International Scholars Journals

International Journal of Virology and Parasitology, Vol. 6 (6), pp. 001-010, June, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

Bio surfactants produced by *enterobacterial*

Alejandro De Jesús Cortés-Sánchez[1]*, Mayra Diaz-Ramirez[2], Alan Javier Hernández-Álvarez[3], Felipe García-Ochoa[4], Adriana Villanueva-Carvajal[5], Liliana León-López[6], Alejandra L. San Martín-Azocar[7].

[1]Secretaria de Salud. Comisión Federal para la Protección contra Riesgos Sanitarios. Departamento de microbiología, México.

[2]Departamento de Alimentos. División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana (UAM), Unidad Lerma. Edo. México.

[3]Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd. W. St. Hyacinthe, QCJ2S 8E3, Canada.

[4]Departamento de Biofísica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México.

[5] Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Campus Universitario “El Cerrillo” A.P. 435, Toluca, Estado de México C.P. 50200, México.

[8]Programa Regional del Posgrado en Biotecnología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, 80000 Culiacán, México.

[7] Instituto Tecnológico y de Estudios Superiores de Monterrey, Escuela Superior de Ingeniería en Alimentos, Biotecnología, Agronomía. Querétaro-México.

Accepted 08 October, 2016

In spite of being considered to be pathogenic microorganisms, some *enterobacterial* has the ability to produce biosurfactants (BS). These molecules besides their physicochemical and biological characteristics as emulsifiers, dispersants, stabilizers among others, are widely accepted in the industry since they are biodegradable, and present low toxicity. This has generated studies focused on the identification of new producer microorganisms, as well as the search of new carbon sources, improvement of production processes and the generation of a wide variety of patents. Their use is extensively spread in a diversity of industrial areas such as the pharmaceutic, food and environment, as alternatives of the surfactants derived from oil (which are highly pollutant),generating products and services of added value. In this review the properties and applications of the BS are summarized as well as the major genres of this group of *Enterobacteriaceae* that have been used for BS's production to update the actual advances on the benefits that these microorganisms possesses as BS producers.

Keywords: biosurfactants, enterobacterial, glycolipids, biotechnology, secondary metabolites.

INTRODUCTION

The family *Enterobacteriaceae* are a group of bacteria that received this name due to the fact that normally they are located like saprophytes in the digestive tract of animals and man, though it is also possible to find them as transitory microbiota or normal in oropharynx, skin and genitourinary tract. In addition they are considered as well as cosmopolitan organisms isolated from soil, water and vegetables. Among the genre that constitutes this group are: *Escherichia spp., Klebsiellaspp.*, *Salmonella spp.*, *Enterobacter spp.*, *Serratia spp.*, *Hafnia ssp.*, *Citrobacter ssp.*, *Yersiniaspp.*, *Proteus ssp.*, *Rhanella ssp*., *Providenciaspp.*, *Morganella spp.*, *Shigella spp.*,, *Edwarsiella spp.*, *Ewingella ssp., Budvicia ssp*., *Tatumella ssp*., *Erwinia spp.,Koserella ssp., Kluyvera ssp., Hoganella ssp., Moellenella ssp., Leminorella ssp., Buttiauxella ssp*., *Pantoea spp*. Some of them are considered as pathogenic for man and plants, being in some cases in a certain manner as opportunists (Ocaña et al. 2007; Romero, 2007; Ardila, 2010; Puerta and Mateos, 2010; Toth et al. 2003). They are gram negative microorganisms with cane or bacillus form of 1-3 µm of length and 0.5 µm of diameter, generally mobile (flagellum), pili's producers, can be aerobic and facultative anaerobic, do not form spores, able to reduce nitrates to nitrites (some exceptions does exist), do not liquefy alginate, capable to ferment carbohydrates as glucose (Embden-Meyerhoff Route) to pyruvic acid with gas production, are oxidase negative, catalase producers and its growth is not favored by NaCl presence (Romero, 2007; Koneman et al. 2008; Puerta and Mateos, 2010; Ardila 2010),in the Table 1 different biochemical tests for the identification of enterobacterial at laboratory level are shown. These bacteria possess a cytoplasmic membrane, a peptidoglycan cover and a complex cellular wall that comprises the capsule, which contains lipopolysaccharides (LPS) and channels for antibiotics and nutrients penetration. They also possess three different types of surface antigens which serves to identify and serotype them, they are: the O somatic antigen of cellular wall, flagellum antigen H, and the K antigen or capsular (Romero, 2007; Ardila, 2010); They also have showed the aptitude to acquire rapidly resistance to antibiotics even to third generation cephalosporin and in recent years a percentage every time higher for these bacteria, mainly of the *Klebsiella spp. and Enterobacter spp.* genre, due to its capacity to synthetize extended spectrum ß-lactamases (ESBL) turning this situation a topic of great medical importance (Puerta and Mateos, 2010; Ocaña et al. 2007).

The pathogeny of the enterobacterialits due to a wide variety of virulence factors which involves the antigenic variation, endotoxins as the O antigen where in addition certain O antigens might act as adhesion/colonization factors which are necessary for the infection, H antigens that are possibly responsible for its capacity of progression across the urinary tract (Ardila, 2010), exotoxins (thermolabile or thermostables), the capsule that gives them protection against phagocytosis, fimbria that are filamentous prolongations which allows the adherence of bacteria to specific recipients of the respiratory tract cells, digestive and genitourinary, plasmids (fragments of transferable extra chromosomic DNA from bacteria to bacteria, not always of the same specie), which confers them antibiotics resistance (R plasmids), bacteriocins (bactericidal substances against strains of the same specie but not against themselves) as colicine and marceline, and finally the intracellular location as protection against the immunological system and antibiotics (Romero, 2007; Ardila, 2010).

