*American Journal of Microbiological Research, 2016, Vol. 4, No. 5, 147-152*
Available online at http://pubs.sciepub.com/ajmr/4/5/4

DOI:10.12691/ajmr-4-5-4

# Exopolysaccharide (EPS) Production by *Exiguobacterium aurantiacum* isolated from Marchica Lagoon Ecosystem in Morocco

**Y. D. Dah Dossounon*, K. Lee, K. Belghmi, F. Benzha, M. Blaghen**

Laboratory of Microbiology, Pharmacology, Biotechnology and Environment, University Hassan II, Faculty of Sciences Ain-Chock, route El Jadida, B.P. 5366, Casablanca, Morocco
*Corresponding author: djolounivcasa@gmail.com

**Abstract** *Exiguobacterium aurantiacum*, a member of the *Bacilli* class, has the ability to synthesize and secrete exopolysaccharides. The strain, isolated from Marchica lagoon in Morocco, produced exopolysaccharide (EPS), mainly during its exponential growth phase but also to a lesser extent during the stationary phase. The optimum pH and temperature for growth and exopolysaccharide (EPS) production were 8 and 37°C respectively, the dry weight of the exopolysaccharides products and biomass was found to be 259. 05 ± 1.48 mg/100ml and 150.25 ± 0. 35 mg/100ml respectively. The partially purified exopolysaccharide (EPS) samples were chemically analyzed. The results showed that the temperature and pH have no effect on the protein amount produced by *E. aurantiacum* while the carbohydrate amount varied. The functional groups in the partially purified exopolysaccharides were determined by the FT-IR. Because of its ability to produce large quantities of exopolysaccharides; this bacterium may prove to be an excellent model species for the development of biotechnology products.

***Keywords:*** *exopolysaccharide, exiguobacterium aurantiacum, marchica lagoon, FT-IR*

**Cite This Article:** Y. D. Dah Dossounon, K. Lee, K. Belghmi, F. Benzha, and M. Blaghen, "Exopolysaccharide (EPS) Production by *Exiguobacterium aurantiacum* isolated from Marchica Lagoon Ecosystem in Morocco." *American Journal of Microbiological Research*, vol. 4, no. 5 (2016): 147-152. doi: 10.12691/ajmr-4-5-4.

## 1. Introduction

Many species of bacteria possess the ability to synthesize the polysaccharides in the form of a capsule surrounding the cell (capsular polysaccharides, CPS) or completely excreted into the environment (exopolysaccharides, EPS). It has been shown that bacterial EPS provide protection from various environmental stresses such as desiccation, effect of antibiotics and predation [12]. Exopolysaccharides generally consist of monosaccharides and some non-carbohydrate substituents (such as protein, nucleic acids, lipids). In recent years, the EPS are positioned as a privileged field of investigation because of the variety of their chemical structures and their many properties. The functional properties of bacterial exopolysaccharides have been demonstrated in a wide range of applications, including pharmaceuticals, environment [1,14], the biosorption of heavy metals [16], food products, cosmetics and bionanotechnology. Several researchers are carried on the EPS production by the bacteria and their biotechnological applications. However, it is important to study the optimal culture conditions for a good production of exopolysaccharides, because factors such as the culture medium, pH, temperature and agitation influence the production of EPS and their composition. The influence of the initial pH of the culture medium on the EPS production by several bacteria has been clearly demonstrated [20].

*Exiguobacterium aurantiacum* is an alkaliphilic extremophile [10]. Extremophiles can be used in various applications due to the presence of rare enzymatic activities which enable them to gain resistance to xenobiotics; to produce exopolysaccharide and biosurfactants [18]. *E. aurantiacum* has been reported to degrade aliphatic hydrocarbon, phenol, pyridine, naphtaléne [17]. Many authors have studied the biodegradation of diverse organic compounds by *Exiguobacterium aurantiacum* and their extracellular biosurfactant production. However, only a few studies have been carried on EPS production by *E. aurantiacum.* The relationship between exopolysaccharide production and the physiology and growth of the organism has not been studied.

This work aims to establish the yield of exopolysaccharide (EPS) in *E. aurantiacum* isolated from Marchika lagoon in Morocco. The influence of the incubation temperature and pH on the amount of EPS were determined for the first time. To understand better the characteristics of EPSs and thus be able to apply them successfully to biotechnological ends it is essential to optimize their production.

