Pak. j. life soc. Sci. (2017), 15(2): 72-83 E-ISSN: 2221-7630;P-ISSN: 1727-4915

Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

REVIEW ARTICLE

# The genus Staphylococcus*:* Harmful and Beneficial Microorganisms in the Environment

Alejandro De Jesús Cortés Sánchez[1]*, Mayra Díaz Ramirez[2], Raquel Garcia Barrientos[3] and Ashutosh Sharma[4]

[1]Consejo Nacional de Ciencia y Tecnologia, Centro de Investigaciones Biológicas del Noroeste, Unidad Nayarit. (UNCIBNOR+). Calle Dos No. 23. Cd. del Conocimiento. Av. Emilio M. González Cd. Industrial. C.P. 63173.Tepic, Nayarit, México

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

[3]Lab de Procesos Biotecnológicos. Universidad Politécnica de Tlaxcala. Av. Universidad Politécnica No. 1 San Pedro Xalcaltzinco, Tepeyanco Tlaxcala, México

[4]Escuela de Ingeniería en Alimentos, Biotecnología y Agronomía. Instituto Tecnológico y de Estudios Superiores de Monterrey. Epigmenio González 500, Santiago de Querétaro, Querétaro, México

***ARTICLE INFO***

Received: May 09, 2017
Accepted: Jul 15, 2017

***Keywords***
Biosurfactants
Food safety
Pathogen
*Staphylococcus*

***Corresponding Author:**
alecortes_1@hotmail.com

## ABSTRACT

The staphylococci, specially the *Staphylococcus* (*S.*) *aureus*, have been regularly considered in food borne problems due to its pathogenic nature in human beings and animals. Besides this, it has also developed resistance to multiple antimicrobial agents which make the condition more adverse. However, some years ago, different species of *Staphylococcus* including *S. haemolyticus*, *S. saprophyticus*, *S. xylosus* had also been reported as excellent producers of bioactive compounds such as the biosurfactants (BS). The BS are amphiphilic molecules mainly synthetized by microorganisms (bacteria, fungi and yeasts) and have acquired importance for its friendly character (biodegradability and low toxicity), and its different functional properties (surface activity, emulsifier, sparkling and humectant etc.) that make them to be considered potential substitutes of chemical surfactants apart from other potential applications in multiple industrial areas such as environmental, food and pharmaceuticals. Therefore, article provides a general review of the genus *Staphylococcus spp.*, from two points of view: a negative one since a focus of clinical, as the producers of food borne diseases, and a positive one, mentioning the capacity of various strains isolated from the environment with the ability to produce biosurfactants.

## ***Staphylococcus spp.*** **and the foodborne illnesses**

The foods may be the source of generating infections due to the consumption of contaminated foods/ beverages that affect the consumer's health. Diarrhea and vomiting are the most general symptoms in foodborne illnesses while septic shock, headaches, fever, double vision and other symptoms can also be present (Garcia-Huidobro et al., 2012; Zamudio et al., 2011; Flores and Herrera, 2005). Foodborne Illnesses (FI) are considered by the World's Health Organization (WHO) as one of the most extended health problems in the world, being an important cause of sicknesses and deaths in under-developed countries, a decrease in terms of productivity, health services related costs, implementtation and tracking of food safety policies (López-Aday et al., 2013; Olea et al., 2012). Approximately 250 microbial agents including various species of bacteria, viruses, fungi, parasites and toxins have been reported as potent source of foodborne illnesses (Garcia-Huidobro et al., 2012; Olea, 2012; Prado et al., 2002). Some of the factors that have been related to the incidence of foodborne diseases in the population in recent years are changes in lifestyle, food consumption habits (fresh or minimally processed foods), increase of risk groups (obesity, diabetes, immunosuppression, among others) and the commercial globalization of food commodities (Garcia-Huidobro et al., 2012; Muñoz et al., 2011; Prado et al., 2002).

According to WHO estimations, 600 million, almost 1 in 10 people around the world, get sick after eating contaminated food and 420,000 die each year. Children under 5 years of age carry 40% of the burden of foodborne diseases, with 125,000 deaths per year. Foods contaminated with harmful bacteria, viruses, parasites or chemicals cause more than 200 diseases, ranging from diarrhea to cancers; diarrheal diseases are the most common of contaminated food consumption, causing 550 million people to get sick and 230,000 deaths per year (WHO, 2017). Only in the United States, 76 million cases are reported per year, giving place to considerable expenses in the health sector (Olea et al., 2012; Prado et al., 2002). Meanwhile, in countries of Latin America such as Mexico, in 2002, the National System of Health Information (SNIS) reported 3612 cases of food poisonings caused by bacteria (Felix et al., 2005). The foodborne illnesses can be classified in food infections or intoxications, and those are considered an important issue in the public health department due to some factors such as: an increase in the incidence, vulnerable population groups, an increase in antimicrobial resistance and the socioeconomic impact generated (Flores and Herrera, 2005; Zamudio et al., 2011; WHO, 2017). The appearance of such diseases is considered as an indicator of the hygienic-sanitary quality in the foods, where the contamination can occur in any stage of the food chain: elaboration, manipulation, conservation, transportation, distribution or commercialization (Zamudio et al., 2011; Flores and Herrera, 2005). Currently food supply chains are globalized, so collaboration between governments, producers and consumers helps to ensure food safety (WHO, 2017).

This document presents a general perspective of the bacterial genus *Staphylococcus* spp., as well as the duality presented by different species of the genus on the one hand the negative or pathogenic character as producers of various diseases, including those originated through food and on the other the positive or beneficial character, being isolated several species with the capacity to produce compounds with surface activity in addition to other chemical and biological properties, being considered a potential alternative for use to synthetic surfactants or petroleum derivatives and in different industrial areas and environment.

## Staphylococci, foods and health

The foodborne illnesses (FI) can be caused by physical, chemical and biological agents. However, the most of the FI reported around the world belong to a biological origin, especially due to several bacteria of the *Vibrio spp., Campylobacter spp., Salmonella spp., Shigella spp., Escherichia coli, Listeria monocytogenes, Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Yersinia enterocolitica and S. aureus* (Felix et al., 2005; Flores and Herrera, 2005; Tamara et al., 2008; Muñoz et al., 2011; Zamudio et al., 2011). The staphylococci, and more specifically the *S. aureus* specie, have a great interest in food and health aspects, being its pathogenic potential in the human being and animals (Tamara et al., 2008). The *Staphylococcus spp.* has several features, such as: it appears in form of cocci, motionless, Gram positive, facultative anaerobic, positive catalase, non-sporulated, with a 0.5 to 1. 5 μm diameter, grouped in unique cells, pairs, tetrads, short chains, or forming bunches of grapes, and present a proportion of guanine-cytosine (G+C) from 30 to 39% (Romero-Cabello, 2007; Cervantes-García et al., 2014).