The pathogenic character of these microorganisms can bring many harmful effects to health; Nevertheless the saprophytic character of some of them, can lead to different benefits for the man as is the case of fungi and yeasts which are producers of a wide diversity of food and pharmaceutical metabolites use with great economic impact (Montoya,2008),likewise the bacteria such as lactic acid bacteria (LAB) that occurs in the production and food preservation (yogurt, cheeses, pickles, sausages, and ensilages) and as well as probiotics due to the health benefits of whom consume these products in adequate dosages (immune stimulation, reduction of serum cholesterol, colon cancer, intolerance to lactose among others) (Ramírez et al. 2011).

The potential discovery in the micro biodiversity is a part of an important activity known as bio-prospection, which consists on the search and analysis of diverse ecosystems, which goes from thermal waters, glaciers zones, and marine sediments, contaminated zones with hydrocarbons amongst others, in order to select those microorganisms with useful metabolic properties of economic and commercial interest. Leading the search to producer organisms of agricultural, pharmacological and food interests predominantly, as well as its application in bioremediation activities (Piñero-Bonilla, 2013).

In this matter stands out the finding of some enterobacterial which are capable to produce metabolites of biotechnological interest such as the bio surfactants (BS); Which in the recent years, have generated significant expectations of industrial interest for their high potential of applications in diverse areas such as food, agricultural, domestic cleanliness, cosmetics, oil recovery and environment throughout bioremediation processes. Nevertheless, one of the limitations in the commercialization and use of these biological compounds,

*Corresponding author: alecortes_1@hotmail.com

Table 1. Biochemical tests for the identification of microorganisms belonging to the *Enterobacteriaceae* family.

Analysis			
Production of enzymes	Oxidase	Urease	DNase
	Lipase	Gelatinase	Catalase
	Ornitin descarboxilase	Phenylalanine desaminase	
	Arginin dihidrolase	Lysin descarboxilase	
SugarsFermentation	Glucose	Dulcitol	Ramnose
	Lactose	Arabinose	
	Sacarose	Xilose	
	Manitol	Melobiose	
	Adonitol	Sorbitol	
	Rafinose	Celobiose	
Metabolism and locomotion	Metil Red	H_2S production	
	Voges-Proskauer	Indol production and gas	
	Citrate	movility	
	Malonate		

Romero, 2007; Koneman et al. 2008; García and Mendoza, 2014

are the high inherent costs of their large-scale production. Therefore, the studies around the world in the production area of these compounds has gone so far to the search of producer strains, as well as the optimization of the production processes that involves the use of inexpensive substrates diminishing this way the costs (Jimenez et al.2010).

The current review is focused in presenting in general means the properties and applications of the BS, as well as the producer microorganisms, specifically some members of the bacterial group *Enterobacteriaceae*, where some genre and species have shown that from their metabolism certain BS compounds with tenso-active characteristics can be obtained, achieving products of high added value and of biotechnological importance for diverse industries.

Biosurfactants

The bio surfactants (BS) are molecules with surface activity (capable of reducing the superficial and interfacial tension) synthesized by a broad variety of microorganisms generally by bacteria, filamentous fungi and yeasts, in an extra cellular manner or as compounds associated with cellular membranes during its growth in insoluble water substrates (Table 2). These compounds are characterized for being amphipathic due to an extensive variety of chemical structures in their molecules which involves hydrophobic groups (hydro carbonated unsaturated chains, saturated, or fatty acids) and hydrophobic (acids, phosphates, peptidicions, cyclical peptides, mono-, di - or polysaccharides) (Janek et al. 2013; Cameotra et al.,2010; Marchant and Banat, 2012; Fracchia et al. 2012; Jimenez et al.2010; Nitschke and Costa, 2007). They are classified due to its composition and microbial origin under molecules of low molecular weight that includes: a) glycolipids b) phospholipids and fatty acids c) lipopeptides/lipoproteins which are efficient in the superficial and interfacial tension reduction; and molecules of high molecular weight such as: d) Polymeric surfactants and e) Surfactants particles which are effective in the emulsions stabilization (Nuvia et al. 2014; Kapadia and Yagnik, 2013; Nitschke and Costa, 2007). The glycolipids are the more commonly isolated BS`s and are widely studied due to their high production performance and low cost, are constituted by mono-, di-, tri- and tetra- saccharides in combination with one or more aliphatic fatty acids chains or hydroxi-aliphatic acidsby an ester group (Nuvia et al.2014; Kapadia and Yagnik, 2013; Jimenez et al. 2010). The BS are generally synthesized during the secondary metabolism, its main functions during growth and survival of the producer micro organisms are, the adhesion to interfaces in natural environments, emulsification of immiscible substrates in water, biodisponibility, hydrophobic substrate desorption for its degradation, nutrients transport, defense strategies against other micro organisms for its antibiotic effects in eukaryotes and prokaryotes and they also intervene in the microbe-host interactions, as well as in quorum sensing mechanisms (Gudiña et al. 2013; Cameotra et al. 2010; Jimenez et al. 2010).

Table 2.-Producer microorganisms of different BS (Yu et al.2002;Gunter et al.2005;Hewald et al.2005; Nitschke, and Costa,2007;Kapadia and Yagnik, 2013; Fracchia et al. 2012; Morita et al. 2013; Janek et al. 2013; Jimenez et al.,2010; Dinamarca et al.2013; Nuvia et al.2014).

