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Review

A Review: Klebsiella pneumoniae, Klebsiella oxytoca and Biotechnology

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Abstract

Biotechnology, molecular biology and genetic engineering, and bioprospecting play a crucial role in our common future, enabling industrially important microorganisms to ensure sustainable products (fuels, chemicals, pharmaceuticals, food, drug delivery systems, medical devices etc.) and new bioeconomic opportunities. Biotechnological applications are able to provide cost-effective green alternatives to conventional industrial processes, which are currently affecting the nature and biodiversity. *Klebsiella* species are among the well-studied microbes both in medicine field, as ones of the most resilient opportunistic pathogens, and in industry, due to their promising biochemical properties, and their potential as better hosts than other microorganisms, for i.e. in genetic manipulation. *Klebsiella oxytoca* and *Klebsiella pneumoniae* are ubiquitously found in natural environments, but also as commensals in the human gut, and associated with a high-resistance to the first-line antibiotics. However, these specific strains are continuously isolated and studied for different industrial purposes (i.e. bulk chemicals and biofuels production, medical diagnosis, nanoparticles and exopolysaccharides synthesis, plant growth promoting activities, bioremediation and biodegradation agents etc.), and scientific results regarding their biotechnological potential could generate big impact for global bioeconomy development. Recently, research in synthetic biology gained a lot of attention, and new techniques highlight ways to reprogramme these microbial cells in view of high-yield or high-quality new chemicals obtainment. Therefore, some scientific research niches are emerging in biotechnology, and unknown metabolic pathways and genes are identified and further studied, to provide alternative solutions to the global challenges.

Keywords Biotechnology, *Klebsiella*, *K. oxytoca*, *K. pneumoniae*.

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Introduction

Worldwide, there is a major interest to search for novel materials based on natural resources, and biotechnology is considered a key tool for sustainable development, due to its contribution in a wide range of industrial sectors. Developing bio-based products and bioprocesses which can compete in the market with those derived from chemicals, represents a challenge for the 21st century (OECD). Since the 80s and 90s, the interest for biotechnology and bioeconomy had grown, and new generation of technologies emerged into industry. Nowadays, modern biotechnology, the developments in the genetics and molecular biology changed many perceptions and play key roles in the new era of sustainable economy, delivering revolutionary new products and services. A global bio-based economy, with benefits for human well-being and environment is eagerly desired, because at the present, most of the economic sectors depend on energy and materials originating from carbon sources. In this context, the application of biotechnology proves to successfully replace many unsustainable products or to provide innovative solutions in various fields, such as: food and feed, chemicals, agriculture, environment, detergents, textiles, pulp and paper, polymers, pharmaceuticals and cosmetics, etc. (SINGH et al, 2013).

A “Rainbow code” for biotechnology classification was published in 2012, to describe the specific areas of biotechnological focus using colors, as follows: *white* for biotech industry and environmental engineering; *gray* for environment (traditional fermentations); *green* for agriculture; *red* for medicine and human health; *blue* for aquatic resources; *brown* for dessert and arid lands; *yellow* for nutrition, and considered the oldest branch of biotechnology (recently, insect biotechnology for drug discovery has been coded yellow); *gold* for bioinformatics; *violet* for law, ethical and philosophic domains; *dark* for bioterrorism and biological weapons (KAFAESKI, 2012; BARCELOS et al, 2018).

Among all these fields, white biotechnology, known as industrial biotechnology is considered the biggest branch, and a component of the “green chemistry”. It refers to the application of bio-based products (biochemicals, biomaterials and biofuels) which can replace the petroleum-derived chemicals, and also to the biocatalysis processes in industry. In the biotechnology, microorganisms constitute a well-studied base for industrial applications, with origins in antiquity. Some of the most known industrial biotechnological products which are commercially produced or aimed for further development and utilization, demonstrating a scientific progress towards a modern and environmentally-friendly economy, are considered the followings: biodegradable polymers (biopolyesters, polylactic acid, polyhydroxybutyric acid – PHB or polyhydroxyalcanoates – PHAs, which are similar to petroleum derived polymers; diols; dicarboxylic acids; amino acids; hydroxyalkanoic acid); enzymes (amylases, lipases, proteases, cellulases, oxidases, beta-xylanases); food additives (flavours); surfactants; fine chemicals; biofuels, and others (KAFAESKI,

2012; SINGH et al, 2013; BARCELOS et al, 2018; VLADU et al, 2019).

Another important area is green biotechnology, mainly related to agriculture and environment, having some important applications in the field, such as: genetically modified organisms, transgenic organisms, biopolymers of plant origin, seeds, and trees or their exudates, etc. Blue biotechnology is the next area worth to be mentioned, and it is related to the exploration of marine resources, with important applications in the pharmaceutical and energy fields, continuously offering new products with anti-oxidant, antimicrobial, or antitumor activities, cosmetics, additives and pigments, biofuels etc. (BARCELOS et al, 2018).

Microbial diversity has been intensively explored for years, but scientific progress and the recently availability of genomic data made bioprospectation a promising research field, in the context that Earth is a home for more than 1 trillion microbial species. Therefore, discovering microbial communities represents an advantageous opportunity for the sustainable development of biotechnological industries (TANNER and VILANOVA, 2017). Microorganisms are a valuable resource for novel compounds, bioactive natural products, new drugs for the emerging human diseases, and for innovative approaches of genetic engineering. Considering the advances made in the last two decades, a revitalized interest for bacterial secondary metabolites is observed, and a new market is opening for the development in the fields of genetics, genomics, metabolic engineering, bioinformatics and synthetic biology. Bioprospecting for microbial-based products, including the discovery of the wide diversity of microbes and describing their ecology, represent one of the key areas for the future of biotechnological applications, which looks bright (SEKUROVA et al, 2019).

Bioprospecting of new isolates for antibiotics, polysaccharides and enzymes discoveries, with applications in food, pharmaceutical, cosmetics, agricultural, medical and chemical industries, benefits from an increased worldwide interest.

One of the global crises is considered to be antibiotic resistance among bacterial pathogens, and it highlights an urgent need to identify novel drugs (STOICA et al, 2019). The majority of discoveries in this field were made during the ‘50s-‘70s, and with time, a skepticism from the pharmaceutical companies related to natural products was observed; despite of this, scientific community proved that new efficient drugs can be obtained (i.e recently, bioprospecting human microbiota lead to the discovery of two new antibiotics: lugdunin, which is active against Gram-positive strains, including *Staphylococcus aureus*, and lactocillin, active against vaginal pathogenic bacteria) (SEKUROVA et al, 2019). There is a wide known acronym for the pathogens responsible for nosocomial infections, especially in the US, namely ESKAPE, which refers to: *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterococcus* spp. (MARCOLEFAS et al, 2019). To counteract the effects of their antibiotic resistance,

researchers focused on studying these pathogenic strains, their multi-drug resistance, and innovative discoveries were made in the last years with the help of metagenomics, computational analysis of DNA sequences in relation with bacterial metabolites, engineering of biological systems and reorganization of metabolic pathways (SEKUROVA et al, 2019).

Another focus of bioprospection, with multiple applications, including pharmaceuticals and biomedicine, is represented by the microbial exopolysaccharides (EPSs) production. Due to their various chemical structures and functional properties, these EPSs found an extensive range of industrial applications, being synthesized by different microbial genera and, some of them, successfully commercialized at large-scale. However, such compounds represent only a small part from the biopolymer market, and expanding the research on eco-efficiency product development represent a current focus for biotech industry (VENTORINO et al, 2019).

