**Review of Literature**

The name exopolysaccharides (EPS) as proposed by Sutherland (1972) provides a general term for various forms of bacterial polysaccharides found outside the cell wall. These extracellular, long-chain, high-molecular-mass polymers dissolve or disperse in water to give thickening or gelling properties and are indispensable tools in food product formulation. They consist of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose and rhamnose, in different ratios. They are secreted into their surroundings during growth and are not attached permanently to the surface of the microbial cell. This distinguishes them from the structurally similar capsular polysaccharides (CPS), which do remain permanently attached to the surface of the cell.

Microorganisms produce a large number of structurally diverse extracellular polymeric substances (EPS). Bacterial polysaccharides represent a diverse range of macromolecules that include peptidoglycan, lipopolysaccharide, capsules & exopolysaccharides compound whose function range from structural cell wall component (eg. Peptidoglycon) and important virulence factors (eg. Poly N-acetyl glucosamine in S. aureus.) to permitting the bacterium to survive in harsh environment polysaccharides biosynthesis is a tightly regulated, energy intensive process and understanding the subtle interplay between the regulation and energy conservation, polymer modification and synthesis and the external ecological function is a huge area of research.

**Concept of EPS**

Microorganisms (bacteria, phytoplankton and flagellates) produce high molecular weight, hydrated polymeric compounds called exopolysaccharides (EPS) during their life-cycle (Ducklow and Mitchell 1979). EPS may exist as capsules, sheaths, slimes (loosely attached to the cell wall), apical pads or mesh-like fibrils in the natural environment (Beveridge and Graham 1991, Costerton et al., 1992, Herndl 1993, Hoagland et al 1993, Takeda et al 1998).

Capsules are tightly bound to the cell wall by non-covalent linkages or they may be liberated into medium as loose slime (i.e. ropy polysaccharides) where as sheaths are linear EPS-containing structures surrounding chain of cells. Slime layer is a less organized form of capsule or sheath that diffuses into the surrounding environment (Wingender et al., 1999). Microorganisms produce different forms of EPS to perform diverse functions.

The production of EPS is general property to microorganisms innatural environment. EPS fill and from the space between the cell. They are responsible for architecture and morphology of matrix in which cell live.

Microorganisms can synthesize storage polysaccharides such as glycogen that are located in cytoplasm cell wall structural polysaccharides such as peptidoglycon & lipotechoic acids of gram +ve bacteria & the lipopolysaccharides. Anchored in the outer membrane of gram
Moreover, some bacteria can secrete polysaccharide layer on their surface, which together with a few glycoproteins, all grouped with in the general term glycocalyx.

**EPS COMPOSITION**

The composition of EPS largely depends on the extraction method. The chemical structure of polymeric substances is diversified. EPS compounds belong to such different classed of macro molecules as polysaccharides. Proteins, nucleic acids, glycol proteins, lipids uronic acid depending on their chemical composition, the EPS are classified as homopolysaccharides which contain a single type of monosaccharides & heteropolysaccharides which comprise repeating units of different monosaccharides. The ECP layer form by the accumulation of various type of polymeric substances of high viscosity around bacteria cell walls. The chemical composition, chain length & structure of these together with the molar mass and radius of gyration of the EPS molecule determine the physiochemical characteristic and there by their viscosity intensifying properties. (Law and Marshall, 2001) (Tuinier et at 2001) (Ruas-Madiedou et al., 2002)


Almost all bacteria show nonsaccharide component, such as peptidic moieties, acetyl pyruvyl and / or sulphate group (de philippis et al., 2001)

Extracellular polymers synthesized by tropical inertidal biofilm bacteria appears to be a glycoprotein, a true polysaccharide dominated by neutral sugars but with significant concentration of uronic acids and hexosamines.

Extracellular polymeric substance (EPS) extracted from both sludge & pure bacterial strains isolated from waste water sludge characterized in term of proteins & carbohydrate content lipids and zeta potential.

Most EPS produced by marine bacteria are heteropolysaccharides containing three or four different monosaccharides arranged in group of 10 or less to form repeating units. Components most commonly found in marine EPS are monosaccharides. Such as pentose (as D - arabinose, D – Ribose, D-xylose,) hexoes (D – Glucose, D- Glactose, D- Manoosse, D- Allose, L– Rhlamonose ,L - fucose), Amino sugar, (D-Glicosime & D-Glactosamine ) or uronic acid D-
Glucuronic acids, D-Galacturonic acids) Organic & inorganic substituents such as sulphate, phosphate, acetic acid, succinic acid and pyruvic acid may also present. Some EPS are neutral macromolecules, but the majority of them are polyeanionic for the presence of uronic acids or Ketal – linked pyruvate or inorganic residues such as phosphate or sulphate as well.

