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In The Name Of Allah,
The Most Gracious,
The Most Merciful

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MANEL and NOUR-EL-HOUDA

Abbreviation	*Expansion*
C	Cytosine
CFU	Colony Forming Unit
CAT	Catalase
CPS	Capsular Polysaccharide
DNA	Deoxyribonucleic Acid
DPPH	1, 1-Diphenyl-2-picryl hydrazyl
EPS	Exopolysaccharide
G	Guanine
GIT	Gastro-intestinal-tract
GSH-Px	glutathione peroxidase
GST	Glutathione-s-transferase,
GR	Glutathione reductase,
GRAS	Generally Recognized as Safe
HePS	Heteropolysaccharide
HoPS	Homoolysaccharide
LAB	Lactic Acid Bacteria
MRS	Man-Rogosa Sharp
PBS	Phosphate Buffer Saline
ROS	Reactive Oxygen Specie
rpm	Revolutions per minute
SOD	Superoxide dismutase
TCA	Trichloroacetic Acid

Introduction

Oxidation plays a fundamental role in many living organisms within the production of energy necessary for all biological processes. However, this fact is the origin of the disruption of the delicate balance between generation of reactive oxygen species (ROS) and antioxidant scavenging systems, known as oxidative stress which induces serious biological molecules damages **(Lin and Yen, 1999; Heo et al., 2005)**. The term reactive oxygen species is used by investigators to include both oxygen-centered radicals and non-radical derivatives of oxygen that are continuously produced *in vivo* (**Andersen, 2004**). A large variety of reactive oxygen species can be formed in the human body and in food systems **(Lin et and Yen, 1999)**.

According to many researchers, oxidative damage has been confirmed to have a significant pathological role in different kind of human diseases (**Yoshikawa and Naito, 2002; Heo et al., 2005; Lobo, 2010; Shinde et al., 2012**). Even though both humans and other organisms possess antioxidant defense and repair systems which are evolved to protect them against oxidative damage, these systems are not efficient enough to totally prevent the damage. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage **(Lin and Yen, 1999; Al-Dalaen and Al-Qtaitat, 2014)**.

In order to reduce induced oxidative damage, both synthetic and natural antioxidants are used. However, there are doubts about the safety and long-term effects on health of synthetic antioxidants such as butylated hydroxytoluene (BHT), which are suspected to have some toxic effects **(Heo et al., 2005; Li et al., 2014)**. Therefore, it is desirable to develop natural nontoxic antioxidants with possible antioxidant and/or radical scavenger properties as alternatives to synthetic ones in pharmaceutical and food industries **(Heo et al., 2005)**. By far it has been established that some microbial exopolysaccharides (EPS) possessed antioxidant activity **(Li et al., 2014)**.

Lactic acid bacteria (LAB) are useful microorganisms in dairy technology and they also contribute to the benefic effects on the health and their utility is reinforced by their demonstrated probiotic properties **(Liu et al., 2011)**. In addition, they exhibit antioxidant activity in all major way; they may reinforce the inherent cellular antioxidant defense by secreting enzymes like superoxide dismutase (SOD). They also release and promote the production of the major non-enzymatic antioxidant and free radical scavengers. Moreover, they promote the production of certain antioxidant biomolecules, such as the exopolysaccharides (**Afify et al., 2012**). In the same line, during the last few decades exopolysaccharides produced by LAB are the subject of an

increasing number of studies. Due to their generally recognized as safe, EPS from LAB are potentially useful as safe additives to improve texture and viscosity of natural fermented products, moreover it has been suggested that some EPS produced by LAB may confer health benefit to the consumer **(Li et al., 2014)**.

Recent studies focused on the evaluation of the antioxidant activities of LAB have agreed upon the potent antioxidant and free radical scavenging effect of wide range of exopolysaccharides **(Ruas-Madiedo et al., 2002; Xu et al., 2011; Li et al., 2014).** In this context the main objectives of the present study are:

i. Screening of the ability of some lactic acid bacterial strains for EPS production.

ii. Extraction and quantification of EPSs.

iii. Evaluation of antioxidant activity of EPSs.

iv. Evaluation of some probiotic properties of one of the selected isolates.

Part I.

Literature review

I.1. Lactic Acid Bacteria

I.1.1. Introduction

Lactic acid bacteria (LAB) are a relatively heterogeneous group of bacteria which share several common characteristics, e.g. in metabolism and physiology, including: low G+C content; high acid tolerance and production of lactic acid as their major end product **(Klein et al., 1998; Vasiljevic and Shah, 2008; Khalisanni, 2011)**. They are Gram positive, non-sporulating, cocci or bacilli, catalase and oxidase negative, devoid of cytochromes and preferring anaerobic conditions but are aerotolerant. Generally LAB have complex nutritional requirements especially for amino acids and vitamins **(Klein et al., 1998; Khalisanni, 2011)**.

The most important genera of LAB are *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Weissella*, *Carnobacterium*, *Tetragenococcus*, and *Bifidobacterium* **(Klein et al., 1998; Vasiljevic and Shah, 2008)**. Under a biochemical perspective, LAB include both homofermenters; producing mainly lactic acid such as *Lactococcus* and *Streptococcus*, and heterofermenters which, apart from lactic acid, yield a large variety of fermentation products (acetic acid, ethanol, carbon dioxide, formic acid) like *Leuconostoc* and *Weissella* **(Mayo et al., 2010; Khalisanni, 2011)**.

Except for a few species, LAB members are nonpathogenic organisms with a reputed Generally Recognized as Safe (GRAS) status **(Mayo et al., 2010)**, they are generally associated with rich habitats in nutriments including various food products (milk, meat, vegetables), furthermore other species can be found in the human and animal gastro intestinal tract (GIT) **(Klein et al., 1998; Khalisanni, 2011)**.

I.1.2. Lactic acid bacteria as probiotics

The term 'Probiotics' is defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" **(Vasiljevic and Shah,2008)**. The most widely used probiotic microorganisms belong to the groupe of lactic acid bacteria **(Yoon et al., 2006)**, though some other strains such as *Saccharomyces boulardii* has been studied extensively **(Sindhu and Khetarpaul, 2001)**.

LAB are believed to perform a leading part in the health of the host. Several genera and species such as *Lactobacillus* and *Bifidobacteria spp* showed various nutritional and therapeutic benefits and many probiotic effects have been well documented **(Song et al., 2015)**. An effective probiotic

LAB strain must require several basic properties. Survival and adhesion within the gastro intestinal tract, production of antimicrobial substances, antagonism against pathogenic bacteria and safety in human use are considered as the most important selection criteria of a good probiotic strain **(Vasiljevic and Shah, 2008; Yang et al., 2015)**. Various studies on probiotic strains of LAB were reported to involve inhibition of pathogenic microorganisms, protection against gastro intestinal diseases, anti-mutagenic and anti-carcinogenic activities, and enhancement of the host immune response **(Vasiljevic and Shah, 2008; Song et al., 2015)**.

I.2. Oxidative stress

The paradox of aerobic life, or as it is known the 'Oxygen Paradox' is that, biological tissues require oxygen to satisfy their energetic demands, however, about 5% or more of the inhaled O_2 is converted to reactive oxygen species that may lead to damaging effects on cells **(Davies, 1995; Bandyopadhyay et al., 1999)**. Thus, cells under aerobic conditions are always menaced with the insult of ROS which are efficiently taken care of by the highly powerfull antioxidant systems of the cell. Once the balance betweeen ROS production and antioxidant defenses is lost, oxidative stress occurs which through a set of events perturbates the cellular functions leading to various pathological conditions **(Hemnani and Parihar, 1998; Bandyopadhyay et al., 1999).**

I.2.1. Definition of oxidative stress

"Oxidative stress" defined as the shift in the oxidant/antioxidant balance in favour of oxidants, refers to the phenomenon that reflects an imbalance between the production of reactive oxygen species (ROS) and so-called oxidants (Figure 1), and their elimination by protective mechanisms named as antioxidative systems **(Al-Dalaen and Al-Qtaitat, 2014)**. This cell state characterized by excessive production of reactive oxygen species (ROS) and/or a reduction in antioxidant defenses occurs as a result of an increase in oxidative metabolism which is the consequence of several factors **(Singh, 2004; Franco et al., 2008)**. On the one hand, oxidative stress is well known to play a pivotal role in many physiological functions. On the other hand, it has been considered as a serious state because of the oxygen free radicals that are capable of damaging almost all types of biomolecules **(Yoshikawa and Naito, 2002)**.

