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Potential Application of Bacillus subtilis Lipopeptides in Toothpaste Formulation

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Abstract: ***Toothpaste is a gel dentifrice used with a toothbrush as an accessory to clean, keep and promotes oral hygiene. Bio toothpaste is the toothpaste that contains biologically active ingredient. In the present work Bacillus subtilis was used as a source for the production of bio surfactant (lipopeptides) or emulsifiers. After 2 days of production period bio surfactant was extracted using acid alkali precipitation method. And finally obtained powdered bio surfactant was used along with the other chemical ingredients for the formation of bio toothpaste. The physicochemical properties were analyzed considering several tests mainly spreading ability, water activity, and pH, foaming and cleaning tests. The evaluation of the antimicrobial activity of the formulated dentifrice was carried out against different bacteria.***
Keywords: Bio toothpaste, Biosurfactant, Lipopeptides, Bacillus subtilis, physiochemical tests, precipitation

I. INTRODUCTION

A. Toothpaste

Toothpaste is used as an effective agent in the removal of plaque bacteria. They are produced to serve multiple purposes like Slight abrasion, froth, sweetening, bleaching, and prevention of plaque, calculus and decay and toothpaste possess complex chemical structures [1].

Toothpastes have been used since the ancient past and are one of the main irreplaceable components of oral health care. The design of toothpaste formulations began in China and India, as 300-500 BC. During that period, squashed bone, pulverized egg and clam shells were utilized as abrasives as a part of tooth cleaning. Modern toothpaste formulations were developed in the 19th century. Later on, chalk and soap were incorporated to those formulations. After 1945, several formulation advancements of different detergents had begun; sodium lauryl sulfate had been used as emulsifying agent [1]. The first toothpaste was invented by the dental surgeon and chemist Washington Went-worth Sheffield [10].

TABLE I
TOOTHPASTE COMPONENTS and ROLE

Components	Role
SLS(sodium lauryl sulphate)	Emulsifying agent or as detergent
Triclosan (2, 4, 4´trichloro-2´ hydroxydiphenyl ether), Zinc chloride (0.30%)	Antibacterial agent
$Al(OH)_3$,$CaCO_3$	Abrasives
NaF , SnF_2	For preventing tooth decay
Peppermint oil ,ginger ,neem ,vanilla	Flavoring agent
Glycerol ,Sorbitol	Others

Sodium lauryl sulphate (SLS) and sodium lauryl sarcosinate are the two most common surface-active agents. Industrially, SLS is used in hard surface cleansing products, grease cleaners, car washing and detergents, personal hygiene products such as shampoos and shower gels, bath foams, face cleansing soaps, and toothpastes as detergents and foaming agents. The frequent use of this material leads to multiple allergic and toxic reactions. Systemic ingestion of SLS compounds may also exert a carcinogenic effect [11]. Several clinical studies have demonstrated the inhibitory effects of the antimicrobial dentifrices and mouth rinses on plaque-associated gingivitis. In the mid-1980s, the American Dental Association (ADA) Council on Dental Therapeutics (now the Council on Scientific Affairs) drafted a set of guidelines to be used to assess both the benefits and risks of active agents in formulations designed to reduce plaque and gingivitis. Shortly thereafter, the U.S. Food and Drug Administration (FDA) adopted similar guidelines. Triclosan, 2, 4, 4´ trichloro-2´-hydroxydiphenyl ether, is a broad-spectrum antibacterial agent that demonstrates in vitro activity against many of the organisms associated with plaque and plaque-associated gingivitis. Triclosan/ copolymer dentifrice can have a profound effect on oral bacteria in plaque related to inflammation and oral malodor [12]. In recent years, the focus has shifted towards the release of active ingredients during formulation developments to prevent and /or treat oral illness [1].The inconsistent eating habit of different age people and the increased consumption of sugar may continuously raise the frequency of these oral diseases. In fact, as reported by the Centre for Disease Control and Prevention (CDC), children suffer from high dental caries prevalence, with 27% of preschoolers and 42% of school-age children. Moreover, 91% of adults have dental caries experience once in lifetime. This oral problem is due to significant role of microorganisms since several bacteria are present in dental plaque. It can be estimated that around 700 bacterial types exist in the human oral micro biome. Therefore, to maintain ideal oral environment, it is important to control these natural processes and the most common and effective factor for cleaning, removing and preventing plaque is carried out thanks to the mechanical action of the toothbrush and not by the toothpaste. However, for most people, brushing alone will be insufficient to maintain plaque control for long period. Moreover, patients search to have an attractive smile, as it is considered synonymous with health. This growing demand for an enhanced esthetic appearance and an improved oral health has led to a great development of dentifrices [10].