Biosurfactant	Classification	Chemical structure	Microorganism Producer
Ramno lipids	Low molecular weight	Glycolipid	*Pseudomonas aeruginosa Pseudomonas chlororaphis* *Pseudomonas fluorescens* *Pseudomonas putida BD2*
Soforo lipids	Low molecular weight	Glycolipid	*Candida bombicola* *Rhodotorula bogoriensis, Wickerhaminella domercqiae* *Candida batistae*
Trehalo lipids	Low molecular weight	Glycolipid	*Rhodococcus erythropolis, Mycobacterium spp.* *Arthrobacter spp.* *Nocardia spp.* *Corynebacterium spp.* *Brevibacteria spp.*
Mannosylerythritol lipids	Low molecular weight	Glycolipid	*Pseudozyma sp.* *Ustilago sp.*
Celobiose Lipids	Low molecular weight	Glycolipid	*Ustilago maydis*
Mycolics acids	Low molecular weight	Fatty Acids/Neutral lipids	*Corynebacterium insidibasseosum*
Gramicidin	Low molecular weight	Lipopeptide	*Bacillus brevis*
Iturin A	Low molecular weight	Lipopeptide	*Bacillus amyloliquefaciens*
Surfactin	Low molecular weight	Lipopeptide	*Bacillus subtilis* *Bacillus pumilus* *Lactobacillus spp.*
Emulsan	Low molecular weight	Lipo-polysaccharide	*Acinetobacter calcoacetius*
Vesicules	High molecular weight	Surfactant particles	*Acinetobacter calcoaceticus*
Liposan	High molecular weight	Polymeric	*Candida lipolytica*
Mannoprotein	High molecular weight	Polymeric	*Saccharomyces cerevisiae*

The diversity in the functional properties of the BS isextensive such as emulsifying, solubilizing, dispersant, moisturizing, foaming, cleanliness, phase separator, active surface and also able to reduce the viscosity of heavy liquids as oil, making them a potential alternative in many industrial processes and domestic applications. In addition for several years they have been considered to be substitutes to the replacement of synthetic surfactants in diverse industrial areas such as food, pharmacy, cosmetics, textiles, paintings, agrochemicals and in bioremediation environment processes (Figure 1). The aforementioned is due to the fact that these biological molecules exert certain advantages or benefits on its counterparts of chemical origin such as its biodegradability, environment compatibility, low toxicity, high selectivity and specificity in extreme temperatures, pH and salinity, biocompatibility (as an example: not skin irritating), high digestibility, emulsifier activity, demulsifier and antimicrobial (Kapadia and Yagnik, 2013; Ławniczak et al. 2013; Fracchia et al. 2012; Jimenez et al. 2010) besides

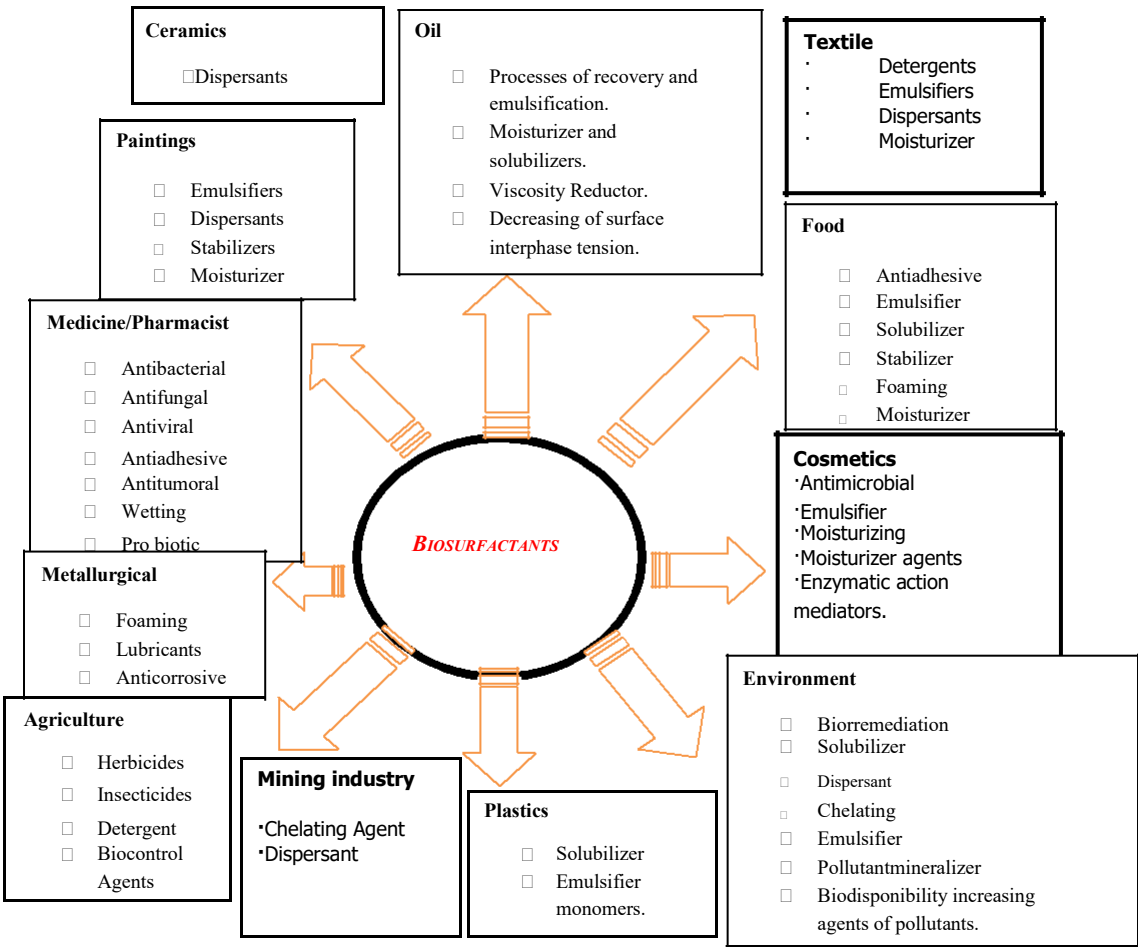

Figure 1.-Potential uses of BS in different industrial areas (Rodrigues, et al. 2006; Nitschke et al. 2007; Banat et al.2000; Jiménez et al., 2010; Banat, et al. 2010; Ławniczak et al. 2013; Kapadia and Yagnik, 2013; Gudiña et al. 2013).

these can be produced from economic raw materials or residual substrates as animal fat, fried oil, molases, soap stock, dairy whey, starch residues, by-products of the oil vegetal refinement and distilleries) (Maneerat et al.2005; Nitschke et al. 2007; Kapadia and Yagnik, 2013).

