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Biosurfactant from marine actinobacteria and its application in cosmetic formulation of toothpaste

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ABSTRACT

Biosurfactant was obtained from Nocardiopsis VITSISB and it was used in cosmetic formulation of toothpaste, replacing Sodium Lauryl Sulphate which is normally used in commercial toothpaste as a surfactant. This biosurfactant toothpaste was qualitatively analyzed by several tests like Spreadability Test, Foaming ability Test, Abrasiveness Test, Brine shrimp Hatchability Test and cleaning ability test. The results indicate that biosurfactant are more efficient and less toxic surfactant compare with chemical surfactant. In conclusion, the biosurfactant obtained from actinobacteria can act as good ingredient in place of chemical surfactant for toothpaste formulation. So with the advantage of environmental compatibility, in future the biosurfactant may be used in other cosmetic formulation like Shampoo formulation, face wash formulation etc. as an alternative for the chemical surfactant.

Keywords: Biosurfactant, Formulation, Spreadability Test, Foaming ability Test, Brine Shrimp Hatchability Test.

INTRODUCTION

Many microorganisms like Actinobacteria are able to produce a wide range of amphipathic compounds which is normally known as biosurfactant. Due to their amphipathic nature, biosurfactant can partition at the interface between difference fluid phases such as oil, water or air interfaces. There are many types (e.g. glycolipids, rhamnolipids, sophorolipids etc) of biosurfactants each produced by specific microorganisms. Glycolipids and lipopeptides are low molecular weight biosurfactants that effectively lower surface and interfacial tensions. High molecular weight compounds include extracellular polysaccharides, lipopolysaccharides, proteins, and lipoproteins that have a high affinity for surface binding [1]. Biosurfactant have special advantage over the chemical surfactants (e.g. Sodium Lauryl Sulphate), such as non toxicity, higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperature, pH, and salinity. Hence, biosurfactant provide highly valued surface properties, they have a higher degree of biodegradability and environmental compatibility which synthetic surfactants lack. Even with potential for several applications, production cost must be lowered, and high yield mutant strains need to be developed for these compounds to be profitable on a commercial scale. Exhibiting unique characteristics including antiradical properties, stimulation of dermal fibroblast metabolism, and hygroscopic properties to support healthy skin physiology, future prospects of biosurfactant based products include several types of facial cosmetics, lotions, beauty washes and hair products [2]. Biosurfactant production and analysis is currently a wide and active field of study and biosurfactant easily can be used in cosmetic formulation replacing the chemical surfactant. Biosurfactant also have enzyme activity and it's known as bacterial lipases. Bacterial lipases are ubiquitous enzymes of considerable physiological significance and industrial potential [3]. Lipases are widely used in cosmetic industry and it was used as an emulsifiers and moisturizers [4]. In a recent study carried out by market researcher, the market for enzyme used in cosmetic is set to grow 5% per year up to 2015 driven by technological progress and consumer awareness of seemingly potent power of enzymes [5]. Normally toothpastes are complex mixtures of abrasives, surfactants, anticaries agents, tartar control ingredients, pH buffers, humectants and binders to provide consistency and shape. Toothpastes are the essential cosmetics of daily uses. The

primary function of toothpaste is the cleaning of accessible surfaces of the teeth [6]. Therefore in the present work, following aspects of toothpaste were planned for the formulation and this study is also carried out to the application of biosurfactant in cosmetic formulation instead of using chemical surfactant.

MATERIALS AND METHODS

Actinobacterial strain:
The biosurfactant was extracted from *Nocardiopsis VITSISB* which was collected from Molecular & Microbiological Research Lab of VIT University. The biosurfactant kept in freeze at 4°C until further use.

Toothpaste formulations
Formulation 1:
In a glass beaker, specific amount of sodium alginate was taken and mixed with glycerine (Solution A). In another glass beaker, specific amount of calcium carbonate, sodium chloride, sodium fluoride and biosurfactant were taken and mixed with methanol (Solution B). Then solution A and solution B were mixed and stir as thoroughly as possible. At last distilled water was added while stirring until the mixture reaches the same consistency as of commercial toothpaste.