Rhodococcus Rhodochocus produced heteropolysaccharides the sugar composition was D-glactose, D-mannose, B-glucose, D-glucosonic acid in 1:1:1:1 ratio and .8% (w/w) Octade Caneic acid and 2.7% (w/w) hexadecanoic acid.

EPS produced by microorganism isolated from cold marine environment (Pseudomonas, strain SM 9913 CAMO25, CAM036) had sulfated heteropolysaccharides, high level of uronic acid with acetyl succinyl groups.

EFFECT OF ENVIRONMENTAL CONDITION

The physical properties of the eps change according to the environmental conditions and growth period. Therefore bacteria may produce a polysaccharide with a specific linkage pattern under one set of environmental conditions but as the environmental cue changes (extreme heat or lack of water), the polysaccharide structure can modify to ensure the viability of the organism.

In a field survey on the effects of a combination of nutrients-nitrogen and phosphorus and operational condition on eps sludge in wastewater treatment plants, it was observed that protein, carbohydrates and total eps differ significantly with a variation in nitrogen concentration (EPS: protein: carbohydrates F=5.747:14.142: 4.912;df =7).however, this ratio was unaffected by different phosphorus content (EPS: protein: carbohydrate F=1.797:0.697:0.785; df=2). Step-wise regression showed that protein was inversely proportional to nitrogen, while unaffected by phosphorus. Whilst both nitrogen and phosphorus were inversely proportional to carbohydrate content, phosphorus had a more pronounced effect on carbohydrate than nitrogen. Protein and carbohydrate content were found to be directly proportional to the eps content (Hoa et al.,2003).

Carbon source concentration, nitrogen supplementation and other nutritional and environmental factors were optimized to obtain maximal eps recovery. Production of eps was strongly influenced by certain environmental factors that is 34c of temperature, neutral or slightly basic media, more than 25% of the carbon source was improved about 42% in comparison to that observed in the initial media (Journal of industrial microbiology and biotechnology vl.27).

“Number of EPS-producing thermophilic LAB strains, i.e. S. Thermophilus and Lactobacillus strains. Not only the influence of physical factors (temperature, pH, and aeration), chemical factors (carbon, source, nitrogen source, carbon/nitrogen ratio, and combinations of carbohydrates), but also the influence of more technological factors (i.e. fed-batch fermentations) were investigated during controlled and non-controlled fermentations.
These industrially relevant factors have led to the conclusion that the maximum EPS production occurs when the maximum bacterial growth was observed, indicating growth-associated EPS production (EU INCO-COPERNICUS Project IC15-CT98-0905)”.

Biopolymers exhibit the required material properties to replace conventional, non-biodegradable, petroleum-based polymer products. They have a closed carbon cycle, making them carbon neutral and environmentally friendly. Biopolymers are produced from non-toxic substrates during in vivo enzymatic reactions (Telana Rapp, 2012).

The C/N ratio is an important factor affecting the biosynthesis of many metabolites. The lower C/N ratio was beneficial for the cell growth, but the EPS yield increased obviously with the increasing C/N ratio.