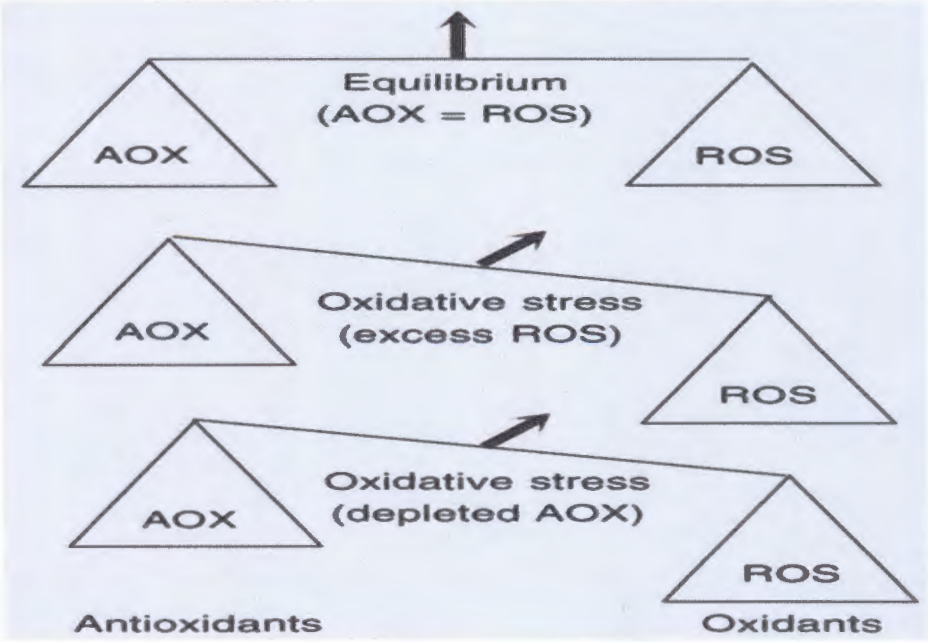

Figure 1. *Balance between oxidant and antioxidant defenses oxidative stress* **(Kunwar and Priyadarsini, 2011).**

I.2.2. Molecular basis of oxidative stress

Reactive oxygen species (ROS) is a collective term used for a group of oxidants, which are either free radicals (containing highly reactive unpaired electrons) such as superoxide ($O_2^{\cdot}$), nitric oxide ($NO^{\cdot}$) and hydroxyl radical ($OH^{\cdot}$) or molecular species capable of generating free radicals such as hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) **(Andersen, 2004; Kunwar and Priyadarsini, 2011).**

A free radical is defined as a molecule with one or more unpaired electrons in its outer orbital, which makes this species very unstable and tending to react with other molecules so as to pair this electron and thereby generate more stable species **(Adly, 2010).** These ROS are usually generated as byproduct of normal aerobic metabolism. Once they appear at low to moderate concentrations, they function in physiological cell processes. However, at high concentrations, they produce adverse modifications to different cell components **(Al-Dalaen and Al-Qtaitat, 2014).** Eventually, along his lifetime, the human body is gradually exposed to potentially harmful oxidative stresses. These may emerge from endogenous as well as exogenous sources **(Adly, 2010).**

ROS are produced from the activation of molecular oxygen, intracellularly through multiple mechanisms, as a result of normal cellular metabolism. Their major sources are mitochondria, peroxisomes, endoplasmic reticulum, inflammation processes, phagocytosis and complex in

cell membranes (Figure 2) **(Adly, 2010; Al-Dalaen and Al-Qtaitat, 2014)**. Furthermore, an excessive level of free radicals can also be generated from other several exogenous sources including exposure to pollutants, strenuous physical activity, alcohol exposure, cigarette smoke, trauma, medications, infections, toxins, radiation, and poor diet **(Singh, 2004; Adly, 2010).**

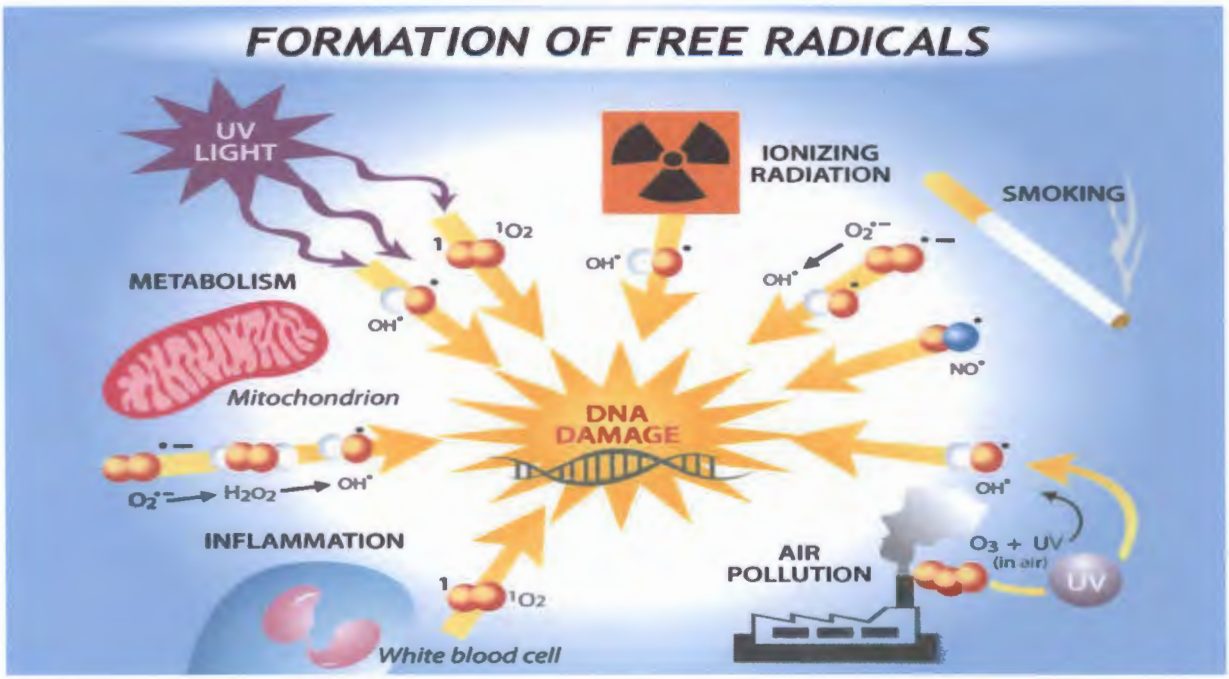

Figure 2. *Common source of oxidative stress* **(Adly, 2010).**

I.2.3. Biological damage of reactive oxygen species

The close association between oxidative stress and human diseases has become well documented where oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, hypertension, diabetes, ischemic diseases, the process of aging, autoimmune disorders, cardiovascular and neurodegenerative diseases **(Yoshikawa and Naito, 2002; Lobo, 2010; Shinde et al., 2012)**. Major findings research have pointed that these various diseases are resulted from that, ROS attack almost all major biomolecular groups regarding: proteins, lipids, nucleic acids and carbohydrates because of their special affinity to them. Thus, this fact leads to promote several changes within their structure and functions **(Lobo, 2010)**, which are discussed below :

- **Protein damage**

At the cellular level, when protein are exposed to reactive oxygen species, modifications of amino acid side chains occur, especially with those containing SH groups (methionine, cystein) and, consequently, the protein structure is altered. These modifications lead to functional changes that

disturb cellular metabolism and can lead to their targeted degradation **(Farr and Kogoma, 1991; Cabiscol et al., 2000; Kohen and Nyska, 2002)**. Proteins can be oxidatively modified in three ways: oxidative modification of specific amino acid, free radical mediated peptide cleavage and formation of protein cross-linkage. Oxidatively damaged protein products may contain very reactive groups that may contribute to damage to membrane and many cellular functions. Peroxyl radical is usually considered to be the essential free radical that causes proteins oxidation **(Cabiscol et al., 2000; Lobo, 2010)**.

- **Lipid peroxidation**

It is well-known that all cellular membranes are vulnerable to lipid peroxidation due to their high concentrations of lipid especially unsaturated fatty acids **(Kohen and Nyska, 2002)**. Lipid peroxidation can cause different arrangement in the membrane lipid bilayer that induce an inactivation of the membrane bound receptors and enzymes and lead to an increase in tissue permeability **(Al-Dalaen and Al-Qtaitat, 2014)**. Actually, this phenomenon generates a wide number of compounds including, alkanes, malanoaldehyde and isoprotanes **(Lobo, 2010)**.

- **DNA Damage**

Nucleic acids, particularly DNA are considered as a major target for oxidative stress **(Al-Dalaen and Al-Qtaitat, 2014)**. ROS can interact with DNA and cause several types of damage such as: degradation of DNA bases, single- or/and double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, DNA-protein cross-linkage and damage to the DNA repair system **(Farr and Kogoma, 1991; Kohen and Nyska, 2002; Kunwar and Priyadarsini, 2011; Rahal et al., 2014)**. Actually, DNA damages are not related to all ROS but, most are attributable to hydroxyl radicals **(Kohen and Nyska, 2002)**. In fact, the degradation of the bases will produce numerous products including 8-OH-guanine, hydroxymethyl urea, thymine glycol **(Adly, 2010)**.

I.3. Antioxidant defense systems

To counteract the harmful effects of reactive oxygen species ROS produced in the course of normal physiological conditions, humans have evolved several complex antioxidant strategies **(Ozbek, 2012; Rahal et al., 2014)**. An antioxidant can be defined as: "any substance that, when present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents the oxidation of that substrate" **(Halliwell, 1995)**. Antioxidants can be either endogenous or obtained exogenously via daily diet or through dietary supplements. An ideal antioxidant should

be readily absorbed, quench free radicals, and chelate redox metals. It should also work in both aqueous and/or membrane domains (**Valko et al., 2006; Al-Dalaen and Al-Qtaitat, 2014**).

I.3.1. Classification of antioxidants

The human antioxidant system can be divided into two major groups, enzymatic antioxidants and non-enzymatic antioxidants (Table 1). Enzymatic antioxidants can be further devided into primary and secondary enzymatic defenses. The former prevents the formation or neutralize free radicals, the latter does not neutralize free radicals directly, but has supporting roles to the other endogenous antioxidants (**Carocho and Ferreira, 2013**). Although its outstanding effectiveness, the endogenous antioxidant system does not suffice, and humans depend on various types of exogenous antioxidants that are present in the diet so as to maintain low levels of free radical concentrations (**Al-Dalaen and Al-Qtaitat, 2014**).

Table 1. *Classification of antioxidant defenses* **(Carocho and Ferreira, 2013).**

Enzymatic antioxidants		**Non-enzymatic antioxidants**
The primary defense	- Glutathione peroxidase - Catalase - Superoxide dismutase	- Uric acid - Vitamins (A, C, E, K) - Flavonoids - Glutathione
The secondary defense	- Glutathione reductase - Glucose-6-phosphate dehydrogenase	

I.3.2. Mechanisms of antioxidants action

Both enzymatic and non enzymatic antioxidant systems are necessary for sustaining life by maintaining a delicate intracellular redox balance and minimizing undesirable cellular damage caused by ROS (**Rahal et al., 2014**). These can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants, and transition metal binding proteins (**Young and Woodside, 2001**).