Many factors have been reported to affect the production, structure and properties of BS, among them are the producer strain nature, culture conditions and culture media composition (type, solubility and carbon source concentration). It`s been reported that BS biosynthesis production it`s more predominant in water insoluble carbon sources instead of hydrophilic substrates (Kiran et al. 2014, Jimenez et al. 2010). Some hydrophobic sources used for this purpose are diesel, kerosene, phenanthrene, crude oil, vegetable oils (corn, soybean, sunflower and olive), glucose, sucrose, fructose, molasses, glycerol and other organic compounds (Nuvia et al. 2014; Fakruddin, 2012). However in some cases a catabolic repression phenomenon could exist due to substrates such as glucose and other primary metabolites as acetate and tri carboxyl acids (Karanth et al. 1999; Jimenez et al., 2010). The nitrogenous source is an elemental component in cellular growth, proteins and enzyme synthesis, showing an effect in BS production, some microorganisms such as P. aeruginosa BS-2 and *U. maydis* showed a BS increase under limited nitrogenous conditions (Saharan et al. 2011). There are a wide variety of nitrogenous sources used in this process as urea, peptone, yeast extract, ammonium sulfate, ammonium nitrate, sodium nitrate, meat extract and malt (Fakruddin, 2012; Saharan et al. 2011). Phosphorus, metal ions, metal ions as nano-particles also affect the BS production, as well as the environmental conditions such as temperature, pH, dilution rate, agitation and aeration which is closely involved in the oxygen transfer from the gas to the aqueous phase (Nuvia et al.

2014; Fakruddin, 2012; Kiran et al. 2014, Jimenez et al. 2010).

BS production by different *Enterobacteriaceae*

Some genera and species of the *Enterobacteriaceae* family that have been isolated and/or identified as BS producers are followed described; these compounds generally are promoted as an application alternative on different industrial areas, mainly in processes from oil industry and environment (bioremediation) due to their pathogen character.

Klebsiella: This genus is formed by different species, among the best studied are *K. ozaenae, K. pneumoniae, K. oxytoca, K. planticola y K. terrigen.* These bacteria are immobile and have an external layer formed by a large polysaccharides capsule (anti-phagocyte factor), this characteristic makes them dissimilar from other *Enterobacteriaceae* genus (Romero, 2007; PAHO, 2014). These microorganisms are mainly isolated from fecal and clinical samples; however, they appear and multiply naturally in aqueous environments and in nutrient-rich waters such as paper mill waste, textile and sugarcane processing where higher concentrations can be achieved (PAHO, 2014).

Rakeshkumar et al., (2013) evaluated the BS production capacity of the alkalophilic strain *Klebsiella sp*. RJ-03, isolated from soil contaminated with oil, using several unconventional carbon sources (*M. indica*, bagasse, wheat straw, rice hull, potato peel powder, corn powder, soy powder, *Jatropha* cake and seaweed sap) at 5% in distilled water, these samples were digested during 1 hr and incubated at 32°C/120rpm for 72h. The BS nature produced was a polysaccharide-protein complex; among the different carbon sources used the highest BS production (15.4g/L) was achieved using corn powder, obtaining suitable viscosity and emulsification properties, surface tension reduction, high temperature stability, pH and salt stress, ability to remove soil and fabrics oil, efficacy, stability and compatibility with different commercial chemical detergents and surfactants (SDS, Tween 80, Triton X-100) improving the yield of washing processes, this study confirms the possibility to obtain an economical industrial production of BS which can be used as an additive for industrial and home detergents.

Moreover Parven et al. (2012) reported the isolation of *Klebsiella pneumoniae* WMF02 strains from hydrocarbons polluted regions in Selangor, Malaysia. This strain was fermented in liquid state, using palm oil sludge (POS) as substrate at 4% and sucrose as co-substrate (5g/L) during 30h at 37°C and at 180rpm. This process enabled the synthesis of a phospolipid BS type, which reduced the surface tension of the fermentation broth from 36.2 mN/m to 26.2 mN/m, furthermore the addition of a water soluble co-substrate (sugars) facilitated the release of immiscible substrates from the cells to the media. Additionally a nutrients optimization analysis (carbon sources, minerals and nitrogen) to synthesize BS was carried out showing as optimal concentrations: saccharose 5 g/L, $MgSO_4$ 0.4 g/L, $FeSO_4$ 0.3 g/L, $NaNO_3$ 2 g/L, K_2HPO_4 4 g/L, y 4 % (v/v) of POS, promoting a slight drop of the culture surface tension from 36.2 mN/m a 25.70 mN/m. These authors concluded that the palm oil sludge is a useful substrate to produce BS by *Klebsiella pneumoniae* WMF02 isolates.