Current concerns regarding climate change, pollution, and availability of natural resources lead to an increased demand for alternative energy sources, production of bio-based bulk chemicals, renewable feedstocks utilization, development of biomass conversion processes, and a worldwide target for sustainable economy. Due to an increased environmental awareness, many efforts and progress were made to search alternatives to traditional energy or chemical products. Petrol fuels are becoming scarce, and in this context, research on biofuels production has attracted a growing attention in the last years. Microbial biotechnology has the potential to replace many from the current production technologies which rely on fossil fuels. Biohydrogen, 1,3-propanediol (1,3-PDO), 2,3-butanediol (2,3-BD), bioethanol, and 2-butanol represent only few examples of bulk chemicals obtained from biosynthesis, and with utilization of renewable resources as substrates (ALMEIDA et al, 2012; ELSHARNOUBY et al, 2013; CHO et al, 2015; YANG and ZHANG, 2018; ROSALES-CALDERON, 2019; MAR et al, 2020).

Biological efforts to find green alternatives to chemical processes are continuously made, and some of them are finding *Klebsiella* species as promising industrial microorganisms, among which *K. pneumoniae* and *K. oxytoca* are the most studied. Biotechnology considered some pathogens (*Klebsiella* spp., *E. coli*, *Clostridium* sp., *Bacillus* sp.) as producers of valuable chemicals compounds (i.e. ethanol, hydrogen, 1,3-PDO, 2,3-BD as biofuels), but also other bioproducts (D-lactate, exopolysaccharides, phytase, auxin, metal-nanoparticles, pectate lyase etc). Moreover, some of the *Klebsiella* spp. are endosymbionts, which greatly contribute to nitrogen-fixing, and have an important contribution in bioremediation processes (i.e cyanide or mercury removal) (GALLO et al, 2018; MITREA and VODNAR, 2019 etc.).

Historical information about *Klebsiella* dates since 1882, when Carl Friedländer, a German pathologist, described a capsulated bacterium causing severe pneumonia, a pathogen commonly known as causing the "Friedländer's pneumonia". In 1887, the microorganism

was described and named *K. pneumoniae* by Trevisan, following a previous description of the *Klebsiella* genus in 1885, which was aimed to honor the German microbiologist Edwin Klebs. Another important mention of *Klebsiella* name appeared when Anton von Frisch, an Austrian urologist, identified and described a causative organism of rhinoscleroma (a disease of upper respiratory tract), as a gram-negative coccobacillus, named *K. rhinoscleromatis* by Trivisan (in 1887), and recognized as a subspecies of *K. pneumoniae* (SAHLY et al, 1997; PONTUAL et al, 2008; ZEDAN, 2017; BENGOCHEA and PESSOA, 2019).

Klebsiella genus belongs to the *Proteobacteria* phylum, *Gammaproteobacteria* class, and to the *Enterobacteriaceae* family, and it is included in the KES subgroup (*Klebsiella*, *Enterobacter*, *Serratia*), while its species are Gram-negative, rod shaped and non-motile. *Klebsiella* spp. are ubiquitously found in natural environments (water, soil, plants), but also in animals and as commensal flora in the gut of humans; many of them have been also isolated from clinical samples, considering that *Klebsiella* spp. are known as the second leading microbes (*K. pneumoniae* in majority, responsible for 70% of infections, and for 1/3 of Gram-negative infections) involved in nosocomial infections, after *E. coli*. (DE JESUS et al, 2015; SINGH et al, 2016; ASHA et al, 2017; MERLA et al, 2019; QUANSAH et al, 2019; EFFAH et al, 2020; HERRIDGE et al, 2020).

Generally, *Klebsiella* species are considered opportunistic pathogens, but they can cause life-threatening infections, such as: pneumonia, bacteremia, bloodstream infections, antibiotic-associated haemorrhagic colitis, neonatal septicemia, meningitis, pyogenic liver abscess, brain abscess (responsible for 16 to 43% of central nervous system infections), urinary tract infections, endocarditis, endogenous endophthalmitis, and sepsis (SAHLY et al, 1997; HO et al, 2011; HSIEH et al, 2012; CHEN et al, 2013; KUMAR et al, 2013; GHASEMIAN et al, 2018; BENGOCHEA and PESSOA, 2019; EFFAH et al, 2020).

Limitation of the study: Although many information was synthesized and analysed (196 articles), it is worth noting that there were a number of scientific papers which were not assessed, due to the huge amount of available data, and mostly with impact in the medical field.

Klebsiella oxytoca

Clinical significance

In taxonomy, *Klebsiella oxytoca* (LAUTROP, 1956) was earlier named and described by Flugge, in 1886, as a *Bacillus oxytocom pernicius* strain isolated from sour milk, and further mentioned as a microorganism associated with *Musca domestica*, or as *Bacterium oxytocom*, a clinical isolate from an abdominal infection (actinomycosis), and also known as *Aerobacter oxytocom* after Trevisan or Bergey (1923) (FLUGGE, 1886; TAVCAR and STROPNIK, 1970; GREENBER, 1971; SINGH et al, 2016). In 1974, Jain and collaborators reported the *Oxytocom* group as distinguish from *Klebsiella* genus, using DNA-DNA hybridization, and

later, in the 1980, a particular interest for *K. oxytoca* was shown, after some strains were found to have a beta-lactam resistance phenotype (GRANIER et al, 2003).

K. oxytoca is known as an opportunistic pathogen, causing septicemia, pneumonia and urinary tract infections in humans, and emerging as an important bacteria in hospital-acquired infections, but also as a beneficial microorganism, an endophytic nitrogen-fixing bacterium, and an exopolysaccharides producing microorganism (GALLO et al, 2012; CHEN et al, 2013; SINGH et al, 2016; GHASEMIAN et al, 2018; PEREZ-VASQUEZ et al, 2019). Despite of being ubiquitous in environment, the strains are commonly isolated from the skin, mucous membranes, oropharynx and intestins of humans or animals, as well as from clinical patients and healthcare facilities (DARBY et al, 2014). From animal lesions, a specific serotype of this species was isolated more often than others, namely the K55, from a total of 79 serotypes recognized for *Klebsiella* genus (BLEICH et al, 2008; HSU et al, 2016; CAI et al, 2019).

This bacterium belongs to a diverse population structure composed from six species and represented by different phylogroups: *K. oxytoca* (Ko2), *K. michiganensis* (Ko1), *K. grimontii* (Ko6), *K. huaxiensis* (Ko8), *K. pasteurii* (Ko4) and *K. spallanzanii* (Ko3). *K. oxytoca* is characterized by cylindrical rod-shaped cells of 2-5 µm dimensions, produces a prominent capsule, and beta-lactamases (including metallo-beta-lactamases), which make it a multi-resistant species to antibiotics with broad spectrum of activity, such as cephalosporins (ceftriaxone, cefotaxime or ceftazidime) and aztreonam (monobactam); moreover, its resistance was identified also to non-beta-lactam antibiotics among clinical isolates, and is supposed to be higher than that of *K. pneumoniae*, being also resistant to the widely used antiseptic, chlorhexidine (FEVRE et al, 2005; TRIVEDI et al, 2015; CHILOM et al, 2016; SINGH et al, 2016; WANG et al, 2017; ZEDAN et al, 2017; GHASEMIAN et al, 2018; HERRIDGE et al, 2020).

