MARINE BACTERIAL EXOPOLYSACCHARIDES AN EMERGING NOVEL BIOPOLYMER: PURIFICATION, CHARACTERIZATION AND MERCURY BIOSORPTION STRATEGIES.

Abstract

Marine terrain represents an unfamiliar diversity of microbial population which has adapted extreme condition of that ecosystem. Bacteria is well known group of organisms which are important and dominant occupant of such harsh environments. Marine bacteria are talk of town nowadays due to their immense production and secretion of some industrial important products such as extremozymes, biopolymers, pigments and biosurfactants. This chapter focuses on exopolysaccharide production from marine bacteria and its classification, biosynthesis, characterization along with EPS mediated heavy metal removal strategies through Biosorption.

Keywords: Extremozymes, Biopolymer, Exopolysacchride, Biosorption

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I. INTRODUCTION

Nowadays, biological materials which are secreted externally by marine bacteria in extreme conditions of marine terrain have developed valuable interest for researchers. Furthermore, bacteria live in such extreme conditions must adopt various special metabolic activities and pathways to survive. In order to survival strategy, bacteria are able to secrete special bioactive compound. (Chi and Fang, 2005) Marine bacteria are able to produce various types of extracellular material, in which Exopolysaccharide is one of most important component. In marine terrain, under uttermost stress bacteria protect themselves with the layer of EPS. EPS have different structural properties that makes it an unique and special in various fields.(Gupta and Diwan, 2017) EPSs are known as important secondary metabolite produced by bacteria, algae and fungi. (Sutherland, 1972). EPS term was firstly coined by Sutherland, 1972. EPS is mainly made up of Carbohydrates specially polysaccharide, proteins and nucleic acids. (Flamming and Wingender, 2001) EPS is physiologically complex macromolecule with high molecular weight. The main reason behind high molecular weight is long polymer chain of carbohydrates linked with glycosidic bond. In marine ecosystem, EPS serves as carbon reservoir for marine bacteria, which helps them to survive in utmost environment. (Poli et al., 2010)This chapter highlights the EPS production by marine bacteria, classification, biosynthetic pathways of EPS, characterization of EPS and possible outcomes for mercury biosorption.

II. CLASSIFICATION & BRIEF BIOSYNTHESIS ASPECTS OF EPS

EPSs are categorized in two categories: *viz* Homopolysaccharides and Heteropolysaccharides. The Homopolysaccharides are divided into four groups depend upon glycosidic linkage as per following α-D-glucans, β-D-glucans, fructans and polygalactan and they all containing single monosaccharide type. The Heteropolysaccharides mostly made up of multiple composite of D-glucose, D-galactose, L-rhamnose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine and glucuronic acid. (Nwodo et al., 2012) Glycosidic bond known as backbone of EPS which is present between monosaccharide units. Normal linkages with different monomers are β-1, 4 or β-1, 3 and α-1, 2 or α-1, 6 linkages as per configuration of carbon atom. There are many variations in the structures of homopolysaccharides and heteropolysaccharides that not only show the chemical nature and glycosidic linkage but also reflect site of synthesis of synthetic enzymes. (Rehm, 2010), (Parker et al., 2017)
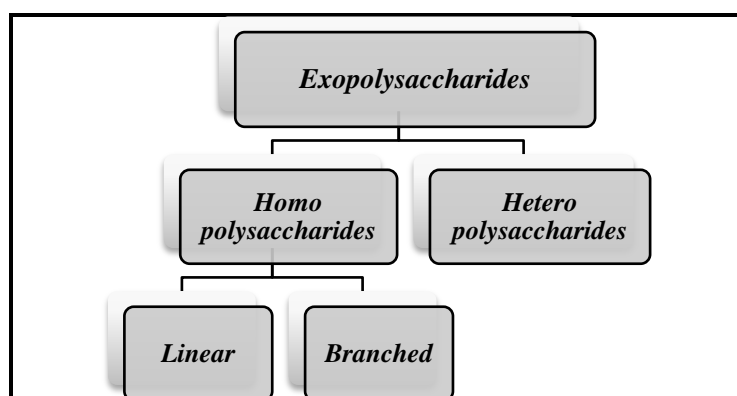
Figure 1: Classification of EPS based on Structure composition

1. **Structural Diversity of EPSs in Marine Territory:** EPSs have broad heterogeneity of structural and functional diversity that reflects their various functions. Structure of EPSs varies from linear to branch as per shown in Figure 1. EPSs in bacteria have different functions based on their monosaccharide composition. Different types of EPSs are categorized as structural polysaccharides, capsular polysaccharides and biofilm-associated polysaccharides. (Chakraborty et al., 2016) Different environmental conditions and circumstances make EPS more concentrate, complex and diverse. (Mayer et al., 1999). Osmotic irregulations in the environment may lead to change the structure of EPS and surface attachment force and capacity. More rough surface more EPS production, so type of surface is a notable parameter. (Flemming and Wingender, 2001)

- **Structural Polysaccharides**: Structural polysaccharides are well known responsible for the structure and shape of cells. (Dumitriu, 2004).. Distinctive feature of these polysaccharides like; elasticity not only protect cell wall but also give shape and integrity. (Matias et al., 2003).

- **Capsular Polysaccharides:** Most of all bacterial species have this type of polysaccharide. Capsular polysaccharide present mainly as heteropolysaccharides or homopolysaccharide along with glycosidic linkages between monosaccharides (Roberts, 1996). Although they are tightly attached to the cell surface, it is loose and not well synchronized structure. (Whitfield, 1988). These polysaccharides have main function to protect bacterial cell from desiccation. (Jenkinson, 1994).

- **Biofilm-Associated Polysaccharides:** Biofilm is known as matrix of EPS producing microorganisms. These types of polysaccharides have novel and diverse material with various physical and chemical properties that make a wall surrounding microorganisms. (Sutherland, 1990). For the formation of biofilm microorganism have adsorption mechanism of nutrients at surface. Eventually whole process starts from attachment and end with EPS synthesis and biofilm formation. (Chakraborty et al., 2016).

2. **Biosynthesis of EPS:** Different environmental and physiological parameters like growth period, pH, and availability of nutrient, temperature and salinity are responsible for biosynthesis of EPS (Harder and Dijkhuizen, 1983). EPS biosynthesis requires consumption of higher amount of energy, the only reason behind it is that EPS differs from one genus to another. (Patel et al., 2010).

There are several steps of EPS biosynthesis listed below:
- Carbohydrate transport into the cell
- Phosphorylation of carbohydrate moiety
- Polymerization of carbohydrate units
- Transport of EPS to the cell surface (Madhuri and Prabhakar, 2014).

