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Starvation Conditions Effects on Carbohydrate Metabolism of Marine Bacteria

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1. Introduction

In the coastal shorelines terrestrial organic materials transported by river runoff represent an important material source to the ocean and is estimated that 0.4×10^{15} g yr⁻¹ organic carbon is discharged to ocean by land flows or rivers (Meybeck, 1982, He et al, 2010). This amount of riverine organic carbon is sufficient to support the entire organic carbon turnover in the ocean (Williams and Druffel, 1987) even though, little terrestrial signal by carbon isotopic ratio ($\delta^{13}\text{C}$) of the bulk DOC in ocean was shown, suggesting that terrestrial organic carbon undergo rapid removal and decomposition within estuarine mixing (Druffel et al, 1992; Hedges et al, 1997).

Carbohydrates are, among others, the major components of identified organic matter in ocean and account for 3% to 30% of the bulk Dissolved Organic Carbon (DOC) (Gueuen et al, 2006; Hung et al, 2003; Pakulski and Benner, 1994), whereas, in estuarine and marine surface waters they are considered the most labile fractions of bulk organic matter and may play key roles in the geochemical cycles as reported by several studies (Benner et al, 1992; Burdige and Zheng, 1998; Middelboe et al, 1995; Murrell and Hollibaugh, 2000).

In complex macro-aggregates mixtures, the carbohydrates are dominant with proteins and lipids, with evidence that in mucilage samples carbohydrates are relevant components of the organic carbon (17–45% on dry weight basis depending on the age of the aggregates) with heavy colonization by several heterotrophic bacteria and autotrophic organisms which embedded the organic matrix (Simon et al, 2002; Urbani and Sist, 2003). Thus high density of micro organisms enriches the matrix in organic and inorganic nutrients in comparison with the surrounding water and such macro flocks are important hot-spots for bacterial growth and carbon cycling (Kaltenbock and Herndl, 1992; Alldredge, 2000 and Azam and Long, 2001) and then significance of bacteria for the formation and decomposition of aggregates appears to be higher than previously estimated (Simon et al, 2002). .

2. Importance of carbohydrates availability for marine bacteria

Marine micro-organisms have important established roles which may reflect large-scale changes within inter tidal systems and heterotrophic bacteria, for example, are crucial to transformations and re-mineralization of organic carbon, nitrogen and other nutrients throughout oceans (Azam, 1998; Azam et al, 1993).

Bacterial utilization of carbohydrates depends heavily on their chemical composition (Aluwihare and Repeta, 1999; Arnosti, 2000, Zoppini et al, 2010) and slow hydrolysis may provide the pathway for the accumulation in aquatic environments (Cowie and Heges, 1984; Cowie et al, 1995; Benner et al, 1992).

Else, bacteria play an important role in regulation of the rate of organic matter mineralization, nutrient cycling, and energy transfer in aquatic environments and demineralization or the turnover of carbohydrates has been used to evaluate the efficiency of the microbial community and the liability of the organic carbon in coastal waters (Azam and Worden, 2004; Lennon, 2007).

Considering that total number of bacteria on Earth, the largest proportion of bacterial cells presumably reside in oceanic subsurface rather than in terrestrial areas (3.5×10^{30} and $0.25\text{--}2.5 \times 10^{30}$ respectively) and therefore bacterial cells are estimated to contain, in total, 350–550 Pg of carbon, up to 60–100% of the total carbon found in plants, as well as large amounts of nitrogen and phosphorous (Whitman *et al* 1998). Thus, despite their modest size as individuals, as a group these organisms not only contribute to the flow of nutrients worldwide, but may also constitute a significant proportion of the nutrients in living biomass (Horner-Devine et al, 2009).

In estuarine areas, biodegradation by heterotrophic bacteria for Organic composition changed rapidly along the estuary, showing a selected removal of carbohydrates and amino acids within the DOC pool in the upper reach and mixing zone, and an autotrophic source of Particular Carbohydrates (PCHO) in the lower estuary, which gave an insight into the DOC estuarine process (He et al, 2010).