➢ **The antioxidant enzymes**

It concerned a large number of enzymes that have a common role in the fact of minimizing the dangerous effect of free radicals. As examples we notice:

- **Glutathione peroxidase (GSH-Px)**, which eliminates peroxides as potential substrate for the Fenton reaction.

- **Catalase (CAT)**, that converts hydrogen peroxide into water and molecular oxygen.
- **Superoxide dismutase (SOD)**, converts superoxide anions into hydrogen peroxide as a subtract for catalase **(Lobo et al., 2010; Carocho and Ferreira, 2013)**.

➢ **The chain breaking antioxidants**

Once a free radical interacts with another molecule, it leads to the formation of secondary radicals that can further react with other targets, thus generating more radical species. Such reactions continue to propagate till the radicals are neutralised by a chain breaking antioxidant **(De Zwart et al., 1999)**. Chain breaking antioxidants are ''small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable byproducts'' **(Young and Woodside, 2001)**. Such antioxidants are divided into lipid phase antioxidants that scavenge radicals in membranes and lipoprotein particles such as vitamin E and aqueous phase antioxidants as vitamin C which will directly scavenge radicals present in the aqueous compartment **(Young and Woodside, 2001; Powers and Jackson, 2008).**

➢ **The transition metal binding proteins**

The major essential transition metals in human diseases are iron and copper that play a key role in the production of hydroxyl radicals through Fenton reaction as demonstrated below **(Stohs and Bagchi, 1995) :**

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$

Transition metal binding proteins such ferritin, transferrin, lactoferrin, and caeruloplasmin act as a pivotal component of the antioxidant defense system by sequestering iron and copper so that they are not available to drive the formation of the hydroxyl radical. For instance, caeruloplasmin may function as an antioxidant via the rapid oxidation of the Fe^{2+} form that drives the Fenton reaction to the less reactive Fe^{3+} form as shown in the reaction below **(Young and Woodside, 2001)**:

$$4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O$$

I.4. Antioxidant potential of exopolysaccharides of LAB

I.4.1. Antioxidant activity of LAB

As well as their several potential health benefits including reduction and prevention of diarrheas of different origin, improvement of the intestinal microbial balance by antimicrobial activity,

alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency, and antitumorigenic activities **(Songisepp et al., 2004; Zhang et al., 2011)**,the antioxidant activitiy of some LAB has been reported **(Kullisaar et al., 2002; Songisepp et al., 2004)**. The antioxidant activity of probiotic LAB strains has been widely studied. Major findings research have agreed upon the potential antioxidant effect of *Lactobacillus* and *Bifidobacteriem* strains which were able to decrease the risk of accumulation of reactive oxygen species in a host organism **(Songisepp et al., 2004;Kim et al., 2006; Zhang et al., 2011)**.

Recent studies indicate that LAB are classified as a good candidates for development of new strains with antioxidant activity, however, little is known about their antioxidant potential mecanisms **(Kullisaar et al., 2012; Wu et al., 2014)**. A study on LAB shows that the antioxidant activity is due to several factors regarding, the presence of wide range of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and glutathione-s-transferase (GST), the production of exopolysaccharides and other free radical scavengers compounds such as glutathione **(Kullisaar et al., 2003; Cinque et al., 2011)** as well as the exhibition of metal ion chelating activity **(Kullisaar et al., 2003; Zhang et al., 2011)**.

I.4.2. Lactic acid bacteria as source of exopolysaccharides

Lactic acid bacteria are useful microorganisms in dairy technology, with along-documented history of use in foods as well as maintenance of the host's health. Their utility is reinforced by their demonstrated probiotic properties **(Liu et al., 2011)**. To date, several probiotic strains are known to produce, extarcellularly, effective bioactive molecules which exert several beneficial effects by different mechanisms **(Kodali and Sen, 2008; Zhang et al., 2013)**. Exopolysaccharides constitute a class of such effective biomolecules that lactic acid bacteria release into the surroundings to protect themselves against sever environmental factors **(Kodali and Sen, 2008)**.

I.4.3. Definition and classification of exopolysaccharides

EPSs are long-chain polysaccharides consisting of branched, repeating units of sugars (mainly glucose, galactose and rhamnose in different ratios) or sugar derivatives, **(Welman and Maddox, 2003)**.They are secreted into their surroundings during growth and are not attached permanently to the surface of the microbial cell **(Laws et al., 2001)**. This distinguishes them

from the structurally similar capsular polysaccharides (CPS), which do remain permanently attached to the surface of the cell **(Welman and Maddox, 2003)**.

Depending on their chemical composition, the EPS from LAB are classified as homopolysaccharides (HoPS) and heteropolysaccharides (HePS). Homopolysaccharides consist of repeating units of only one type of monosaccharide such as cellulose, dextran and mutan, whereas heteropolysaccharides comprise repeating units of two or more types of monosaccharides *e.g.* gellan and xanthan. The total yield of EPS produced by LAB can be influenced by composition of the medium and growth conditions **(Welman and Maddox, 2003; Ruas-Madiedo and De Los Reyes-Gavilan, 2005)**.

I.4.4. Applications of exopolysaccharides

Due to their GRAS status and unique properties, EPS derived from LAB have recently received increasing interest indicating that a broad range of these biomolecules can be applicable for a wide range of industries **(Tallon et al., 2003)**. In the food industry, EPS obtained from LAB have been known to improve the rheology of fermented foods as natural biothickeners, emulsifiers, gelling agents and physical stabilizers **(Ruas-Madiedo and De Los Reyes-Gavilan, 2005; Kumar et al., 2007)**.

Moreover EPSs can be used as bioflocculants, bioabsorbants, encapsulating materials, heavy metals removing agents and drug delivery agents **(Kanmani et al., 2011).** In addition to these technological benefits, it has been suggested that some EPSs produced by lactic acid bacteria are claimed to have beneficial physiological effects on consumer's health. Several studies have indicated that these EPSs may have anti-tumor, anti-ulcer, immuno-modulating, cholesterol-lowering activity and prebiotic effect **(Ruas-Madiedo et al., 2002; Ruas-Madiedo and De Los Reyes-Gavilan, 2005).**

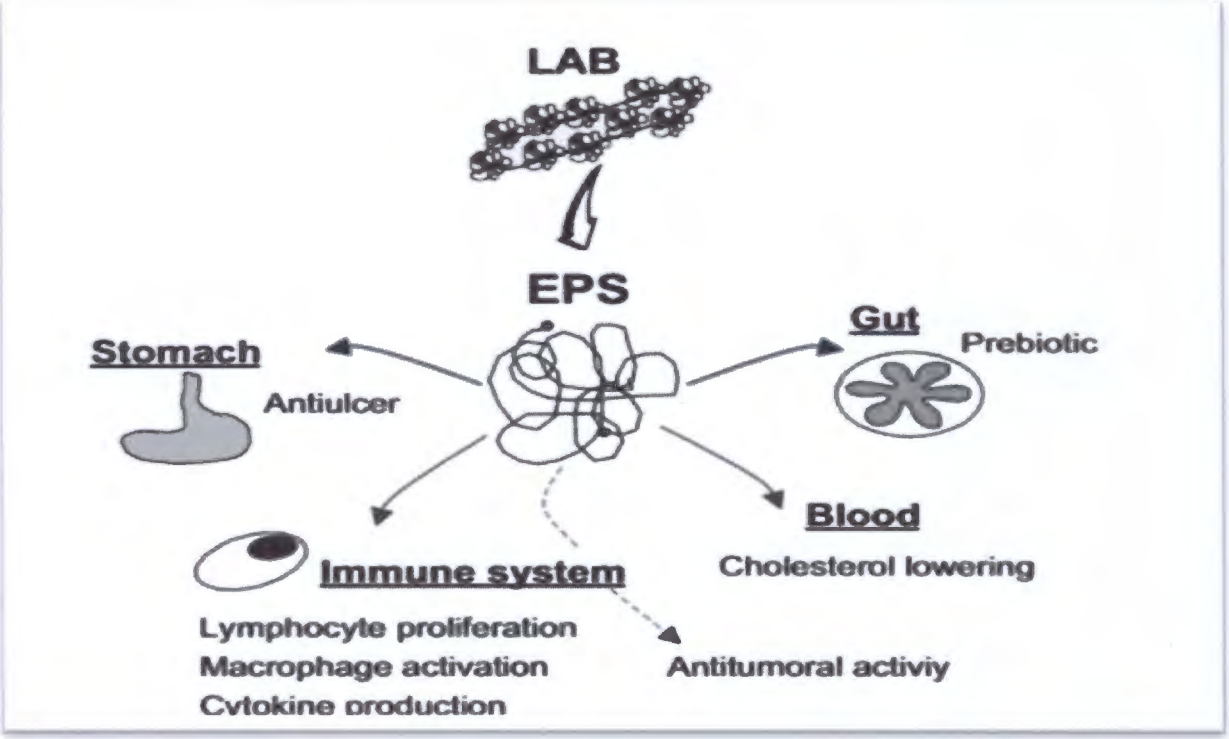

Figure 3. *Putative health-pormoting effects of LAB exopolysaccharides* **(Ruas-Madiedo et al., 2002).**

I.4.5. Antioxidant potential of exopolysaccharides

Actually, the antioxidant properties of plant and fungal polysaccharides have been widely studied and well documented. During the last few decades, much attention has been focused on LAB EPSs for their potential therapeutic activities including antioxidant properties **(Baruah et al., 2016)**. Free radicals and other reactive oxygen species (ROS) are supposed to be highly potent oxidants while they react with all biomacromolecules in living cells (DNA, lipids, proteins and carbohydrates), they lead to carcinogenesis and mutagenesis. LAB producing EPSs have been suggested to help alleviate cancers by different mechanisms. The antioxidant (free radical scavenging) activity of their EPSs has been confirmed to be one of such mechanisms **(Liu et al., 2011)**. Current research have been focused on the study and the evaluation of the potential antioxidant activity of a wide range of exopolysaccharides from LAB. The table below shows some of LAB strains that acquire high potential of exopolysaccharides antioxidant activity.