This system is different in both homopolysaccharides and heteropolysaccharides. For homopolysaccharides (α-D-glucans, β-D-glucans, fructans and polygalactan), glycosyl-transferase is an enzyme that responsible for the polymerization reaction and also responsible for the formation of glycosidic bonds. (Werning et al., 2014) For heteropolysaccharides with long repeating units, this set of repeating units takes place in the cytoplasm; glycosyl-transferases are responsible for the continuous transfer of sugar

residues to a lipophilic carrier. (Finore et al., 2014) Transport of bacterial EPS have four various mechanisms of synthesis: - (1) Wzx/Wzy dependent pathway (2) ABC (ATP-binding cassette) transporter dependent pathway (3) synthase dependent pathway and (4) Extracellular polymerization (Schmid et al., 2015; Parkar et al., 2016).

- **Wzx/Wzy Dependent Pathway:** The Wzx/Wzy-dependent pathway has been mainly studied in Gram-negative bacteria for heteropolysaccharides production.(Delbarre-Ladrat et al., 2014) In this pathway, individual repeating unit of sugar monomer is connected by diphosphate anchor at inner membrane, and set by small group of glycosyl-transferases and translocated across the cytoplasmic membrane by the protein Wzx which is also called as flippase. Before transportation of EPS takes place to the surface of cell, polymerization happens at the periplasmic space with the help of Wzy protein. Many of the sugar polymers are mustered by the Wzx/Wzy-dependent pathway. Two types of enzymes are used in this pathway, flippase (Wzx) and polymerase (Wzy) (Schmid et al., 2015); (Parkar et al., 2017); (López-Ortega et al., 2021)).

- **ABC (ATP-binding cassette) Transporter Dependent Pathway:** This pathway has more similarities with Wzx/Wzy-dependent pathway. This pathway is used for the synthesis of capsular polysaccharide. (Whitney et al., 2013) capsular polysaccharide synthesized through this path way convey maintained glycolipid at the reducing terminus made of phosphatidylglycerol and a poly2 keto3 deoxyoctulosonic acid. This appears one of the main variations of the Wzx/Wzy and the ABC dependent pathways. (Willis and Whitfield, 2013)

- **Synthase-Dependent Pathway:** The main advantage of this pathway is, it is flippase independent pathway that synthesize complete polymer over cell wall and membrane. Homo polysaccharide can be made through this pathway. The polymerization and translocation process is done by a single synthase protein; else in some cases like alginate, cellulose is a subunit of an envelope-spanning multi-protein complex (Rehm, 2010); Parkar et al., 2017).

- **Extracellular Polymerization:** Homopolysaccharides like dextran, levan and mutan are extracellulerly synthesized polysaccharides. The secretion of dextran and levan are directly induced in the presence of sucrose (Schmid et al., 2015). Dextran is the most common example of sucrase activity, which has α (1-6) glycosidic linkage of glucose. The dextran sucrase is the key enzyme responsible for dextran to release outside. Similarly, levan-sucrase responsible for levan to release outside. (Schmid et al., 2015)

Table 1: Bacterial EPS Components

Components	Examples
Pentose Sugars	D-Xylose , D-Arabinose, D-Ribose
Hexose Sugars	D-Glucose, D-Galactose, D-Mannose, L-Rhamnose, L-Fucose
Organic substances	Acetate, Glycerate, Succinate, Pyruvate,
Inorganic substances	Sulfate, phosphate
Uronic acids	Glucuronic acids, Galacturonic acids,
Amino Sugars	D-Glucosamine, D-Galactosamine

Table 2: Various Exopolysaccharides produced by Bacteria

Bacteria	EPS	Linkage	Substrate	Reference
Acetobacter xylinum	Cellulose	β-(1,4) linked homopolymer	Fructose and Glucose	(Choi, Choi and Lee, 1996)
Pseudomonas aerugina	Alginate	β-(1,4)-linked non-repeating heteropolymer	Xylose	(Celik et al., 2008)
Leucomostoc sp.	Dextran and derivatives	α-(1,2) and α-(1,3) / α-(1,4)-branched α-(1,6)-linked homopolymer	Sucrose	(Santos et al., 2000)
Agrobacterium	Curdlan	β-(1,3)-linked homopolymer	Glucose and Sucrose	(Shih et al., 2009)
Alcaligenes faecalis	Curdlan	β-(1,3)-linked repeating heteropolymer	Glucose	(Wu et al. 2008)
Xanthomonas campestris	Xanthan	β-(1,4)-linked repeating heteropolymer	Molasse	(Kalogiannis et al. 2003)
Bacillus sp.	Levan	Not reported	Sucrose	(Shih et al. 2010)
Enterobacter sp.	Fucopol	Not reported	Glycerol and Glucose	(Alves et al., 2009) and (Torres et al., 2011)

Table 3: Characterization of Exopolysaccharide producing Marine bacteria

Bacteria	Culture Conditions	Medium Composition	Monomers of EPS	Reference
Alteromonas sp. JL2810	pH 7.6 and tem 25°C for 2 days	1% Glucose, 1.9% NaCl, 0.03% NH_4Cl, 0.03% KCl, 0.04% K_2HPO_4, 0.05% $MgSO_4$ $7H_2O$, and 0.003% $CaCl_2$ $7H_2O$	Galacturonic acid ,Mannose, Rhamnose and Galactose	(Zhang et al., 2015)
Enterobacter cloacae Z0206	pH 7.0 and tem 28°C for 2 days	Potato juice (200 g potato); 3 g/L bactopeptone; 3 g/L yeast extract; 20 g/L sucrose	Glucose, Galactose and Mannose	(Xu et al., 2009)
Enterobacter sakazakii.	pH 7.0 - 7.2 and tem 36 °C for 48 h	Glucose (20 g/L), $(NH_4)_2SO_4$ (0.3 g/L), Na_2HPO_4 (10 g/L), KH_2PO_4 (3 g/L), K_2SO_4 (1 g/L), NaCl (1 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $CaCl_2 \cdot 6H_2O$ (0.02 g/L), and $FeSO_4$ (0.001 g/L	Fucose, Glucose and Galactose	(Xiao et al., 2021)
Enterobacter cloacae	Not Reported	Not Reported	Glucose, Fucose and Glucuronic acid	(Wang et al., 2013a)
Enterobacter A47	pH 6.0 to 8.0 and tem 28 to 35°C	Not Reported	Glucose, Galactose, Fucose and Glucuronic acid	(Torres et al., 2012)
Enterobacter A47	Tem 30 ± 0.1 °C and pH 7.00 ± 0.05 for 72 h	Glycerol and Ammonia	Glucose, Galactose, Fucose and Glucuronic acid	(Torres et al., 2011)
Alteromonas pelagimontana	pH 6.0 and tem 35°C for 48 h	5 g Peptic digest, 1 g Yeast extract, 0.1 g $C_6H_5FeO_7$, 8.8 g $MgCl_2.6H_2O$, 3.24 g Na_2SO_4, 1.8 g $CaCl_2.2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 0.034 g $SrCl_2$, 0.022 g H_3BO_3, 0.004 g Na_2O_3Si, 0.0016 g NH_4NO, 0.008 g Na_2HPO_4 and 0.0024 g NaF	Not reported	(Sinha et al., 2017)

