GENERAL INTRODUCTION

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I. Historical perspectives

The formation of exopolymeric substances enables single-cell organism to live an impermanent multicellular lifestyle, in which "collective behavior" facilitates survival in adverse environments. The switching from planktonic growth to biofilm occurs in response to external stimuli that involves various regulatory networks (O'Toole et al., 2000; Monds & O'Toole 2009). This cellular reprogramming equips bacteria with a weapon that enables their survival in unfavorable conditions (Whiteley et al., 2001; Vuong et al., 2004). Microbial biofilm formation is known to be a sequential bacterial development process (O'Toole et al., 2000) and is regulated by a series of genetic and phenotypic determinants (O'Toole et al., 2000). Bacteria take shelter in an extracellular matrix (self-produced) inside biofilm, accounting ~90% of the biomass (Flemming & Wingender, 2010). The matrix is composed of extracellular polymeric substances that along with carbohydrate-binding proteins (Branda et al., 2006), pili, flagella, other adhesive fibers (Cegelski et al., 2009), and extracellular DNA (eDNA) (Qin et al., 2007), act as a scaffold for the three dimensional biofilm structure. There was a period (1978-1990) when biofilms were considered as unstructured accretions of bacterial cells, surrounded by the exopolysaccharide (EPS) matrices. Inside the matrix, nutrients are trapped for metabolic utilizations by the resident bacteria and through H-bond interactions with hydrophilic polysaccharides; water is efficiently retained (Flemming &

Wingender, 2010). There are speculations that production of exopolymeric substances (EPSs) by bacteria growing under extremely nutrient-poor conditions (where these nutrients are available at levels below threshold concentrations) might aid in concentrating the nutrient for sustenance (Pahm & Alexander, 1993). Enzymes secreted by the bacteria modify EPS composition in response to changes in nutrient availability (Sauer et al., 2004). Hence, the structural components of the matrix provides a highly hydrated, robust structure which keeps bacteria in close proximity, enabling intimate cell-to-cell interactions and DNA exchange (Flemming & Wingender, 2010). Moreover, it protects the biomass from desiccation, predation, oxidizing molecules, radiation, and other detrimental agents (Flemming & Wingender, 2010). The matrix, more particularly EPS, protects biofilm bacteria from exposure to innate immune defenses (such as opsonization and phagocytosis) inside the host, and against various antibiotics (Walters et al., 2003; Cerca et al., 2006). Inter bacterial interactions within the matrix can promote the spread of drug resistance markers and other virulence factors (Vuong et al., 2004). As a result, biofilm-forming pathogens (*Pseudomonas aeruginosa*) establish chronic and recalcitrant infections like upper respiratory infections (Govan & Deretic, 1996), urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* [UPEC] and *Klebsiella pneumoniae* (Foxman, 2010), periodontitis (Kuramitsu & Wang, 2011), catheter-induced and other device-associated infections (Venditti et al., 1993). In immuno-compromised patients, the symptom of infections by opportunistic biofilm-forming or EPS forming pathogens can be shocking, leading to harsh symptoms and, in many cases, death. Formation of biofilm or colony by bacteria is a behavior that allows prompt utilization of the substrate. The combined exo-enzymatic action of growing colony provides benefits to

the individual cells when growing in nutrient rich (copiotrophic) condition. Alternatively, an oligotrophic bacterium growing slowly in nutrient-poor condition may have a life strategy in which dispersal is promoted to optimize cell access to substrates. In the latter scenario colony formation is possibly not adaptive. While aquatic ecologists have had an interest in oligotrophic bacteria (Costerton et al., 1981; Yokoi et al., 1995), these organisms are still relatively unknown to many microbiologists, especially clinical microbiologists. Growth and continued existence of bacteria are often influenced by EPS produced by them.