Table 2. *Examples of LAB strains with potential antioxidant effect of exopolysaccharides.*

Strain	References
Bifidobacterium animalis RH	**(Xu et al., 2011; Shang et al., 2013)**
Lactococcus lactis sub sp. *lactis 12*	**(Pan and Mei, 2010)**
Lactobacillus paracasei sub sp. *paracasei* NTU	**(Liu et al., 2011)**
Lactobacillus plantarum NTU 102	**(Liu et al., 2011)**
Lactobacillus plantarum C88	**(Zhang et al., 2013 ; Baruah et al., 2016)**
Lactobacillus helveticus MB2-1	**(Li et al., 2014)**
Streptococcus phocae PI80	**(Kanmani et al., 2011)**
Lactobacillus rhamnosus E/N	**(Polak-Berecka et al., 2013)**
Lactobacillus plantarum YW32	**(Baruah et al., 2016)**
Enterococcus faecium BDU7	**(Abdhul et al., 2014)**

Part II.

Materials and methods

This work was realized in the laboratory of Microbiology, Department of Applied Microbiology and Food Sciences, University of Jijel, between April and June 2016. It was dedicated to the evaluation of antioxidant potential of exopolysaccharides from some LAB strains.

II.1. Materials

II.1.1. Probiotic strains

The present study was concerned with nine bacterial strains isolated from different origins. These probiotic bacteria were kindly provided by Dr. Tayeb Idoui, laboratory of Biotechnology, Environment and Health, University of Jijel.

Table 3. *LAB used in the study and their origins.*

Strains	**Code**	**Origin**
Lactobacillus delbruecki	Lb. del	-
Lactobacillus curvatus	Bj432	Jijelian butter
Lactococcus lactis ssp. cremoris	CHT24	Camel milk
Streptococcus salivarius ssp thermophilus	St.sa	Milk
Lactobacillus plantarum	15	Milk
Lactobacillus sp.	45	-
Lactobacillus confusus	Lb. Con	Human feces (API50+Log)
Lactobacillus sp.	05	-
Lactobacillus plantarum	G1	Gizzard

II.1.2. Media and buffers

Several culture media and buffers were used in this experimental work which were:

Man-Rogosa Sharp (MRS broth and MRS agar) .

Hyper-sucrose agar.

Phosphate Buffered Saline (PBS, pH 7.4, PBS, pH=2.0, PBS 1% bile salt).

II.1.3. Apparatus

- Autoclave (pbi brand);
- Oven (Memmert);
- pH meter (Hanna);
- Spectrophotometer (JENWAY);
- Water bath (Gerhardt Bonn);

- Vortex (VWR);
- Hot plate;
- Balance (KERN);
- Centrifuge (Sigma);
- Shaker incubator (INFOS HT ECOTRON);
- Analytical balance (KERN);
- Micropipettes (SMART);
- Fridge (ENIEM).

II.1.4. Chemicals and reagents

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 1,10-phenanthroline, Trichloroacetic acid solution (TCA), hydrogen peroxide (H_2O_2), ethanol, $FeSO_4$, phenol, sulfuric acid, ascorbate, HCl, NaOH.

II.2. Methods

II.2.1. Revivification : Preparation of bacterial cultures

Nine LAB strains chosen for the present study were maintained as frozen stocks in MRS medium at -20°C. All bacterial strains were revitalized in Man Rogosa Sharp broth (MRS) and incubated at 37°C for 24 h before use.

II.2.2. Screening of bacterial strains producing EPS

The production of exo-polysaccharides was investigated on hyper-sucrose agar medium (Annexe1) at 37°C for 24h **(N'tcha et al., 2016)**. EPS positive isolates were defined as those displaying slimy colony morphology, these colonies were tested for compactness or ropiness by touching them with a sterile inoculation loop **(Mostefaoui et al., 2014)**. Four strains were selected for the next step according to their large amount of viscosity production among nine strains. The selected strains were: *Lb. curvatus* Bj432, *Lc. lactis ssp cremoris* CHT24, *St. salivarius ssp thermophilus* St.sa and *Lactobacillu ssp* 05.

II.2.3. EPS production

The isolate St.sa, CHT24, Bj432 and 05 were grown in Erlenmeyer flask of 200 ml containing 50 ml of modified MRS broth (20g/l of glucose was replaced by lactose), incubated in shaking incubator at 37°C for 24h **(Savadogo et al., 2004).** Total EPS was estimated in each sample by the phenol–sulfuric acid method, using glucose as standard **(Mozzi et al., 2000)**.

II.2.4. EPS extraction

EPS was extracted from the production medium as the protocol described by **Li et al., (2014)** with slight modifications. After 24 h of incubation period, the cells was separated by centrifugation at 12,000 x g for 15 min at 4°C. Then, trichloroacetic acid (TCA) solution was added to the supernatant to give final concentration of 4% (w/v). The precipitated proteins were removed by centrifugation (12,000 × g for 30 min at 4°C), and three volumes ice cold ethanol were added to the supernatant, stirred vigorously and kept at 4°C for overnight. Crude EPS was collected by centrifugation at 15,000 × g for 15 min, then the EPS pellet was dissolved in distilled water.

II.2.5. EPS quantification

Exopolysaccharides were estimated as total carbohydrates by phenol-sulfuric acid method. To 800 µl of sample (EPS solution), 40 µl of phenol reagent (80% w/v) was added followed by 2 ml of concentrated sulfuric acid, vortex was applied after addition of each reagent. The absorbance was measured at 490 nm against the reagent blank that contains 800 µl of distilled water instead of sample. The EPS content of each sample was then calculated using the glucose standard curve (Annexe 3). For the preparation of standard curve, the same procedure was investigated using glucose solution (1mg/ml) as standard reagent instead of EPS solution. The results were expressed as milligrams (mg) of EPS per liter **(Mozzi et al., 2000)**.

II.2.6. Determination of antioxidant activity

Sterile MRS broth was inoculated with the four strains (CHT24, St.sa, Bj432, 05) and incubated at 37°C for 18 hours. Cells of the four strains were harvested by centrifugation at 6000 rpm for 10 min. Both intact cells (total cell numbers were adjusted to 10^9 CFU/ml) and supernatant obtained after centrifugation as well as EPS already extracted were subjected to different antioxidant assays namely DPPH free radical scavenging assay, hydroxyl radical scavenging assay and ferrous ion chelating assay so as to evaluate their antioxidant potential (**Lin and Chang, 2000**).

- **DPPH free radical scavenging activity**

The DPPH radical-scavenging capacity was evaluated according to the method described by **Li et al. (2014)**. Briefly, 1.0 ml of sample solution was added to 0.2 ml of freshlly prepared ethanolic DPPH solution (0.4 mM), and then, 2 ml of distilled water was added. The mixture was mixed vigorously and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm against a blank where ethanolic DPPH solution was replaced by ethanol.

Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The scavenging ability was defined as **(Marinova and Batchvarov, 2011)**:

(%) DPPH scavenging = $[(A_{control} - A_{sample}) / A_{control}] \times 100$.

- **Hydroxyl radical scavenging ability**

The hydroxyl radical scavenging activity was performed with the method described by **Li et al. (2014)**. The hydroxyl radical was generated in the mixture of 1 ml of 0.75 mM phenanthroline, 1 ml of 0.75 mM $FeSO_4$, 1 ml of H_2O_2 (0.01%, v/v) and 1.5 ml of 0.15 M PBS (pH 7.4). After the addition of 1.0 ml of sample solution, the mixture was incubated at 37◦C for 30 min. After the period of incubation, the absorbance of the mixture was measured at 536 nm. The scavenging activity on hydroxyl radical (%) = $(A_{sample} - A_{blank})/(A_0 - A_{blank}) \times 100$, where A_0 was the absorbance of the control in the absence of the sample and A_{blank} was the absorbance of distilled water instead of H_2O_2.

- **Measurement of the chelating capacity of ferrous ion**

Iron ion chelating assay was performed as described by **Zhang et al. (2011)** with some modifications. Briefly, 0.5 ml of sample was mixed with 0.1 ml of ascorbate (1%, w/v), 0.1 ml of FeSO4 (0.4 g/l), and 1 ml of NaOH (0.2 M). The mixture was incubated at 37°C in a water bath, and 0.2 ml of trichloroacetic acid (TCA) (10%) was added into the mixture and incubated for 20 min. Supernatant was obtained by centrifugation at 6000 rpm for 20 min, and 0.5 ml of phenanthroline (1g/l) was added. After the 10 min reaction, the absorbance was measured at 510 nm. The chelating capacity of ferrous ion was determined as described below:

Chelating capacity % = $[1-(A_{sample}/A_{control})]\times 100$.

II.2.7. Determination of probiotic viability in different conditions

Among the four bacterial strains used in our study, the strain 05 that has showed high antioxidant potential has been selected in order to determine its acid and bile salt tolerance as described below.