Bacillus licheniformis	Tem 30±2 °C for 72 days. pH - 7.0	Nutrient broth with 3.5% NaCl (w/v) and 0.02% (w/v) Glucose	Glucose, Galactose , Mannose and Arabinose	(Singh et al., 2011)
Streptomyces carpaticus No. 3	pH 7, 28 °C	Glucose 30.0, $NaNO_3$ 3.0, Yeast extract 5.0, NaCl 4.0, $MgSO_4$ 0.5, K_2HPO_4 1.0, and $CaCO_3$ 1.0	Galacturonic acid, Glucose, Xylose, Galactose, Mannose, and Fructose	(Selim et al., 2018)
Enterobacter cloacae WD7	Tem 30 °C for 72 h	Glucose, 10, $(NH_4)_2SO_4$, 0.5, Polypeptone, 2, Yeast extract, 0.5, K_2HPO_4, 2, $MgSO_4.7H_2O$, 0.5 and NaCl, 0.1	Not reported	(Prasertsan et al., 2008)
Enterobacter cloacae MBB8	pH 7.2 for 48 h	6.1 g Tris base, 12.3 g $MgSO_4$, 0.74 g KCl, 0.13 g $(NH_4)_2HPO_4$, 17.5 g NaCl, and 0.14 g, $CaCl_2$	Glucose and Fructose	(Prakash Shyam et al., 2021)
Geobacillus thermodenitrificans ArzA-6	pH 7.0 and tem 65 °C	Glucose, 6; Yeast extract, 0.2; Peptone, 0.1; $MgSO_4$, 0.1; KCl, 0.2.	Galactose, Arabinose, Fructose and Glucose	(Panosyan et al., 2018)
Shewanella livingstonensis	20 °C for 24 h	1 g Yeast extract, 5 g Bacteriological peptone, 32 g Artificial sea salts and MA supplemented with 3% (w/v) Glucose	Arabinose and Xylose	(Nichols et al., 2005)
Paenibacillus jamilae	Tem 30 °C , pH 7 for 120 h	NH_4Cl (20 to 190 mM), KH_2PO_4 (5 to 50 mM), $MgSO_4$ $7H_2O$ (40 mg/L), $CaCl_2$ (10 mg/L), $SO_4Fe.7H_2O$ (20 mg/L), $MnSO_4$ H_2O (10 mg/L), and KCl (50 mg/L)	Not reported	(Morillo et al., 2006)
Bacillus altitudinis MSH2014	Tem 37 °C for 48 h	Glucose (20); Yeast extract (0.1); NH_4NO_3 (0.8); $CaCO_3$ (1); K_2HPO_4 (0.6); KH_2PO_4 (0.5); $MgSO_4.7H_2O$ (0.05), $MnSO_44H_2O$ (0.1)	Mannuronic acid and Glucose	(Mohamed et al., 2019)

Idiomarina fontislapidosi F32	32 °C	7.5%. (w/v) Salts;1% (w/v) Glucose	Glucose, Mannose and Galactose	(Mata et al., 2008)
Idiomarina ramblicola R22	32 °C	7.5% (w/v) Salts; 1% (w/v) Glucose	Glucose, Mannose and Galactose	(Mata et al., 2008)
Alteromonas hispanica F23	32 °C	7.5% (w/v) Salts; 1% (w/v) Glucose	Glucose, Mannose and Xylose	(Mata et al., 2008)
Salipiger mucosus A3	32 °C for eight days	7.5% (w/v) Salts and Glucose 10 g/L	Fucose, Galactose, Glucose and Mannose	(Llamas et al., 2010)
Pseudoalteromonas sp. Strain SM20310	pH 7.5 and tem 15 °C for 72 h	(5 g/L Peptone , 1 g/L Yeast extract, ,Artificial sea water) supplemented with 3 g/L Glucose	Xylose, Mannose, Glucose, Galactose & Rhamnose	(Liu et al., 2013)
Paenibacillus Spp.	pH 8.0, tem 24 °C for 60 h	(Sucrose 188.2 g/L, Yeast extract 25.8 g/L, K_2HPO_4 5 g/L, $CaCl_2$ 0.34 g/L	Glucose, Mannose, Galactose and Glucuronic acid	(Liang and Wang, 2015)
Kosakonia sp. CCTCC M2018092	pH 7.0 and tem 30 °C	Not reported	L-Fucose, D-Glucose, D-Galactose, D-Glucuronic acid	(Li et al., 2020)
Halomonas elongata S6	pH 7 and tem 37°C for 5 days	(g/L): NaCl, 50; $MgCl_2 \cdot 6H_2O$, 13; $MgSO_4 \cdot 7H_2O$, 9; KCl, 1.3, $CaCl_2 \cdot 2H_2O$, 0.2; NaBr, 0.15; $NaHCO_3$, 0.05; Yeast extract, 0.3; Peptone 0.2 and Glucose 10	Glucose, Rhamnose, Mannose and Glucosamine	(Joulak et al., 2020)
Enterobacter cloacae Z0206	30°C for 2 days	Dextrose, 2.5%; Peptone, 0.5%; Yeast extract, 0.5%, K_2HPO_4, 0.2%; KH_2PO_4, 0.1% and $MgSO_4 \cdot 7H_2O$, 0.05%.	Glucose, Mannose and Galactose	(Jin et al., 2010)
Halorubrum sp.	pH 8 and tem 30°C	Glucose, 10 g; $MgCl_2 \cdot 7H_2O$, 5.9 g; $MgSO_4$, 3.24 g; $CaCl_2$, 1.8 g; KCl, 0.55 g; $NaHCO_3$,	Mannose, Glucosamine, Galacturonic acid,	(Hamidi et al., 2018)

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TBZ112		0.16 g; KBr, 0.08 g; SrCl$_2$, 34.0 mg; H$_3$BO$_3$, 22.0 mg; Na$_2$O$_3$Si, 4.0 mg; NaF, 2.4 mg; NH$_4$ NO$_3$, 1.6 mg; Na$_2$HPO$_4$, 8.0 mg; Peptone, 5 g and Yeast extract 1 g	Arabinose, and Glucuronic acid	
Halomonas sp. *TG39*	Not reported	3/4-strength Seawater, 0.5% Peptone, 0.1% Yeast extract	Not reported	(Gutierrez et al., 2009)
Enterobacter strain A47 (DSM 23139)	temperature 30.0±0.1 °C pH 6.80±0.05	Not reported	Fucose, Galactose, Glucose, Pyruvate, Succinate and Acetate	(Freitas et al., 2011)
Klebsiella sp	Not reported	Not reported	Glucose, Fructose, Galactose, Fucose and Uronic acid	(Essawy et al., 2017)
Vibrio harveyi strain VB23	pH 7 and tem 28°C for 48 h	MSM medium supplemented with NaCl 1.5% (w/v), 0.2% Glucose	Galactose, Glucose, Rhamnose, Fucose, Ribose, Arabinose, Xylose and Mannose	(Bramhachari and Dubey, 2006)
Klebsiella oxytoca KY498625	37 °C for 24 h	0.2g KH$_2$PO$_4$; 1.5g K$_2$HPO$_4$; 0.2g MgSO$_4$.7H$_2$O; 0.1g CaSO$_4$.2H$_2$O; 2.0 mg FeCl$_3$; 0.5g Yeast Extract, 20g Sucrose	Not Reported	(Am Moghannem et al., 2017)
Enterobacter sp.	pH 6.8 and tem 30 °C	Not reported	Fucose, Galactose and Glucose	(Alves et al., 2009)
Enterobacter sp. strain ACD2	pH 7.0	3% Glucose, 0.25% Casein hydrolysate, 0.4% K$_2$HPO$_4$, 0.07% MgSO$_4$.7H$_2$O, and 0.005% ZnSO$_4$.4H$_2$O	Fucose, Galactose, Glucose, and Glucuronic acid	(Almutairi and Helal, 2021)
Pseudoalterom onas sp. AM	pH-7.0	(g/L): Casein hydrolysate 2.5; K$_2$HPO$_4$ 4.0; MgSO$_4$.7H$_2$O 0.7; MnSO$_4$.7H$_2$O 0.05; and Glucose 30.0	Glucose	(Al, 2011)