For hydrothermal marine compartment, heterotrophic thermophiles in culture far exceeds the number of their autotrophic counterparts, and many are known to metabolize carbohydrates *via* fermentation or respiration nevertheless, the energetic associated with these high-temperature microbial processes have been all but ignored (Amend et al, 2001).

In Coastal marine regions, sediments receive organic matter from a variety of sources, and its reactivity towards anaerobic fermentation and respiration is determined by the relative content of e.g. carbohydrates, lipids and proteins (Kristensen et al, 1995; Kristensen and Holmer, 2001). Proteins and labile carbohydrates derived from algae usually constitute the main organic sources for marine sediments, but coastal areas may also receive detritus rich in structural carbohydrates (e.g. cellulose) from vascular plants (Cowie and Hedges, 1992; Leeuw and Largeau, 1993; Kristensen, 1994). Furthermore, sediments of anthropogenic point sources, such as marine fish farms, may concentrate high loads of protein and lipid rich materials of animal origin (Ackefors and Enell, 1994). Proteins and simple carbohydrates are

generally labile towards anaerobic degradation (Arnosti and Repeta, 1994; Holmer and Kristensen, 1994; Arnosti and Holmer, 1999), whereas anaerobic degradation of lipids and structural carbohydrates occur at much lower rates (Boetius and Lochte, 1996; Canuel and Martens, 1996). This difference is most likely caused by differential efficiency of extra-cellular enzymes produced by anaerobic fermenting bacteria (Valdemarsen et al, 2010)

2.1. Bio mineralization of carbohydrates by marine bacteria and production of carbohydrases

Bacteria are the most abundant and most important biological component involved in the transformation and mineralization of organic matter in the biosphere (Cho et al, 1988; , Pomeroy et al, 1991). Heterotrophic bacteria contribute to the cycles of nutrients and carbon in two major ways: by the production of new bacterial biomass (secondary production) and by the re mineralization of organic carbon and nutrients. Understanding this dual character of planktonic bacteria in aquatic ecosystems is a central paradigm of contemporary microbial ecology (Billen et al, 1984; Ducklow et al, 1992 and Del Giorgio and Cole, 1998).

Further, bacteria are capable of out competing, other organisms for organic compounds at low concentrations since they possess high substrate affinities, surface-to volume ratios, and metabolic rates. Natural DOM sources are heterogeneous mixtures of compounds, and bacteria can utilize several molecules simultaneously (Bott et Kaplan, 1985).

Extracellular enzymes, term generally utilised to define enzymes located outside the cytoplasmic membrane, are important catalyst in the decomposition of particulate organic matter (POM) and dissolved organic matter (DOM) including marine snow (Cho and Azam, 1988; Smith et al, 1992) and only small molecules, around 600 Da (Weiss et al, 1991), can be transported across the bacterial membrane and thus bacteria secrete enzymes to hydrolyze high-molecular-weight organic matter. Moreover extra cellular enzymes play an important role in nutrient cycling as they may be produced in order to acquire the limiting nutrient (Hoppe, 1983; Zoppini et al, 2010).

In the aggregates, the activity of the extracellular enzymes transforms non utilizable POM in DOM, thus bringing small nutrient molecules into solution (Smith et al, 1992; Simon et al, 2002). The metabolic activity of colonizing bacteria drives sinking particulate organic matter into non sinking DOM (Azam and Long, 2001; Smith et al, 1992) fueling free-living bacteria (Kiørboe and Jackson, 2001). This bun coupled solubilisation causes rapid POMYDOM transition with important biogeochemical implications for carbon flux in the oceans (Smith et al, 1992; Simon et al, 2002).