Formulation 2:
In a glass beaker, 5gm of baking soda, 1.5 gm of common table salt (sodium chloride), 1.5 gm of calcium carbonate and 0.5gm of biosurfactant were taken and mixed with 4 ml of glycerine and was stir as thoroughly as possible. Distilled water was added with a dropper while stirring until the mixture has about the same consistency as commercial toothpaste.

Efficiency tests of formulated Toothpastes:
Abrasiveness Test:
Pea sized amount of commercial toothpaste, formulation1 toothpaste and formulation 2 toothpaste were placed on three different clean slides and one drop of distilled water was added on each toothpaste sample. With the help of cotton swab each toothpaste sample was rubbed in a back and forth motion for 25 times using short 1cm stroke. Then carefully the slides were rinsed off and dried with soft tissue paper. Each slide was examined under a dissecting microscope and the amounts of scratches are determined on the surface of the slide [7].

Determination of Spreadability:
One gm of commercial toothpaste was placed on the centre of the glass plate and another glass plate is placed over the sample. 1kg weight on top of the glass plates was placed. After 10 min, the weight is removed and the diameter of the paste is measured in centimetre [7]. The experiment was carried out in triplicate.

Determination of pH:
In 250 mL beaker 2.0 gm commercial toothpaste was taken and 80 mL of distilled water was added and the solution is stir well. After 30 mins, the pH of the solution is measured with the help of pH meter. This test was repeatedly done for formulation1 and formulation2 toothpaste. Buffer tablets of pH 4, 7 and 9 were used to calibrate the pH meter during measurement.

Determination of foaming ability:
In a test tube 5 mL of distilled water was taken and followed by 0.5 gm of commercial toothpaste was added. The top of the test tube was covered with cork and the test tube is shaken properly. The nature and stability of the foam thus formed were studied [8] and the height of the foam above the water is measured in centimetre. This test was repeated for formulation1 and formulation2 toothpaste.

Cleaning ability Test:
In a small beaker, chocolate and coffee were mixed with hot water and little amount of food colour was added and mixed properly. The baked egg shell and human teeth were stained with this solution for 1hour. The stained egg shell and human teeth were brushed with the wet tooth brush for 5 to 10 strokes (back and forth motion) and there was no change in colour of stain. After that small amount of commercial toothpaste placed on tooth brush and the stained egg shell and human teeth were brushed by 5 to 10 strokes and hence the cleaning ability of specific toothpaste is observed. Whole test is repeated for formulation1 and formulation2 toothpaste to check their cleaning ability. The results were interrupted as follows '+++' 95% cleaning ability, '++' 85-95% cleaning ability, '>85%' cleaning ability.

Brine Shrimp Hatchability Test:
The brine shrimp (*Artemia salina*) eggs were added in sterile seawater (1gm cyst per litre) at 28°C. Again eggs were added separately in different concentration of commercial toothpaste and formulated toothpastes (25, 50, 70 and 100 µg/mL). The toxicity was determined after 12h, 24h and 48h of exposure [9]. The numbers of survivor were counted and percentage of deaths was calculated. The results were interrupted as follows '××' high toxicity, '×××' medium toxicity and '××××' low toxicity.

RESULTS AND DISCUSSION

The biosurfactant toothpaste efficiency results are shown in Table 1.

Table: 1. Efficiency tests result of formulated toothpastes

Experiments	Commercial toothpaste	Formulation 1 toothpaste	Formulation 2 toothpaste
Abrasiveness Test (scratch)	4	1	2
Spreadability Test (cm)	3.4±0.2	3.9±0.1	3.2±0.4
pH	8.37	8.89	8.67
Foaming ability(cm)	2.9±0.1	3.1±0.3	2.4±0.2
Cleaning ability**	+ + +	+ + +	+ +
Brine shrimp Hatchability Test***	××	××××	×××

*** '+++' indicate 98% cleaning ability.*
'++' indicate 85-95% cleaning ability.
**** '××' indicate high toxicity.*
'×××' indicate medium toxicity.
'××××' indicate low toxicity.