II. Oligotrophic style of growth

Oligotrophic environments usually deficient in exogenous supply of nutrients and are defined by a low nutrient flux, <1 mg carbon per litre per day (Schut et al., 1993) as well as by low absolute concentrations of nutrients (Morita, 1997). The aquatic environment is the largest habitat on Earth, accounting for >90% of the biosphere by volume and harbouring microorganisms responsible for ~50% of total global primary production. Despite of highest cellular production rate of any ecosystem on the planet, aquatic environments has vast oligotrophic (e.g., nutrient-limited open ocean water) situation. As a result, the aquatic environment is often considered a marine desert. Oceanic ecosystems are far more productive than terrestrial ecosystems in terms of per unit of biomass, and as a result, the turnover rate of nutrients per unit of biomass is several hundred times higher. But, due to limited nutrient availability and low population densities, the effective overall turnover rate is still so low (Munster, 1993) that the ocean classifies as oligotrophic with organic carbon fluxes of a milligram of carbon l^{-1} d^{-1} as

well as by low ambient concentrations of nutrient (Poindexter, 1981). However, high productivity is predominantly due to the phototrophic prokaryotic primary-producers, and the heterotrophic prokaryotes caused nutrient alteration and remineralisation. The carbon, nitrogen and phosphorus fixation by these bacteria and their subsequent conversion into particulate matter are critically important processes in aquatic environments. Heterotrophic bacteria are major contributors to oceanic and terrestrial biogeochemical cycles (Whitman et al., 1998). As reservoirs of nutrients in oligotrophic marine ecosystems, these ultramicrobacteria exhibited impacts on the productivity of all marine life by means of interacting with all trophic levels and control the nutrient fluxes via mineralisation. With predictions of increasing ocean oligotrophy as an outcome of global warming (Matear & Hirst, 1999) without any doubt it is vital to know the physiology of this class of bacteria in order to determine their impact on primary production in aquatic ecosystem. A major portion of terrestrial and marine environments are oligotrophic (nutrient poor) and thus, the routine situation for almost all bacteria is that they are in inadequate supply of one or more vital nutrients. In spite of low levels of nutrients the pelagic marine environment is dominated, in terms of biomass and activity, by small bacteria, variously referred to as ultramicrobacteria, microcells, nanoplankton or picoplankton depending on the terminology adopted by microbial ecologists and physiologists (Table I).

Table I. Description of terms relating to the small size of bacteria.

Ultramicrobacteria	**Microorganism with a cell volume of <0.1 μm^3 that maintains its size with only minor changes, irrespective of growth conditions. Observed by light microscopy.**
Ultramicrocells	Smaller forms (usually starved) of microorganisms that are larger when actively growing. Usually associated with reductive cell division during starvation. Observed by light microscopy.
Nan(n)obacteria	Possible synonym for UMB. In the literature usually associated with structures in geological samples with sizes ranging from 0.01 – 0.1 μm. Usually associated with uncultured and unsubstantiated descriptions of microorganisms. Observed by electron microscopy.
Femtoplankton	Marine microorganisms 0.02-0.2 μm
Picoplankton	Marine microorganisms 0.2-2.0 μm
Nanoplankton	Marine microorganisms 2.0-20 μm
Microplankton	Marine microorganisms 20-200 μm

Other allied terms	Dwarf cells/bacteria, lilliputian cells, femtobacterioplankton, miniature cells/bacteria, nanocells, nanosized, nanobe, nanoorganisms

Local variations in nutrient content can occur because of physical processes, including upwelling of nutrient rich deep waters or aeolian and riverine deposition, or biological processes such as phytoplankton blooms or aggregation of particulate organic matter. In addition, heterogeneity in ocean waters is not limited to gross differences in nutrient concentrations, but extends to microscale patchiness that occurs throughout the continuum of ocean nutrient concentrations (Azam & Malfatti, 2007). Bacteria, in ecological terms, are generally defined as r-strategists, having a small body, short generation time, and highly dispersible offspring that continuously challenged by conditions of nutrient limitation and starvation in natural environment (Morita, 1997). As bacteria cannot escape the environment they undergo both phenotypic and genetic changes in order to face that adverse situation. Bacteria have evolved a wide range of growth and survival strategies to maximize reproductive success. In particular, nutrient type and availability have provided strong selective pressure for defining lifestyle strategies among marine bacteria. However, although a large number of copiotrophic marine organisms (and fewer oligotrophs) have been cultured, the study of trophic strategy has been impaired by a lack of understanding of the molecular basis of adaptation. Nevertheless, oligotrophs are omnipresent in the environment and have been isolated from soil (Hattori, 1984), rivers (Yanagita et al., 1978), lakes (Lango, 1988), oceans (Deming, 1986), and tap water lacking organic substances (Jaeggi & Schmidt-