III. PURIFICATION AND CHARACTERIZATION OF EPS

For any fermentation, the downstream process (Figure 2.2) is the most valuable and notable process to purify the product. In case of marine EPS purification, EPS is associated with different metals, Proteins, Amino acids and many salts. So, downstream process take an account for safely isolation of EPS from mixture of broth.(Delbarre-Ladrat et al., 2014)

1. **Cell Removal:** Mainly the first step of downstream process is to remove bacterial cell from fermented broth. Cell removal is one of the important steps including in downstream process. Mainly Centrifugation and Filtration which are widely used to isolate cell bound EPS from cell without any functional damage. The physical separation techniques will able to separate the EPS from cells.(Donot et al., 2012) Capsular and Loosely bound EPS can be removed to surround of the cell mainly with different duration and speed of centrifugation.(Dave et al., 2020) Filtration is known as a good method to separate high molecular weight molecules from small bounded components.(Delbarre-Ladrat et al., 2014)

2. **EPS Precipitation:** Separation of the EPS can be achieved by precipitation with the addition of organic solvents like alcohols and acetone. As EPSs are naturally hydrophilic because of hydroxyl groups and carboxyl groups, it will become more difficult to separate by chemical methods. Some chemical, enzymes and deprotenization procedures may affect yield and recovery of EPS (Delbarre-Ladrat et al., 2014) Acetone and alcohols *viz* methanol, ethanol and isopropanol can be added to the fermentation medium to decrease the solubility of EPS and also to remove some protein and DNA contamination.(Dave et al., 2020)

3. **Chemical Characterization of EPS for Structure Elucidation:** The primary characterization of EPS includes various parameters like the determination of total carbohydrate, protein contents. The monosaccharide composition is usually identified by hydrolysis with trifluoroacetic acid (TFA) (2–4 M), HCl and H_2SO_4 (1 M) (Casillo et al., 2018) Carbohydrate is non volatile due to lack of chromophores and luminophore groups within it. Thus, sugars are often going for chemical derivatization. Derivatised glycoconjugates converted into volatile nature and can be easily identified by analytical methods such as GC, GLC (Concórdio-Reis et al., 2021), LC-MS by GC–MS (Corsaro et al., 2004). Hydrolysed product can be identified through paper, thin layer chromatography and High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), HPLC-RI. (Panosyan et al., 2018)

4. **Various biotechnological and microbiological applications of Marine EPS:** Marine ecosystem harbors enormous microbial biodiversity which produces structurally diverse EPSs. They are used various fields *viz* in food, textile, detergents, pharmaceutical, agricultural, paper, paint and oil industries, in bioremediation processes, in drug delivery and cancer therapy.

   - **In Food industries:** Most of marine EPS used as emulsifying or stabilizing agents in food industry. Many EPS have hydrophobic nature with high molecular mass can be encouraged surface activity and hence can be used in process of emulsification.(López-Ortega et al., 2021) *Halomonas* sp. H96 has acidic EPS

exhibits high amount of uronic acid may affect the viscosity of EPS. (Béjar et al., 1998) *Sphingomonas elodea a* non-halophilic bacteria secreted EPS gellan which has similar uronic acid content as halophilic bacteria. Gellan from moderately halophile *Sphingomonas paucimobilis* ATCC 31461was used as food additive which was approved by the FDA. (Prajapati et al., 2013)

- **In medical field:** EPSs have been applied in medical field due to their distinct properties compared to other biocomponents secreted from bacteria. EPSs have been used in various biomedical applications like as ophthalmic, tissue regeneration, antitumor, anti-inflammatory, fibrinolytic agents etc. (Shih, 2010). Glycosaminoglycans one type of EPSs are notably put in application in field of glycobiology, which is used as therapeutic drugs.(Delbarre-Ladrat et al., 2014) EPS of *Geobacillus thermodenitrificans* strain B3-72 can be used as antiviral agent on immuno-competent cells.(Arena et al., 2009)

- **Metal bioremediation:** EPSs are mainly made of different negatively charged functional groups and differ in interaction with various ionic compounds (Zhang et al., 2010). Nowadays bacterial EPS is used in for metal remediation in industrial as well as environmental waste water sources. EPSs have some non-carbohydrate components such as proteins and nucleic acids. They are unique because of their tertiary structure and rigidness. Furthermore presence of uronic acid which leads to negative charge on the EPS that makes it acidic also. (Iyer et al., 2005) Various bacterial EPS mediated Heavy metal biosorption shown in table 4
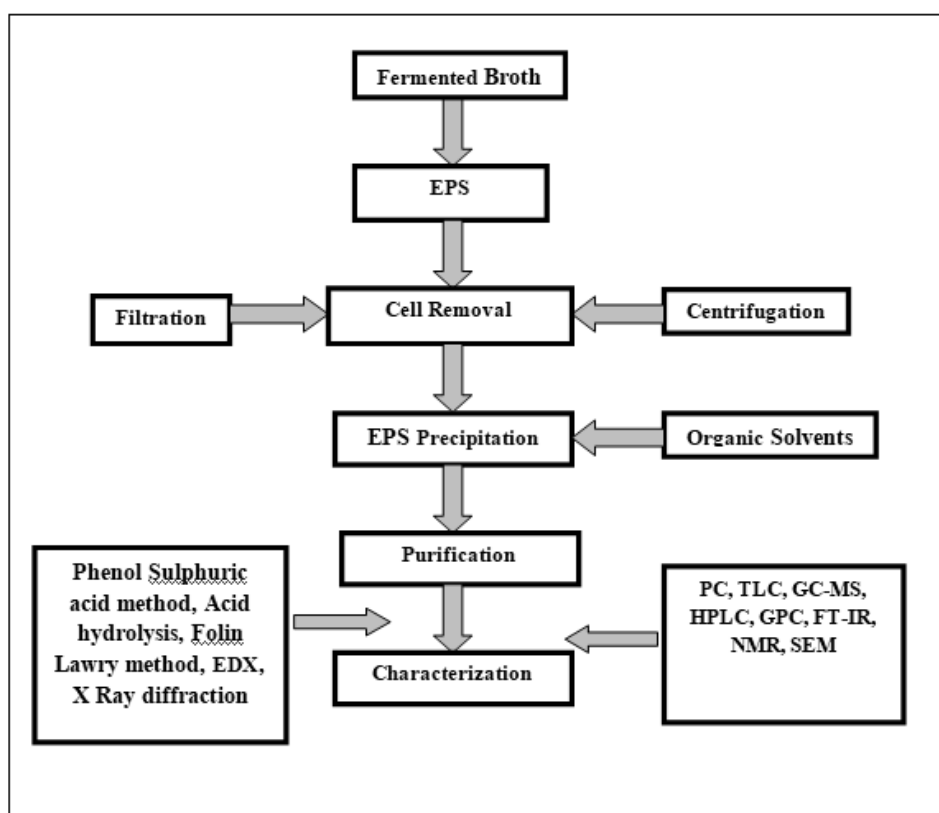
Figure 2: Schematic diagram of downstream process and characterization of EPS