Therefore, this microorganism is naturally resistant to amino- and carboxypenicillins, producing a K1 enzyme (KOXY type), a molecular class A chromosomal beta-lactamase; scientific reports mention its resistance to carbapenems (due to the carbapenemases production – i.e imipenemases), such as ipinem, ertapenem, and meropenem (while remaining susceptible to tigecycline, ciprofloxacin and colistin) and the presence of a molecular class-A serine beta-lactamase, which is capable to hydrolyze them, namely KPC, usually found in *K. pneumoniae*, which still remain sensitive to clavulanic acid and tazobactam (GHEORGHIU et al, 1997; LABRADOR et al, 2014; IZDEBSKI et al, 2015; SINGH et al, 2016; WANG et al, 2017; ZEDAN et al, 2017).

Besides, *K. oxytoca* produces cytotoxins associated with antibiotic-associated hemorrhagic colitis (AAHC, including pediatric infections), and also with the cell apoptosis, which could be strain specific; these compounds, namely tillivaline, a non-protein cytotoxin (a pentacyclic peptide toxin, member of pyrrobenzodiazepine family), and kleboxymycin, as a primary product of the nonribosomal

peptide synthesis pathway, are considered important pathogenic factors, playing a role in the virulence of the strains (JOAINIG et al, 2010; Tse et al, 2017; VALIDI et al, 2017; GHASEMIAN et al, 2018; DALLAL et al, 2019; HERRIDGE et al, 2020).

Biotechnological importance

Agricultural biotechnology is one of the key actors involved in global sustainable development, a tool which provides benefits for crop production, but still it is considered a controversial topic, due to the utilization of genetically-modified organisms, for which potential ecological and health risks involved need to be very clear for everyone. However, scientists demonstrated that understanding of gene functions lead to innovative biotech solutions, with better results, which could be sustainable for environment (BJÖRNBERG et al, 2015; KUMAR and POULOSE, 2015).

Therefore, in the last decades alternative strategies were developed to increase the agricultural productivity, and environmental microbes played a key role. Rhizospheric microorganisms are used as bioinoculants for supplying nutrients and/or stimulating plant growth, because they are well known as producers of siderophores, phytohormones and antibiotics, involved in macro-nutrients uptake (i.e. phosphorous, nitrogen, phosphate, zinc, potassium and iron) or nitrogen fixation, which are very important for plant-growth. The role of metagenomic and metabolomic approaches is considered very important (SATYANARAYANA et al, 2012; SAPRE et al, 2018; EGAMBERDIEVA et al, 2019).

Among the rhizospheric bacteria, known as plant growth-promoting (PGPR), *Klebsiella* genus is recognized to be a relevant contributor, as well as *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Serratia*, *Xanthomonas*, *Bradyrhizobium* and many other microorganisms. Some diazotrophs are reported to improve the productivity of crops under salt-stress condition, such as: *Pseudomonas*, *Bacillus*, *Enterobacter*, *Agrobacterium*, *Streptomyces*, *Klebsiella*, and *Ochromobacter* (with a demonstrated salinity tolerance of 4-8% NaCl, and in the case of *Klebsiella* sp., a tolerance up to 20%). Soil salinity is a problem of global food security, but using salt-tolerant rhizospheric microbes as plant-growth promoters (ST-PGR) could alleviate this issue, enhancing productivity and improving soil health as well (SACHDEV et al, 2009; YABIN et al, 2012; EGAMBERDIEVA et al, 2019).

Phytohormones production is another advantage of these microorganisms, because they are essential for plant growth (EGAMBERDIEVA et al, 2019). Plant-associated microorganisms synthesize various plant hormones, such as gibberellins, cytokinins, jasmonic acid, abscisic acid, ethylene, and indole-3-acetic acid (IAA). The role of auxins in agriculture was known since 1934, when it was published a report on auxin application in root formation, as a stimulator. Among the strains which produce auxins, IAA especially (*Erwinia herbicola*, *Pantoea agglomerans*, or fungi like *Mucoromycotina*, *Basidiomycota*, and *Ascomycota*), known as a key auxine, an important plant

hormone, which stimulates cell proliferation, elongation and enhance the minerals and nutrients uptake from soil, *K. oxytoca* was well studied, and recognized as a producer, and proved its potential to be applied in biological treatments in agriculture (i.e immobilized cells on SiO₂/TiO₂ matrix) (LEOPOLD, 1963; CELLOTO et al, 2012; HOFFMAN et al, 2013; JASIM et al, 2014; PAVLOVA et al, 2017). Sapre and collaborators reported that 1-aminocyclopropane-1-carboxylic acid ACC deaminase activity characteristic to a *Klebsiella* sp. strain isolated from the rhizosphere of wheat plants is responsible for the growth promoting and increased salt-tolerance effects, but also for the IAA production under salt-stress conditions (SAPRE et al, 2018).

Some of the *Klebsiella* species are known as plant growth promoting rhizobacteria and model systems, which have been studied for their contribution to nitrogen fixation (i.e. strains isolated from sugarcane, soybean, rice, pearl millet, Kentucky bluegrass, pioneer grass *Pennisetum polyantha*), but also for solubilization of phosphate or for their antifungal activity (i.e. phytopathogenic fungi) (SACHDEV et al, 2009; YABIN et al, 2012; YU et al, 2017). Being able to convert atmospheric nitrogen to ammonia, an enzymatic conversion which is strictly limited to prokaryotes (or in some methanogenic strains belonging to *Archaea*) and catalyzed by the nitrogenase enzyme complex, *K. oxytoca* is an endophytic diazotroph organism, with agricultural importance (SCHMITZ et al, 2002; BAO et al, 2013; CHEN et al, 2013). Furthermore, this microbe could be applied to maintain the phosphorus level in extreme environment conditions or as a potent phytase producer, a soil fertilizer in crop production or as an animal feed supplement (i.e. poultry or fish industries); other microorganisms known as phytase producers are: *B. subtilis*, *Yersinia rohdei*, *Shigella* sp., *K. pneumoniae*, *K. terrigena* etc. (SATYANARAYANA et al, 2012; WALPOLA et al, 2014; EBRAHIMIAN et al, 2019).

Due to existing environmental concerns, alternatives for fossil fuels are gaining more attention, and efforts to produce bio-based bulk chemicals and biofuels are continuously made to meet the global demand; microbial processes represent an alternative to the petroleum-based compounds obtainment (CHO et al, 2015; JIANG et al, 2016; ROSALES-CALDERON et al, 2019; HAKIZIMANA et al, 2020).

Worldwide, biological hydrogen is developed as a renewable energy source, replacing the traditional technologies which rely on fossil fuels, and among them, electrolysis of H₂O and steam reformation of CH₄ are the most used. The biohydrogen production (BHP) was initially described in the 19th century, and two biological methods are widely known, photosynthetic production and dark fermentative production (BHP – which in general, involves metabolic engineering), and numerous microorganisms were studied to utilize a variety of organic substrates, especially forestry and agricultural wastes (i.e. corn stalk, rice and wheat straw, sugarcane bagasse). The most utilized substrates in microbial production of hydrogen are glucose, sucrose and starch, but other cheap

feedstocks are currently in use, such as: industrial waste (including food and municipal waste), molasses, biodiesel waste and lignocellulosic biomass. Fermentative biohydrogen can be produced by different microbes, and *Klebsiella* species are among those which are preferred due to their cultural adaptability, simple growth conditions and utilization of various carbon sources, including lignocellulosic substrates, which usually contain cellulose, hemicelluloses, and lignin (NIU et al, 2010; ABREU et al, 2012; RITTMANN et al, 2012; ELSHARNOUBY et al, 2013; REGINATTO et al, 2015; YANG and ZHANG, 2018).