The *Staphylococcus spp.* is widely spread in the environment as they are microorganisms with an easy adaptation to various environmental conditions. This genus contains 32 species, 16 of which are found in the human beings as an opportunistic microbe, forming part of the skin's microbiota and the mucous of mammals and birds (Cervantes-García et al., 2014; Zendejas-Manzo et al., 2014; Elika, 2013; Todd, 2005).

From the clinical and food safety perspective, the following species are those identified as infectious agents and cause of intoxications in humans and animals: *S. aureus, S. lugdunensis, S. intermedius, S. haemolyticus, S. epidermidi, S. saprophyticus S. hominis subsp. hominis, S. warneri, S. simulans, S. capitis subsp. ureolyticus, S. auricularis, S. cohnii subsp. urealyticum* (Teran et al., 2014; Cervantes-García et al., 2014; Zendejas-Manzo et al., 2014; Romero-Cabello, 2007; Elika, 2013;).

A chemical characteristic of the pathogen staphylococci is its capability of producing the coagulase enzyme (responsible for the conversion of fibrinogen into fibrin). Coagulase production is an important virulence factor, and it is used as an indicator of such feature, letting the scientists differentiate *S. aureus* (positive coagulase) from other staphylococci species (negative coagulase) (Winn et al., 2006; Romero-Cabello, 2007; Bustos-Martinez et al., 2006). Although, this characteristic is not a limiting one, as species like *S. epidermidis, S. haemolyticus* and *S. saprophyticus* are negative coagulase and, along with *S. aureus* positive coagulase, they have been reported as common causal agents of human infections being the last point the most relevant of all (Winn et al., 2006; Predari, 2007; Romero-Cabello, 2007; Cervantes-Garcia et al., 2014).

In the last years, the incidence of *Staphylococcus spp.* has increased significantly, being the species *Staphylococcus aureus* the one with the highest rate of pathogenicity and frequency, and also the main cause at hospitable and community levels of infections such as osteomyelitis, septic arthritis, sepsis, abscesses, pneumonia, empyema, endocarditis, pericarditis, meningitis, scarlet fever, scalded skin syndrome, toxic shock, and foodborne poisonings (Todd, 2005, Bustos-Martinez et al., 2006; Zendejas-Manzo et al., 2014; Chavez et al., 2015). The *S. aureus* is a pathogen of worldwide importance, which possess an opportunist character, being part of the human microflora (around 20 & 50% are carriers in nostrils permanently, and another 30% in skin and intestinal tract permanently). This feature emerges when combined its virulence factors and a decrease in the defenses of the host, leading to a colonization and diseases named "virulence factors"; some are from protein nature such as hemolysis (alpha, beta, gamma and delta), nucleases, proteases, lipases, hyaluronidase, catalase, coagulase, collagenase, leucocidin; different toxins like the toxic shock syndrome (TSST-1), staphylococci factors (SE), and exfoliative toxins (ETA & ETB), besides the structural virulence factors such as the peptidoglycan, protein A, adhesion factors, teichoic acids and capsular polysaccharides (Bustos-Martinez et al., 2006; Zendejas-Manzo et al., 2014; Cervantes-Garcia et al., 2014).

*S. aureus* and other coagulase positive species are the causative agents of food poisoning, one of the most common foodborne illnesses. These intoxications originate from consuming food with the toxins generated by microbial metabolic processes during their growth in food. These are classified as acute gastro enteric character, whose symptoms depend on the amount of ingested poisoned food, toxin's concentration and individual susceptibility. Such symptoms are: nauseas (vomit) and diarrhea, which appear after the consumption of contaminated foods. It is estimated that enterotoxins are produced in food when microbial growth is in proportions above $10^5$ CFU/g (Bustos-Martinez et al., 2006; Tamara-Diaz et al., 2008; Brizzio et al., 2013; Zendejas-Manzo et al., 2014). The staphylococci toxins are proteins considered as super-antigens and pyrogens composed of considerable amounts of lysine, tyrosine, aspartic and glutamic acid. Their molecular weight is around 28 and 35 KDa. The production by *S. aureus* is affected by the type of nutrients, the substrate's pH, temperature, atmosphere, NaCl, and competitor microorganisms. A great variety of toxins is known, such as the SEA, SEB, SEC, SED & SEE, which are more frequent in food poisonings, and SEF, SEG, SEH, SEI SEJ, SEK, SEL, SEP, SEM & SEO; all of them are water-soluble, thermostable, and resistant to the host's digestive enzymes (Lombard et al., 1996; Bustos-Martinez et al., 2006; Manfredi et al., 2010; Brizzio et al., 2013; Cervantes-Garcia et al., 2014; Demirci et al., 2016). It has been reported different manners for its sighting in foods by immune and molecular methods that detect coding genes in strains coming from foods associated to intoxication outbreaks (Lombard et al., 1996; Bustos-Martinez et al., 2006; Manfredi et al., 2010; Brizzio et al., 2013; Demirci et al., 2016). Food contamination by staphylococci is due, mostly, to bad or absent hygiene practices and elaboration in any of the food chain phases, easing the presence and development of the pathogen. The humans are considered the main ecologic niche; therefore, the contamination can occur commonly by the direct contact of the manipulators with the foods. Dairy products, chicken, eggs, meats and vegetables are the most related to the intoxications (Elika, 2013; Brizzio et al., 2013; Puig-Pena et al., 2015; Zendejas-Manzo et al., 2014).

## Susceptibility to antimicrobials

The infections by *S. aureus* in the last years have emerged and acquired some relevance in the public health aspects, not only their incidence in hospital and community cases, but also the development and later spreading through the exchange and acquisition of genes due to mobile genetic elements (plasmids, transposons and insertion sequences) that codify the resistance to different antimicrobials: penicillin, methicillin (*mec*A), vancomycin (*VanA*), fluoroquinolones (*gyr*A or *par*E), among others, giving room to negative aftermaths such as: long hospital stays, high health costs, high death rates, seek and reduction of therapeutic alternatives (Bustos-Martinez et al., 2006; Perazzi et al., 2010; Casellas, 2011; Zendejas-Manzo et al., 2014; Cervantes-Garcia et al., 2014; Chávez et al., 2015). Now a day, all the *S. aureus* isolations of hospital origin and more of the 85% of that communitarian originated are resistant to the penicillin (Casellas, 2011). The resistance phenomenon owes its incidence to the microorganisms they get coding enzymes that inactivate the penicillin or other β-betalactam antibiotic and block the antibacterial agent to prevent the synthesis of the cell wall by not allowing it to unite the penicillin binding proteins (PBP) that rule the intersection of the components (carbohydrates and peptides) in cell wall (Casellas, 2011; Monge, 2013). In the USA, the surveillance project of pathogen agents epidemiologic indicates that 60% of the nosocomial bacteria are caused by positive Gram cocci; meanwhile, numbers of the Pan-American Association of Infectiology have a tendency to match for all the Latin American region, being the negative coagulase staphylococci (mostly the *Staphylococcus epidermis* strains) the causal agents of bacteremia, followed by strains of *Staphylococcus aureus* and, with a less frequency in Latin America, the enterococci (Casellas, 2011).