Basically, toothpaste acts three main functions, they removing strain on tooth through abrasion, cleaning oral cavity through detergents and acting as a carrier for releasing therapeutic compounds [10]. Cleaning particles are an important component of toothpastes, if the toothpaste is too abrasive, the tooth enamel can be damaged over time. The damage can be even more pronounced in the dentin. Toothpaste's abrasiveness is measured by its Relative Dentin Abrasively (RDA). According to RDA value 0 to 70 RDA value indicates low abrasive, 70 to 100 values indicate medium abrasive, 100 to 150 showed highly abrasive and 150 to 250 indicates regarded as harmful limit. So, here in case of abrasiveness taste the result showed that commercial tooth paste has more abrasive particle because of that reason the scratches are coming more compare to other two formulations. In case of formulation1 and formulation2 less scratch are observed because calcium carbonate and baking soda are used in those formulations respectively as an abrasive and baking soda have RDA value 7, so it is low abrasive and calcium carbonate also have less RDA value compare to other abrasive [11]. The standard RDA values of some commercial toothpaste are showed in Table 2. [12].

Table: 2. RDA values of several commercial Toothpaste

Toothpaste	RDA values****[8]
Straight Baking Soda	7
Colgate Regular	68
Colgate Total	70
Sensodyne	79
Colgate Platinum	106
Close up	120
Colgate Whitening	124
Colgate Tarter control	165
Pepsodent	150
Colgate Herbal	110

***** 0-70 RDA values = Low abrasive*
70-100 RDA values = Medium abrasive
100-150 RDA values = highly abrasive
150-250 RDA values = Regarded as Harmful limit

The pH value of toothpaste also plays a crucial role. The pH value gives an indication of inorganic constituents in toothpaste. Acidic pH encourages the growth of oral bacteria that cause dental carries [13]. The pH test result showed that formulation1 and formulation2 toothpaste are more basic in nature compare to commercial toothpaste. Hence, the pH and abrasiveness test indicates that formulation1 and formulation2 toothpaste are more efficient than commercial toothpaste. All toothpaste usually has foaming property. The foaming ability test of toothpastes showed that the efficiency and detergency of a particular toothpaste. Here commercial toothpaste and formulation1 showed almost same result in foaming ability test. So, this result indicates that biosurfactant are efficient greater than equal to chemical surfactant and biosurfactant also act as a good detergent in toothpaste Fig.1.