In biotechnology, lignocellulosic biomass and its fermentation by microorganisms is connected with second-generation of biofuels. *K. oxytoca* is recognized as an ethanologenic bacterium (and many recombinant strains have been adapted), utilizing a variety of sugars, including cellobiose and celotriose, xylobiose, xylotriase, and arabinoside maltodextrines and cyclodextrines. Bioethanol is currently obtained from sugar or starch-containing feedstocks, as a first-generation of biofuel (1G). However, *K. oxytoca* is also considered as a robust hydrogen producer, and a good candidate for BHP, which can easily utilize lignocellulosic material (i.e. from the pulp and paper wastewaters), and especially xylose as the sole carbon source (a pentose derived from the hydrolysis of hemicelluloses). Nowadays, corn stalk is the most often used as substrate for hydrogen production in fermentation processes. Still, this microbe belongs to an ethanol/acetate type, because it produces ethanol and acetate during the xylose fermentation, which suggests a formate cleavage pathway, a formate-hydrogen lyase being involved in this mechanism. With a recognized ability to produce biohydrogen, *K. oxytoca* is utilized i.e by Nanologix, a company from Ohio, US, which patented a method for gas production, at bioreactor scale, and with a ratio of 1:1 (hydrogen: carbon dioxide), using organic material as substrate. Yet, *K. pneumoniae* is better known to produce biohydrogen from xylose (WOOD et al, 2005; DIZ et al, 2007; LIN et al, 2010; MAINTINGUER et al, 2011; REGINATTO et al, 2015; YU et al, 2017; JOSHI et al, 2019; ROSALES-CALDERON et al, 2019).

K. oxytoca is also able to utilize glycerol as an alternative substrate (instead of glucose, molasses or sucrose) for the production of other valuable chemicals, such as 2,3-butanediol (2,3-BD) and 1,3-propanediol (1,3-PDO) (BIANCO et al, 2017; HAKIZIMANA et al, 2020).

Glycerol is a byproduct generated mainly from biodiesel industry, and it can be utilized in chemicals biosynthesis using microbial fermentation, with 1,3-PDO production as the most promising option for its bioconversion. Renewable sources are generally preferred to produce bulk chemicals, due to the environmental concerns. *Klebsiella* species metabolize it through both, oxidative and reductive pathways, glycerol being dehydrogenated to dihydroxyacetone (DHA) and dihydroxyacetone-phosphate (DHAP) or to 3-hydroxy-propionaldehyde (3-HPA), which is further reduced (YANG et al,

2012; WOJTUSIK et al, 2015; JIANG et al, 2016; LEE et al, 2018; YANG and ZHANG, 2018).

1,3-PDO or trimethylene glycol production was studied more than 100 years, and it is industrially obtained through chemical processes. The global market is estimated to reach 620 million USD by 2021, with a demand of 225.9 kilo tons by 2022. Its properties make 1,3-PDO a very attractive raw material, and an alternative source of energy (JIANG et al, 2016; NETO et al, 2017; ROSALES-CALDERON et al, 2019). It can be used as a monomer for polyesters (i.e. polytrimethylen terephthalate – PTT or polybutylene terephthalate – PBT), polyethers, and polyurethanes production or it can be used as a solvent (i.e. in the cosmetic industry); therefore, it is a necessary bulk-chemical commonly found in the manufacture of resins, adhesives, inks, anti-freezers, carpets and special textile fibers (ALMEIDA et al, 2012; NETO et al, 2017; LEE et al, 2018; ROSALES-CALDERON et al, 2019). During the 1,3-PDO production, *K. oxytoca* was reported to synthesize some other compounds such as 2,3-BD, lactate, acetate, and formate, or succinate and ethanol (RODRIGUEZ et al, 2012).

2,3-butanediol or 2,3-butylene-glycol (2,3-BD) is another important chemical feedstock, and it is commonly obtained from petroleum. It is used in chemicals, food and pharmaceutical industries (i.e. manufacture of 1,3-butadiene for synthetic rubber, diacetyl for flavouring, or printing inks, perfumes, fumigants, antifreeze solutions, solvent, polymers synthesis – polyurethane, liquid fuel, resins, additives – methyl ethyl ketone, etc.) or, if it is converted to octane, it could be used as aviation fuel. Among the microorganisms which are able to produce this alcohol (genera of *Bacillus*, *Klebsiella*, *Serratia*, and *Enterobacter*) and to utilize waste products (i.e. glycerol), *Klebsiella* species are the most efficient natural 2,3-BD producers, their pathogenic factors being removed through genetic modifications. Therefore, *K. oxytoca* is a native producer of 2,3-BD, and glucose, glycerol and molasses are used as common substrates (ANVARI et al, 2009; ALMEIDA et al, 2012; CHO et al, 2015; BIALKOWSKA, 2016; YANG and ZHANG, 2018; CHA et al, 2020; HAKIZIMANA et al, 2020). Not only glycerol is an attractive substrate for 2,3-BD production, but also sugarcane molasses and lignocellulosic biomass are as well (*Jatropha* hulls, corn stover or corncobs, artichoke tubers), and *K. oxytoca* is known to utilize glycerol as a sole carbon source for 2,3-BD synthesis, with acetoin as a precursor in the metabolic pathway, as reported by Cho et al, 2013.

Industrial activities and excessive use of agricultural chemicals are causing a serious environmental pollution problem. Some of the biotechnological applications in natural environment include biosorption, biodegradation, bioaccumulation, rhizoremediation, and biotransformation. A great interest is given to bioremediation, and various microorganisms are used in technological processes, such as: bacteria (*Bacillus*, *Pseudomonas*, *Serratia*, *Klebsiella*, *Alcaligenes*, *Acinetobacter*, *Escherichia*, *Enterobacter*, *Rhizobium*, *Microbacterium*, *Arthrobacter*, *Micrococcus*,

Corynebacterium, *Flavobacterium* etc.), fungi (*Aureobasidium*, *Cunninghamella*, *Trichoderma*, *Penicillium*, *Aspergillus* and *Rhizopus*), and yeasts (*Saccharomyces*, *Candida*, *Sporobolomyces*, *Rhodotorula*), (JOUTEY et al, 2013; ARANSIOLA et al, 2017; GULATI and MEHTA, 2017; AVCIOGLU et al, 2019; KUMAR et al, 2019).

Heavy metals bioremoval involves biosorption, and bacterial strains are the most often utilized to capture metal ions. Cadmium, lead, mercury, chromium, cobalt, nickel, copper, and zinc need particular attention in their removal from the ecosystems, some of them being hazardous for environment and human health. Cadmium, mercury and arsenic are commonly removed through chemical processes (including evaporation and electrochemical treatment) or membrane technology, but these are mostly ineffective for concentrations under 100 mg/L. Therefore, innovative biotechnologies which involve the use of microbes are preferred in these situations.

Among the bacterial strains which possess tolerance for heavy metals, *K. pneumoniae* and *K. oxytoca* are often found as promising microorganisms for research developments, being known as highly tolerant in cadmium and arsenic media. Metallothioneins are involved in bacterial metal tolerance, and they have a role to bind metals, including those which are non-essential for microorganisms' development. *Klebsiella* strains were often isolated as potential microbes to use in the bioremediation processes, their metabolic properties being exploited in mercury and cadmium removal, pesticide degradation and other similar approaches (ARANSIOLA et al, 2017; MUTIAT et al, 2018).