Lorenz, 1990). Some oligotrophic isolates can even grow in distilled water (Favero et al., 1971). Two different types of oligotrophs can be distinguished. Those oligotrophs that can grow on only a low concentration of carbon are called obligate oligotrophs (Fry, 1990). Those that are able to grow at both low and high concentrations of organic substances are called facultative oligotrophs (Ishida et al., 1982). There is a confusing variety of definitions for oligotrophic bacteria (Table II). Some researchers define oligotrophs as organisms able to grow at nutrient concentrations of 5 mg C 1^{-1} but not at concentrations of 7.5 g C 1^{-1} (Yanagita et al., 1978). Others define oligotrophs as those bacteria that are able to grow on media with 1 to 15 mg C 1^{-1} as well as on media with a higher nutrient content (Kuznetsov et al., 1979). Ishida et al. (1982) call such organisms facultative oligotrophs, as opposed to obligate oligotrophs (Yanagita et al., 1978) that cannot grow at substrate concentrations >0.3 g C 1^{-l}. Horowitz et al. (1983) postulated the term euryheterotroph for facultative oligotrophic bacteria and Baxter & Sieburth (1984) replaced 'facultative oligotroph' by 'eurytroph'. The confusion on the proper definition is strengthened by the fact that some researchers use the term oligotroph only for those organisms that are restricted to growth on low nutrient media, while others employ the term to broadly speak about both the obligate and facultative oligotrophs. A general characteristic of oligotrophs, and one that is currently used in all definitions, is the ability to grow in low nutrient media (0.2 to 16.8 mg C 1^{-l}). But is this truly such a remarkable characteristic? In the ocean's euphotic zone, food often comes in waves (Mopper & Lindroth, 1982) and the availability is short lasting.

Table II. Definition used to characterize oligotrophic bacteria.

Definition	Source
"Oligotrophic bacteria are heterotrophic bacteria capable of growth in the presence of organic nutrients equivalent to 16.8 mg C l^{-1}".	Akagi et al., (1977)
"Bacteria capable of growth on unamended BWA (agar-solidified Chesapeake Bay water)".	Mallory et al., (1977)
"Oligotrophic bacteria are capable to grow on media containing only minerals, and they meet their carbon and energy requirements from trace amounts of organic substances [. . .] found in the air".	Moaledj, (1978)
"A trophic group of bacteria that can grow only in the presence of a minor amount of nutrients and not in the presence of a large amount".	Yanagita et al., (1978)
"Those [bacteria] that develop at the first cultivation on media with the minimal content of organic matter of about 1-15 mg C $l^{-1}$and that grow on such media at subsequent recultivation though they can grow on richer media".	Kuznetsov et al., (1979)
"Organisms that grow in media containing organic matter at a concentration of 1 mg C per litre. [. . .] . Obligate oligotrophs may decrease in number or disappear with the onset of man-made eutrophication, facultative oligotrophs can tolerate or rapidly adapt to the higher concentrations of organic substances".	Ishida & Kadota, (1981)

"Oligotrophic bacteria can [. . .] be conceived of as those whose survival in nature depends on their ability to rnultiply in habitats of low nutrient fluxes (approaching zero to a fraction of a mg C/litre/day)"	Poindexter, (1981)
"Bacteria which grow at substrate concentrations < 1 mg C 1^{-l}".	Ishida et al., (1982)
"Bacteria that can be isolated on a low-nutrient medium (unsupplemented Bushnell Haas agar) and that are restricted to growth at low nutrient concentrations".	Horowitz et al., (1983)
"Oligotrophs are defined as those organisms known to be able not only to survive but particularly to multiply under conditions of extremely low and often discontinuous supply of nutrients. In other words, organisms adapted to low and irregular fluxes of substrates".	Van Gemerden & Kuenen, (1984)
"Among oligotrophs we tentatively define the obligate oligotroph as an organism which does not grow in rich (200 mg C l^{-1}) media, and the facultative oligotroph as an organism which grows in not only poor (0.2 mg C l^{-1}) but also rich media".	Ishida et al., (1989)
"Oligotrophic bacteria can broadly be defined as organisms that grow on low concentrations of organic substrates". Obligate oligotrophs cannot grow at substrate concentrations above 6 g C 1^{-1}".	Fry, (1990)
"…oligotrophic microorganisms are prokaryotic and eukaryotic organisms that are evolutionarily adapted to exploit ecological niches characterised by low substrate concentrations and low energy fluxes.	Semenov, (1991)

Oligotrophs, [. . .] may develop in rich as well as in poor environments [. . .]."