Further, almost heterotrophic bacteria have developed several mechanisms which allow the preferred utilization of the most efficiently metabolised carbohydrates when are exposed to a mixture of carbon sources. Interestingly, similar mechanisms are used by some pathogens to control various steps of their infection process. The efficient metabolism of a carbon source might serve as signal for proper fitness. Alternatively, the presence of a specific carbon source might indicate to bacterial cells that they thrive in infection-related organs,

tissues or cells and that specific virulence genes should be turned on or switched off. Frequently, virulence gene regulators are affected by changes in carbon source availability. Thus, the activity of PrfA, the major virulence regulator in *Listeria monocytogenes*, seems to be controlled by the phosphorylation state of phosphotransferase system (PTS) components. In *Vibrio cholerae* synthesis of HapR, which regulates the expression of genes required for motility, is controlled via the Crp/cAMP CCR mechanism, whereas synthesis of *Salmonella enterica* HilE, which represses genes in a pathogenicity island, is regulated by the carbohydrate responsive, PTS-controlled Mlc (Poncet et al, 2009).

2.2. Use of carbohydrates mineralization for marine bacteria identification

The metabolic diversity of bacteria is perhaps as remarkable as their taxonomic and evolutionary diversity. Although culture-based studies are limited in their ability to estimate bacterial diversity, and several studies demonstrated varieties of modes of energy conversion of bacteria and wide ranges of substrate uses and metabolic pathways.). The flexibility of bacterial metabolism is similarly best illustrated by their abilities to degrade xenobiotic compounds such as malathion (an insecticide) and 2,4,5-trichlorophenoxyacetic acid (a herbicide), which are toxic to many other organisms (Brock et al, 1987 and Horner et al, 2003).

Metabolic assays mainly based on carbohydrates degradation on either aerobic or anaerobic conditions still effectively used to identify environmental bacteria besides molecular methods. Among these methods, the most widely used commercial identification systems are the API, the Biolog systems and more recently Microgen systems (Awong-Taylor et al, 2007).

The API 20 system included profiles of approximately 40 groups and species of bacteria in addition to the *Enterobacteriaceae* group in the API data set segregated mainly on assimilation of several carbohydrates with varied number depending on the type of API test kit used. (MacDonell et al, 1982; Bertone et al, 1996).

The Biolog identification system (Biolog, Inc., Hayward, CA, USA) is a bacterial identification method that establishes identifications based on the exchange of electrons generated during respiration, leading subsequently to tetrazolium-based color changes. This system tests the ability of micro organisms to oxidize a panel of 95 different carbon sources (Truu et al, 1999).

3. Effects of starvation conditions in carbohydrates metabolism

In their natural environment especially in marine ecosystems, bacteria are frequently exposed to major changes in growth conditions. These changes can include temperature, salt concentrations, essential nutrients, oxygen supply, pressure...etc. In order to sustain these changes, bacteria have developed mechanisms which allow them to adapt to drastic environmental alterations. Mainly, response to carbon source availability seems to be of special importance, because many bacteria dispose of more than one mechanism to adapt Carbon Metabolism and Virulence to changes in carbohydrate composition. These mechanisms include induction of specific carbohydrate transport and utilization system by

the presence of the corresponding carbon source and their repression when a more efficiently utilizable carbohydrate is present in addition. The latter phenomenon is called carbon catabolite repression (CCR) and is often mediated by more than one signal transduction pathway, which can include regulation of transcriptional activators or repressors, anti terminators, carbohydrate transporters or metabolic enzymes (Poncet et al, 2009).

3.1. *In vivo* starvation effect on catabolic carbohydrates profiles of *E.coli*

Escherichia coli, a fecal coliform, was found to survive for longer periods of time in non sterile natural seawater when sediment material was present than in seawater without sediments, and growth was observed to occur by one occasion (Gerba et al, 1976). This enteric bacterium was found to increase rapidly in number in autoclaved natural seawater and autoclaved sediment taken from areas receiving domestic wastes, even when the seawater had salinities as high as 34 g/kg. Else, longer survival of *E. coli* in sediment is revealed and attributed to greater content of organic matter present in sediment than the seawater.