Klebsiella species have also demonstrated high resistance to other heavy metals: i.e. Pb, Zn or Ag in wastewaters, which makes them a potential biosorbent with high efficiency for Pb uptake, especially. In addition, *K. oxytoca* was reported to remove manganese from wastewaters (through oxidation), which makes it useful for mine waters bioremediation or to reduce molybdenum (while utilizes glyphosate as a carbon source), the last being known as a pesticide used in transgenic crops, and as a major soil pollutant (MASDOR et al, 2015; MUÑOZ et al, 2015; SABULLAH et al, 2016; BARBOZA et al, 2018). Moreover, *K. oxytoca* could be used to degrade carben-dazim, a fungicide and a pollutant, which is commonly identified in water reservoirs and organochlorine pesticides in soils, like endosulfan, which can be utilized as a sulfur source in microbial development. Other *Klebsiella* sp. to degrade organophosphorous pesticides, especially chlorpyrifos, which is a very toxic insecticide (IFEDIEGWU et al, 2015; KAFILZADEH et al, 2015; ALVARADO-GUTIÉRREZ et al, 2020).

Hydrocarbons pollution is another major problem, for which bioremediation processes are successfully developed to replace traditional technologies (mechanical, dispersion, washing burying). Various microorganisms are capable to utilize hydrocarbons as carbon sources, and the metabolic pathways are widely studied. Among oil-degrading bacteria, species of *Klebsiella* (i.e. *K. oxytoca* showed a biodegradation rate of 61-98% for n-alkanes – C13-C30),

Serratia, *Enterobacter*, *Enterococcus*, *Alcaligenes*, *Acinetobacter*, *Escherichia*, *Corynebacterium*, *Staphylococcus*, and *Streptococcus* were isolated from petroleum contaminated soil (CHAMKA et al, 2011; JOUTEY et al, 2013; GULATI et al, 2017). Polycyclic aromatic hydrocarbons (PAHs) are commonly found as organic pollutants in soils (i.e. phenanthrene), and they attracted a specific interest due to their mutagenic and carcinogenic properties, and persistence in the environment. Remediation using bacterial strains is considered as an environmentally safe process. *Klebsiella* sp. demonstrated that could be applied to remediate the phenanthrene-rich contaminated sites (HASSAN et al, 2015).

Phytoremediation emerged as a sustainable alternative to remediate contaminated soils, and more specifically, rhizoremediation, which refers to microbial degradation of organic pollutants (i.e. petroleum hydrocarbons – alkanes and PAHs), as a result of biostimulation activities of the plant-growth promoters. *Klebsiella* sp. was found to enhance plant growth, while remediates the hydrocarbons pollution of the soil (JOUTEY et al, 2013; CORREA-GARCÍA et al, 2018). Among the endophytic and rhizobacterial species (*Bacillus*, *Pseudomonas*, *Hafnia*, *Enterobacter*, *Serratia*, *Xanthomonas*, etc.) known for their contribution in toxic compounds bioremediation, *Klebsiella* species have been shown to have resistance to phenol, which is another pollutant in soil, originating from plastics, pharmaceuticals or petrochemical industries, being capable to utilize it as the sole carbon source (RADULOVIĆ et al, 2019).

Cyanide pollution is another worldwide problem (14 million kg per year of cyanogens waste are produced), in which biotechnology offers microbial tools to remediate it. Various metabolic pathways (hydrolytic, oxidative, reductive and substitution/transfer reactions) are involved in cyanide removal, and metabolic engineering could be used to enhance the native capacities of microbes. Genetics studies in the bioremediation field date since 1970s, when some bacterial genes encoding catabolic enzymes were cloned and characterized. Genetically engineered microorganisms are gaining more attention, but at the present time there are some ecological concerns and regulatory obstacles regarding their field applications. Currently, wastewaters which contain cyanide are treated by chemical oxidation methods (alkaline chlorination, ozonation, wet-air oxidation), but biological treatment would be recommended due to its cost-effectiveness and environmentally-friendliness processes. Highly toxic for living organisms, cyanide content in electroplating and metal plating wastewaters, respectively, was found to be degraded by *K. pneumoniae* and *K. oxytoca* (cyanide was transformed to methane and ammonia), but also some other microorganisms are known to contribute in cyanide degradation, such as *Pseudomonas fluorescens*, *Bacillus nealsonii*, *Serratia marcescens*, *Azotobacter vinelandii* or *Rhodococcus* sp. (KAO et al, 2003; JOUTEY et al, 2013; AVCIOGLU et al, 2019).

K. oxytoca, which is known to degrade organic pollutants, has been also shown to break down the sodium

dodecyl sulphate (SDS), but in a mix of cultures, which included *Acinetobacter calcoaceticus*, *Serratia odorifera* and *Burkholderia* sp. (OTHMAN et al, 2019).

If bioremediation field is left aside, another area of research focus for biotechnological developments using *K. oxytoca* strains, is worth considering as well taking into account some results of Lam and the collaborators; they studied oviposition ecology of *Musca domestica* and hypothesized that *K. oxytoca* along with other bacteria which grow on housefly eggs lead to an inhibition of fungal growth, and due to this antagonistic relation it has been observed a significant suppression of fungi development (*Phoma* spp., *Fusarium* spp., *Rhizopus* spp.) in chicken manure. Therefore, bacterial symbionts in the context of insect-microbe systems proved some benefits, but this area is not well studied, and exploring these relations, especially the antifungal activity of *K. oxytoca* could be of future interest and biotechnological potential in agriculture (LAM et al, 2009; LAM, 2010).

Research on microbial polysaccharides has been evolved during years, genes responsible for exopolysaccharide (EPS) production being identified and manipulated to obtain recombinant strains, high quality and better yields of bioproducts (DLAMINI et al, 2007). Bacterial EPSs gained the biotechnologist's interest, mainly due to their potential futuristic applications, and to their new and/or enhanced properties, given by the genetic manipulation (SENGUPTA et al, 2018).

Physical, chemical and rheological properties of *Klebsiella*'s polysaccharides have attracted the scientists' interest since decades; these complex molecules, composed of tri- to heptasaccharide units, have found applications in food, pharmaceuticals, cosmetics, biomedical, textile and coating industries, etc. (LAPASIN and PRICI, 1995).

K. oxytoca is known to produce EPSs of environmental and pharmaceutical interest; these polysaccharides have been found to be composed of rhamnose, galactose, mannose, glucose, arabinose, and glucuronic or galacturonic acid, and most of them have metal-chelating properties and biofloculant activity (FARRES et al, 1997; LEONE et al, 2007; QIANG et al, 2013; ARCON et al, 2015; YU et al, 2016; MOGHANNEM et al, 2017; GALLO et al, 2018).

K. oxytoca was extensively studied for its capability to produce metal-nanoparticles, under anaerobically conditions, and as a result of some peculiar polysaccharides produced in the presence of heavy-metals (lead, zinc, cadmium, arsenium, mercury), considered as a mechanism of cell protection, polymers which precipitate with metals embedded in their structure. Applications such as nanofertilizers, innovative chemical catalysts, antimicrobial and anticancer agents were found to be suitable. Moreover, some of these biogenerated nanoparticles, like Fe(III)-EPS were tested as nutraceuticals, with action on the *Tuber borchii* fungus, or in the prevention of arthritis and anemia, or bimetallic Fe-Pd EPS complexes, utilized in the hydrodechlorination processes (BALDI et al, 2009; ARCON et al, 2015; GALLO et al, 2018). Other studies are strengthening the biotechnological potential of *K. oxytoca*

as a biogenic nanoparticles producer (ferrihydrite or silver nanoparticles), especially in the field of biomedicine (cancer therapy, drug delivery systems, magnetic recording media, transducers etc.), (BALAŞOIU *et al*, 2009; ISHCENKO *et al*, 2011; ANGHEL *et al*, 2012; BALDI *et al*, 2016; CHILOM *et al*, 2017).