In river-outflow regions, or during senescence of massive phytoplankton spring blooms, conditions may actually be eutrophic. By definition such regions are characterised by a more than 50-fold higher nutrient flux than that in oligotrophic regions (Poindexter, 1981). Yet, rapid growth of bacteria and consumption of substrates will again result in low steady state substrate concentrations and growth limitation. Therefore, eutrophic environments select temporarily for K-strategists when nutrients become depleted (Andrews & Harris, 1986). The fluctuations in nutrient availability in the ocean and the presence of microniches result in the coexistence of copiotrophic and oligotrophic bacteria in an oligotrophic environment. Therefore, the r/K-selection concept is not completely synonymous with the copiotrophic/oligotrophic concept (Andrews & Harris, 1986), and this is probably the reason why the r/K-strategy concept has not been broadly adopted in marine microbiology. Total prokaryote numbers in the ocean are estimated at 10^{29} (Whitman et al., 1998), as a result, marine microorganisms contribute a large proportion of the world's biosphere in terms of carbon, nitrogen and phosphorus. Furthermore, of the three largest microbial habitats (seawater, soil and sediment/soil subsurface), the rates of cellular activity and turnover are highest in the open ocean (Whitman et al., 1998). By virtue of their abundance and biomass heterotrophic prokaryotes in the ocean play an essential role in nutrient transformation and remineralisation. In addition, picophytoplankton (phototrophic prokaryotes and

eukaryotes), contribute significantly to global primary production (Campbell et al., 1994), with estimates as high as 50% of global carbon fixation attributed to this size class (Partensky et al., 1999). Thus, together the smallest heterotrophic and phototrophic cells play an essential role in regulating the accumulation, export, re-mineralisation and transformation of the world's largest pool of organic carbon (Cole et al., 1988) resulting in an ecosystem composed primarily of a microbial food web where prokaryotes and picoeukaryotes represent the most important biological component.

III. An overview of the marine microbial community

When observed directly, indigenous bacterial communities are rich in carbon and nitrogen and exhibit a low protein and DNA content, they display typical cell volumes in the range from 0.02-0.12 μm^3, around an order of magnitude smaller than commonly studied bacteria such as *Escherichia coli* (Fuhrman, 1981; Strehl et al., 1999; Button & Robertson, 2000). Initial attempts to isolate marine bacteria on nutrient rich agar plates revealed a discrepancy of up to three orders of magnitude between plate counts and the observed total number of cells in marine samples (Ferguson et al., 1984). Taken together with the observation that a high proportion of early ocean isolates were typically larger (0.34-6.4 μm^3) and undergo starvation induced miniaturisation processes (Schut et al., 1993), it was believed that indigenous microcells represent starved and dormant forms of isolates that could not form colonies on agar plates. It was therefore assumed that in the environment starvation was the natural state of microorganisms. This assertion, however, does not account for a number of observed phenomena listed here. (i) On a per unit volume basis, oceanic microcells exhibit higher activity than the atypically large cells

(Ouverney & Fuhrman, 1999). (ii) More than 90% of the productivity in pelagic regions is due to free-living, rather than substrate-attached, cells (Cho & Azam, 1988). (iii) Bacteria that remain small when actively growing have been observed and isolated (Schut et al., 1993). (iv) The global significance and activity of ultramicrobacterial phototrophic cyanobacteria is well established (Partensky et al., 1999), and (v) Starved, or dormant, bacteria may not become predominant in the ocean while in the non-growing state. More recently molecular techniques suggest low *in situ* abundance of typically isolated bacteria (Eilers et al., 2000). Developments with molecular methods enabled the relative abundance of specific prokaryotic taxa to be determined without the need to cultivate microorganisms. Initial studies based on SSU rRNA sequence libraries found that the most abundant rDNA sequences obtained did not correspond to cultured species and were distantly related to other rDNA sequences in databases (Giovannoni & Rappe, 2000). These results clearly demonstrated that natural bacterial communities were composed of unknown species that were incapable of forming colonies on commonly used microbiological media.