In Europe, countries like Spain have reported an incidence of resistance between 25 & 30% of the *S. aureus to* antimicrobials like methicillin, and for the negative coagulase Staphylococcus between 50 & 60%, giving space to new challenges (Cercenado, 2016). Meanwhile, in Latin America as Cuba, an analysis carried out by Puig-Pena et al., 2015 to isolated food strains of positive coagulase (89%) and manipulators (11%), demonstrated that the *S. aureus* was the most common isolated bacteria; 56.1% of all the positive coagulase Staphylococcus were resistant to, at least, one of the tested antibiotics. Penicillin (52.9%), erythromycin (30.3%) and the tetracycline (24.4%) represented the highest percentages of resistance. A fifth of the *S. aureus* strains (86.4%) was resistant to the methicillin, 62.9% were multi-resistant. The antimicrobial resistance patterns involved one to five drugs, concluding it exists a high proportion of resistant isolations to the regular drugs; and also, a wide variety of resistance patterns and a high percentage of identified multi-resistant strains, which means a need of promoting the rational use of antibiotics, the implementation of hygiene measures and the prevention against foodborne illnesses, and detailed epidemiological surveys.

**Sanitary regulation and microbiological analysis of foods for the detection and isolation of staphylococci**

For the matters of isolation and identification of the *S. aureus* and the detection of toxins in foods, it has been reported several methods involving biochemical tests and special cropping ways for the isolation and identification, among which are the Bennett-Reginald and Lancette-Gayle, 2001 & Bennett-Reginald and Hait-Jennifer, 2011 presented in the Bacteriological Analytical Manual (BAM) in the U.S. Food and Drug Administration (FDA), the ones of the International Organization for Standardization (ISO) ISO 6888 part 1 y 2 under the general title of Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species), or the UK Standards for Microbiology Investigations of the department of Public Health of England (PHE, 2014). https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and consistency-in-clinical-laboratories. Meanwhile, in Mexico, the official Mexican health regulations, such as the "NOM-210-SSA1-2014" and "NMX-F-310-1978", establish the method of microbiological analysis for the isolation and estimation of *S. aureus* in foods, which involves an isolation through differential and selective culture media and later confirmation through different biochemical tests (figure 1 and table 1); and the Official Norm "NOM-243-SSA1-2010" establishes the method for detection of staphylococci toxins in foods like dairy products and derivatives. In the figure 1, a flowchart for isolation and detection of *S. aureus* in the microbiological lab in foods through the usage of selective cropping along with differential and biochemical confirmation tests. In regard to the regulation for production and food safety referring to *S. aureus* and its toxic metabolites, there are microbiological specifications around the world about the presence of staphylococcal enterotoxins in several foods. In the European community, the specifications in the regulation (EC) 178/2002 of January 28th, 2002 established the principles and the general requirements of the food legislation, the European Food Safety Authority (EFSA) is created and procedures are established regarding the food safety; subsequently the regulation (CE) 2073/2005 of the commission of November 15th, 2005, mentions that the microbiological criteria applicable to food and the corresponding modifications, such as cheeses, milk powder and whey powder, enterotoxins of staphylococci must not be detectable in 25 g of food. Likewise, some hygiene guidelines are established and must be followed in the manufacturing processes related to the coagulase-positive staphylococcus producers of the intoxications.

In Mexico, the sanitary regulation focused on the staphylococci and toxins for various foods is the Official Mexican Norm "NOM-242-SSA1-2009". Meanwhile, to foods like dairy products, milk formula, combined milk products and derivatives, the provisions, safety specs and testing methods are in the Official Mexican Norm "NOM-243-SSA1-2010". In both cases, the maximum limit allowed is the absence of staphylococci toxins in all the foods. Also, through the Official Mexican Norm "NOM-251-SSA1-2009", the minimum requirements for appropriate hygiene practices were established and they must be applied in the processes of foods, beverages or food supplements and their raw materials, in order to avoid contamination throughout the process.

**Staphylococci producing biosurfactants**

Biosurfactants (BS) are amphiphilic molecules synthetized mainly by different microorganisms (bacteria, funguses and yeasts), many of them isolated from a wide variety of environments: soil, ocean water, marine sediment and oil fields. These molecules present the capacity of reducing the surface tension and interfacial between two immiscible liquids, increasing the solubility of hydrophobic compounds in aqueous environments (Siñeriz et al., 2001; Satpute et al., 2010; Soberon-Chavez and Maier, 2011; Toribio et al., 2014; Cruz et al., 2014; Becerra-Gutiérrez and Horna-Acevedo, 2016). Bio-surfactants are from amphiphilic nature due to in their structure present a hydrophilic fraction responsible of their grade of solubility that might be constructed by carboxyl groups of fatty acids, phosphate groups, amino acids, peptides (anions or cations), mono- or polysaccharides, and a hydrophobic region, which consists of saturated fatty acids and hydroxylated unsaturated, or ramified and united by ester, amide or glycosidic bonds (Siñeriz et al., 2001; Toribio-Jiménez et al., 2014; Cruz et al., 2014; Becerra-Gutiérrez and Horna-Acevedo, 2016). These compounds are produced, mostly, during the growth

