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Research Article

IN VITRO PHARMACOLOGICAL ACTIVITY OF BIOSURFACTANT

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ABSTRACT

Biosurfactants or surface active agents are produced by microorganisms. These molecules reduce surface tension both aqueous solutions and hydrocarbon mixtures. Although a large number of biosurfactant producers have been reported in the literature, biosurfactant research, particularly related to production enhancement and economics, has been confined mostly to a few genera of microorganisms such as *Bacillus, Pseudomonas* and *Candida*. Biosurfactants are not only useful as antibacterial, antifungal and antiviral agents but also have the potential for use as major immunomodulatory molecules, adhesive agents and even in vaccines and gene therapy. In the present study we have examined the effect of biosurfactant *in vitro*. For this purpose we have choosen frog as an experimental animal and the tissue was frog isolated heart. Biosurfactant elicited dose-dependent cardiac depressant activity. Atropine (ATP), a muscarinic blocker could not antagonize the effects of biosurfactant which indicate that the activities are not mediated through muscarinic receptors.

Key words: Biosurfactant, Heart rate, Cardiac output, Cardiac depressant.

INTRODUCTION

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively[1]. They are a structurally diverse group of surface active molecules synthesized by microorganisms[2]. Rhamnolipids from *Pseudomonas aeruginosa*, surfactin from *Bacillus subtilis*, emulsan from *Acinetobacter calcoaceticus* and sophorolipids from *Candida bombicola* are some examples of microbial-derived surfactants. Originally, biosurfactants attracted attention as hydrocarbon dissolution agents in the late 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry[3]. The reason for their popularity as high value microbial products is primarily because of their specific action, low toxicity, higher biodegradability, effectiveness at extremes of temperature, pH, salinity and widespread applicability, and their unique structures which provide new properties that classical surfactants may lack[4]. Biosurfactants possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as chemical surfactants. Unlike chemical surfactants,

which are mostly derived from petroleum feedstock, these molecules can be produced by microbial fermentation processes using cheaper agro based substrates and waste materials. During the past few years, biosurfactant production by various microorganisms has been studied extensively. Also various aspects of biosurfactants, such as their biomedical and therapeutic properties[5], natural role[6], production on cheap alternative substrates[7] and commercial potential[1], have been recently reviewed. No attempt has been made, to describe the research and development strategies of making the biosurfactant production process cheaper and commercially attractive. Most of the work on biosurfactant applications has been focusing on bioremediation of pollutants[8] and microbial enhanced oil recovery[3]. However, these microbial compounds exhibit a variety of useful properties and applications in various fields. Biosurfactants has many applications in the areas such as food and food related industries (as emulsifiers, foaming, wetting, solubilizers, antiadhesive agents), biomedicine and therapeutics (as antimicrobial agents, immunoregulators and immunomodulators, their possible role in signalling and cytotoxic activity). With these specialized and cost-effective applications in biomedicine, we can look forward to biosurfactants as the molecules of the future.

MATERIALS AND METHODS

Preparation of nutrient broth

Weigh 10 gm of Beef extract, 10 gm of Peptone, 5 mg of Sodium chloride and dissolve in sufficient amount of distilled water. Dissolve with the aid of heat. After complete dissolution make the volume upto 1 litre with distilled water. Adjust the p^H to 8.0 to 8.4 with 5M NaOH and boil for 10 mins, filter, sterilize by maintaining at 115^{0}C for 30 mins and adjust the p^H to 7.3+0.1. 250 ml of nutrient broth was prepared in conical flask. It was sterilized in the autoclave at 15 lb pressure for 15 mins. Under aseptic conditions nutrient broth was inoculated with a loop of *Bacillus Subtilis* and kept for incubation at 35-37^{0}C for 24 hrs.

Preparation of basal mineral salt media[9]

Weigh 4 gm of NH_4NO_3, 5.9 gm of Na_2HPO_4, 4.1 gm of KH_2PO_4, 0.5 gm of NaCl, 0.096 gm of $MgSO_4$, 7.74 x 10^{-4} $CaCl_2$, 1.43 X 10^{-3} Na_2EDTA, 2 % w/v dextrose and make the volume upto 1 litre. 150 ml was transferred to each conical flask and to it 3 ml kerosene was added and kept in autoclave for sterilization at 15 lb pressure for 15 mins. Then 24 hrs sample of bacillus subtilis (1 ml) was inoculated into each mineral media and kept for incubation for 3 days. Incubation temperatures were varied between 30^0 and 70^0 C. After 3 days the mineral media was centrifuged at 5000 rpm. The centrifuged sample was adjusted to p^H 2 and kept in refrigerator at 4–6^{0}C for 2 days for the formation of biosurfactant.

Detection of Biosurfactant

The biosurfactant formed was detected by its emulsifying activity. 0.5 ml of sample fluid and 0.5 ml of kerosene are added to 4.0 ml of distilled water. The tube was vortexed for 10 sec, hold stationary for 1 min and then visually examined for turbidity of a stable emulsion. Emulsification power was measured by vortexing equal volumes of the centrifuged culture with kerosene for 1 min and determining the % of volume occupied by the emulsion. The mixture was allowed to settle for 24 hrs and the height of the emulsion was measured.

Physiological solution

Frog Ringers solution Weigh 9 gm NaCl, 0.42 gm KCl, 0.12 gm $CaCl_2$, 0.50 gm $NaHCO_3$, 1 gm dextrose and dissolve in sufficient amount of distilled water. After complete dissolution make the volume upto 1 litre with distilled water. Either $CaCl_2$ or $NaHCO_3$ should be added at the end inorder to prevent the formation of $CaCO_3$ which forms a precipitate.