IV. Predicated properties of oligotrophs

According to the fundamental roles of nutrient uptake and utilisation a list of predicted properties were advanced for a model oligotroph at the Dalhelm Conference (Hirsch et al., 1979). The proposed characteristics include: (i) the high surface per volume ratio (cells are expected to be small), (ii) favoured usage of metabolic energy for nutrient uptake especially during periods of non-growth, (iii) constitutive nutrient uptake ability, (iv) having high affinity, low-specificity transport systems for simultaneous

uptake of mixed substrates, and (iv) the organization of nutrient reserves following nutrient uptake. The small size of cells would provide a distinct advantage in terms of grazer avoidance and increased efficiency of nutrient uptake, while nutrient uptake mechanisms were expected to have a broad specificity, be inducible and subject to a minimal amount of catabolite repression in order to ensure simultaneous utilisation of the broadest range of substrates (Poindexter, 1979). Oligotrophs were also expected to regulate their biosynthetic rate in line with nutrient uptake rates (Poindexter, 1979). Finally, oligotrophs were predicted to have the ability to store diverse nutrients in reserves (Hirsch et al., 1979). Since the proposal of these characteristics was advanced a range of physiological studies have been conducted to test their validity (Schut et al., 1997). Unfortunately very few of these studies were conducted with oligotrophs, highlighting the need to obtain relevant oligotrophic isolates for laboratory studies.

V. Bioflocculation as a microbial response to substrate limitations

Bacteria have extraordinary capability for survival in the presence of extremely reduced amount of energy and nutrient sources. In the year 1995, Tada et al. isolated and identified 66 strains of facultative oligotrophs from the clinical samples out of which 27 strains were *Klebsiella pneumoniae*, 18 strains were *Pseudomonas aeruginosa*, 10 strains were *Enterobacter aerogenes*, 6 strains were *Serratia marcescens*, and 2 strains were *Klebsiella oxytoca*. *K. pneumoniae* and *P. aeruginosa* made up the majority of the bacteria identified (70%). Oligotrophic strain like *K. pneumoniae* strain MB45 was isolated from river water on diluted Luria agar (Kumar et al., 2011). In a separate study from India eight oligotrophic isolates, MB19, MB26, MB29, MB42, MB45, MB49,

MB51 and MB72 were recognized as the member of genus *Klebsiella* (Chakraborty et al., 2013). The attachment of microorganisms to the surfaces and other microorganisms is ubiquitous. Microbes preferentially grown on the surface and attached microorganisms are frequently dominant compared to freely suspended cells ranging from human digestive system to natural streams (Costerton et al., 1978). Gravitational settling, centrifugation, and filtration are enhanced if larger, faster settling aggregates. While biological and inorganic particles can be aggregated by addition of chemical coagulants, the ability of organisms to self associate or bioflocculate. Why microorganisms bioflocculate? Understanding this phenomenon is important for operating suspended growth in the biological reactors. From colloidal properties of inorganic particles, the chemical control of particle aggregation is well established (O'Melia, 1972). Various mechanisms for microbial aggregation were reported which includes polymeric materials, cell appendages like pili, fimbriae, cilia, filaments, fuzz and hairs. Phenomena of attachment involve combination of hydrogen and ionic bonds along with dipolar and hydrophobic interactions (Calleja, 1984). Finally growth within an aggregate may increase nutrient uptake compared to freely dispersed cells. For a pure culture of a bacterium to bioflocculate when substrate is nearly depleted implies that cell association may confer some advantage over freely dispersed cells. A common feature shared by *K. pneumoniae* is the ability to form biofilms, a major virulence factor contributing towards disease of the host (NIH, 2002). Some species of the genus *Klebsiella* were reported to produce exopolysaccharides in the culture medium (Baldi et al., 2009). Gallo et al. (2012) showed that *Klebsiella oxytoca* BAS-10 producing a biotechnologically relevant exopolysaccharide during Fe(III)-citrate fermentation. A strain of *Klebsiella oxytoca* was