B. Bio Surfactants and Lipopeptides

Surfactants are amphipathic molecules consisting both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases having different degrees of polarity and hydrogen bonding; e.g., oil and water or air and water interfaces[4]. The surface and Interfacial tension reducing properties of surfactants confer excellent detergency, emulsifying, foaming and dispersing traits; those make them some of the most versatile process chemicals [2]. Bio surfactants are considered as “green” alternatives to synthetic surfactants [5]. Bio surfactants are surface-active compounds synthesized by microorganisms with pronounced surface and emulsifying activities. Surfactins are not toxic for fungal pathogens by themselves but sustain some synergistic effect on the antifungal activity of iturin A. They act on phospholipids and are able to form selective ionic pores in lipid bilayers of cytoplasmic membranes [9].Some lipopeptides have been studied in greater detail, including surfactin, iturin, bacillomycin, fengycin lichenysin etc. [6]. It possesses various biological activities; anti-microbial, anti-viral, anti-tumor, blood anticoagulant and fibrinolytic activities [5]. Surfactins are especially potent surface-active compounds exhibiting some biological properties such as cytolytic activity and antiviral properties. Iturin A is a strong antifungal agent. Both show hemolytic activities. Surfactin S1 and Iturin A interact in synergism on biological and surface-active properties [26].

II. PRODUCTION OF BIO SURFACTANT

A. Production by bacteria

Bacteria are the main group of biosurfactant-producing microorganisms, although they are also produced by some yeasts and filamentous fungi. These compounds can be synthesized by microorganisms growing on water-immiscible hydrocarbons, as well as on water-soluble compounds such as glucose, sucrose, glycerol, or ethanol, and can be excreted or remain attached to the cell wall [4] very few anaerobic biosurfactant-producing bacteria have been characterized, interest in anaerobic biosurfactant production[8]. A survey of literature shows that biosurfactants are produced by a wide variety of microorganisms; however the chemical nature of biosurfactant is dependent on the producing species. The cell growth and the accumulation of metabolic products of a lipopeptide biosurfactant process are strongly influenced by medium compositions such as carbon sources, nitrogen sources, phosphorous sources, growth factors, and inorganic salt concentrations [5].Among the biosurfactant producing potential microbes, Bacillus subtilis are known to produce cyclic lipopeptides (CLPs) including Surfactins, iturins, fengycins, and lichenysins, as the major classes of biosurfactants. Surfactin-cyclic lipopeptide biosurfactant produced by B. subtilis is one of the most effective biosurfactant which can lower the ST and IFT of water and water-n-hexadecane system from 72 to 27 mN/m and 43 to 1 mN/m respectively [3] Bushnell and Hass (1941) were among the first to demonstrate bacterial production of biosurfactants by isolating Corynebacterium simplex and a strain of Pseudomonas in a mineral media containing either kerosene, mineral oil or paraffin[14]. Bacillus subtilis and Bacillus licheniformis are well- known producers of surface active metabolites. They not only produce good biosurfactants but are also capable to grow under facultative or anaerobic conditions, and have also been reported to be non-pathogenic, which permits their use in food and pharmaceutical industries, apart from environmental applications. The success of biosurfactant production at an industrial scale depends on several strategies, which involved the development of cheaper scaled-up processes, strain improvement, optimization of media components and conditions using statistical methods or the use of inexpensive raw materials [3]