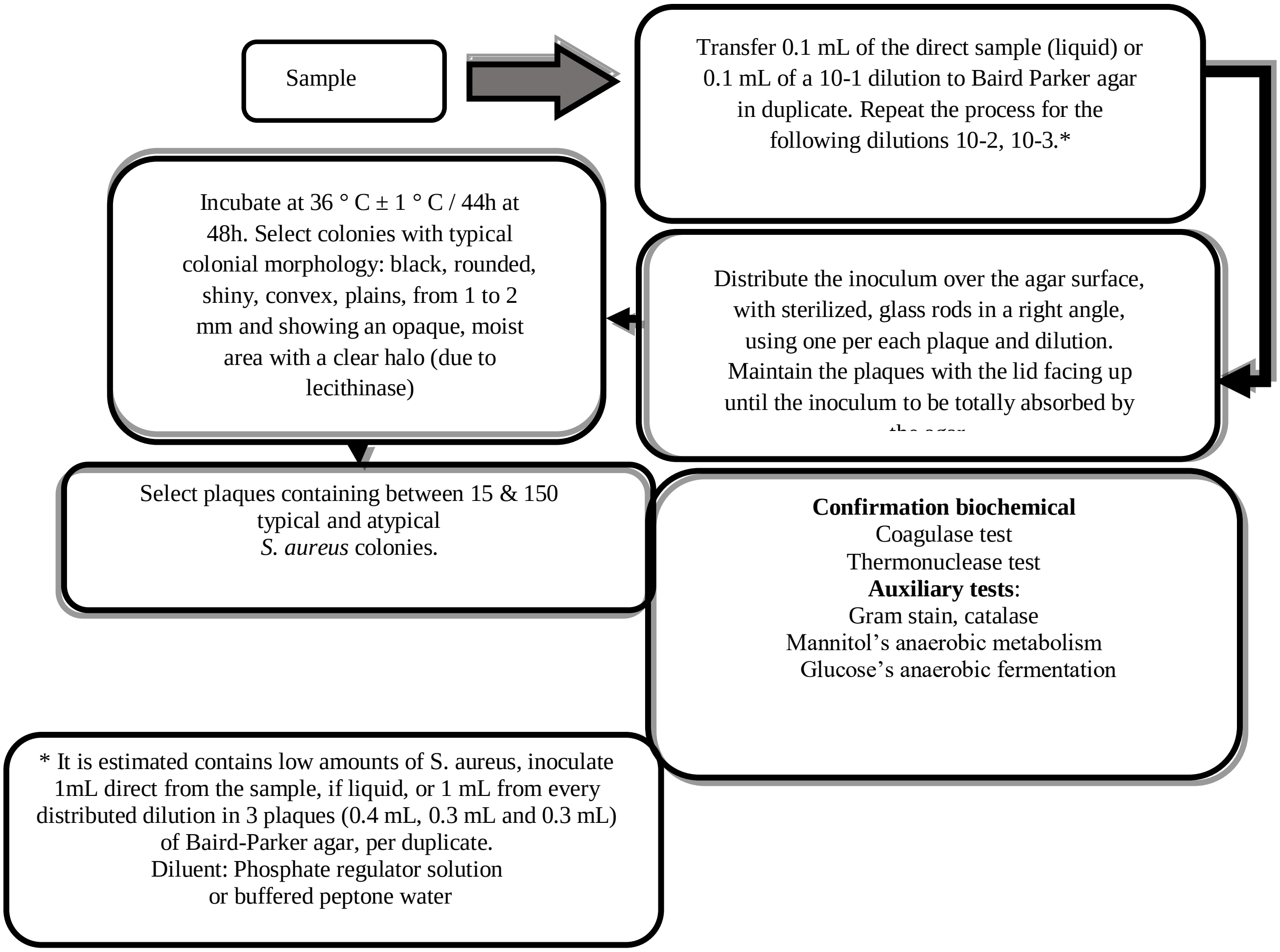
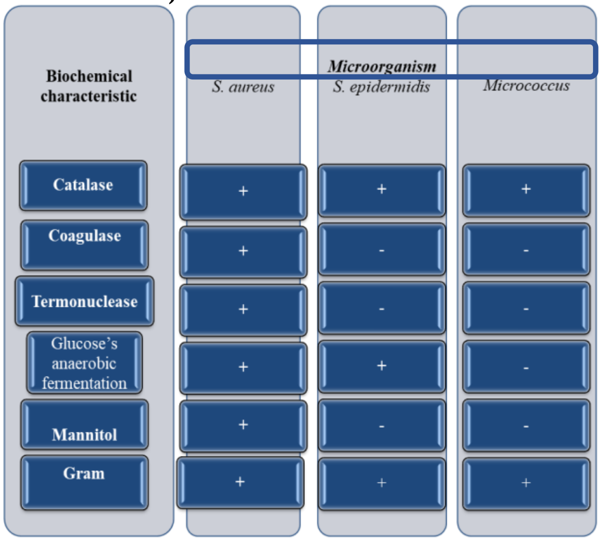


**Fig. 1: Flowchart for isolation and identification of *S. aureus* in foods (NOM-210-SSA1-2014).**

**Table 1: Analysis and characteristics of biochemical identification for *S. aureus* (NOM-210-SSA1-2014).**



| Biochemical characteristic | Microorganism | | |
|---|---|---|---|
| | *S. aureus* | *S. epidermidis* | *Micrococcus* |
| Catalase | + | + | + |
| Coagulase | + | - | - |
| Termonuclease | + | - | - |
| Glucose's anaerobic fermentation | + | + | - |
| Mannitol | + | - | - |
| Gram | + | + | + |

Positive reaction (+), negative reaction (-)

over water-immiscible substrates and are secreted outside the cell or can be bound to cellular components (Okoliegbe and Agarry, 2012; Cruz et al., 2014; Becerra-Gutiérrez and Horna-Acevedo, 2016). Compared to their synthetic-origin peers, the bio-surfactants possess different properties which give them an advantage over the first, like biodegradability, biocompatibility, specificity, foaming, emulsifying and humectant capacity, structural diversity, low toxicity, stability at different temperature conditions, pH and ionic strength, can be produced from organic waste, and show surface and interphase activity (by decreasing the water surface tension and interfacial water/ hexadecane), low critical micellar concentration and biological activity (antimicrobial, antitumor and immunomodulatory) (Siñeriz et al., 2001; Raiger and Lopez, 2009; Okoliegbe and Agarry, 2012; Sánchez et al., 2014; Cruz et al., 2014; Becerra-Gutiérrez and Horna-Acevedo, 2016; Satpute et al., 2017). Nowadays, it exists a growing interest in the substitution of surfactant agents of chemical origin by those of biological origin due to the aforementioned properties

(Satpute et al. 2010; Soberon-Chavez and Maier, 2011; Becerra-Gutiérrez and Horna-Acevedo, 2016; Satpute et al., 2017); besides, in the last years it has been reported their potential applications in several industrial areas, such as: agriculture, medicine, oil, foods and environment (Figure 2) (Nitschke and Costa, 2007; Raiger and Lopez, 2009; Satpute et al., 2010; Okoliegbe and Agarry, 2012; Cruz et al., 2014; Sanchez et al., 2014; Becerra-Gutiérrez and Horna-Acevedo, 2016).

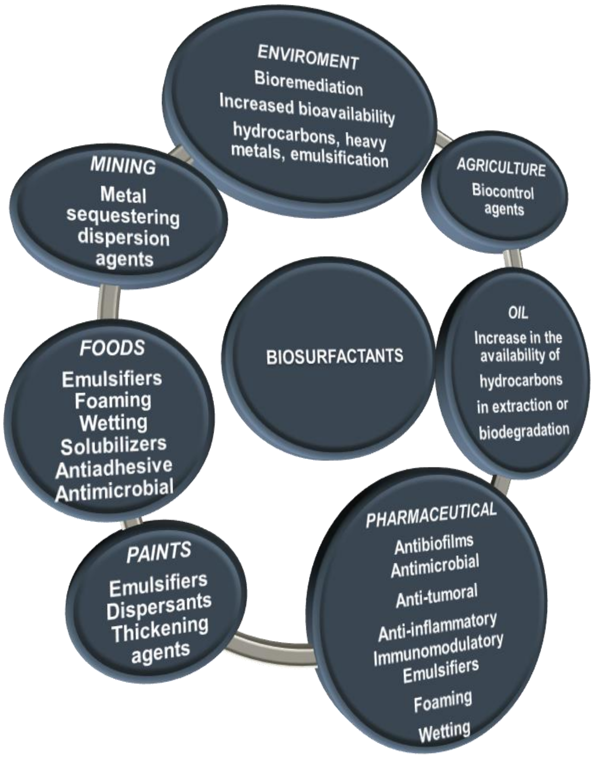


**Fig. 2:** **Applications and industrial areas of the biosurfactants** (Banat et al., 2000; Nitschke and Costa, 2007; Raiger and López, 2009; Ibrahim et al., 2010; Fracchia et al., 2012; Cruz et al., 2014; Banat et al.,2014; Sarubbo et al. 2015).