isolated from acid pyrite-mine drainage, typically produces a ferric hydrogel, comprising of branched heptasaccharide repeating units; xopolysaccharide, which consists of 4 rhamnose (Rha), 2 glucuronic acids (GlcA) and 1 galactose (Gal) (Arcon et al., 2012). Dlamini et al., 2007, reported that the major monosaccharide constituents of the polysaccharide produced by whey utilizing bacteria *Klebsiella oxytoca,* were rhamnose (37%, w/w) and glucose (34%, w/w) along with the residues of cellobiose suggesting that the polysaccharide had a cellulose backbone. Fucogel, a polysaccharide produced by *Klebsiella pneumoniae* I-1507 was found to compose of galactose, 4-*O*-acetyl-galacturonic acid and fucose (Guetta et al., 2003). Rättö et al. (2001), isolated EPS from two similar *K. pneumoniae* strains and found that each contained approximately mannose, galactose, and GalA in a ratio of almost 3:1:1. In a different study, *K. pneumoniae* EPS isolated from ESKAPE organisms comprised of 1.3% glucose, 49.4% mannose, and 5.0% GlcA (Bales et al., 2013).

VI. Description of the bacterium *Klebsiella pneumoniae*

The genus *Klebsiella* was named after the German bacteriologist Edwin Klebs (1834–1913). *Klebsiella pneumoniae* is a gram negative, nonmotile, encapsulated, lactose-fermenting, facultative anaerobic, rod shaped bacterium. Although found in the normal flora of the mouth, skin, and intestines (Ryan & Ray, 2004), it can cause destructive changes to human lungs if aspirated, specifically to the alveoli resulting in bloody sputum. In the clinical setting, it is the most significant member of the *Klebsiella* genus of Enterobacteriaceae. *K oxytoca* and *K rhinoscleromatis* have also been demonstrated in human clinical specimens.

VII. Clinical Significance of *K. pneumoniae*

K. pneumoniae can cause destructive changes to human lungs via inflammation and hemorrhage with cell death (necrosis) that sometimes produces a thick, bloody, mucoid sputum (currant jelly sputum). These bacteria gain access typically after a person aspirates colonizing oropharyngeal microbes into the lower respiratory tract. As a general rule, *Klebsiella* infections are seen mostly in people with a weakened immune system. Most often, illness affects middle-aged and older men with debilitating diseases. This patient population is believed to have impaired respiratory host defenses, including persons with diabetes, alcoholism, malignancy, liver disease, chronic obstructive pulmonary diseases (COPD), glucocorticoid therapy, renal failure, and certain occupational exposures (such as paper mill workers). Many of these infections are obtained when a person is in the hospital for some other reason (a nosocomial infection). Feces are the most significant source of patient infection, followed by contact with contaminated instruments.

The common condition caused by *Klebsiella* bacteria is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients possess an increased tendency to develop lung abscess, cavitation, empyema, and pleural adhesions. It shows high death rate of about 50%, even when antimicrobial therapy is used. The mortality rate can be nearly 100% for people with alcoholism and bacteremia (Forner et al., 2006). In addition to pneumonia, *Klebsiella* can also cause infections in the urinary tract, lower biliary tract, and surgical wound sites. The range of clinical diseases includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea,

upper respiratory tract infection, wound infection, osteomyelitis, meningitis, and bacteremia and septicemia. For patients with an invasive device in their bodies, contamination of the device becomes a risk; for example, respiratory support equipment and urinary catheters put patients at increased risk. Also, the use of antibiotics can be a factor that increases the risk of nosocomial infection with *Klebsiella* bacteria (Podschun & Ullmann, 1998). Sepsis and septic shock can follow entry of the bacteria into the blood. Two unusual infections of note from *Klebsiella* are rhinoscleroma and ozena. Rhinoscleroma is a chronic inflammatory process involving the nasopharynx. Ozena is a chronicatrophic rhinitis that produces necrosis of nasal mucosa and mucopurulent nasal discharge. Research conducted at King's College, London has implicated molecular mimicry between HLA-B27 and two Klebsiella surface molecules as the cause of ankylosing spondylitis (Rashid & Ebringer, 2007). *Klebsiella* ranks second to *E. coli* for urinary tract infections in older people. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma.

