Journal of Plant Science and Research, 5(4): 47-56.

Sneath, P.H.; Mair, N.S.; Sharpe, M.E. and Holt, J.G. (1986). Bergey's manual of systematic bacteriology. Volume 2. Williams & Wilkins. ISBN 978-0-387-28022-6.

Theron, H.; Enter, P.V. and Lues, J.F.R. (2003). Bacterial growth on chicken eggs in various storage environments. Food Research International, 36(9): 969-975.

Velraeds, M.M.; Van der Mei, H.C.; Reid, G. and Busscher, H.J. (1996). Inhibition of initial adhesion of uropathogenic Enterococcus faecalis by biosurfactants from Lactobacillus isolates. Applied Environmental Microbiology, 62(6): 1958-1963.