Proteus: This genus involves several microorganisms in the shape of very mobile bacilli of which the most known species are *Proteus mirabilis, P. vulgaris* and *P. myxofaciens*. These species are considered pathogens and are associated with urinary tract infections which are prone to generate some opportunistic infections (Romero, 2007). Padmapriya et al. (2011) reported the ability of an isolated strain from *Proteus inconstans* to grow and produce BS on different carbon sources (glucose, saccharose and glycerol), temperature and pH conditions, evaluating its biodegradation and emulsification hydrocarbons ability. A BS glycolipid nature was obtained, showing a productivity of 0.8 mg/mL after 72h in a culture supplemented with glucose at pH 7 and 35°C. This *Enterobacteriaceae* was identified as a potential microorganism to be used for bioremediation processes on soils polluted with oil as well as in the enhanced oil recovery (EOR). Otherwise Ibrahim et al. (2013) reported the production of bacterial isolates from the soils through enrichment methods in agar-oil at 30°C/5 days, identifying among them some *Enterobacteriaceae* like *Proteus vulgaris, Proteus mirabilis y Serratiamarcescens,* showing a higher emulsification index (87%) of BS produced by *Serratiamarcescens*. The nature of the BS generated was lipopeptidic and could be used in the EOR process under different environmental conditions.

Serratia: These microorganisms are bacillus which live in the gut, and furthermore can produce pathologies and also affect other tissues. The most common species are *Serratialiquefasciens, S. marcescens, S. rubidea, S. Ficaria y S. odorífera. Serratiamarcescens* is the most known and it was considered as an important tracking environmental pollution signal due to its red pigmentation in culture media. Also, these microorganisms can be found in stagnant water, plants and soil (Romero, 2007; Koneman et al. 2008). *Serratia*strains are able to generate different BS such as the BS ionic serrawettinas produced by *Serratiamarcescens,* presented in three species, W1, with a cycle structure (D-3-hydroxydecanoyl-L-seryl) 2, W2 D-3-hydroxydecanoyl-D-leucyl-L-seryl-L-threonyl-D-phenylalanyl-L-isoleucyl lactone, and W3, cycle depsipeptide formed by five amino acids and by dodecanoic acid. *Serratiarubidaea* produces rubiwettina R1, formed by D-3-hidroxi fatty acids and RG1, β-glucopyranosyl ring bound to D-3- hydroxylated fatty acids. These molecules are secondary metabolites which are

produced at 30°C above culture surfaces in a liquid state and at 37°C in solid media secreted by extracellular vesicles. These BS facilitates the grow diffusivity and the colonization of the solid surface environment being more noticeable in deficient nutrimental conditions in order to find more nourishing areas; moreover serrawettinas with antibiotic activity have been reported acting as chemical carriers in the predator-prey relation and also have shown to be inductors in the apoptosis cycle of various cell lines derived from human tumors and cell B-chronic lymphocytic leukemia (Toheyet al. 2011). Devendra et al. (2011) reported the isolation of *S. marcescens* strain from marine environments which is able to produce BS in a Zobell marine broth during 48h/30°C/120rpm. The BS generated of glycolipid nature and also formed by glucose and palmitic acid demonstrated an inhibitory activity in the molding of biofilms and in the growth of pathogen agents as *C. albicans, P. aeruginosa y B. pumilus,* showing that the marine microflora is a source of bioactive compounds with many applications in the clinical and environmental areas.

Providencia: These microorganisms have similar characteristics to the *Proteus* genus; these are *P. rettgeri, P. alcalifaciens, P. heimbache* and *P. stuartil*; all of them are capable to remove the amine group in the phenylalanine. Furthermore, the infections produced by these microorganisms are uncommon in humans (Koneman et al. 2008; Romero, 2007). Parveen et al. (2014) found that *Providencia alcalifaciens* SM03 strain is able to produce a BS glycolipid-type when the residual palm kernel cake- (PKC) is degraded as substrate at 2% in liquid fermentation at 37ºC/180 rpm during 36h with yields of 8.3g/L, concluding that the PKC is a potential substrate in the BS production using this novel strain. Moreover Azizollah et al. (2011) reported that microorganisms isolated from the ear canal and groin areas of goats and cows which belongs to the genus *Providentia spp*. were able to generate BS through hemolytic activity assays and oil diffusion technique (O.S. SD). Also these authors observed that when the inoculation was performed in Lauria Bertani broth at 37 °C/72 h/ 50 rpm, the supernatants showed an emulsification index at the 24h (E24%), 72h (E72%) and O.S. SD of 54%, 54% and 71 cm for goat isolates and 50%, 63.6% and 5.55 cm for cow isolates, respectively. The authors suggested even though the function and composition of the BS is not well known, its probable function could be as an auxiliary in the surface on the fat layer elimination process by the hydrophobic fat solubility or in the prevention of the destructive function in the skin due to lytic substances; also it could contribute to the organic matter dissolution of the skin surface secreted by different systems in the body and also plays an important role in the formation of bacterial communities in the skin surface. Finally the ruminant's skin fat zones could be a good source of these BS producers, however more in depth research about phylogenetic of the strains producers and chemical composition of the BS obtained must be developed.