VIII. Biotechnological applications of exopolysaccharides

Polysaccharides are polymers of carbohydrates with huge structural diversity, from long linear repetition of the same monomer to highly branched structures of different sugars. This high structural diversity reflects the functional diversity of these molecules. There are two types of polysaccharides, storage polysaccharides (i.e. glycogen) and structural polysaccharides, which are normally secreted by the cell and form different cell structures (i.e. cellulose, chitin). Extracellular polysaccharides or

exopolysaccharides belong to this last group. EPS are produced not only by microorganisms, but also by algae, plants and animals (Sutherland, 2005). Bacterial exopolysaccharides are a major component of the EPSs or matrix of biofilms, and mediate most of the cell-to-cell and cell-to-surface interactions required for biofilm formation and stabilization (Flemming & Wingender, 2010). The matrices of biofilms from natural environments, such as marine and fresh water, soil, or chronic infections, contain a ubiquitous composition of polysaccharides. More than 30 different matrix polysaccharides have been characterized so far. Several are homopolysaccharides (i.e. glucans, fructans, cellulose), but most of these are heteropolysaccharides consisting on a mixture of sugar residues. Exopolysaccharides can even differ between strains of single species, as exemplified by strains of *Pseudomonas aeruginosa*, which can produce one, two or three different exopolysaccharides (alginate, Pel and Psl) (Ryder et al., 2007). Since most mutants deficient in the synthesis of exopolysaccharides are impaired for biofilm formation it was assumed that bacterial exopolysaccharides play only a structural role in biofilms. However, in recent years an unexpected function for these molecules as inhibitors of biofilm formation has been described. This new function was meticulously reviewed by Rendueles et al. (2012). Oligosaccharides with the ability to inhibit and/or destabilize biofilm formation are referred to as antibiofilm polysaccharides, and their production appears to be a well-conserved ability among living organisms. Interestingly, antibiofilm polysaccharides do not have biocidal activity, a property that could increase the technological applications of these molecules as antibiofilm agents in industry and medicine by diminishing the emergence of resistance by natural selection. EPS occur in two basic forms: as a capsule, where the polymer is closely associated with the cell

surface, and as slime loosely associated with the cell surface. Their composition and structure vary greatly: they can be either homo- or heteropolysaccharides and may also contain a number of different organic and inorganic substituents. Most homopolysaccharides are neutral glucans, whilst the majority of heteropolysaccharides are polyanionic due to the presence of uronic acids. Furthermore, charge can be conferred by the presence of sulphate and phosphate groups, pyruvate ketals or succinyl hemiesters (Sutherland, 1990a; Freitas et al., 2011). EPS production involves a significant expenditure of carbon and energy by microorganisms, an expenditure which must afford them some benefits. EPS act as an adhesin and favour interactions and cellular associations amongst microorganisms, creating micro-environments within which the transfer of genes and metabolites is very common. Moreover, the production of EPS provides a way for microorganisms to ensure their survival in nutrient-starved environments (Sutherland, 1990b; Wolfaardt et al., 1999). They have aroused great interest among biotechnologists because of their wide range of potential applications in such fields as pharmacy, foodstuffs, cosmetics and the petroleum industry, in which emulsifying, viscosifying, suspending and chelating agents are required (Freitas et al., 2011). During the past 50 years a considerable number of bacterial EPS have been described but, with the exception of xanthan produced by *Xanthomonas campestris* and gellan produced by *Sphigomonas paucimobilis*, few have achieved great commercial success due either to their being unable to offer better properties than those already on the market or to difficulties in finding new applications (Sutherland, 2002). Purified *Vibrio* sp. A101 polysaccharide showed decrease in the minimum biofilm eradication concentration (MBEC) of amikacin, tobramycin and gentamicin against *P. aeruginosa*

biofilms (Jiang et al., 2011), suggested potential applications of antibiofilm polysaccharides or oligosaccharides as adjuvants in traditional antibiotic treatment.

The present study dealt with the following objectives:

IX. Objectives of the study

1. To screen and identify EPS-producing bacteria from river water samples.
2. To select an EPS-producing facultative oligotrophic strain from the pool of EPS-producing bacteria for further studies.
3. To reveal basic physiology of the selected strain.
4. To standardize extraction and purification of EPS from the batch culture and quantify EPS yield by the test strain at different phases of growth.
5. To determine the composition of EPS followed by an attempt to reveal the biological properties.
6. To determine the Flocculating and Emulsifying activity of the purified EPS.
7. To optimize EPS dosage, temperature, pH and metal cations on flocculating rate for biotechnology applications.