Experimental, mainly *in vitro*, studies have been performed on the survival of different enteric bacterial species in seawater and results showed solar radiation is the most adverse factor for enteric bacteria. Its effect was found to be wavelength-dependent (most inactivation caused by UVB solar spectrum with a range of dose from 0.98 to 4 kJ/m²) and thus restricted to shallow (45 to 90 cm) or clear waters (Rozen and Belkin, 2001; Sinton et al, 1994; Villarino et al, 2003; Whitman et al, 2004). Moreover, in cases of its release from wastewater into marine environment, enteric bacteria are subject to osmotic stress with effects depending on water salinity (a high loss of viability for *E. coli* occurred for salinities between 15 and 30 g/L) (Anderson et al, 1979; Pereira and Alcantara, 1993). Most coastal waters provide low concentrations of nutrients essential to the growth and survival of enteric bacteria (Barcina et al, 1997). These stresses induce different resistance mechanisms for at least part of the cellular population (Martin et al 1998; Rozen et al 2002). As a consequence, different cellular states occur in the stressed population, including the viable but nonculturable state (VBNC). The ability of such cells to recover their growth capacity is still under debate (Arana et al, 2007).

Thus, we investigated survival and virulence of *Escherichia coli* strains (*E.coli* O126:B16 and *E.coli* O55:B5) exposed to natural conditions in brackish water by incubation in water microcosms in the Bizerte lagoon in Northern Tunisia and exposed for 12 days to natural sunlight in June (231 to 386 W/m², 26.61 uC, 30 g/L) and in April (227 to 330 W/m², 17.61 uC, 27 g/L) or maintained in darkness for 21 days (17.61 uC, 27 g/L). The results revealed sunlight as the most significant inactivating factor (decrease of 3 Ulog within 48 hours for the two strains) compared to salinity and temperature (in darkness). Survival time of the strains was prolonged as they were maintained in darkness. Local strain (*E. coli* O55:B5) showed better survival capacity (T₉₀ 552 hours) than *E. coli* O126:B16 (T₉₀ 511 h).

For both, modifications were noted only for some metabolic activities of carbohydrates hydrolysis (Table 1). Thus, *E. coli* O126:B16 culturable cells lost the ability to assimilate

amygdalin, and *E. coli* O55:B5 lost ability to assimilate melibiose, amygdalin, rhamnose, and saccharose.

Although, in previous data revealed exposure increase to sunlight, particularly UV radiation, alleviates some of the negative effects of altered organic matter quality through selective photolytic degradation. Both ultraviolet radiation (UV-B, 280–320 nm and UV-A, 320–400 nm) and visible light (photo synthetically active radiation, PAR, 400–700 nm) and induce major photolytic changes to complex organic molecules and generate large quantities of readily utilizable substrates for bacterial metabolism (Lindell et al 1995, Espeland & Wetzel 2001, Tietjen & Wetzel 2003). Thus, rates of carbon and nutrient cycling are often accelerated, stimulating ecosystem productivity. Interestingly, this enhanced microbial respiration may lead to increased CO₂ production and evasion to the atmosphere, creating a potential positive feedback to atmospheric CO₂ concentrations (Wetzel 2001; Lingo et al, 2007)

Strains	ONPG	ADH	LDC	ODC	CIT	H ₂ S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	CAT
Sunlight incubation (April and June) (n=2)																						
<i>E.coli</i> O126B16 ^a	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	-	+	+	-	+
after 3 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	-	+	+	-	+
<i>E.coli</i> O55B5 ^a	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	+
after 12 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	+
Darkness incubation (n=2)																						
<i>E.coli</i> O126B16 ^a	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	-	+	+	-	+
after 2 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	-	(-)	+	-	+
after 21 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	-	(-)	+	-	+
<i>E.coli</i> O55B5 ^a	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	-	+
after 2 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	(-)	(-)	+	-	+
after 16 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	(-)	(-)	(-)	(-)	+	-	+
after 21 days	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	(-)	(-)	(-)	(-)	+	-	+

(+) positive reaction; (-) : negative reaction; *n*: number of colony tested. ONPG: β-galactosidase, ADH: arginine dihydrolase, ODC: ornithine decarboxylase, CIT: use of citrate, URE: urease, TDA: tryptophane desaminase, IND: indole production, VP: acetoin production, GEL: gelatinase, GLU: glucose assimilation, MAN: mannitol, SOR: sorbitol, RHA: rhamnose, SAC: saccharose, MEL: melibiose, ARA: arabinose