The physiological activity of the bio-surfactants is not fully understood yet, but it is estimated that its function is to allow the microorganisms to grow onto immiscible substrates of water by reducing the surface tension in the phase's limit, increasing the availability of the substrate for its uptake and metabolism (Okoliegbe and Agarry, 2012). Besides, the bio-surfactants are involved in the mobility and microbial adhesion, so if the producer microorganism is in the interface, these compounds reduce the surface tension and allow the movement in the search for new environments for the growth, reproduction, and colonization. On the other hand, when the producer microorganisms are pathogens, the bio-surfactants act like humectants, or dispersion agents, in the surface of the host cells; antimicrobial activity is another function in the biosurfactants, considered as a form of competition of the producers in the survival and stability in adverse biological and environmental conditions (Okoliegbe and Agarry, 2012; Sánchez et al., 2014). Biosurfactants can be classified according to several characteristics, from chemical composition, function, microbial origin, or molecular weight (Siñeriz et al., 2001; Raiger and Lopez, 2009; Okoliegbe and Agarry, 2012; Cruz et al., 2014). Based on the molecular weight, they are grouped in: 1. Low molecular weight bio-surfactants that include glycolipids (rhamnolipids, trehalose lipids, sophorolipids, fructose lipids, cellobiose lipids and mannosylerythritol lipids); these are the most studied due to their great performance in production and cost. Other types are the lipo-peptides (surfactin, polymyxin and gramicidin), modified amino acids and fatty acids, showing good efficiency in the decrease of the surface and interfacial tension; and 2. High molecular weight bio-surfactants, which are extracellular polymers, defined as bio-emulsifiers including polysaccharides, lipo-polysaccharides, lipo-proteins, or complex mixtures of the aforementioned, being stabilizers of water-oil emulsions (Table 2) (Morita et al., 2007; Raiger and Lopez, 2009; Okoliegbe and Agarry, 2012; Cruz et al., 2014).

Bio-surfactants show a structural diversity in their hydrophobic and hydrophilic parts; therefore, the understanding of the biosynthesis and regulation mechanisms is necessary. It has been established, for the biosynthesis, four possible mechanisms, which are:

a. The hydrophilic and hydrophobic parts are synthesized *de novo*
b. The hydrophilic is synthesized *de novo* and the substrate induces the hydrophobic fraction.
c. The synthesis of the hydrophilic fraction depends on the substrate; meanwhile the hydrophobic fraction is synthesized *de novo.*
d. The synthesis of both structures depends on the source of carbon used (Siñeriz et al., 2001).

On the other hand, it has been reported that several factors affect the production, proportion and composition of bio-surfactants. Those factors are the carbon's source which can be divided into three types: carbohydrates, vegetal oils and hydrocarbons, that can be used either individually or combined; another one is the source of nitrogen needed as a compound of proteins during the cell growth in the forms of inorganic ammonium salts, yeast, meat extract, malt or urea; some researchers have pointed that some producer strains can generate an overproduction and changes in the bio-surfactants' composition when under limited nitrogen conditions, likewise the generation of those molecules often occurs during the growth's stationary phase. Finally, the environmental factors have been

**Table 2: Biosurfactants (BS) produced by diverse microorganisms and classification.**

| | Type of biosurfactants according to the composition | Biosurfactants | Producer microorganism |
|---|---|---|---|
| Low molecular weight | Glycolipids | Rhamnolipids | *Pseudomonas sp., Serratia rubidaea* |
| | | Mannosylerythritol lipids | *Candida antartica*<br>*Ustilago maydis* |
| | | Trehalose lipids | *Rhodococcus sp., Arthrobacter sp., Rhodococcus spp., Nocardia spp., Corynebacterium spp., Tsukamurella spp., Gordonia spp., Mycobacterium spp.* |
| | | Sophorolipids | *Candida batistae, Candida lypolytica,Candida bombicola, Candida bogoriensis, Wickerhaminella domercqiae* |
| | | Liposomes of cellobiose | *Ustilago maydis* |
| | Fatty acids, Phospholipids and Neutral lipids | Corinomicolic acid | *Corynebacterium lepus, Corynebacterium insidibasseosum, Penicillium spiculisporum, Arthrobacter paraffineus* |
| | | Phospholipids | *Thiobacillus thiooxidans* |
| | | Neutral lipids | *Nocardia erythropolis* |
| | Lipopeptides | Surfactin | *Bacillus subtilis, Bacillus pumilus A Serratia marcescens* |
| | | Serrawettin | *Pseudomonas fluorescens* |
| | | Viscosin | *Bacillus brevis* |
| | | Gramicidin | *Bacillus polymyxia* |
| | | Polymyxin | |
| High molecular weight | Polymeric | Emulsan | *Acinetobacter calcoaceticus* |
| | | Biodispersan | *Acinetobacter calcoaceticus* |
| | | Liposan | *Candida lipolytica* |
| | | Mannanprotein | *Saccharomyces cerevisiae* |
| | | Alasan | *Acinetobacter radioresistens* |

Nitschke and Costa, 2007; Rhaman and Gakpe, 2008; Raiger and López, 2009; Soberon-Chavez and Maier, 2011; Fracchia et al., 2012; Cruz 2014; Sarubbo et al., 2015; Becerra-Gutiérrez and Horna-Acevedo, 2016

reported exerting an effect in the bio-surfactants' production through growth, and cell activity like pH, temperature, agitation, concentration of metallic ions and oxygen availability (Gautam and Tyagi, 2006; Rahman and Gakpe, 2008; Saharan et al., 2011).

Nowadays, before the great biotechnological potential of the bio-surfactants, researches around the world are being carried out searching for producer strains and bio-surfactants' identifiers. The bio-prospection of producer microorganisms can be done using different methods like: hemolysis of erythrocytes, aximetric drop shape analysis (ADSA), cell surface hydrophobicity, drop collapse, oil spread, tilted glass slide, blue agar plate method, emulsification activity, agar plate method and direct colony chromatographic (TLC) technique. Meanwhile, the extraction, purification and characterization can range from the conventional ones to the most sophisticated, depending on the characteristics of the surfactant to obtain. The methods are: solvent extractions (ethanol, acetone, among others), acid precipitation, filtration, centrifugation, ion exchange, adsorption–desorption, Thin Layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC), Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), Gas Chromatography and Mass Spectroscopy (GC-MS), Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), Fast Atom Bombardment- Mass Spectroscopy (FAB–MS), microscopic techniques, Transmission Electron Microscopy (TEM), Cryo-TEM, Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) (Satpute et al. 2010).

**Production of biosurfactants by species of the genus *Staphylococcus spp.***

The genus *Staphylococcus* spp. is generally known for its pathogenic character in humans and animals, but it is also known of some species that are considered as commensals. On the other hand, there is little information on the isolation of staphylococcus strains from both the clinical environment and from different natural environments producing secondary added-value metabolites and biotechnology versatility such as biosurfactants (Eddouaouda et al., 2012; Mani et al., 2016; Rossi et al., 2016).