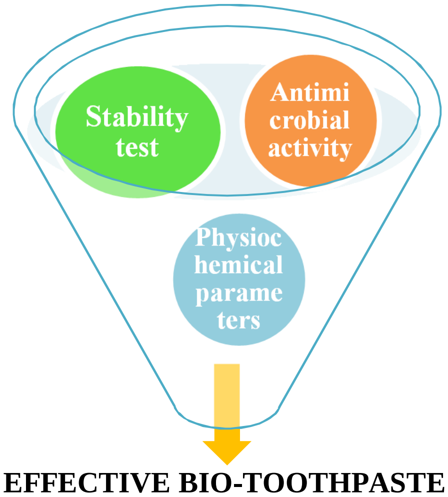

Fig.1 Biotoothpaste

B. Production by different Organisms

In order to reduce the Production costs, surfactin production by *B. subtilis* strains has been studied using different substrates, such as molasses, potato peels, whey powder, and cashew apple juice or cassava waste water[29].

Table ii
Lipopeptide productions

Sr. no.	Organism's name	Growth medium	Production medium	Source of organisms	Reference paper no.
1	Lactobacillus strains	MRS medium	MRS-Lac medium	Dairy	[2]
2	Bacillus subtilis	LB medium	Molasses medium	Petroleum contaminated garbage site	[3]
3	Bacillus subtilis	Nutrient agar medium	Potato peel as substrate+M9 medium	Fermented food and oil contaminated soil	[4]
4	Bacillus subtilis	Nutrient broth	Minimal medium	Pure culture	[5]
5	Bacillus subtilis	LB medium	Growth medium	Oil field	[6]
6	Bacillus mojavensis and Pseudomonas stutzeri	----	Anaerobic fermentation medium	Oil field	[8]
7	Bacillus subtilis	LB medium	M9 medium	Soil	[9]
8	Bacillus subtilis	LB medium	Minimal medium	Soil	[10]
9	Lactobacillus paracasei	MRS media	Fermentation medium	Dairy	[13]
10	YEAST STRAINS:- Saccharomyces	PDA agar medium	MS medium +crude oil	Polluted lagoon water	[14]

	cerevisiae and Candida albicans				
11	Actinomycetes strains	YMD agar medium	Optimized production medium	Laterite soil sample	[20]
12	Bacillus subtilis	Nutrient agar medium	Cassava effluent medium	Pure culture	[25]
13	Bacillus subtilis	Nutrient agar medium	863 medium And optimized medium	Pure culture	[26]
14	Bacillus subtilis	LB medium	Solid-state fermentation production medium	South Tunisian soil	[28]
15	Bacillus subtilis	LB medium	CSL in demineralized water at different conc. [5, 10, and 15% (v/v)]	Pure culture	[29]

LB= Luria-Bertani medium
MRS= medium introduced by DeMan, Rogosa and Sharpe
PDA= Potato dextrose agar
MS= Mineral salts medium
YMD= Yeast extract malt extract dextrose agar
CSL=Corn steep liquor

C. *Sources for the production*

Different agro industrial wastes are studied and reported to be suitable for biosurfactant production: Molasses, sludge palm oil, Cashew apple juice, Cassava waste water. Date molasses can be used as the sole carbon and energy source for biosurfactant production using *Bacillus subtilis* B20 [3].

D. *Extraction Methods*

Produced lipopeptide can be extracted by acid alkali method[10], with phosphate-buffered saline (PBS)[2],by HCl Precipitation[3],[9],centrifugation, filteration[6],[14],acetone precipitation[14].

E. *Physicochemical Evaluation Tests*

The formulated bio toothpastes were subjected to various evaluation tests like pH, Spread ability, abrasiveness, foaming ability, cleaning ability, fineness, moisture and volatile content, tube inertness, Test for F-, Pb, As, and stability studies. Antimicrobial activity of the formulated toothpaste was also determined against mouth common flora (*Bacillus subtilis*) by disc diffusion method and cork borer method.