- **Low pH tolerance**

To determine the transit tolerance to low pH, the method of **Kim et al., (2006)** was used with slight modifications. Strains were grown in MRS broth for 24h, 1ml aliquote of the bacterial culture was inoculated in 20ml of PBS adjusted to pH 2.0 with 1M HCL. Cultures were incubated at 37°C. After 0, 2 and 4h incubation, cells were serially dilluted to 10^{-9} and the viable cells were

enumerated on MRS agar plate at 37°C for 48h. The bacterial survival rate was evaluated as described below (**N'tcha et al., 2016**) :

Survival rate (%) = [log CFU T_nh / log UFC T_oh] x 100.

T_oh: Initial time (0h)

T_nh: Time after (n) hours (2h, 4h)

- **Bile salt tolerance**

The test was carried out using the method described by **Mishra and Prasad, (2004)**. A concentration of 1% bile salts was prepared in PBS, sterilized and stored at room temperature. The pellet obtained after centrifugation of fresh culture was suspended in PBS then transferred to 20 ml of PBS with 1% bile salt. After 0, 2 and 4h incubation at 37°C the viable count was performed.

Various tests were carried out *in vitro* for the evaluation of the antioxidant activity of LAB strains. Three antioxidant models namely DPPH radical scavenging activity, hydroxyl radical scavenging activity and metal ion chelating ability were used to reflect the antioxidant capacities of intact cells, supernatant and extracted EPS, moreover one strain was selected for the evaluation of its probiotic properties.

III.1. Screening of bacterial strains producing EPS

All tested bacterial strains were capable to grow on hyper-sucrose agar indicating a production of exopolysaccharides. Table (4) shows the results after 4 h of incubation. Among the nine tested strains, EPS production showed important level with four strains (*Lactobacillus* sp. 05, *St.salivarius ssp thermophilus* St.sa, *Lb. curvatus* Bj432, *Lc. Lactis* ssp *cremoris* CHT24) that have shown large and slimy colony (Figure 4).

Our results are correlated with those obtaind by **N'tcha et al., 2016** in which, five strains of LAB have also formed large and slimy colony indicating EPS production. The production of exopolysaccharides by LAB is a favorable phenomenon for many food industrial processes. The main advantage of the use of EPS producing lactic acid bacteria in the lactic ferments during the production of fermented products is to improve the texture and viscosity. It was also suggested that some of EPS produced by LAB may confer beneficial effects for consumer's health **(Li et al., 2014)**. However, the EPS produced by *St. mutans* and S*t. salivarius* are involved in bacterial colonization and dental plaque formation **(N'tcha et al., 2016).**

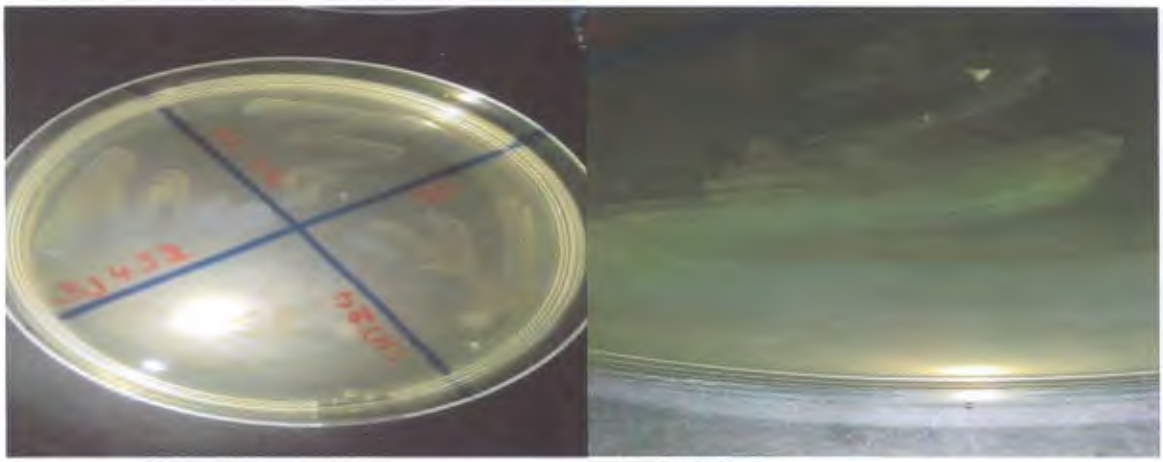

Figure 4. *Exopolysaccharides produced by LAB strains on hyper sucrose agar.*

Table 4. *Capability of the tested LAB strains to produce exopolysaccharides.*

Species	Test conclusion
Lc. lactis ssp *cremoris* **CHT24**	+++
Lb. curvatus **Bj432**	+++
Lactobacillus sp. **05**	+++
St. salivariu sssp thermophilus **St.sa**	++
Lactobacillus sp. **45**	+
Lactobacillus delbruecki **Lb.del**	+
Lb. plantarum **15**	+
Lb. Plantarum **G1**	+
Lb. confusus **Lb.con**	+

+ + +: high EPS production

+ +: middle EPS production

+: low EPS production

III.2. EPS quantification

Exopolysaccharides were estimated as total carbohydrates by phenol-sulphuric acid method using glucose as standard (Annexe 2). This method is simple, rapid, and sensitive, and gives reproducible results **(Dubois et al., 1956).** The method detects virtually all classes of carbohydrates, including mono-, di-, oligo-, and polysaccharides. Although the method detects almost all carbohydrates, the absorptivity of the different carbohydrates varies (**Nielsen, 2010**). The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color (**Albalasmeh et al., 2013**).

In the present study, tested strains have shown EPS production in the range of 23.4-35.4 mg/L (Figure 2). The highest level of EPS production was obtained with *Lb. curvatus* Bj432 followed by *St. salivarius ssp thermophilus* St.sa (34.4 mg/l) and *Lc.lactis* ssp. *cremoris* CHT24 (34 mg/l), whereas *Lactobacillus* sp. (05) has shown the lowest EPS amount. Other similar studies have obtained high concentrations of EPS compared with our results. **Savadogo et al. (2004)** has obtained a range of 100-600 mg/l of EPS production with *Lb.lactis* subsp. *lactis.* In the same topic, **N'tcha et al., (2016)** have found greater EPS, *Lb.s casei* (1130 mg/l), *St. thermophilus* (750 mg/l), *Lb. fermentum* (700 mg/l) and *Enterococcus faecium* (330 mg/l). The amount of EPS production

differs between genera ,also, these differences may probably due to the production coditions such a temprature, pH and medium composition **(Lai, 2014).**

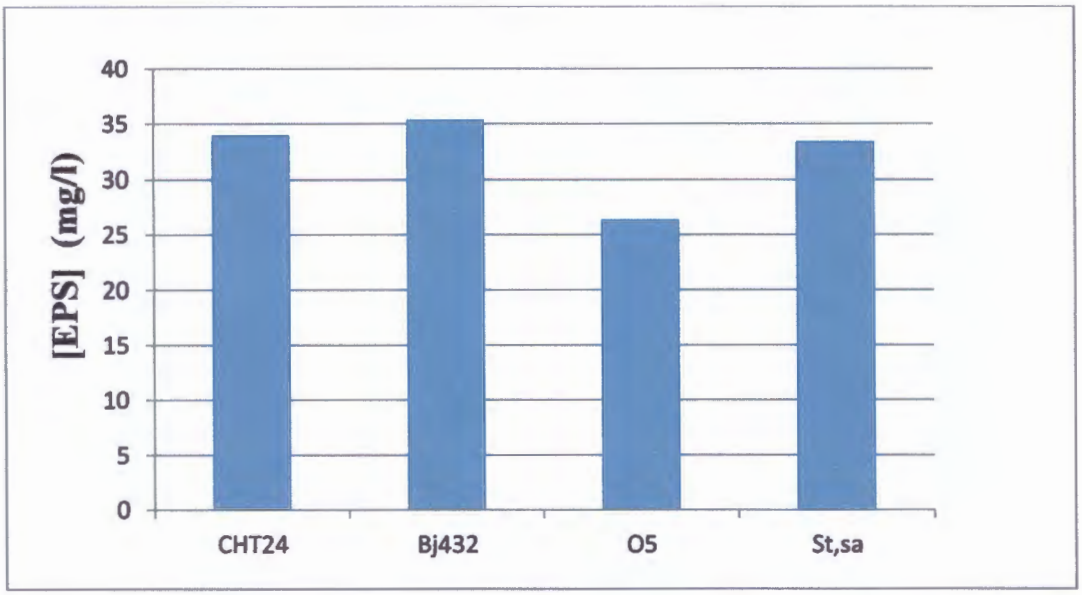

Figure 5. *Total sugar content of EPS samples of tested strains.*

III.3. Determination of antioxidant activity

III.3.1. DPPH free radical scavenging activity

Due to its simplicity, rapidity, sensitivity and reproducibility, the DPPH free radical scavenging method has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants. The principle of the assay is based on the reduction of ethanolic DPPH solution in the presence of a hydrogen-donating antioxidant, thus neutralizing its free radical character and leading to the formation of non-radical form DPPH-H **(Zhang et al., 2011)**. When DPPH radicals are scavenged, the color of the reaction mixture changes from purple to yellow and absorbance at 517nm decreases **(Liu et al., 2011)**.

As shown in the figure 6, the scavenging DPPH rate of supernatant was higher than that of both intact cells and crude EPS. The intact cells showed DPPH free radical scavenging activity in the range of 44% - 50%, the strain CHT24 has shown the higher level following by the strain 05 with 48% activity. These results were similar to those obtained by **Lin and Chang (2000)**, which reported that *Lb. acidophilus* showed the ability to scavenge DPPH free radical by 43.2%–52.1%. The higher level of scavenging ability was found in supernatant samples in the range of 80% and 92%, this may probably due to the fact that, in addition to EPS polymers, other antioxidant components such as proteins, peptides and microelements were present in the supernatant where

several interactions and some synergistic effects for antioxidant properties were occurred. Moreover, the scavenging abilities of EPS samples on DPPH free radicals were in the range 43% - 61%.The strain Bj432 exhibited the stronger EPS scavenging effect followed by the strain 05, however St.sa and CHT24 strains have shown the lower rate about 44% and 43% respectively.