Enterobacter: These microorganisms are mobile bacillus, which produce decarboxylation of ornithine and large quantities of gas; they are part of the native intestines microflora; some of them are pathogenic and sometimes they can generate opportunistic infections. The most known species are *E. agglomerans, E. cloacae, E. aerogenes, E. asburiae, E. dissolvens* (Koneman et al. 2008; Romero, 2007). Toledo et al. (2008) reported the isolation of *Enterobacter sp.* from crude oil wastes which was able to produce a polymeric BS growing on different hydrophobic substrates (n-octane, toluene, xylene, light mineral oil, heavy mineral oil and crude oil) at 1% during 8 days at 32°C and 100rpm, showing a higher yield of 0.86 ±0.23 g/L in heavy oil. These authors concluded that this strain is able to be used as emulsifier of the evaluated substrates with potential applications in bioremediation processes. On the other hand, Jadhav et al. (2011) reported the isolation of a bacterial strain from diesel contaminated soil, which was identified through genetic analysis of 16S rRNA, as member of the genus *Enterobacter* and named *Enterobacter sp.,* MS16. This strain was able to produce BS and grew on unconventional substrates such as molasses, sunflower oil and peanut oil residues, being the latest the one with highest BS production (1.5g/L) and with a 68% surface tension decrease. Moreover the composition of this BS was mainly composed of glucose, galactose and arabinose as well as well as C_{16} and C_{18} hydroxy fatty acids; the BS also showed antifungal activity and inhibition of fungal spores' germination. The conclusion of this work was that the *Enterobacter sp.,* MS16 production and the use of the residual industrial substrates are an economical way to produce BS with biological activity and with excellent surface activity properties. Hošková et al. (2013) analyzed the production of the BS rhamnolipid type (composed of rhamnose and β-hydroxylated fatty acids) produced by *Enterobacter asburiae* which was isolated from culture collections. This process (200h at 30°C / 100rpm) used 200 mL of mineral basic medium, a variety of carbon sources (glycerol, sunflower oil and sodium citrate), two sources of nitrogen ($NaNO_3$ and $(NH_4)_2SO_4$) and different phosphorus concentrations in order to observe their effect on production. The higher BS concentration was 0.51g/L during the late stationary growth phase using as a carbon source sodium citrate, then glycerol and sunflower oil. It was also observed that when sodium nitrate was used as a nitrogenous source the highest production was achieved while the phosphorous content did not have any significant effect. A higher percent of decanoic acid was presented in these BS compared with those produced by *P. aeruginosa* showing a better emulsification activity (22 mg/L) at values of critical micelle concentration (CMC) (the CMC is

considered an important surfactants physicochemical property, which indicates its emulsification ability) than the ramnolipids generated by *P. aeruginosa* which is the most known microorganism as a producer of these compounds with CMC of 60 mg/L.

Citrobacter: This genus has 11 species, among the most important are *C. freundii, C. diversus y C. amalonaticus*. These microorganisms are ubiquitous and frequently cause urinary tract and respiratory infections in immune-compromised organisms. Biochemically are characterized by decarboxylating lysine, producing H_2S and using citrates as unique carbon source (Koneman et al. 2008; Romero, 2007). Santi et al., (2013) reported from the BLAST (Basic Local Alignment Search Tool) gen 16rRNA sequencing analysis the identity of the *Citrobacter* and *Enterobacter* strains isolated from soils contaminated with fecal matter being specifically *Citrobacterfarmeri* and *E. cloacae subsp. Dissolvens*. The phylogenetic analysis showed a close relationship with *C. farmeri y C. amalonaticus, E. hormaechei y E. mori*. The isolated strains were capable to produce termostablelipo-peptides (100°C /30 min) with antibacterial activity against gram positives and negative strains. These authors concluded that there is presence of producer and co-producer strains of different antimicrobial lipo-peptides which are part of the human intestinal flora and frequently are isolated from foods, these microorganisms have showed potential applications in biotechnology as well as pharmaceutical and food uses, specifically in the preservation of dairy products. However, the large scale production of antimicrobial lipo-peptides is still expensive, being of high importance the optimization of the physical and chemical culture parameters and the transcriptional regulation of the lipopeptidesynthetases genes.

Escherichia: For a long time, it was believed that *coli* was the unique specie of the *Escherichia* genus, this specie has been widely studied and recognized as a saprophytic from intestinal native flora and as human pathogen which causes many clinical trials, both intestinal and extra intestinal (Romero, 2007;Herrera et al. 2001). However, the identity systems (galleries API, Vitek and Micro Scan) have allowed the species number to increase. Now, this genus involves species like *E. coli, E coli inactiva, E. hermannii, E. vulneris, E. fergusonii y E. blattae* (Herrera et al., 2001). Species as *fergusonii* producing molecules of biotechnological interest.Sriram et al., (2011) reported the isolation of *Escherichia fergusonii* from the soil contaminated with oil, capable to degradate hydrocarbons, tolerate heavy metals and synthesize lipo-peptides type emulsifiers, which are temperature stable, pH and salt conditions of NaCl, $CaCl_2$ and $MgCl_2$ from oil as the unique source of carbon and energy. This study shows the emergence of new strains as producer of bio surfactants with potential environmental applications in bioremediation of hydrocarbons and heavy metals.

Pantoea: Recently this genus has been described within *Enterobacteriaceae* due to taxonomic difficulties associated with members of *Enterobacter agglomerans* complexes. Seven species composed this genus, *P. agglomerans, P. dispersa, P. stewartii, P. punctata, P. citrea, P. terrea y P. ananas*. Some species are reported as pathogen in plants and occasionally in humans as opportunists (Perez, 2008; Segado-Arenasa et al. 2012). On the other hand, these strains have shown ability to synthesize BS; Vasileva-Tonkova and Gesheva (2007) reported the isolation from ornithological soils in Dewart Island (Frazier Islands), Antarctica, of *Pantoea sp*. A-13 which is able to produce a BS glycolipid type as the ramnolipids during its growth in *n*-paraffins or kerosene as unique carbon sources. These results were tested through hemolytic activity studies, growth inhibition of microorganisms as *Bacillus subtilis*, and fine layer chromatographic studies. This strain has been proposed as BS producer with potential application in bioremediation.