Frog Heart perfusion by Syme's technique

The effect of biosurfactant on isolated frog heart was done by Syme's technique. Frog (Rana tigrina) was stunned by head-blow using a steel rod and pithed. The skin and abdomen were cut and opened. The pectoral girdle was cut using a bone cutter

and pericardium was removed. Syme's cannula was connected to the reservoir of frog Ringers solution and introduced immediately into the sinus venosus of the heart. The connecting blood vessels were cut and heart was isolated from the animal and mounted on to a stand. Heart was then covered with thin layer of cotton wool to prevent drying. Frog Ringer solution was used to wet the heart frequently to prevent drying. Heart was connected to Starlings lever and adjusted to mark on the smoked drum for recording the responses of the heart.

The level of Frog Ringer solution in the Syme's cannula was maintained by fixing a glass tube into the cork fixed to the reservoir (Marriott bottle) tightly. The heart was allowed to stabilize and when the heart rate and cardiac output were taken, the recordings were made on a slow rotating sooted drum, to which a sooted kymograph paper was affixed. The effects of biosurfactant was studied on isolated perfused frog heart. The parameters studied include the force of contraction, heart rate and cardiac output. Minimum 5 mins time was allowed between the additions of samples *per se* (in frog Ringers solution) and its fractions. When a blocker was used, it was diluted with known amount of frog Ringer solution in the syringe itself and added slowly. The heart rate (HR), cardiac output (CO) and force of contraction were the parameters used for the study. The dilutions were prepared in frog Ringers solution. No suspending agents were used. The heart was moistened with frog Ringers solution from time to time.

RESULTS AND DISCUSSION

Effect of Biosurfactant on isolated frog heart at the dose range of 1 µg to 300 µg, dissolved in frog Ringer solution

The normal HR and CO were 83 per min and 7 ml/min respectively. 1µg biosurfactant increased the HR from 83 to 85 per min and there was no change in CO. 3 µg biosurfactant increased the HR from 83 to 85 per min and there was no change in CO. 10µg biosurfactant decreased the HR from 83 to 82 per min and CO was decreased from 7 to 6 ml/min. 30µg biosurfactant decreased the HR from 83 to 80 per min and CO was decreased from 7 to 6 ml/min. There was decrease in force of contraction. 100µg biosurfactant decreased the HR from 83 to 78 per min and CO was decreased from 7 to 6 ml/min and there was decrease in force of contraction. 300µg biosurfactant decreased the HR from 83 to 74 per min and CO was decreased from 7 to 6 ml/min and there was decrease in force of contraction. The decrease in force of contraction produced by increase in doses of biosurfactant was dose-dependent.

Effect of Biosurfactant and influence of Atropine on the action of biosurfactant on isolated frog heart

The normal HR and CO were 83 per min and 7 ml/min respectively. 10µg biosurfactant decreased the HR from 83 to 82 per min and CO was decreased from 7 to 6 ml/min. There was decrease in force of contraction. Atropine 50µg diluted sufficiently was administered slowly. The HR and CO are 83 per min and 7 ml/min respectively. Later when 10µg biosurfactant was given the HR and CO are 82 per min and 6 ml/min respectively. There was decrease in force of contraction. When 80µg Atropine was given the HR and CO are 83 per min and 7 ml/min respectively. Later when 10µg biosurfactant was given the HR decreased from 83 to 82 per min and CO was 7 ml/min. There was decrease in force of contraction. Atropine 50µg to 80µg had no influence on the effect of biosurfactant on isolated frog heart.

CONCLUSION

Biosurfactant has antimicrobial activity, anticancer activity, immunomodulatory activity, sperm immobilizing activity and antiadhesive activity. They are also used as agents in surgical, agents for respiratory failure, agents for skin fibroblast metabolism. Biosurfactant obtained from Bacillus elicited dose-dependent cardiac depressant activity. Atropine (ATP) a muscarinic blocker could not antagonize the effects of biosurfactant which indicates that the activity is not mediated through muscarinic receptors.

Futher work in this direction might reveal the mechanism involved in these pharmacological actions and it is hoped that a systematic and exhaustive work is likely

to yield some agents of therapeutic value. This work is first of its kind so we wish that more attention be paid inorder to conclude the activity of this new category of compounds.

REFERENCES

1. Karanth NGK, Deo PG and Veenanadig NK. Microbial production of biosurfactants and their importance. Curr Sci. 1999;77:116-123.
2. Lu JR, Zhao XB, Yaseen M. Biomimetic amphiphiles: Biosurfactants. Curr Opin Colloid Interface Sci. 2007;12:60–67.
3. Banat IM, Makkar RS and Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol. 2000; 53: 495–508.
4. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiol. Mol Biol Rev. 1997;61:47–64.
5. Cameotra, SS and Makkar RS. Recent applications of biosurfactants as biological and immunological molecules. Curr Opin Microbiol. 2004;7:262–266.
6. Ron EZ and Rosenberg E. Natural roles of biosurfactants. En-viron Microbiol. 2001;3:229–236.
7. Makkar RS and Cameotra SS. An update on the use of un-conventional substrates for biosurfactants production and their new applications. Appl Microbiol Biotechnol. 2002;58:428–434.
8. Mulligan CN. Environmental applications for biosurfactants. Environ. Pollut. 2005;133:183-198.
9. Fareh ND, Adibah Y, Madihah MS, Norazurah S and Chan HX. Production of biosurfactant by locally isolated thermophilic facultatively anaerobic bacteria strain B160. 2009;133:183-198.