Table 1. Characteristics of *E.coli* O55B5 and *E.coli* O126B16 before and during seawater incubation

3.2. *In vitro* starvation effect on catabolic carbohydrates profiles of *E.coli*

Depending on the nature of aquatic environmental receptor (seawater, wastewater or brackishwater), different physiological, metabolic and pathogenic modifications related to stress conditions have been reported by much studies (Gauthier *et al*, 1993; Dupray and Derrien, 1995; Baleux *et al*, 1998; Troussellier *et al*, 1998; Monfort and Baleux, 1999). And different studies showed that adaptation forms of enterobacteria to drastic conditions

occurring gradually during discharging in wastewater (Dupray and Derrien, 1995), or during the transfer from a previous culture to a saline medium or to mixed wastewater/seawater medium (Munro *et al*, 1987), allowing better survival in seawater conditions. In this report, *E. coli* was chosen as a representative strain of Thermotolerant Coliform (TTC), used as fecal contamination indicator. Most of *E. coli* strains are normal intestinal flora components; however, certain pathogenic strains are important disease factors. Those pathogenic strains are classified into at least six distinct groups: enteropathogenic *E.coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroinvasive *E.coli* (EIEC), diffuse-adhering *E.coli* (DAEC), enteroaggregative *E.coli* (EAEC), enterohemorrhagic *E.coli* (EHEC) (Schroeder *et al*, 2004). Thus, we studied the behavior of these 6 different types of *E.coli* when discharged in seawater with or without a previous incubation in wastewater microcosms. Else, we examined their metabolic, antibiotic and virulence resistance patterns after exposure to salinity gradient.

Therefore, the studies were carried out using membrane chambers with 120ml capacity, constituted by assembly of glass tubes allowing dissolved matter to penetrate, through 0.2 µm- pore- size, 25 mm-diameter filters, fixed between two Teflon joints. For each *E.coli* strains two types of microcosms were prepared: one type was inoculated by seawater and the second with wastewater.

For microcosm inoculation, the seawater used was brought from Bizerte lagoon (northern part in Tunisia of Tunis). Wastewater was brought from sewage treatment station (near Tunis town).

The results obtained (Table 2) showed most important modifications noted for the strains previously inoculated in wastewater and O126B16 *E.coli* strain lost ability of fermenting glucose and mannitol, producing indole and to use citrate, but it acquired urease and gelatinase activities. All the other pathogenic *E.coli* strains maintained their major metabolic characteristics except for the ability to use citrate and to produce indole.

In further study, we compared the behavior of *E.coli* (ATCC 14948), *Vibrio paraheamolyticus* and *Salmonella Typhymurium* (ATCC 17802) when discharged in seawater with or without previous incubation in wastewater microcosms and we examined mainly their metabolic, patterns after exposure to salinity gradient. The results obtained showed metabolic profiles changes both for *E.coli* and *Salmonella Typhymurium* and loss for production of several carbolases mainly glucose, mannose, rhamnase, sorbitol, sucrose, melibiose and arabinose hydrolases whereas for *Vibrio paraheamolyticus* no changes were observed.

Similar results were found previously by Ben Kahla –Nakbi *et al* (2007) for *V. alginolyticus* strains incubated in seawater microcosms for long period of starvation and for what the result of metabolic carbohydrates profiles tested by results of API system tests, showed no modification. These findings were explained by preparation of cells to enter into VBNC state characterized by low metabolic rate, reduction in size, morphological changes or synthesis of specific proteins. This adaptation strategy developed by bacteria in aquatic environments allows them to survive during long period of time. Modifications of cellular morphology should be related to modifications with carbohydrates metabolites contained in parietal membranes for these species.