CONCLUSION

The family *Enterobacteriaceae* are a variety of bacteria which may be pathogenic, saprophytic or opportunistic. Some members have been isolated and identified by their ability to produce compounds BS with physical, chemical, biological properties with potential benefits for humans and the environment, such as its emulsifier, antimicrobial, chelating solubilizing capacity, among others. However, today there is still a wide variety of studies related to the isolation and identification of new strains of producers molecular mechanisms of synthesis, physical, chemical or biological properties of the biosurfactants generated, effectiveness of such properties, production optimization and Industrial Scaling. So the challenges of the scientists in the area still remain in search of a better quality of life, environmental protection and sustainable development.

REFERENCES

Azizollah E, Najmeh T, Saeid K (2011). Biosurfactant Producing Bacteria on Oily Areas of Ruminant Skin. Iranian Journal of Pharmaceutical Sciences 7(2): 117-121.

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Marchant R (2010). Microbial biosurfactants production, applications and future potential. Applied Microbiology and Biotechnology, 87(2), 427-444.

Banat IM, Makkar RS, Cameotra SS (2000). Potential commercial applications of microbial surfactants. Applied microbiology and biotechnology, 53(5), 495-508.

Cameotra SS, Makkar RS., Kaur, J., & Mehta, S.K. (2010). Synthesis of biosurfactants and their advantages to microorganisms and mankind. In Biosurfactants (pp. 261-280). edited by Ramkrishna Sen. Landes Bioscience and Springer Science+Business Media. Springer New York.

Devendra HD, Vinay S, Pawar YV, Nancharaiah V, Venugopalan P, Ameeta Ravi K, Smita SZ (2011). Anti-biofilm potential of a glycolipid surfactant produced by a tropical marine strain of Serratiamarcescens. Biofouling: The Journal of Bioadhesion and Biofilm Research, 27:6, 645-654.

Elmer W, Koneman S, Allen (2008). Koneman. Diagnostico Microbiológico/ Microbiological diagnosis: Texto Y Atlas En Color/ Text and Color Atlas. 6ta edición Ed. Médica Panamericana, 1691p.

Fakruddin Md (2012). Biosurfactant: Production and Application. J Pet Environ Biotechnol 3:124. doi:10.4172/2157-7463.1000124.

Fracchia L, Cavallo M, Martinotti MG, Banat IM (2012). Biosurfactants and bioemulsifiers biomedical and related applications–present status and future potentials. Biomedical science, engineering and technology, 14, 326-335.

Gakpe E, Rahman PKSM, Mohamed HAA (2007). Microbial Biosurfactants – Review. J. Mar. Atmos. Res. 3(2): 1-17.

García BP, Mendoza MA (2014). Pruebas bioquímicas tradicionales y de alta resolución para identificación manual de enterobacterias. Acta bioquímica clínica latinoamericana, 48(2), 249-254.

Gudiña EJ, Rangarajan V, Sen R, Rodrigues LR (2013). Potential therapeutic applications of biosurfactants. Trends in pharmacological sciences, 34(12), 667-675.

Herrera M, Moya T, Vargas A, Herrera M, Herrera JF y Marín JP. (2001). Aislamiento de cepas de Escherichiaspp. diferentes de Escherichiacoli en el Hospital Nacional de Niños de 1995 a 2000. Rev. Méd. Hosp. Nac. Niños (Costa Rica) vol.36 n.1-2 San José.

Hošková M, Schreiberová O, Ježdík R, Chudoba J, Masák J, Sigler K, Řezanka T (2013). Characterization of rhamnolipids produced by non-pathogenic Acinetobacter and Enterobacter bacteria. Bioresource technology, 130, 510-516.

Ibrahim ML, Ijah UJJ, Manga SB, Bilbis LS, Umar S (2013). Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. International Biodeterioration and Biodegradation, 81, 28-34.

Jadhav M, Kagalkar A, Jadhav S, Govindwar S (2011). Isolation, characterization, and antifungal application of a biosurfactant produced by Enterobacter sp. MS16. European Journal of Lipid Science and Technology, 113(11), 1347-1356.

Janek T, Łukaszewicz M, Krasowska A (2013). Identification and characterization of biosurfactants produced by the Arctic bacterium Pseudomonas putida BD2. Colloids and Surfaces B: Biointerfaces, 110, 379-386.

Jiménez ID, Medina MSA, Gracida RJN (2010). Propiedades, aplicaciones y producción de biotensoactivos: una revisión. Revista internacional de contaminación ambiental, 26(1), 65-84.

Kapadia SG, Yagnik BN (2013). Current Trend and Potential for Microbial Biosurfactants. Asian Journal of Experimental and Biological Sciences, 4, 1-8.

Karanth NGK, Deo PG, Veenanadig NK (1999). Microbial production of biosurfactants and their importance. Current Science, 77(1): 116-126.

Kiran GS, Nishanth LA, Priyadharshini S, Anitha K, Selvin J (2014). Effect of Fe nanoparticle on growth and glycolipid biosurfactant production under solid state culture by marine Nocardiopsis sp. MSA13A. BMC Biotechnology, 14:48.

Ławniczak Ł, Marecik R, Chrzanowski Ł (2013). Contributions of biosurfactants to natural or induced bioremediation. Applied microbiology and biotechnology, 97(6), 2327-2339.

Maneerat S (2005). Production of biosurfactants using substrates from renewable-resources. Songklanakarin J. Sci. Technol., 27(3): 675-683.

Marchant R, Banat IM (2012). Biosurfactants: a sustainable replacement for chemical surfactants?. Biotechnology letters, 34(9), 1597-1605.

Montoya VHH (2008). Microbiología básica para el área de la salud y afines. 2da edición. Editorial. Universidad de Antioquia. 282 p.