## 2. Material and Methods

### 2.1. Microorganism and Culture Media

*Exiguobacterium aurantiacum* was isolated from water samples taken from the lagoon of Marchica from Morocco.

This bacterium was isolated in the context of the project of the 7th PCRD research ULIXES « Unraveling and exploiting Mediterranean Sea microbial diversity and ecology for the xenobiotics and pollutants cleanup » in Morocco. Bacterial identification was done by sequence method using ***GenElute™ Bacterial Genomic DNA Kit*** and ***ABI 3130xl*** Genetic Analyzer. The identification of isolated strain was performed by direct sequencing of PCR amplified 16S rDNA gene fragments. The bacterium have been purified and maintained in glycerol at -20°C.

For EPS detection, experiments were conducted using Petri dishes containing the nutrient agar and the bacterium was maintained at 37°C for 48h. The appearance of the bacterium and mucoid character is visually detected. The second test is the coloration with ruthenium red. The detection of mucoid character is made according to the method of Bouzar. The color of the bacterium on the ruthenium nutrient agar indicates if the strain is productive of EPS or not [4].

For the evaluation of EPS production, the bacterial strain was cultivated in YPMG medium. Composition per liter: Glucose: 20g; Peptone: 5g; yeast extract: 5g; $(NH_4)_2S0_4$: 0,6g; $KH_2PO_4$: 3,18g; $K_2HPO_4$: 5,2g; $MgSO_4$: 0,3g; $CaCl_2$: 0,05g; $ZnSO_4$: 0,2mg; $CuSO_4$: 0,2mg; $MnSO_4$: 0,2mg; $FeSO_4$: 0,6mg).

## 2.2. Culturing Procedures

To establish the kinetics of EPS production, 200mL of the YPMG medium was placed in 500ml Erlenmeyer flasks and 1mL of 16 hours bacterial pre-culture was inoculated. The culture was maintained at a temperature of 37 ° C, pH 7 for 6 days. Some samples are taken during culture to monitor microbial growth and EPS production. 20 ml of culture was taken each time for EPS extraction in 50ml centrifuge tubes and the cell was dried and weighed.

To establish the influence of physicochemical parameters, we assayed the following variables: incubation temperature (30°C, 37°C, and 40°C); initial pH (6, 7, 8, 9 and 10) and incubation either in a rotating shaker (100 rpm) for 4 days. All Experiments were done using 250 ml flasks each containing 100ml of the YPMG medium. 1mL of 16 hours bacterial pre-culture was inoculated. The test was done in triplicate.

## 2.3. Analytical Determinations

*E. aurantiacum* growth was measured based on the dry weight per 100ml of the culture. The cell dry weight (CDW) was determined by centrifugation (8600 x g , 4°C, 30min) followed by drying to a constant weight in an oven at 100°C overnight.

EPS was quantified by dry weight determinations by the method of Castellane *et al*. [8] modified. Briefly, the culture was centrifuged (8600 x g, 4°C) and the supernatant was filtrated, precipitated with cold 96% ethanol at a 1:3(v/v) supernatant: ethanol ratio. The mixture was refrigerated at 4°C overnight. After this period, the samples were centrifuged once again (8000 x g, 4°C) to separate the precipitate from the solvent. The precipitated product was dried at 37°C. For the purification, The EPS was dissolved in the solution of NaCl (1M and 0,5M) respectively. It was re-precipitated with two volumes of absolute ethanol and centrifuged (8000g, 4°C, 20 min). This operation was carried out four times. The solvent precipitation achieved a partial purification of the polymer by eliminating the soluble components of the culture media. The precipitated product was dried at 37°C until a constant weight was observed, and a precision balance used to verify the quantity of EPS obtained. The weight of the EPS is expressed in milligrams per 100 ml of culture (mg / 100ml). The values shown for EPS were calculated by subtracting the amount of background interference in uninoculated medium (approximately 10mg of carbohydrate/100ml) from the amount in fermented broth. This test was triplicated.

For the estimation of carbohydrate and protein content in the EPS, Polysaccharide sample (4 mg in 1 ml of distilled water) was placed in a 100- by 15-mm screw-top glass tube, then 1 ml trifluoroacetic acid 8 N was added, and the tube was tightly capped (Teflon-lined cap) and heated for 2 h at 100°C. The hydrolyzed sample was then cooled to 25°C and uncapped, and samples were dried under reduced pressure for 48 h until only a residue remained [7]. The total carbohydrate content was estimated by phenol sulphuric acid method proposed by Dubois [13]. The amount of protein present in the hydrolyzed EPS was estimated by Bradford method [5].