Strains	API 20E*															
	ONPG	ADH	ODC	CIT	URE	TDA	IND	VP	GEL	GLU	MAN	SOR	RHA	SAC	MEL	ARA
O126B16	+	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+
S.W.	-	-	-	-	-	+	+	-	-	+	+	+	-	-	+	+
W.W.	-	-	-	+	+	+	-	+	+	-	-	-	-	+	-	-
ECEAgg	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+
S.W.	+	-	+	-	-	+	-	-	-	+	+	+	+	+	+	+
W.W.	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	+
ECEI	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	+
S.W.	+	-	+	-	-	+	+	-	-	+	+	+	+	-	+	+
W.W.	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-
ECEH	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	+
S.W.	+	-	-	-	-	+	+	+	-	+	+	+	+	-	-	+
W.W.	+	+	-	+	-	+	-	-	-	+	+	+	+	-	-	+
ECEP	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	+
S.W.	+	-	-	-	-	+	+	+	-	+	+	+	+	-	-	+
W.W.	+	-	-	+	-	+	-	-	-	+	+	+	+	-	-	+
ECET	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+
S.W.	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+
W.W.	+	+	+	+	-	+	-	-	-	+	+	+	-	+	-	+

*ONPG: β -galactosidase, ADH: arginine dihydrolase, ODC: ornithine decarboxylase, CIT: use of citrate, URE: urease, TDA: tryptophane desaminase, IND: indole production, VP: acetoin production, GEL: gelatinase, GLU: glucose, MAN: mannitol, SOR: sorbitol, RHA: rhamnose, SAC: saccharose, MEL: melibiose, ARA: arabinose.

Table 2. Biochemical patterns. Modifications of some biochemical characters on APi 20E strips of different *E.coli* strains after survival in seawater with previous incubation in seawater (SW) or in wastewater (WW). O126B16 (reference *E.coli* strain), EaggEC (Enteraggregative *E.coli* strain), EIEC (Enteroinvasive *E.coli* strain), EHEC (Enterohemorrhagic *E.coli* strain), EPEC (Enteropathogenic *E.coli* strain), ETEC (Enterotoxigenic *E.coli* strain).

Keymer et al (2007) described similar results for *V.cholerae* and pointed that environmental parameters, measured *in situ* during sample collection, should correlated to the presence of specific dispensable genes and metabolic capabilities, including utilization of mannose, sialic acid, citrate, and chitosan oligosaccharides. Thus, gene content identified and metabolic pathways that are likely selected for in certain coastal environments and may influence *V. cholerae* population structure in aquatic environments.

Our previous data described *A.hydrophila* cultured for 30 days in marine water microcosms which maintained the ability to metabolize all the carbohydrates and continued to produce several enzymes with differences of its homologous cultured in waste water previous to incubation in marine water microcosm which failed to produce numerous enzymes and metabolize carbohydrates (El Mejri et al, 2008). Thus, for marine heterotrophic bacterial populations as *Vibrio sp* and *Aeromonas sp* halotolerant conserve major potential of their carbohydrate metabolism.

For high salinity or halotolerant bacteria, many strains that grew fermentatively on carbohydrates in the presence of air (determined by the acidification of the medium) grew especially well on glucose, totally by substrate- level phosphorylation in the absence of air. Tomlinson and Hochstein (1976) demonstrated that O₂ consumption by *Halobacterium saccharovorum* in presence of glucose was 18% of the theoretical amount required for its

complete oxidation it was for 83% in presence of galactose or fructose which like galactose, requires more oxygen for its oxidation and therefore was generally a poorer substrate for fermentation (Javor et al, 1984). The measurement of O₂ consumption, growth rates, and fermentation products in the presence of glycerol, pyruvate, and acetate by strains that demonstrated relatively good anaerobic growth on these substrates would provide further evidence of the importance of fermentative metabolism in extreme halophiles.