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**Effect of desiccation**

Bacteria may use EPS production to alter their microenvironment to enhance survival of desiccation. Cultures subjected to desiccation while growing in sand matrix contained more eps and less protein than those growing at high water potential. (Emily B. Roberson et al., 1992) Bacteria possess ability to adapt to the extremes of environmental stress including tolerance of freezing, desiccation, freeze-thaw cycle, and oligotrophic conditions (Scanlan, 2001). They dominate microbial populations of many extreme environments including soda lake (sulfate reducing bacteria; desulfovibrionales), deep sea hydrothermal vent (Pseudomonas, Alteromonas macleodii), thermal springs (Thermococcus litoralis, Synechococcus and Mastigocladus), hot springs (Geobacillus sp. Bcillus licheniformis), hyper saline (Haloferax mediterranei, Hahell chejuensis) and dry desert (Ramlilbacter tataouinensis). In such harsh environments the bacteria produce copious amount of extracellular polysaccharides (EPS) in the form of sheath, slimes and capsules possibly as contrivance to stress (Whitton, 1987). The equisite preservation of these bacteria in the stress situation is thought to reflect the intrinsic stability of the ECP and its ability to bind heavy metals as well as resist degradation. In a variety of ecological niches, organisms compete with each other for survival and through evolution from unique flora. Bacteria are able to produce antimicrobial compounds against competing flora as an exopolysaccharides, which also protect themselves against desiccation, bacteriophages and protozoan attack (Whitfield 1998, Roberts 1995, Weiner et al., 1995).
EPS synthesis represents a focal point of the ability of some bacteria to express desiccation tolerance (Potts, 1999). Its cells produce large amounts of an unusual excreted polysaccharide that contributes to the marked stabilization of cells during prolonged storage in the air dried state, at low or high temperature. The glycan inhibits fusion of membrane vesicles during desiccation and freeze-drying (Hill et al., 1997) and act as an immobilization matrix for a range of secreted enzymes which remain fully active after long term air dried storage (Hill et al., 1994, Schere and Potts, 1989, Shirkey et al., 2000). It also provides a structural and a molecular scaffold with rheological properties that can accommodate the rapid biophysical and physiological changes in the community upon rehydration and during recovery from desiccation. It swells from brittle dried crust to cartilaginous structures within minutes of rehydration (Hill et al., 1994). The EPS layer tend to be more hygroscopic, often contain more water than the bulk environment and may decrease the rate of water loss from the cells (Potts, 1994).

**ECOLOGICAL SIGNIFICANCE**

Although not an essential feature, EPS does provide structural and functional stability to microbial assemblages in the natural environment (Decho 2000).

Soil fertility is combined effect of physio chemical, biological and biochemical properties of the soil salinity on these characteristics render these soil to support the soil microbial process and growth of crop plants (Rahman Banzegar et al., 1996, Cresimanno et al. 1995, Garcia and Hernandez, 1996, Siddiqui et al., 1996, Weigand et al., 1996).

Most microorganisms produce EPS either for attachment to substratum (adhesion), formation of micro-consortium/biofilms or binding to other particulate matter (cohesion or aggregation). EPS produced for attachment by microorganisms may influence biofouling by conditioning the substratum Characklis and Escher 1988). Other functions like gliding motility, protection against osmotic shock, predation, desiccation and detoxification of toxic compounds, nutrient sequestering, chelation of metals, horizontal transfer of 101 genetic material etc. have also been attributed to microbial EPS (Decho 1990, Hoagland et al., 1993). EPS also contain divalent metal cations that act as ionic bridges linking adjacent polysaccharide chains (Rees 1969 & 1972, Fletcher 1980). The presence of side-linkages and organic molecules influence the overall charge stability, binding capacity, rheology and solubility of the polymer (Hoagland et al., 1993).

Soil bacteria are known to excrete a variety of extracellular polymeric substances especially polysaccharides which may plan an important role for their vital function as suggested by many authors (Mazor et al., 1996). The ECP activities of soil microorganisms are thought to enhance soil formation and water retention, stabilize soil, increase the availability of nutrients of plant growing nearby and reduce soil erosion (Johansen, 1993). Because of their
benefit to agriculture, they have been introduced as soil conditioners in many countries (Metting, 1981) and have also been suggested for use as bio-fertilizers (Painter, 1993, Hokputsa et al., 2003).

Sinorhizobium meliloti, the rhizobial symbiont of Lucerne (Medicago sativa) is a widely used inoculant in agricultural industry. In comparison to Rhizobium leguminosarum bv. Trifolii, S. meliloti has much greater survival on pre-inoculated seed.

It has also been suggested that extracellular polymers can bind metal ions and are mobile in the soil environment. Extracellular polymers also appear to be relatively slowly degraded by soil microorganisms. These properties and the supporting model calculations indicate that extracellular polymers of microbial origin merit consideration as agents that may be applied to contaminated soils to enhance trace metal mobility (Chen et al., 1995).

Applications

Extracellular polysaccharides of microorganisms have been used as additives in the food, biotechnology and pharmaceutical field for several years. Microbial EPSs use in foods, drug delivery, oil recovery, water purification and metal removal in mining and industrial waste treatments. Most of these applications take advantages of their rheological properties, ability to form hydrogels and stability at high temperatures and variable PH conditions (Marine Polysaccharides, FoodApplications, Vazhiyil Venugopal CRC Press 2011).