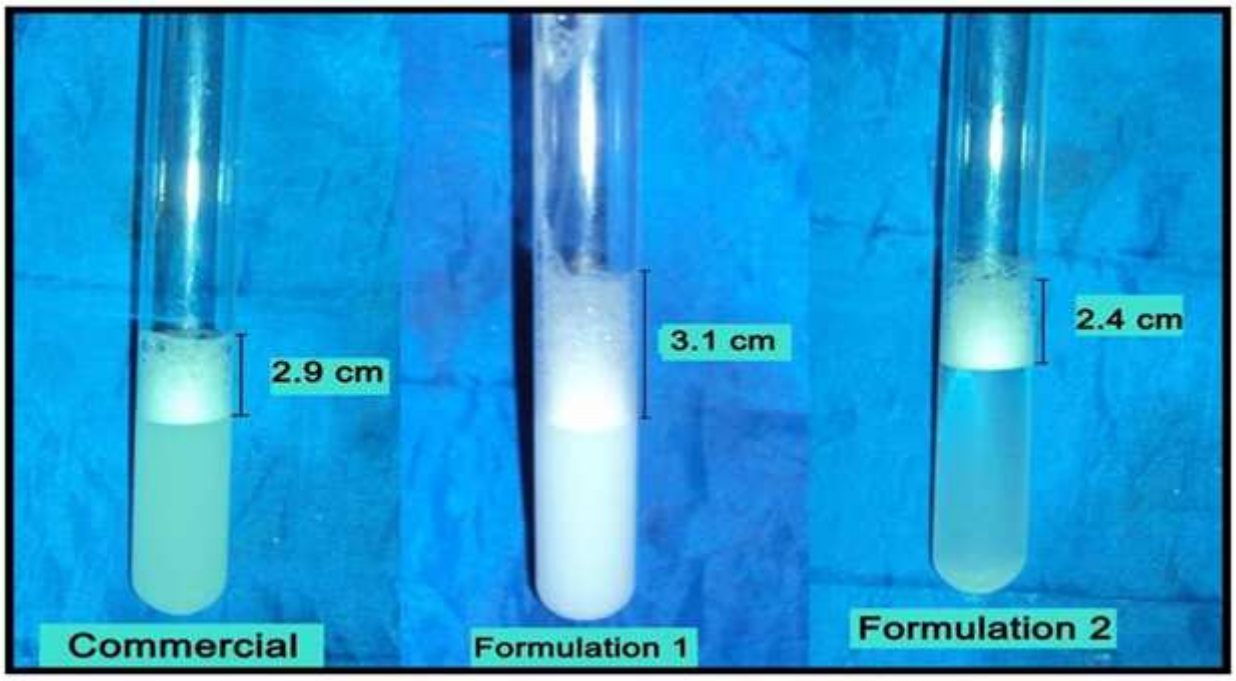

Fig 1. Foaming ability Test

Spreadability was another characteristic of toothpaste. It showed the consistency of paste. Basically the toothpastes are homogenous in nature and it should not separate into liquid and solid ingredients [14]. The large spread area has shown the good consistency of toothpaste. In Spreadability test formulation1 toothpaste showed more consistency (3.9 cm^2) compared to formulation2 (3.2 cm^2) and commercial toothpaste (3.4 cm^2). Another important characteristic of toothpaste is cleaning ability. In case of human, when organic pellicle which is secreted by the saliva and pellicle reacts with colouring agent such as cigarette smoke, beverages, coloured fruits, chocolates etc and those things stained the teeth. Removing spots and stains faces serious problems. Stained pellicle sticks firmly to the teeth and resist against removing. Those stained pellicle can be removed by toothpastes [15]. In commercial toothpaste chemical surfactant are responsible ingredients for removing the stain from teeth. The cleaning ability test results reported that biosurfactant had equal ability to remove the stain from teeth Fig.2.

The brine shrimp hatchability test result shows that commercial toothpaste is highly toxic compare to formulated toothpastes. In commercial toothpaste, Sodium Lauryl Sulphate is used as surfactant but according to the Environmental Working Group's Skin Deep: Cosmetic Safety Reviews [16], research studies on sodium lauryl sulphate have shown links to organ toxicity, developmental toxicity or reproductive toxicity, neurotoxicity, endocrine disruption, biochemical or cellular changes and oral cancer. A study at the Stern College for Women at Yeshiva University in New York in 1997 found that sodium lauryl sulphate in mouth rinses caused desquamation of oral epithelium and a burning sensation in human volunteers [17]. Sodium lauryl sulphate is also associated with increased aphthous ulcers (canker sores) due to the denaturing effect and irritation of the oral mucosa [18]. According to Judi Vance, sodium lauryl sulphate can cause cellular DNA damage [19]. A dental association in Japan tested the effects of sodium lauryl sulphate on bacteria, finding it to be mutagenic. Due to these adverse effects on human health, the use of sodium lauryl sulphate in commercial toothpaste should be avoided. Biosurfactant could replace chemical surfactants (e.g. sodium lauryl sulphate) that are currently used due to reports of adverse reactions with long term use [20].