III. APPLICATIONS OF BIO SURFACTANTS

Many applications have been proposed for biosurfactants including in the bioremediation of contaminated sites or in the food industry due to their ability to solubilize hydrophobic substances in oil–water interfaces [13]. Comparing with chemical surfactants, these compounds have several advantages such as lower toxicity, higher biodegradability, and effectiveness at extreme temperatures and pH values. The diversity of biosurfactants makes them an attractive group of compounds for use in a wide variety of industrial and biotechnological applications such as agriculture, food production, chemistry, cosmetics, and pharmaceutics [2]. B. subtilis, has an average of 4– 5% of its genome devoted to antibiotic synthesis and has the potential to produce more than two dozen structurally diverse antimicrobial compounds. Among these anti-microbial compounds, cyclic lipopeptides (LPs) of the surfactin, iturin and fengycin (or plipastatin) families have well-recognized potential uses in biotechnology and bio-pharmaceutical applications because of their surfactant properties. Surfactin production is not only necessary for root colonization but also for reduction of the infection caused by Pseudomonas syringe on Arabidopsis plants. This disease control was associated with the production of inhibitory quantities of surfactin at the root level [7]. Bio surfactant from anaerobes has been potentially used in enhanced oil recovery [8]. Widespread use of agrochemicals has certainly decreased the outbreak of fungal diseases, but at the same time has contributed to the development of resistant pathogens moreover such chemicals can be lethal to beneficial microorganisms in the rhizosphere and useful soil insects, and they may also enter the food chain and accumulate in the human body as undesirable chemical residues To overcome the above problems, a non-hazardous alternative such as biological control has been extensively studied, and various microorganisms have been reported in the literature to suppress the phytopathogenic fungi[9]. Surfactants play important roles in cosmetic formulations due to their diverse properties. According to the European Commission regulation 2006/257/CE, a surfactant "lowers the surface tension of cosmetics, as well as it aids the uniform distribution of the product when used". Two types of surfactants can be found in the market, those that are produced by chemical synthesis and those that are obtained from microorganisms by biotechnological processes. Currently, the market for beauty and personal care products is seeking for natural ingredients as alternatives to the commonly used chemicals. In this sense, microbial surfactants (also known as biosurfactants) could be among those alternatives given that they are more biodegradable and less cytotoxic than their chemical homologs with the advantage that they can be produced using renewable substrates [13].For many commercial applications in the petroleum and food processing industries biosurfactants play an important role. The potentials of biosurfactants with antimicrobial properties in combating diseases in a world plagued with an increasing prevalence of antibiotics resistance are certainly enormous[14].*Bacillus subtilis* is also used to produce PHA, PHA is biodegradable, water insoluble, non-toxic, bio-compatible, piezoelectric, thermoplastic, and/or elastomeric. These features make them suitable for applications in the packaging industry and as substitute for hydrocarbon-based plastics[30].

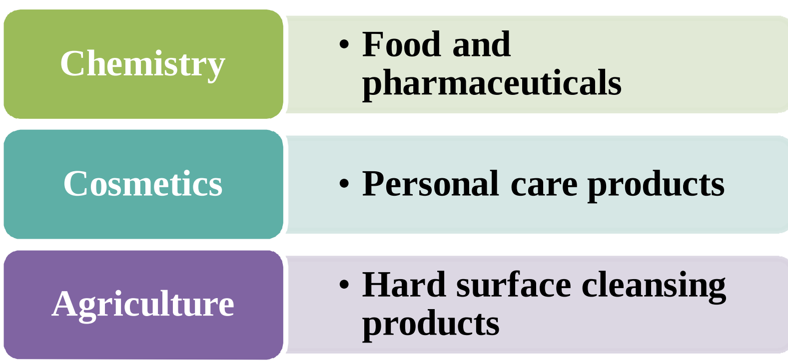

Fig. 2 Applications of Biosurfactant

A. *Disadvantages of biosurfactant*

The costs involved in biosurfactants biotechnological production and recovery can hamper their application in those areas. The cost price can be reduced by optimizing biosurfactant production and by reducing the time of downstream processing [26].

IV. CONCLUSION

The lipopeptides obtained from bacteria is easily isolated, less harmful, having strong antimicrobial activity and thus it can be used for serving the purpose of bio surfactant in many household applications like in the formulation of toothpaste. Mainly bio surfactant is produced using bacillus subtilis bacteria but it can also be produced by some yeast strains. The production of bio surfactant was carried out in minimal agar medium and also with some carbohydrate sources the media was formulated. The extraction was mainly carried out by acid precipitation. The crude bio surfactant/lipopeptides/emulsifier can be used as bio detergent. The toothpaste formulated from bio surfactant was named as bio toothpaste. The physicochemical tests showed satisfactory results and bio toothpaste also showed effective antimicrobial activity.