The differences of the scavenging ability of the four EPS samples may be due their concentrations as well as chemical composition **(Li et al., 2014)**. The EPS of Bj432 that showed the high antioxidant activity has shown also the higher amount of EPS which confirm the relation between the antioxidative activity and the EPS concentration, in addition, according to their chemical properties EPS of Bj432 could be better advantageous than the other tested EPS for reacting with DPPH radicals to convert them to more stable products. Previous studies reported by **Li et al., (2014) and Xu et al., (2011)** have postulated that the free radical scavenging activity of EPS produced by LAB was associated with the molecular weight of EPS fractions that is, the smaller molecular weight, the stronger antioxidant activity, the monosaccharide composition as well as the glycosidic linkage.

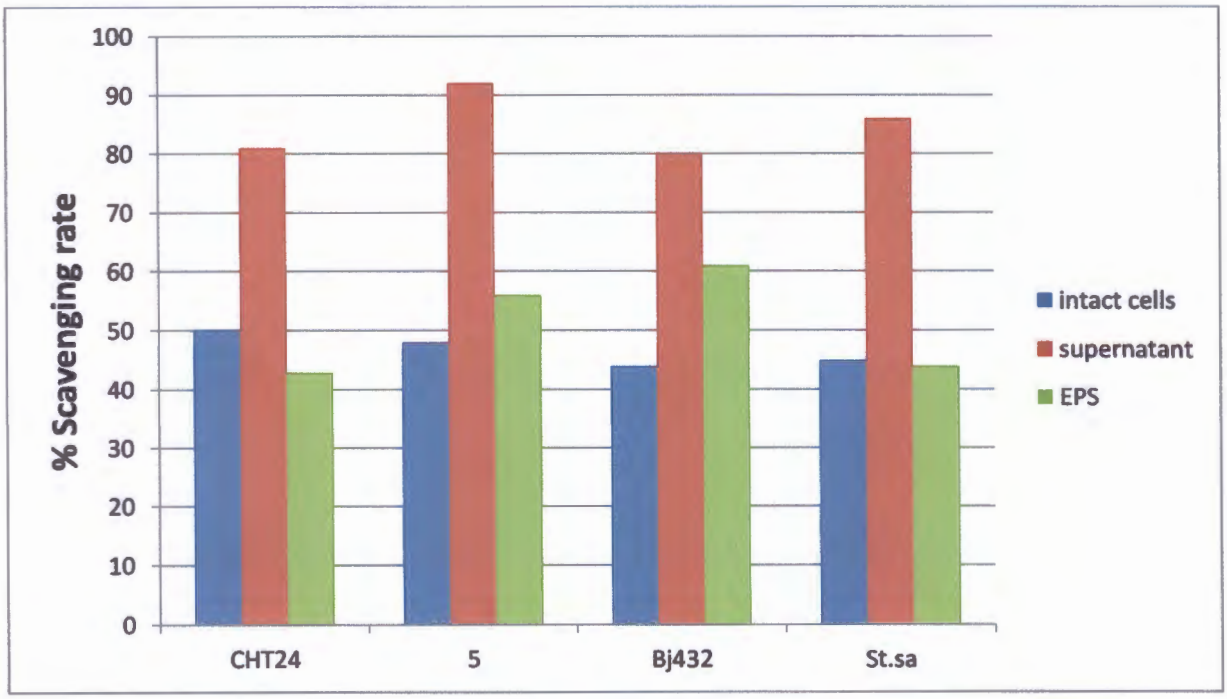

Figure 6. *Scavenging ability of LAB strains on DPPH radical.*

III.3.2. Hydroxyl radicals scavenging activity

Among reactive oxygen species, hydroxyl radical is considered to be the most reactive oxygen radicals, which can react with all biomacromolecules functioning in living cells and can induce severe damage to cells. Therefore, scavenging of hydroxyl radical plays a critical role in reducing oxidative damage **(Xu et al., 2011; Zhang et al., 2011).** The results showing scavenging activity of hydroxyl radicals are shown in figure 7.

As shown in the figure, all tested samples (intact cells, supernatant and EPS) possessed the eliminating ability on hydroxyl radicals generated by the Fenton reaction in the system. The ability of EPS to scavenge hydroxyl radical was aranged of 22%-42% in which St.sa strain showed the highest level, however the supernatant demonstrated a strong scavenging ability on hydroxyl radical with 60%, 64%, 67% and 62% by St.sa, CHT24, 05 and Bj432 strains respectively. This may be explained as the fact of the presence of other antioxidant components in the supernatant such as protein, amino acids, peptides, organic acids and microelements. Moreover, the intact cells showed low hydroxyl radicals scavengingactivity in the range of 17% - 29% where the 05 strain showed the highest level.

The results obtained by **Li et al. (2014)** demonstrated that EPS *L.helveticus* MB2-1 had strong scavenging acticity on hydroxyl radical, the EPS minimized the concentration of ferrous ion in the Fenton reactionand, the sccavenging effect of the EPS might be due the active hydrogen donating ability of the hydroxyl substitutions of EPS.

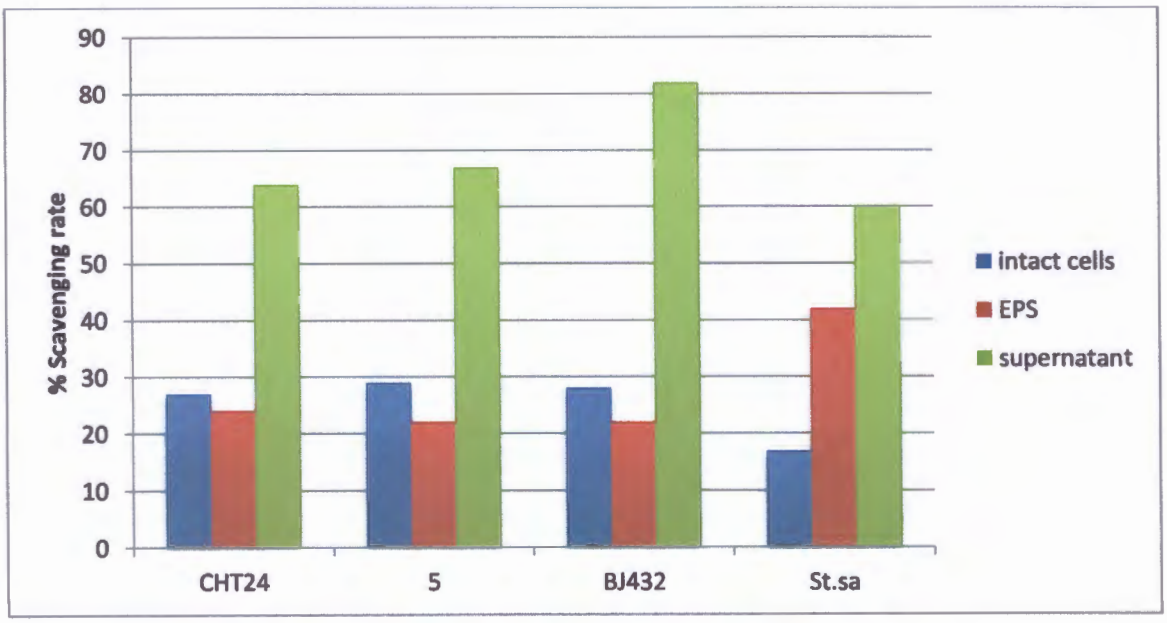

Figure 7. *Hydroxyl radical scavenging ability of LAB strains.*

III.3.3. Metal ion chelating ability

Since it mediates catalyzing transition metals during lipid peroxidation, metal chelating activity is claimed as one of antioxidant mechanism. Among the transition metals, iron is considered as the most important lipid oxidation pro-oxidant owing to its high reactivity **(Liu et al., 2011).**

It was reported that chelating agents, which form s-bonds with metal are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the iron ion **(Li et al., 2014).** The ferrous ion chelating effects of intact cells, supernatant and EPS samples was investigated and the antioxidant activity was evaluated. Figure 8. Shows an excellent level of Fe^{2+} chelating ability with the three samples. Supernatant samples have demonstrated the stronger ferrous ion chelating ability by 99%, 98%, 97% and 93%, reffering to CHT24, Bj432, 05 and St.sa strains respectively followed by the intact cells activity in the range of 78% - 95% where the strains CHT24 and Bj432 have shown the maximum level of chelating ability. However,theEPS iron ion chelating ability was weaker than that of intact cells and supernatant in the range of 70% - 75%.

Previous studies focused on ferrous ion chelating ability of EPS have illustrated that the metal chelating activity increased with the increase in the EPS concentrations **(Li et al., 2014)**, this fact do not confirm the results obtained in our experience in which both Bj432 and CHT24 strains that have shown the higher EPS concentrations, have not shown the higher metal chelating activities, this contradiction can be justify as, probably EPS for 05 strain that showed the highest metal ion chelatig ability may exhibited some properties which make it better advantageous to react with metal ion than that of CHT24 or Bj432.

In addition the intact cells ability to chelate metal ions have been also reported by **Zhang et al., (2011)**, in which *Lactobacillus* strains isolated from yogurt have showed the highest chelating ability by 50.55%, besides **Lee et al., (2005)** have reported that *L. casei KCTC 3260* exhibited high metal ion chelating ability.