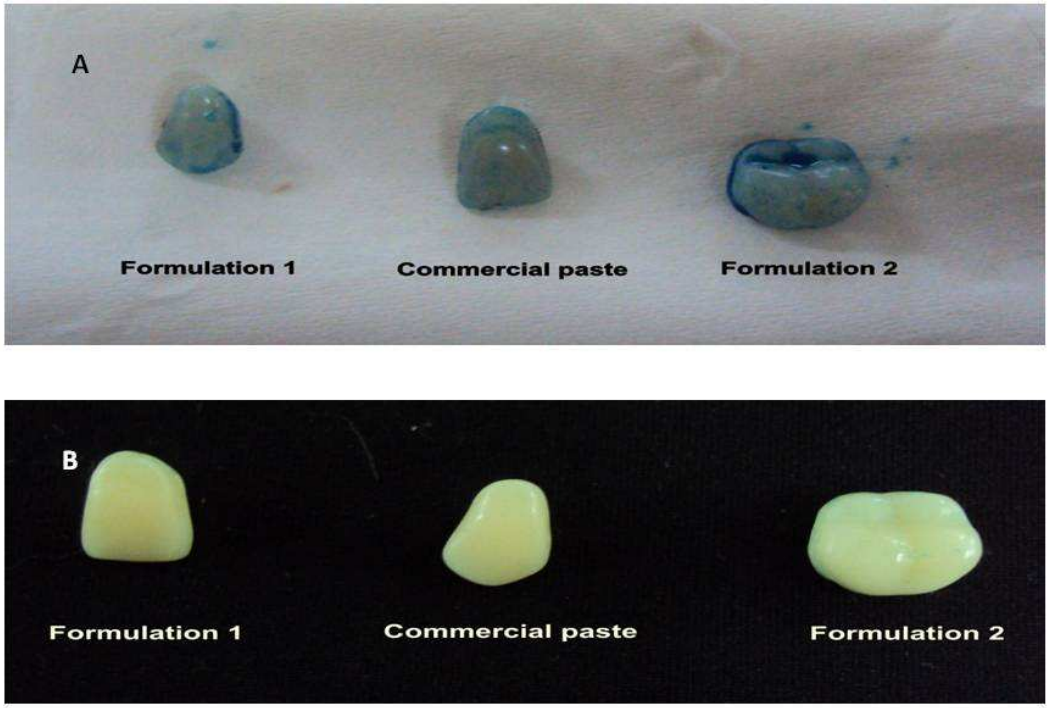

Fig 2. Cleaning ability Test (A. Before cleaning; B. After cleaning)

CONCLUSION

Like chemical surfactants, biosurfactant are excellent emulsifiers and maintain wetting and foaming properties, non toxic in natures that are valued in several applications including the cosmetics industry. Unlike chemical surfactant, biosurfactant are readily biodegradable contributing to environmental compatibility. Overall it may conclude that biosurfactant can act as a replacement candidate for chemical surfactant in cosmetic formulation.

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REFERENCES

[1] EZ Ron and E Rosenberg. *Environ Microbiol*, **2001**, 3:229-236.
[2] AM Shete; G Wadhawa; IM Banat and BA Chopade. *J Sci Ind Res (India)* **2006**, 65:91-115.
[3] R Sharma; Y Chisti and U C Banerjee. *Biotechnology advances* **2001**, 19:627-662.
[4] EN Vulfson. In Industrial Application of Lipases, Cambridge univ. Press 1994, 271-288.
[5] S Li; X Yang; S Yang; M Zhu and X Wang. *CSBJ* **2012**, 2:1-11.
[6] http://australianprescriber.com/magazines/vol17no2/toothpaste.htm
[7] http://www.chymist.com/toothpaste.pdf
[8] C.R Pawar; A.A Gaikwad and R.B Kadtan. *Pharmacologyonline*, **2001**, 2:663-670.
[9] L. Karthik, Gaurav Kumar, Kokati Venkata Bhaskara Rao, *Asian pacific journal of tropical biomedicine*, **2013**, 6(4): 325-332.
[10] http://www.pakshoomerident.com/.../articles/showattachment.aspx?id=10.
[11] I. M. C. Camargo, M. Saiki, M. B. A. Vasconcellos, and D. M. Avila. *J. Cosmet. Sci*, **2001**, vol.52, 163-167.
[12] http://www.dentalprofessionalsonwhitesburg.com/.../**RDA_Val**... - United States.
[13] A O Oyewale. *Journal of Scientific & Industrial Research*, **2005**, vol.64, 101-107.
[14] http://www.caiindia.org.
[15] S Mukherjee; P Das and R Sen. *Trends Bio-technol*, **2006**, 24:509-515.
[16] Environmental Working Group: Skin Deep Cosmetic Safety Database: Sodium Lauryl Sulfate.
[17] H Babich and J.P Babich. *Toxicology letters*, **1997**, vol.91:189-196(8).
[18] B B Herlofson and P Barkvoll. *Acta Odontol Scand*, **1994**, 257-259.

[19] Vance J. "Deathtraps in the cosmetics we use," Consumer Health Organization of Canada.
[20] N Lourith, M Kanlayavattanakul, *Int J Cosmet Sci*, **2009**, 31:225-261.