The following will present in general only some of the research done by various authors around the world that have demonstrated the isolation of strains of the *Staphylococcus spp.*, from different environments with the capacity to produce bio-surfactants which, as already mentioned, have multiple properties with biotechnological potential to be used in different industrial areas.

Eddouaouda et al., 2012 studied the production of a BS of lipo-peptidic nature produced by the *Staphylococcus* *spp.* 1E strain isolated from soil contaminated with crude oil in Algeria; this, by using separate sources of 1% carbon as separate fuels, crude oil, hexadecane and olive oil being the latter the best source of carbon for the generation of BS. In addition, the generated BS presented antimicrobial capacity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* and solubilizing capacity (phenanthrene / water). Meanwhile, Hamed et al., 2012 mention that seawater is a specific medium to harbor complex bacterial communities adapted to adverse conditions, as well as the search for bio-surfactant producing strains using four different methods (Blue agar plate method, blood agar lysis, collapsed test and emulsification index). The investigator reported the isolation of several microbial strains of seawater biofilms formed in the pipelines of a refinery of Bizerte, Tunisia, which are used in the refrigeration of petroleum products after the extraction process at high temperatures (50 °C) and saline conditions (35g / L). The genera identified in the biofilm collected were *Staphylococcus, Bacillus, Micrococcus, Aeromonas, Pseudomonas, Photobacter, Chrysomonas* and *Neisseria,* where the genus *Staphylococcus* in species such as *aureus, saprophyticus,* and *epidermidis* were cataloged as producers of bio-surfactants as adhesion function substances according to the results of the preliminary study. In both studies carried out by these researchers in 2012 they conclude that these compounds are attributed a potential application in the environmental area in bioremediation processes of sites contaminated with hydrocarbons or in MEOR as well as in the biomedical area as antimicrobials subject to the need to carry out thorough biochemical and molecular studies for the identification and characterization of these compounds. On the other hand, San Keskin et al., 2015 found that *Staphylococcus xylosus* STF1 isolated from oil contaminated soils in Ankara, Turkey, being capable of producing a bio-surfactant of lipo-peptidic nature when growing in a medium supplemented with motor oil as the only source (xylene, diesel, kerosene, naphthalene, anthracene, among others) as well as antimicrobial properties against pathogens such as *P. aeruginosa*, *K. pneumonia*, *E. coli* and bacteria of the same genus as *S. aureus*. Concluding that more research is needed to find practical and economic ways such as hyper productive strains, economic substrates, to produce in higher proportion and quality these natural microbial products with those of synthetic products toxic to the environment. Rossi et al., 2016 reported the isolation of strains of *S. haemolyticus* in patients from the Naval Hospital in Rio de Janeiro, Brazil. This microorganism is considered a commensal microorganism and opportunistic pathogen of humans. However, the researcher mentioned this bacterium presented the ability to produce BS which inhibited the cell growth and biofilm formation of human bacterial pathogens, in addition to having synergistic effects with tetracycline due to the increase of the cellular permeability of BS in bacteria. He concluded that the production of bio-surfactant by *S. haemolyticus* can be an important means for competition, survival and biofilm formation by the bacterium, besides a virulence factor that needs more detailed studies together with the inherent interest of the biotechnological potential presented by these compounds. Nwachi et al., 2016 performed an analysis of isolation and production of bio-surfactants by microorganisms from contaminated hydrocarbon soils in the Abakaliki, metropoli of Ebonyi, Nigeria, using different methods including oil spreading test, emulsification test and blood hemolysis test, in addition to its cultivation in medium supplemented with 2% glucose as a source of carbon and energy. He reported the isolation of six producing strains including *Staphylococcus spp.* strains, which had a 50% paraffin oil emulsification index. He concluded that, although the study was carried out on a small scale, bio-surfactants can be produced in greater proportion, with potential application in the oil industry, as well as in the bioremediation of sites contaminated with oil. Mani et al., 2016 reported the isolation of the *Staphylococcus saprophyticus* SBPS 15 strain from coastal contaminated petroleum sites in Puducherry, India, and is also a producer of bio-surfactants of a glycolipid nature. The BS mentioned by the author presented properties of surface activity, temperature and pH stability as well as antimicrobial properties towards human pathogens (*V. cholerae, E. coli, K. pneumonie, P. aeruginosa, Candida albicans, S. paratyphi, C. Neoformans,* among others) evidencing its potential application in the field of biomedical sciences.

## Final comments

The genus *Staphylococcus spp. i*s commonly known, and related, for its pathogenic character in aspects of human and animal health. On the other hand, as previously mentioned, the isolation from different natural environments of species of the genus are capable of producing compounds with functional and biological properties, such as BS, which may open another utilitarian approach to this genus for its potential application in areas such as environmental and biomedical when used in bioremediation processes and as antimicrobial agents (Why not supporting to control the incidence of antimicrobial resistance by different microorganisms? It has been considered several years ago a global health problem). Knowledge of the metabolic diversity of microorganisms can not only focus on the negative character, but also find the beneficial side this from the production of value-added molecules such as bio-surfactants. However, in the field of the generation of BS of microbial origin, including those produced by staphylococci. There are still different limitations that, despite the structural diversity, reported physical and biological properties, are not widely used in various industrial areas due to deeper studies for a greater knowledge in the biosynthesis routes, physicochemical and biological properties, search and selection of producing and highly producing microorganisms are needed, as well as the optimization of production processes and their large-scale extrapolation.

**Authors' contributions**

ADJCS conceived idea, designed, organized project, wrote and revised the manuscript. MDR, RGB and AS wrote and revised the manuscript. All the authors read and approved the final manuscript.

## REFERENCES

Banat IM, MAD De Rienzo and GA Quinn, 2014. Microbial biofilms: biosurfactants as antibiofilm agents. Applied Microbiology and Biotechnology, 98: 9915-9929.

Banat IM, RS Makkar and SS Cameotra, 2000. Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology, 53: 495-508.

Becerra-Gutierrez LK and Horna-Acevedo MV, 2016. Isolation of biosurfactant producing microorganisms and lipases from wastewaters from slaughterhouses and soils contaminated with hydrocarbons. Scientia Agropecuaria, 7: 23-31.

Bennett-Reginald W and M Hait-Jennifer, 2011. Staphylococcal Enterotoxins: Micro-slide Double Diffusion and ELISA-based Methods. Bacteriological Analytical Manual. Chapter 13A. U.S. Food and Drug Administration. https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm073674.htm.

Bennett-Reginald W and Lancette-Gayle A, 2001. *Staphylococcus aureus*. Bacteriological Analytical Manual. Chapter 12. U.S. Food and Drug Administration. https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071429.htm

Brizzio AA, FA Tedeschi and FE Zalazar, 2013. Estrategia de PCR múltiple para la caracterización molecular simultánea de Staphylococcus aureus y enterotoxinas estafilocócicas en aislamientos de brotes de origen alimentario. Biomédica, 33: 122-127.