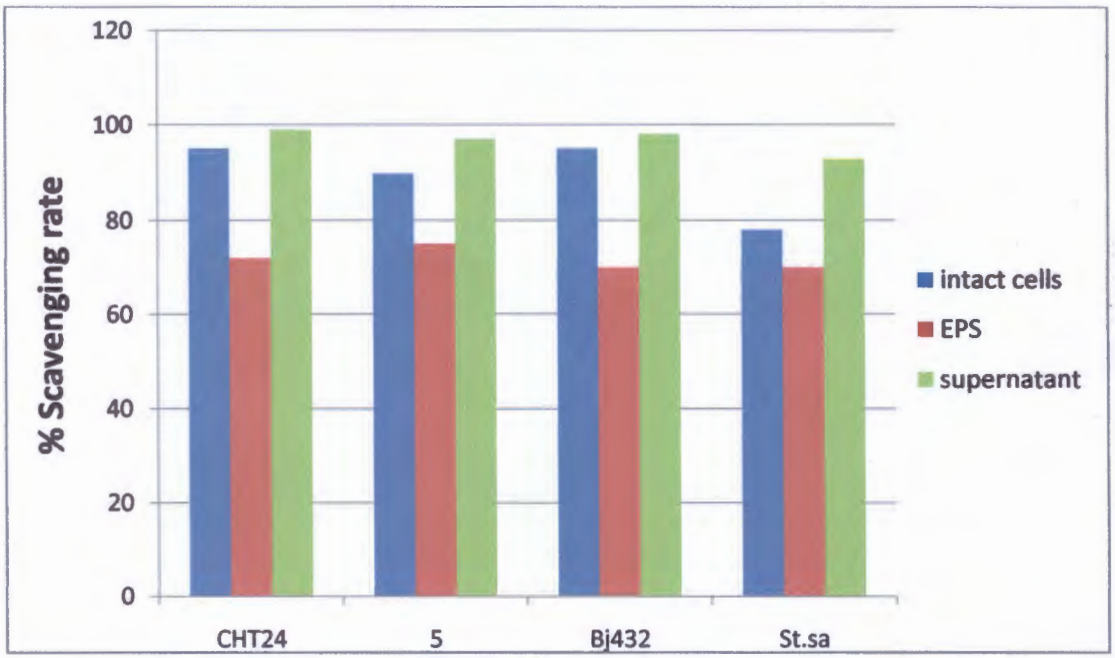

Figure 8.*Chelating capacity of ferrous ion.*

After the evaluation of LAB antioxidant activity, It has been shown that some lactobacilli possess antioxidative activity and are able to decrease the adverse effect of ROS. Both intact cells and EPS produced by the strains showed high free radical scavenging activity and metal ion chelating ability, these results revealed that EPS is one of the mechanisms responsible for the antioxidant activity of LAB. Antioxidant activity of intact cells of LAB is probably due to two types of antioxidant pathways: enzymatic and non enzymatic defense systems, superoxide dismutase (SOD) and glutathione peroxidase (GPx) are widely implicated in the defense against ROS, however to prevent excessive oxidative stress, cells had to develop non enzymatic defense mechanisms **(Lee et al., 2005)**.

Lactobacillus sp., *St. salivarius ssp thermophilus*, *Lb. curvatus* and *Lc.lactis* ssp *cremoris* used in this study may be candidate as antioxidative strains that showed high resistance to ROS. Actually, exopolysaccharides produced by such antioxidative strains, with desirable properties, may be a promising material for both applied microbiology and scientific food industry.

4.Bile salt and low pH tolerance

For a strain to be qualified as probiotics, it must have fulfilled certain physiological characteristics, including survival in the GIT, tolerance to low pH and tolerance to bile salt **(Ji et al., 2015).** The results of the survival rate of the tested strain *Lactobacillus* sp. 05 under low pH and bile salt conditions after the incubation period are shown in figure 9. As seen in

the figure, *Lactobacillus* sp. 05 displayed high tolerance to low pH value (pH 2.0) wich was around 95% and 85% after 2 h and 4 h incubation respetively. On the other hand, the resistance of the strain to high bile conditions (1%) was determined. The result showed high survival rates of about 98 % and 90 % after 2 h and 4 h incubation with 1% bile salt respetively.

These results are in agreement with other studies reported by **N'tcha et al., (2016)** and **Song et al., (2015),** which have demonstrated that *Lactobacillus* strains remain viable when exposed to acid pH values of 2.5-4.0, as well as bile salt concentration. The term 'probiotic' means 'for life', and describes microorganisms that survive passage through the GIT and have beneficial effects on the host. In one hand, acid-tolerant strains have an advantage in surviving in the low pH conditions of the stomach (as low as pH 2.0), where hydrochloric and gastric acids are secreted, on the other hand, the physiological concentration of human bile ranges from 0.3% to 0.5%. Therefore, resistance to bile acid is an important characteristic that enables *Lactobacillus* to survive, grow, and remain active in the small intestine **(Song et al., 2015).** In the same line, the differences observed between our results and other studies of LAB tolerance to acid and bile conditions may probably due to several reasons namely the origins of tested strains, the culture conditions or bile salt concentrations **(N'tcha et al., 2016).**

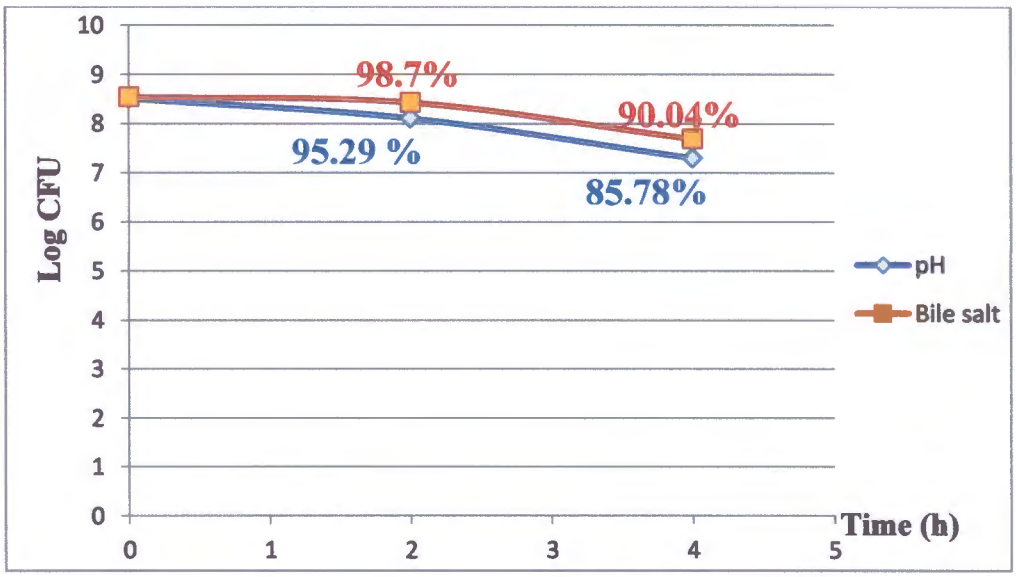

Figure 9. *Bile salts and pH tolerance of 05 strain (numbers reffer to survival rate).*

The strain evaluated demonstrated sufficient acid and bile tolerance to be resistant to physiological stomach and intestinal conditions, making it potentially useful candidate for the development of probiotic.

Conclusion

Referring to the results obtained in the present study, it may be concluded that the nine tested LAB isolated from different origins were capable to produce exopolysaccharides in different levels, whereas, four strains (*Lactobacillus* sp. 05, *St. salivarius ssp thermophilus* St.sa, *Lb. curvatus* Bj432, *Lc. Lactis* ssp. c*remoris* CHT24) were chosen in accordance to their high level of EPS production. The production and extraction of EPS and the evaluation of their antioxidant activities *in vitro* as well as intact cells and supernatants of the selected strains were investigated. The maximum yield of EPS (35.4mg/l) was obtained from *Lb. curvatus* Bj432. The evaluation of antioxidative potential was investigated through DPPH free radical assay, hydroxyl radical assay and metal ion chelating ability. All samples have shown good DPPH scavenging capacity, where the highest ability was obtained from CHT24 intact cells and Bj432 EPS. Although both intact cells and EPS have shown weak scavenging abilities towards hydroxyl radicals in comparison with supernatants, it has been noticed that St.sa EPS was the most efficient one. However, results obtained from metal ion chelating abilities have shown strong capacity with almost all samples upon which BJ432 and CHT24 intact cells were the greatest. Finally, *Lactobacillus* sp. 05 was determined as probiotic strain after being grown under bile salt and low pH conditions .

Further research must be investigated for better illustration of the antioxidant mechanisms of exopolysaccharides from lactic acid bacteria and their involvement in other human health applications. Furthermore, determination of the nature of the extracted EPS after a complete purification is recommanded.

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Annexes

Annex 1

Man-Rogosa Sharp (MRS broth and agar)

Peptone……10g/l

Yeast extract……04g/l

Beef extract……08g/l

Glucose……20g/l

Dipotassique Phosphate……02g/l

Sodium acetate……05g/l

Ammonium citrate……02g/l

Manganous sulfate……0.05g/l

Magnesium sulfate……0.2g/l

Tween 80……1ml/l

Agar ……15g/l

pH = 6.2

Autoclavage 120°C/ 20 min.

Hyper-sucrose agar

Peptone……2.5g/l

Yeast extract……03/l

Beef extract……10g/l

Sucrose……150g/l

Dipotassique Phosphate……02g/l

Magnesium sulfate……0.2g/l

NaCl ……01g/l

Agar ……15g/l

pH = 6.8

Autoclavage 120°C/ 20 min.