Table 4: Reported various metal biosorption by bacterial EPS

EPS producing Bacteria	Metal Biosorption	Reference
Azotobacter chroococcum XU1	Pb and Hg	(Rasulov et al., 2013)
Enterobacter cloacae	Cr	(Iyer et al., 2005)
Paenibacillus jamilae	Pb (II), Cd (II), Cu (II), Zn (II), Co (II), and Ni (II)	(Morillo et al., 2006)
Pseudarthrobacter oxydans strain MM20	Hg	(Mechirackal Balan et al., 2018)
Pseudomonas frederiksbergensis strain SS18	Hg	(Mechirackal Balan et al., 2018)
Enterobacter sp.	Pb	(Li et al., 2021)
Klebsiella sp. J1	Pb	(Wei et al., 2016)
Pseudomonas putida	Cd	(Ueshima et al., 2008)
Rhizobium radiobacter	Pb, Zn	(Wang et al., 2013b)
Sinorhizobium meliloti	As, Hg	(Nocelli et al., 2016)
Stenotrophomonas maltophilia	Cu, Cr	(Kiliç et al., 2015)
Bacillus thuringiensis PW-05	Hg(II)	(Dash and Das, 2016)
Bacillus sp.	Pb, Cd, Cu	(Shameer, 2016)
Klebsiella sp.	Hg(II)	(Xia et al., 2020)

IV. MERCURY POLLUTION & MERCURY BIOSORPTION STRATEGIES

Mercury (Hg) the most dangerous, strong toxic heavy metal which can affect badly the nervous system. This heavy metal has typical property of remains liquid at room temperature, which leads to distinguish it from other elements. It is highly tenacious pollutant that is distributed worldwide because of its powerful bioaccumulative property in the ecosystem. Most danger feature of Hg is that it is a non-decaying element and this is the main reason behind it remains in the environment for many years.(Singh and Kumar, 2019) Mercury pollution, which may have several consequences like cell apoptosis, defects in birth, neurological disorders and many more in humans. Mercury is mainly discharged into the environment from industry. The main case study of Minamata convention has been affected researchers worldwide and forced them to think regarding removal and recovery of the mercury as a pollutant from the environment.(Xia et al., 2020)

1. **EPS mediated heavy metal removal by Biosorption:** In the environment heavy metals like Ni, Cu, Zn, Cd, Cr, Pb, and Hg are known as major pollutants which enter into any water, air and in the soil through various types of industries. Due to their various characteristics *viz* non-biodegradability, toxicity and persistent, they assemble in the different ecosystem and that leads to serious health conflicts. To overcome this problem many conventional methods have been used. There are many methods like chemical precipitation, ultra-filtration, ion exchange, reverse osmosis, electro winning, and phytoremediation. Despite all methods are conventional, they have many disadvantages

and many expensive. In order to resolve the heavy metal contamination, there was demand for one method which has good outcomes with alternatives and cost-effective technologies. In recent biosorption method has been recommended as advanced, profitable, well structured, and environment friendly treatment technology for the removal of heavy metals from contaminated sites.(Kanamarlapudi et al., 2018)

2. **Biosorption:** Biosorption is a simple process in which heavy metals (biosorbate) bind to the surface of the biosorbent of biological material. (Joshi NC, 2017) There are many types of biological material which can be used as biosorbent like microorganisms, plant based derivatives, agricultural waste, extracellular polymeric substances. This process functionally work with various functional groups present on the biosorbent and heavy metal in aerobic as well as anaerobic metabolism.(Davis et al., 2003) This method is conventional method which have many advantages over to the other methods, these include method is simple, no nutrient need, low operational expense and biosorbent regeneration capacity.(Kanamarlapudi et al., 2018)

V. BIOSORPTION MECHANISM

The mechanism of biosorption is a complex process which involves the binding of sorbate onto the biosorbent. Many biological and non biological materials can be used as biosorbents. They can bind to the heavy metals through physical as well as chemical bonding. Biosorbents have various functional groups like carboxyl, ester, carbonyl, sulphate, phosphate, phosphodiester, phenolic and etc that can help to bind metal ions.(Park et al., 2010) Biosorption of heavy metals goes through various interactions such as ion exchange, complexation, physical adsorption and precipitation showed in figure 3.

1. **Cell Surface Adsorption:** The Non metabolic process in which heavy metal will bind to the cell wall of microbial biomass, it is one type of physical adsorption (Joo et al., 2010). Basically, biosorption is based on the cell wall structure of microorganisms. Many anionic groups are present on the cell surface of microorganisms, which help to create negative charge that can attract the metal positive ions to bind on the surface. There are many anionic groups which are notably involved in this process, and they are mainly alcohols, amines, carboxyl, hydroxyl, ester, phosphoryl, sulfonate, thioester and thiol etc. (Beveridge, 1989).