Bustos-Martínez JA, A Hamdan-Partida, M Gutiérrez-Cárdenas, 2006. *Staphylococcus aureus*: La reemergencia de un patógeno en la comunidad. Revista Biomedica, 17: 287-305.

Casellas JM, 2011. Antibacterial drug resistance in Latin America: consequences for infectious disease control. Rev Panam Salud Pública, 30: 519-528.

Cercenado E, 2016. Epidemiology of the infection by resistant gram-positive microorganisms. Revista española de quimioterapia: publicación oficial de la Sociedad Española de Quimioterapia, 29: 6-9.

Cervantes-García E, R García-González and PM Salazar-Schettino, 2014. Características generales del Staphylococcus aureus. Revista latinoamericana de patología clínica y medicina de laboratorio, 61: 28-40.

Chávez M, NC Erazo, DA Reina and M Esparza, 2015. Métodos de tipificación y epidemiología molecular de *Staphylococcus aureus* con resistencia a la meticilina. Revista Biosalud, 14: 81-90.

Cruz NLDR, E Sánchez-Salinas and ML Ortiz-Hernández, 2014. Biosurfactantes y su papel en la biorremediación de suelos contaminados con plaguicidas. Revista Latinoamericana de Biotecnología Ambiental y Algal, 4: 1-21.

Demirci M, F Tombul, A Yiğin, and SK Altun, 2016. Investigation of *Staphylococcus aureus* enterotoxins A to E via real-time PCR from various food samples in Turkey. Pakistan Veterinary Journal, 37: 100-104.

Eddouaouda K, S Mnif, A Badis, SB Younes, S Cherif, S Ferhat and S Sayadi, 2012. Characterization of a novel biosurfactant produced by Staphylococcus sp. strain 1E with potential application on hydrocarbon bioremediation. Journal of Basic Microbiology, 52: 408-418.

ELIKA, 2013. Staphylococcus aureus. Fundación vasca para la seguridad agroalimentaria. http://www.elika.net/datos/pdfs_agrupados/Documento95/7.Staphylococcus.pdf.

Félix-Fuentes A, ON Campas-Baypoli, and M Meza-Montenegro, 2005. Calidad sanitaria de alimentos disponibles al público de ciudad Obregón, Sonora, México. Revista de Salud Pública y Nutrición RESPYN, 6: 1-14.

Flores TG and RAR Herrera, 2005. Enfermedades transmitidas por alimentos y PCR: prevención y diagnóstico. Salud Pública de México, 47: 388-390.

Fracchia L, IM Banat, MG Martinotti and M Cavallo, 2012. Biosurfactants and bioemulsifiers biomedical and related applications-present status and future potentials, INTECH Open Access Publisher, UK, pp: 325-370.

García-Huidobro D, M Carreño, S Alcayaga and J Ulloa, 2012. Clinical and epidemiological description of severe outbreak of foodborne infection by Salmonella Enteritidis. Revista Chilena de Infectología, 29: 132-137.

Gautam KK and VK Tyagi, 2006. Microbial surfactants: A review. Journal of Oleo science, 55: 155-166.

Hamed SB, L Smii, A Ghram and A Maaroufi, 2012. Screening of potential biosurfactant-producing bacteria isolated from seawater biofilm. African Journal of Biotechnology, 11: 14153-14158.

Ibrahim MB, A Franzetti, I Gandolfi, G Bestetti, GM Martinotti, L Fracchia, TJ Smyth and R Marchant, 2010. Microbial biosurfactants production, applications and future potential. Applied Microbiology and Biotechnology, 87: 427–444.

ISO 6888:1999. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)-Part 1: Technique using Baird-Parker agar medium. Part 2: Technique using rabbit plasma fibrinogen agar medium.

Lombard AB, LM de la Garza and CL Ortiz, 1996. Importancia de la detección de enterotoxinas estafilocócicas. Revista Cubana de Alimentación y Nutrición, 10: 2.

López-Aday D, E Rivero-Álvarez, A Martínez-Torres, and M Alegret-Rodríguez, 2013. Foodborne diseases in Villa Clara. Revista Cubana de Higiene y Epidemiología, 51: 203-213.

Manfredi EA, GA Leotta and M Rivas, 2010. PCR múltiple para la detección de los genes sea, seb, sec, sed y see de Staphylococcus aureus: Caracterización de aislamientos de origen alimentario. Revista Argentina de Microbiología, 42: 212-215.

Mani P, G Dineshkumar, T Jayaseelan, K Deepalakshmi, CG Kumar and SS Balan, 2016. Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine *Staphylococcus saprophyticus* SBPS 15. 3 Biotech, 6: 163.

Monge KMM, 2013. Carbapenemicos: tipos y mecanismos de resistencia bacterianos. Revista Médica de Costa Rica Y Centroamerica, 70: 599-605.

Morita T, M Konishi, T Fukuoka, T Imura and D Kitamoto, 2007. Microbial conversion of glycerol into glycolipid biosurfactants, mannosylerythritol lipids, by a basidiomycete yeast, *Pseudozyma antarctica* JCM 10317 T. Journal of Bioscience and Bioengineering, 104: 78-81.

Muñoz AI, M Vargas, L Otero, G Díaz, and V Guzmán, 2011. Presencia de Listeria monocytogenes en alimentos listos para el consumo, procedentes de plazas de mercado y delicatessen de supermercados de cadena, Bogotá, DC, 2002-2008. Biomédica, 31: 428-439.

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

NMX-F-310-1978. Determinación de cuenta de Staphylococcus aureus coagulasa positiva, en alimentos. method of test for count of staphylococcus aureus in food. Normas mexicanas. Dirección general de normas. http://www.economia-noms.gob.mx/noms/ inicio.do.

NOM-210-SSA1-2014 (NORMA Oficial Mexicana), Productos y servicios. Métodos de prueba microbiológicos. Determinación de microorganismos indicadores. Determinación de microorganismos patógenos. http://www. economia-noms.gob.mx/noms/ detalleXNorma Action.do.

NOM-242-SSA1-2009 (NORMA Oficial Mexicana), Productos y servicios. Productos de la pesca frescos, refrigerados, congelados y procesados. Especificaciones sanitarias y métodos de prueba. http://www.economia-noms.gob.mx/ noms/detalleXNormaAction.do.

NOM-243-SSA1-2010 (NORMA Oficial Mexicana), Productos y servicios. Leche, fórmula láctea, producto lácteo combinado y derivados lácteos. Disposiciones y especificaciones sanitarias. Métodos de prueba. http://www. economia-noms.gob.mx/noms/detalleXNorma Action.do.

NOM-251-SSA1-2009 (NORMA Oficial Mexicana), Prácticas de higiene para el proceso de alimentos, bebidas o suplementos alimenticios. http://www.economia-noms.gob.mx/noms/detalleXNormaAction.do.