Rheological behaviour of the bacterial EPS is similar to low viscosity Arabic gum. Its viscosity increased in viscosity at longer hydration time. More rheological and toxicological studies are required in order to analyze their possible applications in food industries (Vincente–Garcia et al., 2003). Several microbial extra cellular polymers are used in industries because their physiochemical properties are similar to plant (cellulose, pectin, and starch) and see weed (aginate and carrageenan) polysaccharides. In the food industry, extra cellular polysaccharides produced by lactic acid bacteria (LAB) and other bacteria are used as viscosifiers, stabilizers, emulsifier, or gelling agents to modify the rheological properties and texture of products.

Bacteria released polysaccharides show promise as thickening or suspending agents, emulsifying or cation chelating compounds and the residual capsulated bacterial biomass, following ECP removal, could be an effective cation – chelating material. The microorganisms used as industrial or technical producers of extra polysaccharides. Species of Xanthomonas campestris, Leuconostoc mesenteroides, Pseudomonas alcaligenes, Sphingomonas paucimovilis, which produce xanthan, dextran, gellan, curdlan, are the most known and industrially used (sutherlandan, 1990. Van kranenburg et al., 1999). EPSs produced by microorganisms from extreme habitats show biotechnological promise ranging from pharmaceutical industries, for their immunomodulatory and antiviral effect, bone regeneration and cicatring capacity, to food-processing industries for their peculiar gelling and thickning properties.
Some polysaccharides have unique physiological activities as anti tumor, antiviral and anti-inflammatory agents as well as an inducer of interferons, platelets aggregation inhibition and colony stimulating factor synthesis. EPS isolated from LAB may have anti-tumor activities, cholesterol-lowering ability (Pigeon et al., 2002), immunomodulating (Kitazawa et al., 1998) (Chabot et al., 2001), and could be considered as probiotics (Oda et al., 1983). A probiotic is a mono- or mixed culture of living microorganisms which, applied to animal or man, beneficially affect the host by improving the properties of the indigenous population of gastrointestinal microorganisms. ECPs contributes at the gum structure of bacterial colonies on solid media (biofilm) and at the increase of viscosity in liquids (Wecker, 1992) (Schuster, 1996).

ECPs have specific functions like adhesion to surfaces, protective barrier, symbiotic relationships with plants and animals, structural elements of biofilms, retention of water, sorption of exogenous organic and inorganic compounds and interaction with enzymes and enzymatic activities (Wingender et al., 1999)(Wolframnt et al., 1999). Actually, high attention is accorded to the exopolysaccharides produced by lactic acid bacteria (Sutherland, 2002) (Van Casteren et al., 1998) which are already accepted as GRAS(Generally Recognised As Safe) and the most adequate for the food industry. Another group of microorganisms producing of exopolysaccharides are the cyanobacteria (Moreno et al., 2000)(Bejar et al., 1998).

Properties of EPS (gums) that give them useful performance in the food and non food areas are physical, although sometimes part of the added value is due to organoleptic improvements. Physical effects drive from the interaction of polysaccharide molecules with themselves and with the molecules of their environment. If the biogum is intended for use in these food industry, consideration must be given to acceptability by government agencies. Bacterial polysaccharides if are non toxic, may be used in food industry for following purposes:

1/ In frozen food → As a binder and stabilizer.
2/ In backed food → As a moisture retention agent.
3/ In processed cheese → To improve texture.
4/ In dairy products → As stabilizer.
5/ In dressing and sauces → As an emulsion stabilizer.
6/ In beverages → To stabilize carbonate and bicarbonate.

EPS can also have several usage in industries, viz.

1/ As textile thickener : For sizing printing and finishing of cotton, rayon, silk etc.
2/ In paper industry : To prevent gelation.
3/ In mining industry : As depressant and auxiliary reagent and foam stabilizer.
4/ In oil well drilling: For water loss control, friction reduction, lubrication and drilling of the drill bits.

5/ In explosive industry: As a gelling agent.

6/ In Industrial water treatment: To remove heavy metal ions. A novel extracellular biopolymer was prepared from Pseudomonas fluorescens C-2 for the removal of nickel (II) ions from aqueous solution. (Yanli et al., 2012).

7/ In Fire fighting: As a friction reducing agent.

8/ In agriculture: As an hydrophilic colloid in soil treatment for reclaiming and improving saline and alkaline soil.