2. **Extracellular Assemblage:** This mechanism has more advantages nowadays as many bacteria are able to secrete different types of metabolites such as EPSs. Mainly they are made up of carbohydrates, proteins, uronic acids, lipids, capsules and slimes. (Ayangbenro and Babalola, 2017) It is high molecular weight macro molecule and very moisture absorptive in nature. EPS contains large amount of hydroxyl group which makes EPS versatile to bind with metal ions. Microbe's species variation and nutritional requirement may change metal binding property (Gupta and Diwan, 2017)

3. **Intracellular Assemblage:** In this mechanism, cellular constituents help metal to bind and accumulate inside the cell and it is energy dependent process. In many instances, the metals may be settled in their original elemental form.(Gupta and Diwan, 2017)

4. **Precipitation:** In many instances, metal will be precipitated on the surface of microbial cell and mostly in insoluble form. Most of the EPSs secreted by the bacteria are involved in the generation of organic precipitates. (Kanamarlapudi et al., 2018)

VI. PROCESS FACTORS INFLUENCING BIOSORPTION

The biosorption ability of each and every biosorbent riles on various influencing factors like pH, initial metal ion concentration, biosorbent concentration of biosorbent and contact time. pH plays an important role for metal ion solubility and charging of biosorbent. (Torres, 2020) Alkali pH helps the metal ions for binding to active sites of biosorbent surface. At acidic pH, the binding of metal ions is insignificant. (Feng et al., 2011). The initial metal ion concentration is also known as major factor in biosorption. Increase in the initial metal ion concentration leads to increase in the biosorption capacity (Naiya et al., 2009).The biosorbent concentration regulates its prospective to remove metal ions at a given metal concentration. The increased bioadsorbent concentration will provide better surface circumference and availability of active sites that leads to the improvement sorption of metal ion. (Kumar et al., 2011); (Torres, 2020)
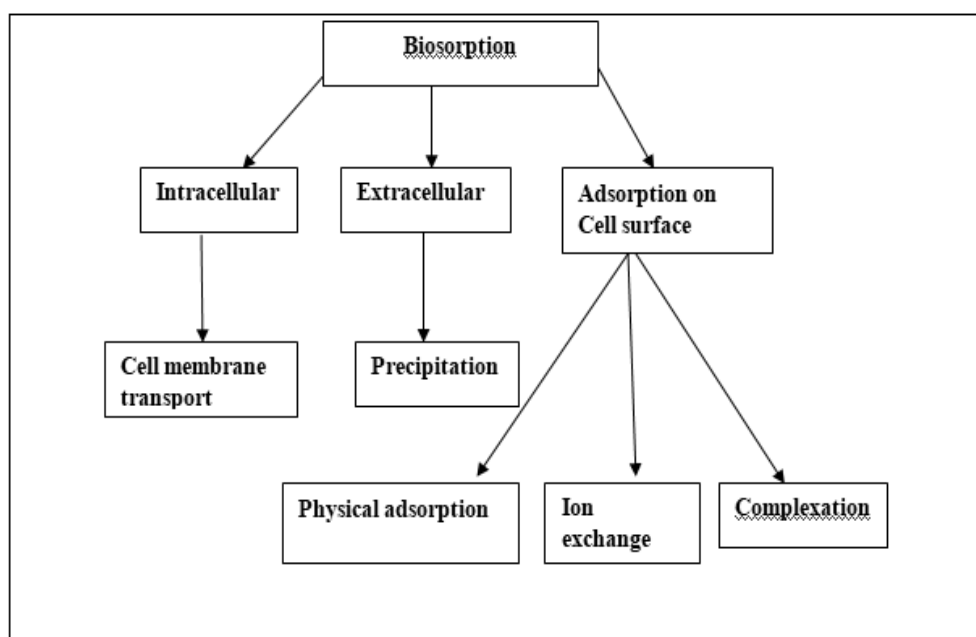
Figure 3: Biosorption types according biosorption location (Park et al., 2010)

REFERENCES

[1] AL, N. 2011. Characterization of an exopolysaccharide-producing marine bacterium, isolate Pseudoalteromonas sp. AM. *African Journal of Microbiology Research,* 5.

[2] ALMUTAIRI, M. H. & HELAL, M. M. I. 2021. Exopolysaccharide production from isolated Enterobacter sp. strain ACD2 from the northwest of Saudi Arabia. *Journal of King Saud University - Science,* 33**,** 101318.

[3] ALVES, V. D., FREITAS, F., TORRES, C. A. V., CRUZ, M., MARQUES, R., GRANDFILS, C., GONÇALVES, M. P., OLIVEIRA, R. & REIS, M. A. M. 2009. Rheological and morphological

characterization of the culture broth during exopolysaccharide production by Enterobacter sp. *Carbohydrate Polymers*.

[4]   AM MOGHANNEM, S., M.S.FARAG, M., SHEHAB, A. M. & SALAH AZAB, M. 2017. Media Optimization for Exopolysaccharide Producing Klebsiella oxytoca KY498625 under Varying Cultural Conditions. *International Journal of Advanced Research in Biological Sciences (IJARBS),* 4**,** 16-30.

[5]   ARENA, A., GUGLIANDOLO, C., STASSI, G., PAVONE, B., IANNELLO, D., BISIGNANO, G. & MAUGERI, T. L. 2009. An exopolysaccharide produced by Geobacillus thermodenitrificans strain B3-72: Antiviral activity on immunocompetent cells. *Immunology Letters,* 123**,** 132-137.

[6]   AYANGBENRO, A. & BABALOLA, O. 2017. A New Strategy for Heavy Metal Polluted Environments: A Review of Microbial Biosorbents. *International Journal of Environmental Research and Public Health,* 14**,** 94.

[7]   BÉJAR, V., LLAMAS, I., CALVO, C. & QUESADA, E. 1998. Characterization of exopolysaccharides produced by 19 halophilic strains of the species Halomonas eurihalina. *Journal of Biotechnology,* 61**,** 135-141.

[8]   BRAMHACHARI, P. V. & DUBEY, S. K. 2006. Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. *Lett Appl Microbiol,* 43**,** 571-7.

[9]   CASILLO, A., LANZETTA, R., PARRILLI, M. & CORSARO, M. M. 2018. Exopolysaccharides from Marine and Marine Extremophilic Bacteria: Structures, Properties, Ecological Roles and Applications. *Mar Drugs,* 16.

[10]   CONCÓRDIO-REIS, P., ALVES, V. D., MOPPERT, X., GUÉZENNEC, J., FREITAS, F. & REIS, M. A. M. 2021. Characterization and Biotechnological Potential of Extracellular Polysaccharides Synthesized by Alteromonas Strains Isolated from French Polynesia Marine Environments. *Marine Drugs,* 19**,** 522.

[11]   CORSARO, M. M., LANZETTA, R., PARRILLI, E., PARRILLI, M., TUTINO, M. L. & UMMARINO, S. 2004. Influence of Growth Temperature on Lipid and Phosphate Contents of Surface Polysaccharides from the Antarctic Bacterium Pseudoalteromonas haloplanktisTAC 125. *Journal of Bacteriology,* 186**,** 29-34.

[12]   DASH, H. R. & DAS, S. 2016. Interaction between mercuric chloride and extracellular polymers of biofilm-forming mercury resistant marine bacterium Bacillus thuringiensis PW-05. *RSC Advances,* 6**,** 109793-109802.

[13]   DAVE, S. R., UPADHYAY, K. H., VAISHNAV, A. M. & TIPRE, D. R. 2020. Exopolysaccharides from marine bacteria: production, recovery and applications. *Environmental Sustainability,* 3**,** 139-154.

[14]   DAVIS, T. A., VOLESKY, B. & MUCCI, A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research,* 37**,** 4311-4330.

[15]   DELBARRE-LADRAT, C., SINQUIN, C., LEBELLENGER, L., ZYKWINSKA, A. & COLLIEC-JOUAULT, S. 2014. Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecules. *Front Chem,* 2**,** 85.