In the water column, biofilm microenvironments in suspended flocks may form a stabilizing refuge that enhances the survival and propagation of pathogenic (i.e., disease-causing) bacteria entering coastal waters from terrestrial and freshwater sources. The EPS matrix offers microbial cells a tremendous potential for resiliency during periods of stress, and may enhance the overall physiological activities of bacteria as it's emphasized by Giller et al (1994) that influences small-scale microbial biofilms must be addressed in understanding larger-scale processes within inter tidal systems. Intertidal systems are a key interface of the ocean, atmosphere, and terrestrial environments, and as such, are characterized by frequent fluctuations in temperature, ion concentration, desiccation, UV-irradiation, and wave action. The relative frequency of these fluctuations poses both physical and biochemical challenges to micro organisms which inhabit this environments such estuarine areas. The characteristics and intensities of such stresses may vary substantially (Decho et al, 2000).

Therefore, in estuarine region with with salinity of 35 ppt it is been reported that the growth rate of lingo-cellulose-degrading populations (50%) is inhibited by NaCl (Liu and Boone, 1991). According to Park et al (2006) the high salinity seems to significantly reduce ecto enzyme activities. Thus, it should be possible that type of bacterial population found at particular station can be influenced by salinity changes. Also, it has been reported in most of the aquatic environments there is a significant relationship between extracellular enzyme activities, their corresponding substrates (polymers) and their hydrolysis products (monomers) (Münster et al, 1992). Thus, presence of cellulolytic and hemi-cellulolytic bacteria in one particular region and their absence in other region should indicated presence of wide-ranging organic matter in each region (Khanderparker et al, 2011). Previous research has also put forth a widely accepted concept that hydrolytic enzymes are induced by presence of polymeric substrates (Chrost, 1991; Vetter and Deming, 1999). It was observed that the genus *Bacillus* and *Vibrio* were the dominant hemicellulase and cellulase producers in both the estuaries. According to Ruger (1989) the genus *Bacillus* comprised phylogenetically and phenotypically diverse species, which are ubiquitous in terrestrial and fresh water habitats and are also widely distributed in seawater ecosystems. While the family of *Vibrionaceae* represent the most important bacterial autochthonous groups in marine environments. Members of this family often predominate in the bacterial flora of seawater, plankton, and fish. In the West Pacific Ocean, , *Vibrios* accounted for nearly 80% of the bacterial population in surface seawater (Simidu et al, 1980) and *Vibrio* phylogenetic diversity of their culturable forms in Bulgarian hot springs as described along with their abilities to metabolize carbohydrates (Derekova et al, 2008)..

For Moari et al, (2011), marine models tested showed, prokaryotic abundance in superficial marine sediments controlled by organic trophic resources, while in sub-surface sediments, prokaryotic activities and abundance were driven by environmental factors and predatory pressure, suggesting that the shift in prokaryotic community structure could be coupled to a change in life-style of microbial assemblages.

Previously, Bouvier et al, (2002), discussed the relationship between the compositional succession and changes in single-cell metabolic activities in the Choptank River, and suggested that profound phylogenetic shifts were linked to cell stress, loss of activity, and death (Del Giorgio and Bouvier, 2002). Similarly both types of succession, i.e., activation/inactivation and replacement, occurred simultaneously, and understanding the relative importance of such processes in determining bacterial succession and community composition remain to be clarified considering environmental conditions that trigger bacterial succession observed within the mixing areas. .

4. Conclusion

Carbohydrates metabolism still basic to marine microbiota mainly for autochthonous communities as *Vibrio*, *Aeromonas* and *Bacillus* genus with high potential of degradation and considerable supply of big amounts of different carbolases and pathways which should take more attention in order to be clarified.

Increase in significant amounts of organic materials are being exchanged between the land and marine ecosystems with higher amounts of dissolved carbohydrates provided especially the polymeric substrates which represent the most abundant molecules used by marine bacteria as structural and /or storage compounds under extreme variations in environmental parametres and biological marine habitats.

The intrinsic ability of bacterial cells to enter in to viable but non culturable state (VBNC) characterized by low metabolic rate and morphological modifications (reduction in cell size and membrane alterations) should be related with carbohydrates metabolism and change observed experimentally. Therefore, investigations in carbohydrates metabolites which continue going on will certainly elucidate more molecular adaptations forms of bacteria (for mainly enteric species) in variable marine ecosystems .

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