Nwachi AC, CC Onochie, IR Iroha, VM Agah, BN Agumah, IB Moses and DO Ogbeja, 2016. Extraction of Biosurfactants Produced from Bacteria Isolated from Waste-Oil Contaminated Soil in Abakaliki Metropolis, Ebonyi State. Journal of Biotechnology Research, 2: 24-30.

Okoliegbe IN and OO Agarry, 2012. Application of microbial surfactant (a review). Scholarly Journals of Biotechnology, 1: 15-23.

Olea A, J Díaz, R Fuentes, A Vaquero, and M García, 2012. Foodborne disease outbreaks surveillance in Chile. Revista Chilena de Infectología, 29: 504-510.

Perazzi B, M Camacho, K Bombicino, Z Flores, C Vay, and A Famiglietti, 2010. *Staphylococcus aureus*: nuevos y antiguos antimicrobianos.

Revista Argentina de Microbiología, 42: 199-202.

PHE (Public Health England), 2014. Identification of Staphylococcus species, Micrococcus species and Rothia species. UK Standards for Microbiology Investigations. ID 7 Issue 3. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories.

Prado V, V Solari, IM Álvarez, C Arellano, R Vidal, M Carreño, and V Muñoz, 2002. Situación epidemiológica de las enfermedades transmitidas por alimentos en Santiago de Chile: Período 1999-2000. Revista Médica de Chile, 130: 495-501.

Predari CS, 2007. Estafilococcos coagulasa negativos: el enemigo silente. Revista Argentina de microbiología, 39: 1-3.

Puig-Peña Y, M Espino Hernández, V Leyva Castillo, N Apórtela López, Y Pérez Muñoz, and P Soto Rodríguez, 2015. Resistencia antimicrobiana en cepas de estafilococos coagulasa positiva aisladas en alimentos y manipuladores. Revista Cubana de Alimentación y Nutrición, 25: 245-260.

Rahman PK and E Gakpe, 2008. Production, characterisation and applications of biosurfactants-Review. Biotechnology, 7: 360-370.

Raiger IILJ and NI López, 2009. Los biosurfactantes y la industria petrolera. Química Viva, 8: 146-152.

Reglamento (CE) No 2073/2005 de la comisión de las comunidades europeas de 15 de noviembre de 2005. Relativo a los criterios microbiológicos aplicables a los productos alimenticios. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2005R2073:20100519:ES:PDF.

Reglamento (CE) No 178/2002 del parlamento europeo y del consejo de la unión europea de 28 de enero de 2002. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:031:0001:0024:ES:PDF.

Romero-Cabello R, 2007. Microbiología y parasitología humana, bases etiológicas de las enfermedades infecciosas y parasitarias. 3ra edición. Editorial Medica Panamericana, México.

Rossi CC, JF Santos-Gandelman, EM Barros, VM Alvarez, MS Laport and M Giambiagi-de Marval, 2016. *Staphylococcus haemolyticus* as a potential producer of biosurfactants with antimicrobial, anti-adhesive and synergistic properties. Letters in Applied Microbiology, 63: 215-221.

Saharan BS, RK Sahu and D Sharma, 2011. A review on biosurfactants: fermentation, current developments and perspectives. Genetic Engineering and Biotechnology Journal, 1: 1-14.

San Keskin NO, D Han, AD Ozkan, P Angun, OCO Umu and T Tekinay, 2015. Production and structural characterization of biosurfactant produced by newly isolated Staphylococcus xylosus STF1 from petroleum contaminated soil. Journal of Petroleum Science and Engineering, 133: 689-694.

Sanchez ADJC, IGT Torres, MEJ y Flores, 2014. Surfactantes biológicos con aplicación potencial en tecnología de alimentos: una revisión. Biológicas Revista de la DES Ciencias Biológico Agropecuarias Universidad Michoacana de San Nicolás de Hidalgo, 15: 16-23.

Sarubbo LA, RB Rocha Jr, JM Luna, RD Rufino, VA Santos, and IM Banat, 2015. Some aspects of heavy metals contamination remediation and role of biosurfactants. Chemistry and Ecology, 31: 707-723.

Satpute SK, AG Banpurkar, PK Dhakephalkar, IM Banat and BA Chopade, 2010. Methods for investigating biosurfactants and bioemulsifiers: a review. Critical Reviews in Biotechnology, 30: 127-144.

Satpute SK, GA Płaza and AG Banpurkar, 2017. Biosurfactants' production from renewable natural resources: example of innovative and smart technology in circular bioeconomy. Management Systems in Production Engineering, 25: 46-54.

Sineriz F, RK Hommel, and HP Kleber, 2001. Production of biosurfactants. Encyclopedia of Life Support Systems, Biotechnology, 5. https://pdfs.semanticscholar.org/60a5/2cd622fbf4c3f943e5e43bdadea63edd1136.pdf

Soberon-Chavez G and RM Maier, 2011. Biosurfactants: a general overview. In Biosurfactants. Springer Berlin Heidelberg, German, pp: 1-11.

Tamara-Díaz L, E Ángel, R Caballero Torres and J Díaz Fernández, 2008. Enfermedades trasmitidas por alimentos. Capítulo 16. In: temas de higiene de los alimentos. Angel E. Caballeros Torres. Editorial Ciencias Médicas. pp: 216-247.

Tamara K, M Zagovalov, VL Castillo and YP Peña, 2008. Principales bacterias patógenas en alimentos. Capítulo 3. In: temas de higiene de los alimentos. Ángel E. Caballeros Torres. Editorial ciencias médicas. pp: 29-41.

Teran KLN, DMS Galvis, GC López, and KV Leal, 2014. Enterotoxins, Biofilm and Resistance to Antimicrobial Agents of Coagulase-Negative

Staphylococcus Strains Isolated from Blood Cultures. Kasmera, 41: 2.

Todd JK, 2005. Infecciones estafilocócicas. Pediatr Revision, 26: 438-43.

Toribio-Jiménez J, JC Velázquez-Aradillas, Y Romero-Ramírez, MA Rodríguez-Barrera, JD Chávez-González, J Guevara-Luna, JL Aguirre-Noyola, A y Fierro-Torres, 2014. Pseudomonas sp productoras de biosurfactantes. Tlamati, 5: 66-82.

Winn WC, DA Stephen, MJ William, WK Elmer, WP Gary, CS Paul and LW Gail, 2006. Koneman diagnostico microbiológico. Texto y atlas en color Editorial Medica Panamericana. 6ta edición. Mexico.

WHO (World Health Organization), 2017. Food safety. Fact sheet. http://www.who.int/mediacentre/factsheets/fs399/es/.

Zamudio ML, A Meza, H Bailón, J Martinez-Urtaza and J Campos, 2011. Experiencias en la vigilancia epidemiológica de agentes patógenos transmitidos por alimentos a través de electroforesis en campo pulsado (PFGE) en el Perú. Revista Peruana de Medicina Experimental y Salud Publica, 28:128-135.

Zendejas-Manzo GS, H Avalos-Flores, and MY Soto-Padilla, 2014. Microbiología general de *Staphylococcus aureus*: Generalidades, patogenicidad y métodos de identificación. Revista Biomédica, 25: 129-143.