[16]   DONOT, F., FONTANA, A., BACCOU, J. C. & SCHORR-GALINDO, S. 2012. Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers,* 87**,** 951-962.

[17]   ESSAWY, A., SHADY, H., KHER, A. & HELAL, M. 2017. Antimicrobial, Anticoagulation, Fibrinolytic and Prebiotic Activities of Exopolysaccharide Produced by Marine Klebsiella Sp. *THE EGYPTIAN JOURNAL OF EXPERIMENTAL BIOLOGY (Botany)***,** 1.

[18]   FINORE, I., DI DONATO, P., MASTASCUSA, V., NICOLAUS, B. & POLI, A. 2014. Fermentation technologies for the optimization of marine microbial exopolysaccharide production. *Mar Drugs,* 12**,** 3005-24.

[19]   FREITAS, F., ALVES, V. D., TORRES, C. A. V., CRUZ, M., SOUSA, I., MELO, M. J., RAMOS, A. M. & REIS, M. A. M. 2011. Fucose-containing exopolysaccharide produced by the newly isolated Enterobacter strain A47 DSM 23139. *Carbohydrate Polymers,* 83**,** 159-165.

[20]   GUPTA, P. & DIWAN, B. 2017. Bacterial Exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies. *Biotechnol Rep (Amst),* 13**,** 58-71.

[21]   GUTIERREZ, T., MORRIS, G. & GREEN, D. H. 2009. Yield and physicochemical properties of EPS from Halomonas sp. strain TG39 identifies a role for protein and anionic residues (sulfate and phosphate) in emulsification of n-hexadecane. *Biotechnol Bioeng,* 103**,** 207-16.

[22]   HAMIDI, M., MIRZAEI, R., DELATTRE, C., KHANAKI, K., PIERRE, G., GARDARIN, C., PETIT, E., KARIMITABAR, F. & FAEZI, S. 2018. Characterization of a new exopolysaccharide produced by Halorubrum sp. TBZ112 and evaluation of its anti-proliferative effect on gastric cancer cells. *3 Biotech,* 9.

[23] JIN, M., WANG, Y., XU, C., LU, Z., HUANG, M. & WANG, Y. 2010. Preparation and biological activities of an exopolysaccharide produced by Enterobacter cloacae Z0206. *Carbohydrate Polymers,* 81**,** 607-611.

[24] JOULAK, I., AZABOU, S., FINORE, I., POLI, A., NICOLAUS, B., DONATO, P. D. I., BKHAIRIA, I., DUMAS, E., GHARSALLAOUI, A., IMMIRZI, B. & ATTIA, H. 2020. Structural characterization and functional properties of novel exopolysaccharide from the extremely halotolerant Halomonas elongata S6. *International Journal of Biological Macromolecules,* 164**,** 95-104.

[25] KANAMARLAPUDI, S. L. R. K., CHINTALPUDI, V. K. & MUDDADA, S. 2018. Application of Biosorption for Removal of Heavy Metals from Wastewater.

[26] LI, S., XIA, H., XIE, A., WANG, Z., LING, K., ZHANG, Q. & ZOU, X. 2020. Structure of a fucose-rich polysaccharide derived from EPS produced by Kosakonia sp. CCTCC M2018092 and its application in antibacterial film. *Int J Biol Macromol,* 159**,** 295-303.

[27] LI, Y., XIN, M., XIE, D., FAN, S., MA, J., LIU, K. & YU, F. 2021. Variation in Extracellular Polymeric Substances from Enterobacter sp. and Their Pb2+ Adsorption Behaviors. *ACS Omega,* 6**,** 9617-9628.

[28] LIANG, T.-W. & WANG, S.-L. 2015. Recent Advances in Exopolysaccharides from Paenibacillus spp.: Production, Isolation, Structure, and Bioactivities. *Marine Drugs,* 13**,** 1847-1863.

[29] LIU, S. B., CHEN, X. L., HE, H. L., ZHANG, X. Y., XIE, B. B., YU, Y., CHEN, B., ZHOU, B. C. & ZHANG, Y. Z. 2013. Structure and ecological roles of a novel exopolysaccharide from the arctic sea ice bacterium Pseudoalteromonas sp. Strain SM20310. *Appl Environ Microbiol,* 79**,** 224-30.

[30] LLAMAS, I., MATA, J. A., TALLON, R., BRESSOLLIER, P., URDACI, M. C., QUESADA, E. & BEJAR, V. 2010. Characterization of the exopolysaccharide produced by Salipiger mucosus A3, a halophilic species belonging to the Alphaproteobacteria, isolated on the Spanish Mediterranean seaboard. *Mar Drugs,* 8**,** 2240-51.

[31] LÓPEZ-ORTEGA, M. A., CHAVARRÍA-HERNÁNDEZ, N., LÓPEZ-CUELLAR, M. D. R. & RODRÍGUEZ-HERNÁNDEZ, A. I. 2021. A review of extracellular polysaccharides from extreme niches: An emerging natural source for the biotechnology. From the adverse to diverse! *International Journal of Biological Macromolecules,* 177**,** 559-577.

[32] MATA, J. A., BEJAR, V., BRESSOLLIER, P., TALLON, R., URDACI, M. C., QUESADA, E. & LLAMAS, I. 2008. Characterization of exopolysaccharides produced by three moderately halophilic bacteria belonging to the family Alteromonadaceae. *J Appl Microbiol,* 105**,** 521-8.

[33] MECHIRACKAL BALAN, B., SHINI, S., KRISHNAN, K. P. & MOHAN, M. 2018. Mercury tolerance and biosorption in bacteria isolated from Ny-Ålesund, Svalbard, Arctic. *Journal of Basic Microbiology,* 58**,** 286-295.

[34] MOHAMED, S. S., AMER, S. K., SELIM, M. S. & RIFAAT, H. M. 2019. Characterization and applications of exopolysaccharide produced by marine Bacillus altitudinis MSH2014 from Ras Mohamed, Sinai, Egypt. *Egyptian Journal of Basic and Applied Sciences,* 5**,** 204-209.

[35] MORILLO, J. A., AGUILERA, M., RAMOS-CORMENZANA, A. & MONTEOLIVA-SÁNCHEZ, M. 2006. Production of a Metal-Binding Exopolysaccharide by Paenibacillus jamilae Using Two-Phase Olive-Mill Waste as Fermentation Substrate. *Current Microbiology,* 53**,** 189-193.

[36] NICHOLS, C. M., LARDIERE, S. G., BOWMAN, J. P., NICHOLS, P. D., J, A. E. G. & GUEZENNEC, J. 2005. Chemical characterization of exopolysaccharides from Antarctic marine bacteria. *Microb Ecol,* 49**,** 578-89.

[37] NWODO, U., GREEN, E. & OKOH, A. 2012. Bacterial Exopolysaccharides: Functionality and Prospects. *International Journal of Molecular Sciences,* 13**,** 14002-14015.