Samples of purified EPS were prepared for I.R. analysis using Perkin-Elmer FT-IR instrument. One part of extract was mixed with ninety nine parts of dried potassium bromide (KBr) separately and then compressed to prepare a salt disc of 3 mm diameter. These discs were subjected to IR-spectra measurement in the frequency range of 400 and 4000 $cm^{-1}$ [25].

# 3. Results

We reported in this study, for the first time, the quantification of exopolysaccharides produced by *Exiguobacterium aurantiacum* according to the pH and temperature of incubation. The 16S rDNA gene sequence of the strain was carefully studied by refereeing to the GenBank database using a BLAST search and was revealed to be identical to *Exiguobacterium aurantiacum* (GenBank Accession N° NR_113666.1).

## 3.1. Phenotypic Evaluation

We focused primarily on the choice of media that a promoted high *Exiguobacterium aurantiacum* EPS yields, because factors such as the culture medium, influences the production of EPS. We found that *E. aurantiacum* grows and produces a significant amount of EPS using glucose as carbon source over the yeast extract and peptone in the liquid medium. This carbon source is inexpensive and easy to obtain and has shown satisfactory results in the production of exopolysaccharides by *E. aurantiacum* strain.

Our observations of *E. aurantiacum* growth and EPS production showed that the colonies of the strain were large, bright and mucoid on nutrient agar (results not shown). The mucoid colonies formed long, viscous filaments when picked with a platinum loop. The colonies were then grown on nutrient agar containing the ruthenium red (pink color) and observed after 24 and 48 hours of incubation. The coloration test showed that the bacterium had a white color on the nutrient agar + ruthenium red (pink color) (results not shown). This

indicates that *E. aurantiacum* has the ability to produce exopolysaccharides.

## 3.2. Growth and EPS Production

To study the synthesis of the EPS as a function of the growth phase, *E. aurantiacum* was grown in YPMG medium. Cell dry weight and exopolysaccharide production are determined during fermentation for six days at 37 °C, 100 rpm and pH 7 without control over pH, as shown in Figure 1. The bacterium has a latency period of 6 hours and then enters to exponential phase. The stationary phase starts from 72h. The kinetics of the EPS production by *E. aurantiacum* showed that the exopolysaccharide is produced during the exponential phase increasingly and reached a maximum value of 0.65 mg/ml after 48h. During the stationary phase, the quantity of EPS in the culture decreased at 60% after 72 hours of incubation (0.4mg / ml).

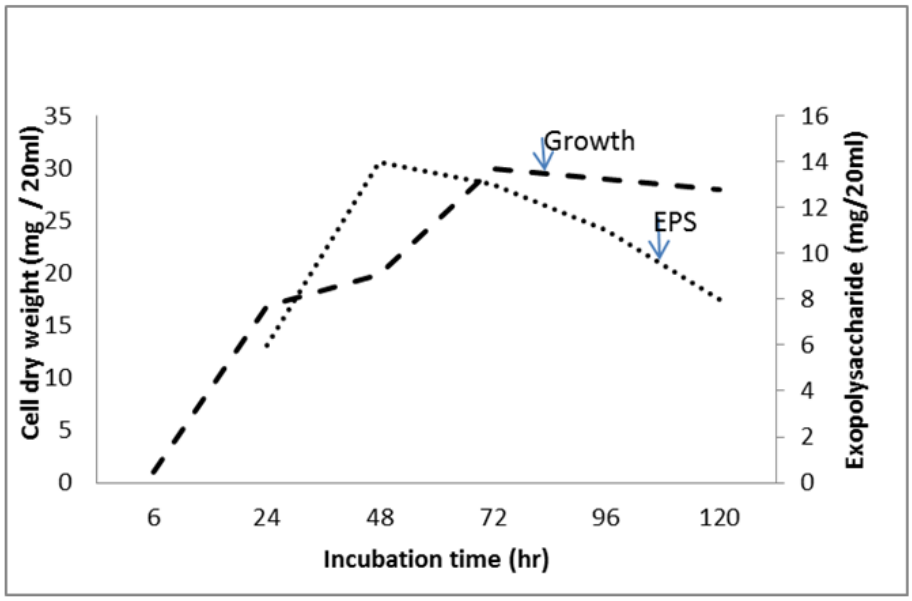


**Figure 1**. Profile of EPS production and cell dry weight by *Exiguobacterium aurantiacum* in YPMG medium (pH 7, 37°C)

We studied the influence of different cultural parameters in order to be able to improve EPS production by *E. aurantiacum strain*. The dry biomass and isolated EPS were weighed and the values obtained are presented in Table 1 and Table 2.