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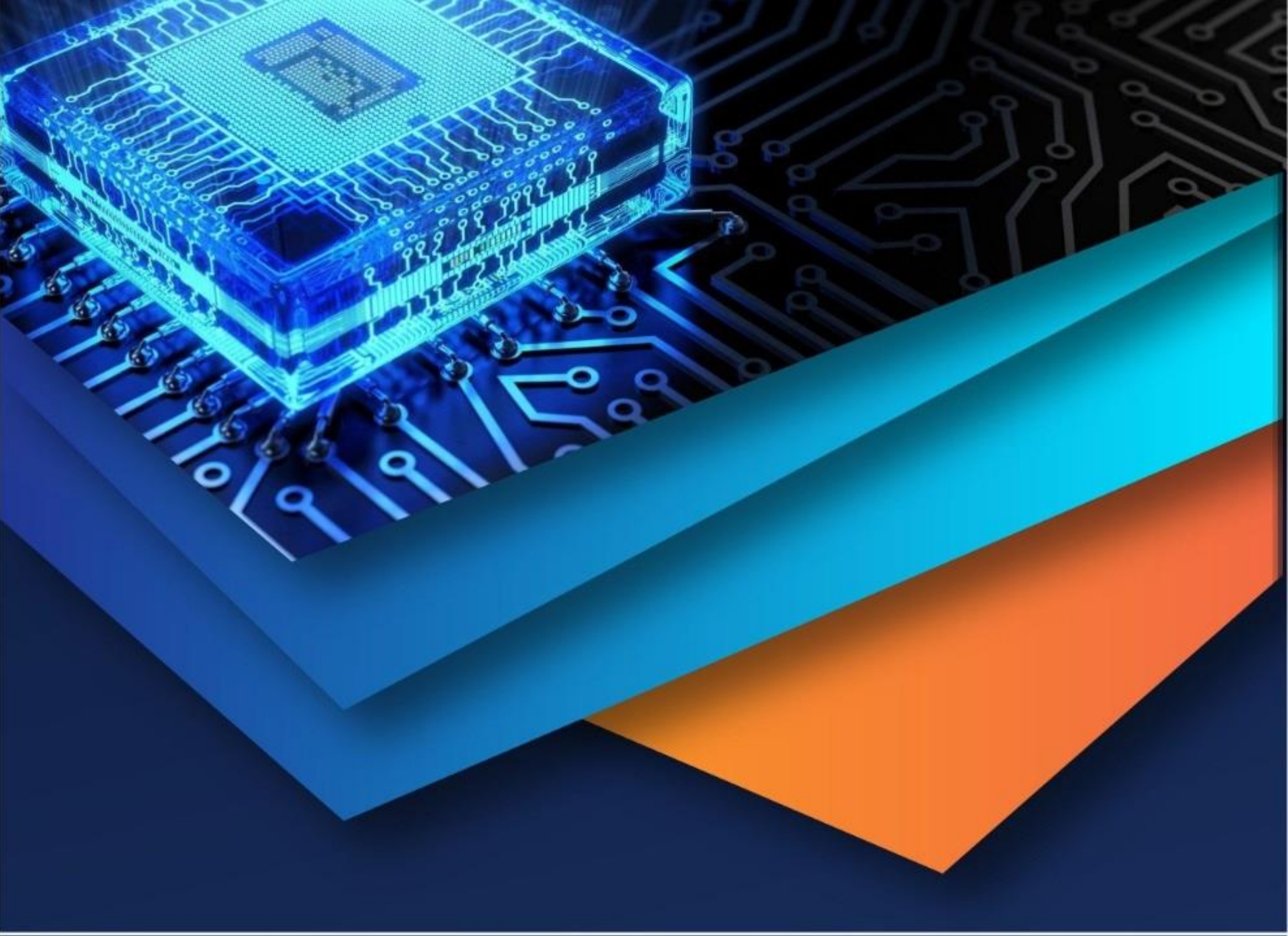

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