Just like *Klebsiella* species are found to possess many distinct and interesting properties (i.e melanin production, as an aromatic polymer with applications in pharmaceuticals, cosmetics and agriculture), *K. oxytoca* shown an ability to produce mannanase, and therefore efficiently hydrolyzing copra meal, a by-product from the coconut industry, which is mainly composed of mannose polysaccharides. In fact, other microorganisms are commercially utilized to produce enzymatic preparations, such as *Aspergillus niger*, *Bacillus* sp. and *Trichoderma* sp. Yet, Chantorn and collaborators demonstrated that copra hydrolysates obtained by using a strain of *K. oxytoca* were effective in improving the growth of *Lactobacillus reuteri*, which signifies that there is a potential for prebiotic use, at the same time preventing the growth of pathogenic bacteria (i.e. *Salmonella Enteridis*), (SHRISHAILNATH *et al*, 2010; CHANTORN *et al*, 2013; THONGSOOK *et al*, 2018).

Klebsiella pneumoniae

Clinical significance

K. pneumoniae is considered as one of the most resilient opportunistic pathogens, recently mentioned by the World Health Organization as a microorganism for which there is an urgency to develop new antibiotics (BENGOECHEA and SA PESSOA, 2019; EFFAH *et al*, 2020). Part of gastrointestinal flora, these strains can cause severe infections, and at the same time demonstrate an increased resistance to the first-line antibiotics (i.e. cephalosporins, fluoroquinolones and aminoglycosides; and recently reported resistance to colistin and tigecycline). Besides, *Klebsiella* species are widely recognized as a major source of carbapenem resistance genes transfer among other species (RIMOLDI *et al*, 2017; EFFAH *et al*, 2020). While carbapenem resistant *K. pneumoniae* (CRKP) represents a challenge in treating nosocomial infections, additional β -lactamase encoding genes are further enhancing the strains pathogenicity, making the problem more serious, increasing the resistance to penicillins, aztreonam, first-, second- and third-generation cephalosporins (except clavulanic acid), (DE JESUS *et al*, 2015; RIMOLDI *et al*, 2017). The major factors involved in the *K. pneumoniae* virulence are fimbrial adhesions (mannose-sensitizing type 1, and type 3), non-fimbrial adhesion (CF29K factor), siderophores, and surface saccharides (capsule – which describe capsular serotypes – to date are recognized 79 serotypes, and LPS – describing O-antigens). Other virulence factors were recently identified, such as systems for iron transportation, genes related to allantoin metabolism or porins, but to date there are no sufficient studies to characterize them accordingly (DE JESUS *et al*, 2015; HSU *et al*, 2016; PACZOSA and MECSAS, 2016; CAI *et al*, 2019).

These facultative anaerobes have 0.3 and 1.8 μm in size, easily ferment lactose, and form mucoid colonies due to their capsules, which are important in the defense mechanism of *Klebsiella* strains, offering resistance to lysis or phagocytosis (higher resistance in K1 and K2 serotypes), or against environmental stress (DE JESUS *et al*, 2015; RIMOLDI *et al*, 2017).

K. pneumoniae can be classified into two subtypes: classical (cKp) and non-classical (ncKp), and hypervirulent (hvKp) or hypermucoviscous (HMKP), among which the hvKp strains are causing diseases, such as pyogenic liver abscesses, pneumonia, meningitis and endophthalmitis in healthy and young people, not only in the immunocompromised individuals (BROBERG *et al*, 2014; DE JESUS *et al*, 2015; EFFAH *et al*, 2020).

Despite of its clinical significance, *K. pneumoniae*, when it is grown on different substrates, it is able to produce useful chemicals; for example, in the case of using glucose and citrate as carbon sources in fermentation processes, the microorganism produces gas and acid (i.e. 2,3-BD, acetoin) or, taking into account its nutritive necessities (i.e. iron), *Klebsiella* will act as when it competes with a host, and it will produce iron-binding molecules (DE JESUS *et al*, 2015). Therefore, from a biotechnological point of view, *K. pneumoniae* is considered as a microbial factory.

Biotechnological importance

Klebsiella genus is mentioned as one of the most proficient in plant-growth promoting together with *Pseudomonas*, *Serratia*, *Erwinia*, *Enterobacter*, *Rhizobium*, *Agrobacterium*, *Phyllobacterium*, *Variovorax* etc., having also a biostimulation potential, due to phosphate solubilization and auxin production. Strains of *Klebsiella* were isolated from i.e. Korean rice cultivars, and used for their siderophore production translated to a biocontrol activity (i.e. affinity for iron chelation or binding, which suppressed the plant-pathogens through competition for Fe^{3+}) (SANGEETHA *et al*, 2020). *K. pneumoniae* does not represent an exception, and some of these strains, free-living diazotrophs and endophytes, have been reported, along with *Azotobacter vinelandii*, and *Enterobacter cloacae* as nitrogen-fixers, but also as advantageous IAA producers, along with other bacteria, such as *Bacillus cereus*, *Rhizobium japonicum*, *Bradyrhizobium japonicum*, *Azospirillum* spp., *Acetobacter diazotrophicus*, *Azotobacter chroococcum*, *A. beijerinckii*, *A. vinelandii*, *Pseudomonas fluorescens*, and fungi like *Aspergillus*, *Penicillium*, and *Trichoderma*. Auxin producing strains of *K. pneumoniae* were more often reported as isolates from the rhizosphere of *Triticum aestivum*, or *Piper nigrum*; yet, the use as potential biofertilizers should be further investigated (SCHMITZ *et al*, 2002; INIGUEZ *et al*, 2004; SACHDEV *et al*, 2009; JASIM *et al*, 2014; KUMAR *et al*, 2017; DHUNGANA *et al*, 2019).

The use of environment-friendly technologies or agents, and bioactive compounds have become a very attractive approach in medicine, industry and agriculture (SANGEETHA *et al*, 2020).

Microbial products have attracted attention during past years, because they could be obtained from green technologies and, therefore the transition towards a low-carbon economy is encouraged (ROSALES-CALDERON et al, 2019). Moreover, the advances in genomics and genetic manipulation of microbial strains represent a renewable alternative which has become commercially competitive with fuels and chemicals production. Many of the biotechnological processes involving engineered strains, were developed and implemented to meet the need for innovative solutions to reduce environmental pollutants (ALMEIDA et al, 2012).

K. pneumoniae, is considered as a microbial cell factory, which can be used for biotechnological purposes, despite of being a pathogenic microorganism. These strains are able to synthesize valuable feedstock chemicals (i.e. diols), among other metabolites (i.e. organic acids), but they are utilized for the 1,3-propanediol and 2,3-butanediol biosynthesis, most often, due to their natural capacity to produce B12 coenzyme (ARASU et al, 2011; KUMAR et al, 2018; MITREA et al, 2019).

Some of these alternatives to petroleum-based chemicals are 1,3-propanediol (1,3-PDO), 2,3-butanediol (2,3-BD), and 3-hydroxypropionic acid (3-HP).