Sodium Phosphate Buffer (PBS)

NaCl……8 g/l

KCl……0.2g/l

Na_2HPO_4……1.44g/l

K_2HPO_4……0.24g/l

pH = 7.4

Autoclavage 120°C/ 20 min.

Annex 2

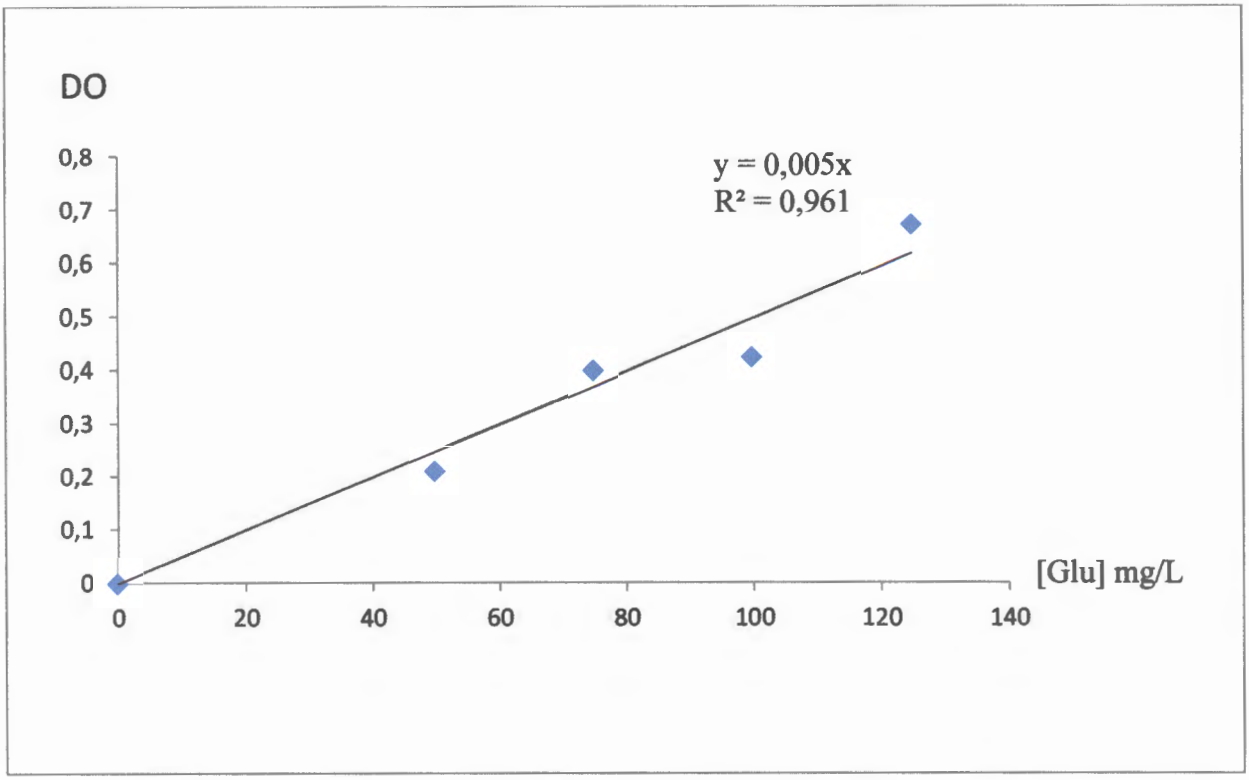

Figure 1. Standard curve of glucose

Annex 3

Table 2. *Results obtained from the quantification of EPS production*

Strains	CHT24	05	Bj432	St.sa
DO	0.170	0.132	0.177	0.167
[EPS] (mg/l)	34	26.4	35.4	33.4

Annex 4

Table 3. *Results obtained from DPPH test*

Strain / DO	CHT24	Bj 432	St.sa	05
Intact cells	0.150	0.168	0.164	0.156
Supernatant	0.170	0.117	0.167	0.132
EPS	0.054	0.060	0.041	0.023
Control	0.299	0.299	0.299	0.299

Table 4. Results obtained from hydroxyl radical scavenging test

Strain / DO	CHT24		Bj 432		St.sa		05	
	blank	sample	blank	sample	blank	sample	blank	sample
intact cells	0.414	0.366	0.401	0.356	0.402	0.375	0.409	0.359
Supernatant	0.344	0.277	0.317	0.253	0.345	0.281	0.368	0.282
EPS	0.336	0.313	0.360	0.333	0.366	0.313	0.359	0.333
control	0.239	0.239	0.239	0.239	0.239	0.239	0.239	0.239

Table 5 . Results obtained from metal ion chelating ability test

Strain / DO	CHT24		Bj 432		St.sa		05	
	blank	sample	blank	sample	blank	sample	blank	sample
intact cells	0.123	0.016	0.109	0.016	0.117	0.071	0.112	0.031
supernatant	0.128	0.001	0.096	0.007	0.104	0.022	0.094	0.009
EPS	0.187	0.093	0.176	0.098	0.192	0.097	0.190	0.082
control	0.363	0.363	0.363	0.363	0.363	0.363	0.363	0.363

Annex 5

Table 6. Effect of pH 2 and bile salts (1%) on the viability of Lctobacillus sp 05.

condition	Initial counts	Survival rate %	
		Tn: 2h	Tn: 4h
pH 2	8.51	95.25	85.78
1% bile salt	8.54	98.7	90.04

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Theme

Antioxidant activity of exopolysaccharides from lactic acid bacteria

Abstract

Despite of the great importance of lactic acid bacteria exopolysaccharides in both food and pharmaceutical industries they also play a critical role as potent antioxidant. In this study, after the screening of nine strains for EPS production, four strains (*Lb. curvatus* Bj432, *St. salivarius ssp thermophilus* St.sa, *Lactobacillus* sp. 05, *Lc. lactis* ssp. *cremoris* CHT24) possessing high level were selected. Extracted EPS were further quantified and their antioxidant activity was evaluated. *Lb. curvatus* Bj432 acquired the highest amount of EPS (35.4mg/l) which exhibited the stronger DPPH free radical scavenging activity (61 %), besides, the higher hydroxyl radical scavenging activity (42%) was obtained by EPS from *St. salivarius ssp thermophilus* St.sa. However, all extracted EPS have shown excellent iron ion chelating ability (70-75%). Furthermore, in order to be qualified as probiotic strain, the survival rate under acidic conditions (pH 2.0) and 1% bile salt during 4h of *Lactobacillus* sp. 05 was evaluated, obtained results showed good tolerance in both cases .

Key words: Lactic Acid Bacteria, Exopolysaccharides, Antioxidant Activity, Probiotics.

Résumé

Malgré la grande importance des exopolysaccharides des bactéries lactiques dans l'industrie alimentaire et pharmaceutique, ils jouent également un rôle essentiel en tant qu'antioxydants puissants. Dans cette étude, après le screening pour la production d'EPS, quatre souches (*Lb. curvatus* Bj432, *St. salivarius ssp thermophilus* St.sa, *Lactobacillus. sp.* 05, *Lc. Lactis ssp. cremoris* CHT24) possédant une très bonne production ont été sélectionnées parmi neuf souches testées. Les EPS extraits ont été quantifiés et leur activité antioxydante a été évaluée. *Lb.*curvatus Bj432 a acquis la plus grande quantité d'EPS (35.4mg /l) dont il a présenté la plus forte activité scavenger du radical DPPH (61%), aussi, l'activité scavenger du radical hydroxyle la plus élevée (42%) a été obtenue par l'EPS de *St. salivarius ssp thermophilus* St .sa. Cependant, les quatres EPS testés ont montré une excellente aptitude à la chélation des ions de fer (70-75%). De plus, dans le but d'être qualifiée comme souche probiotique, le taux de survie dans des conditions acides (pH 2.0) et 1% de sels biliaires pendant 4h de *Lactobacillus* sp. 05 a été évaluée, les résultats obtenus ont montré une bonne tolérance dans les deux cas.

Mots-clés: Bactéries lactiques, Exopolysaccharides, Activité antioxydante, Probiotiques.

ملخص

على الرغم من الأهمية البالغة لمتعددات السكر المنتجة من قبل بكتيريا حمض اللاكتيك في كل من الصناعات الغذائية والدوائية الا أنها تلعب دورا رئيسيا كمواد مضادة للاكسدة . في هذه الدراسة، بعد اجراء فحص إنتاج متعددات السكر ، تم اختيار أربعة سلالات تمتلك مردودية انتاج عالية : *Lb. curvatus* Bj432, *St.salivarius ssp thermophilus* St.sa , *Lactobacillus* sp. 05, *Lc. Lactis ssp.cremoris CHT24* من بين تسعة سلالات تم اختبارها. تم تحديد كمية متعددات السكر و تقييم نشاطها المضادة للأكسدة. *Lb. curvatus* Bj432 حصلت على أكبر قدر من العائد (35.4 مغ / لتر) الذي اظهر أقوى نشاط لمقاومة الجذور الحرة.ل DPPH (61%) الى جانب ذلك، تم الحصول على أعلى نشاط لمقاومة جذور الهيدروكسيل (42٪) من متعددات السكر المنتجة من قبل St .sa. *St. salivarius ssp thermophilus* .في حين جميع متعددات السكر قد أظهرت قدرة ممتازة على إزالة أيونات الحديد (70-75٪).

من أجل أن تصنف ضمن سلالات البروبيوتيك, تم زرع *Lactobacillus sp.* 05 في وسطين الاول حمضي (pH 2.0) و الثاني مزود ب 1% من الأملاح الصفراء مدة 4 سا .أظهرت النتائج نسبة نموجيدة في كلتا الحالتين.

الكلمات المفتاحية: بكتيريا حمض اللاكتيك ، متعددات السكر ، النشاط المضاد للأكسدة، البروبيوتيك.