Nitschke M, Costa SGVAO (2007). Biosurfactants in food industry. Trends in Food Science and Technology, 18(5), 252-259.

Nuvia L, Sánchez-Salinas E, Ortiz-Hernández ML (2014). Biosurfactantes y su papel en la biorremediación de suelos contaminados con plaguicidas. Revista Latinoamericana de Biotecnología Ambiental y Algal, 4(1), 47-67.

Pacwa-Płociniczak M, Płaza Grażyna A, Piotrowska-Seget Z, Singh Swaranjit C (2011). Environmental Applications of Biosurfactants: Recent Advances. Int. J. Mol. Sci., 12(1), 633-654.

Padmapriya B, Rajeswari T, Suganthi S, Rajeswari T, Jayalakshmi S (2011). Biosurfactant Production And Plasmid Isolation From Newly Isolated Hydrocarbonoclastic Bacteria Proteus inconstans. International Journal of Pharmaceutical and Biological Archives; 2(2): 784-790.

Padmapriya B. and Suganthi S (2013). Antimicrobial and Anti Adhesive Activity of Purified Biosurfactants Produced by Candida species. Middle-East Journal of Scientific Research 14 (10): 1359-1369.

PAHO (2014). access 17-february 2015 (http://www.bvsde.paho.org/cdgdwq/docs_microbiologicos/Bacterias%20PDF/Klebsiella.pdf)

Parveen J, Shajrat M, Md. Zahangir A, Wan M, Fazli WN (2014). Isolation and selection of new biosurfactant-producing bacteria from degraded palm kernel cake under liquid state fermentation. J. Oleo Sci. 63, 8, 795-804.

Perez y Terron, R (2008). Evaluación de la diversidad genética y bioquímica de Pantoeasp. en maíz (Doctoral dissertation).Instituto politécnico nacional. Tlaxcala, México.http://tesis.ipn.mx/dspace/bitstream/123456789/3549/1/EVALUACIONDIVERSIDAD.pdf

Piñero-Bonilla J (2013). Importancia biotecnológica de la micro biodiversidad. Los nuevos cazadores de microbios. Revista Venezolana de Ciencia y Tecnología de Alimentos, 4(2), 284-317.

Rakeshkumar M, Jain KM, Nidhi J, Avinash M, Bhavanath J (2013). Effect of unconventional carbon sources on bio surfactant production and its application in bioremediation. International Journal of Biological Macromolecules 62: 52– 58.

Ramírez JCR, Ulloa PR, Velázquez MY, González JAU, Romero FA (2011). Bacterias lácticas: Importancia en alimentos y sus efectos en la salud. Revista Fuente Año, 2(7). 1-16pp.

Rodrigues L, Banat IM, Teixeira J, Oliveira R (2006). Biosurfactants: potential applications in medicine. Journal of Antimicrobial Chemotherapy, 57(4), 609-618.

Romero Cabello R. (2007). Microbiology and Human Parasitology: Etiological Basis of Infectious and Parasitic Diseases. 3ra edición. Ed. Médica Panamericana, 1- 1802 p.

Saharan BS, Sahu RK, Sharma D (2011). A review on biosurfactants: fermentation, current developments and perspectives. Genetic Engineering and Biotechnology Journal,GEBJ-29,p.14.

Salihu A, Abdulkadir I, Almustapha MN (2009). An investigation for potential development on Biosurfactants. Biotechnology and Molecular Biology Reviews Vol. 3 (5):111-117.

Santi MM, Shalley S, Anil KP, Annu K, Suresh K (2013). Isolation and characterization of diverse antimicrobial lipopeptides produced by Citrobacter and Enterobacter. BMC Microbiology, 13:152.

Segado-Arenasa A, Alonso-Ojembarrenaa A, Lubián-Lópeza SP, y García-Tapia AM (2012). Pantoeaagglomerans: a new pathogen at the neonatal intensive care unit?. Arch Argent Pediatr; 110(4):77-79.

Sriram MI, Gayathiri S, Gnanaselvi U, Jenifer PS, Mohan Raj S, Gurunathan S (2011). Novel lipopeptide bio surfactant produced by hydrocarbon degrading and heavy metal tolerant bacterium Escherichia fergusonii KLU01 as a potential tool for bioremediation. Bioresource technology, 102(19): 9291-9295.

Swaranjit SC, Randhir S, Makkar JK, Mehta SK (2010). Synthesis of Biosurfactants and Their Advantages to Microorganisms and Mankind. Chapter 20 in Biosurfactants, edited by Ramkrishna Sen. Landes Bioscience and Springer Science+Business Media.

Tohey M, Taichiro T, Yoji N (2011). Serrawettins and Other Surfactants Produced by Serratia.pp.93-120. In: Biosurfactants from genes to applications. Editor Gloria Soberon-Chavez. Microbiology Monographs Volume 20. Series Editor: Alexander Steinbuchel. Springer.

Toledo FL, Gonzalez-Lopez J, Calvo C (2011). Production of bioemulsifier by Bacillus subtilis, Alcaligenesfaecalis and Enterobacter species in liquid culture. Bioresource Technology 99 (2008) 8470–8475.

Toth Ian K, Bell KS, Holeva MC, Birch PRJ (2003). "Soft rot erwiniae: from genes to genomes". Molecular Plant Pathology 4 (1): 17–30

Vasileva-Tonkova E, Gesheva V (2007). Biosurfactant Production by Antarctic Facultative Anaerobe Pantoea sp. During Growth on Hydrocarbons. Current Microbiology 54(2): 136-141.

Yañez-Ocampo G, Wong-Villarreal A (2013). Biosurfactantes Microbianos, Producción Potencial con Residuos Agroindustriales de Chiapas. Bio Tecnología, 17 (3):12-28.