[38] PANOSYAN, H., DI DONATO, P., POLI, A. & NICOLAUS, B. 2018. Production and characterization of exopolysaccharides by Geobacillus thermodenitrificans ArzA-6 and Geobacillus toebii ArzA-8 strains isolated from an Armenian geothermal spring. *Extremophiles,* 22**,** 725-737.

[39] PARK, D., YUN, Y.-S. & PARK, J. M. 2010. The past, present, and future trends of biosorption. *Biotechnology and Bioprocess Engineering,* 15**,** 86-102.

[40] POLI, A., ANZELMO, G. & NICOLAUS, B. 2010. Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. *Mar Drugs,* 8**,** 1779-802.

[41] PRAJAPATI, V. D., JANI, G. K., ZALA, B. S. & KHUTLIWALA, T. A. 2013. An insight into the emerging exopolysaccharide gellan gum as a novel polymer. *Carbohydrate Polymers,* 93**,** 670-678.

[42] PRAKASH SHYAM, K., RAJKUMAR, P., RAMYA, V., SIVABALAN, S., KINGS, A. J. & MIRIAM, L. R. M. 2021. Exopolysaccharide production by optimized medium using novel marine Enterobacter cloacae MBB8 isolate and its antioxidant potential. *Carbohydrate Polymer Technologies and Applications,* 2**,** 100070.

[43] PRASERTSAN, P., WICHIENCHOT, S., DOELLE, H. & KENNEDY, J. 2008. Optimization for biopolymer production by Enterobacter cloacae WD7. *Carbohydrate Polymers,* 71**,** 468-475.

[44] RASULOV, B. A., YILI, A. & AISA, H. A. 2013. Biosorption of Metal Ions by Exopolysaccharide Produced by <i>Azotobacter chroococcum</i> XU1. *Journal of Environmental Protection,* 04**,** 989-993.

[45] REHM, B. H. A. 2010. Bacterial polymers: biosynthesis, modifications and applications. *Nature Reviews Microbiology,* 8**,** 578-592.

[46] SELIM, M. S., AMER, S. K., MOHAMED, S. S., MOUNIER, M. M. & RIFAAT, H. M. 2018. Production and characterisation of exopolysaccharide from Streptomyces carpaticus isolated from marine sediments in Egypt and its effect on breast and colon cell lines. *J Genet Eng Biotechnol,* 16**,** 23-28.

[47] SHAMEER, S. 2016. Biosorption of lead, copper and cadmium using the extracellular polysaccharides (EPS) of Bacillus sp., from solar salterns. *3 Biotech,* 6.

[48] SINGH, R. P., SHUKLA, M. K., MISHRA, A., KUMARI, P., REDDY, C. R. K. & JHA, B. 2011. Isolation and characterization of exopolysaccharides from seaweed associated bacteria Bacillus licheniformis. *Carbohydrate Polymers,* 84**,** 1019-1026.

[49] SINGH, S. & KUMAR, V. 2019. Mercury detoxification by absorption, mercuric ion reductase, and exopolysaccharides: a comprehensive study. *Environmental Science and Pollution Research,* 27**,** 27181-27201.

[50] SINHA, R. K., KRISHNAN, K. P., SINGH, A., THOMAS, F. A., JAIN, A. & JOHN KURIAN, P. 2017. Alteromonas pelagimontana sp. nov., a marine exopolysaccharide-producing bacterium isolated from the Southwest Indian Ridge. *Int J Syst Evol Microbiol,* 67**,** 4032-4038.

[51] TORRES, C. A., MARQUES, R., ANTUNES, S., ALVES, V. D., SOUSA, I., RAMOS, A. M., OLIVEIRA, R., FREITAS, F. & REIS, M. A. 2011. Kinetics of production and characterization of the fucose-containing exopolysaccharide from Enterobacter A47. *J Biotechnol,* 156**,** 261-7.

[52] TORRES, C. A. V., ANTUNES, S., RICARDO, A. R., GRANDFILS, C., ALVES, V. D., FREITAS, F. & REIS, M. A. M. 2012. Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. *Bioresource Technology,* 119**,** 148-156.

[53] TORRES, E. 2020. Biosorption: A Review of the Latest Advances. *Processes,* 8**,** 1584.

[54] UESHIMA, M., GINN, B. R., HAACK, E. A., SZYMANOWSKI, J. E. S. & FEIN, J. B. 2008. Cd adsorption onto Pseudomonas putida in the presence and absence of extracellular polymeric substances. *Geochimica et Cosmochimica Acta,* 72**,** 5885-5895.

[55] WANG, F., YANG, H. & WANG, Y. 2013a. Structure characterization of a fucose-containing exopolysaccharide produced by Enterobacter cloacae Z0206. *Carbohydrate Polymers,* 92**,** 503-509.

[56] WANG, L., YANG, J., CHEN, Z., LIU, X. & MA, F. 2013b. Biosorption of Pb(II) and Zn(II) by Extracellular Polymeric Substance (Eps) of Rhizobium Radiobacter: Equilibrium, Kinetics and Reuse Studies. *Archives of Environmental Protection,* 39**,** 129-140.

[57] WEI, W., WANG, Q., LI, A., YANG, J., MA, F., PI, S. & WU, D. 2016. Biosorption of Pb (II) from aqueous solution by extracellular polymeric substances extracted from Klebsiella sp. J1: Adsorption behavior and mechanism assessment. *Scientific Reports,* 6.

[58] WERNING, M. L., PÉREZ-RAMOS, A., FERNÁNDEZ DE PALENCIA, P., MOHEDANO, M. L., DUEÑAS, M. T., PRIETO, A. & LÓPEZ, P. 2014. A specific immunological method to detect and quantify bacterial 2-substituted (1,3)-β-d-glucan. *Carbohydrate Polymers,* 113**,** 39-45.

[59] WILLIS, L. M. & WHITFIELD, C. 2013. Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependent pathways. *Carbohydrate Research,* 378**,** 35-44.

[60] XIA, L., TAN, J., WU, P., HE, Q., SONG, S. & LI, Y. 2020. Biopolymers extracted from Klebsiella sp. and Bacillus sp. in wastewater sludge as superb adsorbents for aqueous Hg(II) removal from water. *Chemical Physics Letters,* 754**,** 137689.

[61] XIAO, M., FU, X., WEI, X., CHI, Y., GAO, W., YU, Y., LIU, Z., ZHU, C. & MOU, H. 2021. Structural characterization of fucose-containing disaccharides prepared from exopolysaccharides of Enterobacter sakazakii. *Carbohydrate Polymers,* 252**,** 117139.

[62] XU, C. L., WANG, Y. Z., JIN, M. L. & YANG, X. Q. 2009. Preparation, characterization and immunomodulatory activity of selenium-enriched exopolysaccharide produced by bacterium Enterobacter cloacae Z0206. *Bioresource Technology,* 100**,** 2095-2097.

[63] ZHANG, Z., CHEN, Y., WANG, R., CAI, R., FU, Y. & JIAO, N. 2015. The Fate of Marine Bacterial Exopolysaccharide in Natural Marine Microbial Communities. *PLoS One,* 10**,** e0142690.