2,3-butylene glycol production using microorganisms dates since 1906, when a *K. pneumoniae* strain was utilized in the biosynthesis process, and since then, this microbe is known as among the most efficient in accumulating the compound (along with *Paenibacillus polymyxa*), which is currently utilized as solvent, liquid fuel, precursor of many synthetic polymers and resins (ANVARI et al, 2009; CHAN, 2016; ROSALES-CALDERON et al, 2019). The use of raw materials to produce chemicals is a research focus, and *K. pneumoniae* is known to metabolize xylose, a sugar which is also utilized in biomass conversion for high-value product obtainment. Thus, this bacterium has a specific tolerance to this pentose, which is considered unsatisfactory for 2,3-BD synthesis. Yet, transcriptomics approaches and gene deletion efficiently contribute to the achievement of a higher yield of 2,3-BD, by improving the xylose tolerance at gene level and minimizing the synthesis of undesired biosynthesis byproducts, at the same time leading to a low-cost biological process (GUO et al, 2018). Known as a native hyperproducer of 2,3-BD and of coenzyme B12 (cyanocobalamin), the microorganism can be metabolically engineered for 2-butanone production (i.e. using a diol dehydratase from *Lactobacillus brevis*, while deleting the gene responsible for lactate dehydrogenase, and using glucose as substrate), a compound which is usually obtained through a petrochemical route (CHEN et al, 2015; NETO et al, 2017). 2-butanone synthesis is just another example of chemical (solvent), which can be obtained improving the microbial production in *K. pneumoniae* strains (MAR et al, 2020).

1,3-PDO, other valuable chemical (industrial feedstock) used in cosmetics, pharmaceuticals formulations, and polymers production (including polyesters), can be also obtained from glycerol by fermentation, and with *K. pneumoniae* as producer (most often, engineered strains,

without pathogenicity), (ARASU et al, 2011; JIMÉNEZ-SÁNCHEZ and PHILP, 2016; MORCELLI et al, 2018). This chemical is the main product of glycerol fermentation, whereas lactate, acetate, succinate could be obtained, but also all of these can be controlled by gene inactivation (i.e. genes encoding lactate dehydrogenase, formate acetyltransferase, and alpha-acetone lactate decarboxylase and/or glycerol kinase/dehydrogenase) and/or by over-expression (ALMEIDA et al, 2012; LEE et al, 2018).

Metabolically engineered *K. pneumoniae* strains can be employed to obtain poly(3-hydroxypropionate) (P3HP) or 3-hydroxypropanoic acid (3-HP), a structural isomer of lactic acid, using glycerol or glucose as substrates. Due to its native capacity to synthesize coenzyme B12, *K. pneumoniae* is considered a better host than *Escherichia coli*. Yet, the majority of the wild-type strains of this species are not able to biosynthesize 3-HP, or those which have the capability, produce very low yields, being involved a low activity of aldehyde dehydrogenase. Even though, *K. pneumoniae* and *E. coli* are amongst the most studied engineered microorganisms for 3-HP synthesis; some other bacteria have been investigated (i.e. *Pseudomonas denitrificans*, *Shimwellia blattae*, *Corynebacterium glutamicum*, *Bacillus subtilis*), after some different genes (encoding glycerol dehydratase, glycerol dehydratase reactivase, diol dehydratase and aldehyde dehydrogenase) were introduced to enable efficient production (ARASU et al, 2011; FENG et al, 2015; LI et al, 2016; JIANG et al, 2018; KUMAR et al, 2018; JERS et al, 2019). 3-HP can be further used to obtain 1,3-PDO, acrylic acid, acrylamide, propiolactone or as a precursor for bioplastics (KIM et al, 2017; JERS et al, 2019).

Klebsiella species produce lactic acid during glycerol fermentation, and D-lactate dehydrogenases (ldhA) are the most influencing enzymes in the biosynthesis process, when *K. pneumoniae* is utilized, but with the specific mention that it is an undesired byproduct in the 2,3-BD, 1,3-PDO or 3-HP production; in these cases, CRISPR interface system is preferred as a modern engineering tool to attenuate the lactic acid synthesis. However, this carboxylic acid has found many applications in food, chemical, cosmetics and pharmaceutical industries (i.e. bio-preservative in food-related products and in beverages, antimicrobial agent in food, pH adjusting agent, chemical ingredient for bio-solvents like lactate esters, which are used in paints and coating industry, or it can be used in acrylic acid, 1,2-propanediol and polyurethane manufacturing), (ALMEIDA et al, 2017; KUMAR et al, 2018; WANG et al, 2018; BARDHAN et al, 2019; ROSALES-CALDERON et al, 2019). D-lactate is also produced by *K. pneumoniae*, but with glucose, xylose or arabinose as substrates; it has various applications in cosmetics, spices, coatings, adhesive industries, polylactate production or in herbicide manufacturing (FENG et al, 2017).

Klebsiella species are known to produce both, acidic capsular polysaccharides and exopolysaccharides, as a protective mechanism to environmental stress, and increased yields are observed when the carbon/nitrogen ratio is higher (under nitrogen limitation and carbon excess

conditions). *K. pneumoniae* strains synthesize EPSs (i.e. fucogel) composed mainly of 3-6 sugar units (i.e. galactose, glucose, mannose, galacturonic and glucuronic acids, fucose, rhamnose, glucosamine), and with various properties (pseudoplastic, bioflocculant, biosurfactant, anti-biofilm activity against Gram-negative and Gram-positive microorganisms) and applications (cosmetic, medical and chemical fields), (NAKATA et al, 1999; RAMIREZ-CASTILLO et al, 2004; SABRA et al, 2008; FREITAS et al, 2011; HO et al, 2011; GONCALVES et al, 2014; BEHURIA et al, 2018). Simultaneously, *K. pneumoniae* EPSs producer strains are investigated in green nanotechnology field (synthesis of silver, gold or selenium nanoparticles), which attracts the scientific interest due to some important applications in biomedicine and biotechnology of green nanoparticles (i.e. antimicrobial and sporocidal agents, cancer diagnosis and photo thermal therapy, computed tomography and magnetic resonance imaging), which have low toxicity and high biologic activity (FESHARAKI et al, 2010; MALARKODI et al, 2013; MAITY et al, 2016).

Due to molecular advancements during the recent years, the interest for pullulanase (pullulan α -glucanohydrolase, EC 3.2.1.41) biosynthesis, an enzyme which is capable to hydrolyze α -1,6-glycosidic bonds, started to regain the scientific attention, due to its potential industrial applications, such as starch processing, baking industry or cyclodextrin manufacture. In scientific history, reports on pullulanase production dates since 1961, originating from a *K. pneumoniae* strain, and after, in 1986, the extracellular enzyme was first reported as a lipoprotein synthesized by a Gram-negative bacterium, due to its covalently attached fatty acyls (PUGSLEY et al, 1986; MALAKAR et al, 2010; HII et al, 2012; CHANG et al, 2016).

Environmental pollution is a major problem at global scale. Microbes and biotechnology play an important role in bioremediation and biodegradation processes; among common practices, biostimulation and bioaugmentation are considered eco-friendly and cost-effective (KANAFER et al, 2017). Compared to physico-chemical treatments to remove pollutants from the environment, bioremediation provide various biotechnological alternatives, with limited secondary toxic compounds and less energy consuming, which is why it receives great attention (YOU et al, 2018). Bioremediation is known to be an enzymatic process, when Gram-negative bacteria are involved (NENENG et al, 2018). *Klebsiella* species were reported to have the capacity to degrade different chemical compounds, due to their catabolic activity, and many research studies focused on *K. pneumoniae*' genome analysis (i.e. identification of genes involved in biodegradation processes), (RAJKUMARI et al, 2018).

Various microorganisms are studied for their capabilities to degrade aromatic compounds, which are widely distributed in nature and causing water and soil pollution (*Pseudomonas*, *Bacillus*, *Rhodococcus*, *Pantoea*, *Enterobacter*, *Serratia*, *Flavimonas*, *Klebsiella*, *Streptococcus*, *Micrococcus*, *Pantoea*, *Burkholderia*, *Proteus*, *Arthrobacter*, *Serratia*, *Sphingomonas*, *Gordonia*,

Brevibacterium, *Acinetobacter*, *Alcaligenes*, *Ralstonia*, and *Microbacterium* species), but *Klebsiella* strains were especially investigated for polycyclic aromatic hydrocarbons (PAH) degradation (i.e. pyrene), alkanes from petroleum (mostly hydrocarbons from C₁₀ to C₂₀), kerosene, 3,5-dibromo-4-hydrobenzotrifluoride, nitroaromatic compounds, 1,2-dichloroethane, mono-chlorinated dibenzofuran, dibenzo-p-dioxin, organohalogen and phenolic compounds (i.e. tannic acid), insecticides (endosulfan, an organochlorine pesticide belonging to the polycyclic chlorinated hydrocarbons), herbicides (*s*-triazines, which are endocrine disrupters, chlorsulfuron-ethyl, chlorpyrifos, metsulfuron-methyl, tribenuron-methyl, rimsulfuron, ethametsulfuron, and nicosulfuron), polychlorobiphenyls or even hexahydro-1,3,5-trinitro-1,3,5-triazine – RDX degradation (a toxic explosive, which pollutes terrestrial and aquatic ecosystems), (ZHAO et al, 2002; SEEGER et al, 2010; ALI et al, 2012; PADHI et al, 2013; NWINYI and VICTORY, 2014; PING et al, 2014; KAFILZADEH et al, 2015; NWAGUMA et al, 2016; OAIKHENA et al, 2016; OZYUREK and BILKAY, 2018; PRINCE, 2016; TAHMOURESPOUR et al, 2016; ANGGORO and RATNANINGSIH, 2017; KHANAFER et al, 2017; RAJKUMARIE et al, 2018; YOU et al, 2018; ZHANG et al, 2019). *K. pneumoniae* is studied also for the elimination of heavy metals or metalloids from polluted sites, such as cadmium and mercury (extremely toxic elements and with long elimination half-life), or arsenic, due to its versatility and resistance in different micro-environments, with consideration to its potential pathogenicity (ABBAS et al, 2014; KHAN et al, 2015; BEHURIA et al, 2018; NENENG and GUNAWAN, 2018; KUMAR et al, 2019; MOHAN et al, 2019).

Conclusions

Microbial biotechnological applications have gained great interest, due to the economic and environmental world challenges, and microbial products development promotes innovation as a tool for future sustainability, advancing the scientific results, mainly in the fields of genomics, proteomics and synthetic biology to face the actual problems related to food security and safety, human and environmental health, plant and animal protection (including nature conservation), and revolutionizing the pharmaceutical industry and medicine. However, many of these applications are associated with opportunistic human pathogens (including *Klebsiella* genus), microorganisms also frequently found in natural ecosystems (i.e. endophytic communities), and in these cases, advances in molecular biology are considered as effective approaches to ensure a proper management. Making use of natural resources in a sustainable manner, and also of genetic engineering techniques, new value-added and high-quality microbial products could be largely exploited (i.e. bulk chemicals as precursors, biofuels, biological products for the agribusiness sector – biofertilizers, biostimulants and biopesticides, pharmaceuticals, biomaterials etc.). Therefore, strains with biotechnological potential could be used as microbial factories to produce various compounds to

facilitate the transition towards a sustainable bioeconomy, and to promote the achievement of sustainable development goals (especially SDG no. 2 – “zero hunger”, 3 – “ensure healthy lives and promote well-being for all at all ages”, 9 – “build resilient infrastructure, promote sustainable industrialization and foster innovation” and 12 – “ensure sustainable consumption and production patterns”), due to the cost-effective technologies, and their reduced CO₂ emissions and environmental footprints, when compared with petrochemical-based products. The efforts of modern biotechnology need to be continued, to ensure both the necessities of a sustainable industrial growth and public acceptance, at the same time playing a crucial role for innovation progress and applicability (MOSTTAFIZ et al, 2012; LOKKO et al, 2018; www.un.org).

Petroleum-based hydrocarbons are found among the most significant compounds in environmental polluted sites. Modern biotechnology develops bioremediation strategies which are needed to counteract the adverse effects, because conventional technologies are expensive and they produce secondary toxic chemicals. *K. pneumoniae* strains are often isolated from polluted environments, and especially from hydrocarbon-polluted soils, and these microbes are known for their petroleum degrading properties and their capacity to produce biosurfactants, with proven emulsification activity (i.e. against kerosene, petroleum, toluene, xylene, gasoline, and diesel), which makes them attractive and advantageous to use in biodegradation processes (NWAGUMA et al, 2016; OZYUREK et al, 2018).

Wastewaters from pulp and paper industry contain pollutants, such as polycyclic aromatic hydrocarbons (PAHs), dioxins, furans and chlorophenols, which are toxic for the aquatic ecosystems. Lignin, its derivatives and tannins are responsible for the environmental loading for pollutants, but biological methods which use microorganisms to degrade these compounds (including *K. pneumoniae* which synthesize ligninolytic enzymes – laccases, EC 1.10.3.2 and manganese peroxidase, EC 1.11.1.113) gained much interest due to their eco-friendly characteristics. *K. pneumoniae* is capable to utilize phenolic compounds, which makes it suitable for decolorization and detoxification processes of wastewaters effluents from pulp and paper industry, but it can be also used for other industrial applications, such as fruit juice clarification, bioethanol production or medical diagnostic purposes (NISHA G. et al, NISHA G. et al, 2018).

When compared to other bacterial strains, *K. pneumoniae* is known as a performant producer of 2,3-BD during the glucose fermentation or as 1,3-PDO producer during the glycerol fermentation, but also for other non-natural compounds (i.e. 2-butanone, 2-butanol), when it is metabolically engineered (CHO et al, 2012; CHEN et al, 2015; MITREA et al, 2019).

K. oxytoca is often found in endophytic-associations, and it is considered a plant growth-promoting rhizobacterium, due to its capacity to synthesize auxin (IAA) and siderophore, which contributes to the phosphorus uptake by the plants and nitrogen fixation, being a good candidate as a bio-inoculant, even in environmental stress conditions;

moreover, it has a potential in biocontrol activities, due to its antagonistic effect against fungi (i.e. *Fusarium oxysporum*), (MARTÍNEZ-RODRÍGUEZ and MORA-AMUTIO, 2014; WU et al, 2014). Moreover, these strains are capable to synthesize platform chemicals, among which 1,3-PDO and 2,3-BD are the well-studied, *K. oxytoca* being considered one of the most competitive hosts, due to its higher productivity (WOJTUSIK et al, 2015; CHA et al, 2020). Metal-binding capacity along with EPS production is another biotechnological advantage for this species, which is exploited in view of nanobiotechnology applications (i.e ferrihydrite and silver nanoparticles production), (BALDI et al, 2016; CHILOM et al, 2017).

In conclusion, despite of being a large group of Gram-negative medically important microorganisms, which belongs to the *Enterobacteriaceae* family, *Klebsiella* species are considered normal commensals of the human gut (RIMOLDI et al, 2017), regular and/or opportunistic pathogens, with high-resistance to antibiotics, but also common microbes in natural environments, which have proven their biotechnological potential in various industrial fields, and which are continuously studied for their genome elucidation, metabolic pathways and gene identification, and their natural and possible roles in ecosystems functioning, genetic engineering developments and bioproducts obtainment.

Conflict of Interest

The authors have no conflict of interest to declare.

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