**Table 1. Evaluation of the influence of pH in the exopolysaccharide production and cell dry weight in *Exiguobacterium aurantiacum* strain isolated in Morocco**

| Strain | pH | EPS | Cell dry weight(CDW) | EPS/CDW |
|---|---|---|---|---|
| | | | mg/100ml (mean ± SD) | |
| *Exiguobacterium aurantiacum* | 6 | 26,5 ±0,70 | 58,35 ± 1,90 | 0,45 ± 0 |
| | 7 | 75,2 ±0,28 | 52,55 ± 0,77 | 1,43±0,028 |
| | 8 | 259,05±1,48 | 150,25 ± 0,35 | 1,72±0,014 |
| | 9 | 0 ± 0 | 27,8±2,54 | 0 ± 0 |
| | 10 | 0 ± 0 | 52,4±1,69 | 0 ± 0 |

Mean values (±standard deviation) culture conditions: YPMG medium, 37°C, 100rpm, and 4 days.

**Table 2. Evaluation of the influence of the temperature in the exopolysaccharide production and cell dry weight in *Exiguobacterium aurantiacum* strain isolated in Morocco**

| Strain | Temp (°C) | EPS | Cell dry weight(CDW) | EPS/CDW |
|---|---|---|---|---|
| | | | mg/100ml (mean ± SD) | |
| *Exiguobacterium aurantiacum* | 30 | 57,6 ±1,97 | 43 ± 0 | 1,33±0,049 |
| | 37 | 75,2 ±0,28 | 52,55 ± 0,77 | 1,43±0,028 |
| | 40 | 52,5±0,35 | 40 ± 0 | 1,31±0,007 |

Mean values (± standard deviation) YPMG medium, pH 7, 100 rpm, 4 days.

To evaluate the influence of pH, we grew *E. aurantiacum* in YPMG at 37°C and initial pH 6, 7, 8, 9, and 10. The amount of exopolysaccharides varies considerably in different culture settings. The best production was at pH 8 (259.05 ± 1. 48 mg/100ml) (Table 1) under the conditions used in this study. There was no production in the pH 9 and 10. To evaluate the influence of temperature, we grew *E. aurantiacum* in YPMG at pH 7 at temperatures of 30°C, 37°C and 40°C. The best production was obtained at 37°C (75. 2 ± 0. 28 mg/ 100ml) (Table 2). Low EPS production for *E. aurantiacum* strain is at the temperature of 40 °C.

The value of cellular biomass at pH 8 (150.25 ± 0. 35 mg/100ml) is higher than that of other pH. One note worthy result was that YPMG supported growth at pH 9 and 10 but the cells did not produce exopolysaccharides (Table 1). For the temperatures tested, the value of the biomass at 37° C is higher than that of 30°C and 40°C.

The temperature and pH for the higher production of EPS by *E. aurantiacum* is the same as the optimal growth temperature and pH (Table 2).

We evaluated the specific yield of EPS production. This yield is given by the ratio of the total EPS to the cellular biomass. The best yield was obtained at pH 8 (1.72 ±0,014) followed by pH 7 (1.43±0,028) and pH 6 (0. 45 ± 0). 37 ° C (1.43 ±0.028) is the temperature at which there is a higher production of EPS followed by 30°C (1.33±0.049) and 40°C (1.31±0.007).

## 3.3. Compositional Characterization

Here, Carbohydrate and protein estimation in the hydrolyse EPS was done (Figure 2). The optical density for carbohydrate was higher at pH 8 (2.08 ± 0. 02 mg/100ml) than that of pH 7 (1.377 ± 0.0 1 mg/100ml) and pH 6 (0. 40 ± 0. 003 mg/100ml ). For the influence of température, the optical density for carbohydrate was 0.4 ± 0.06 mg/100ml at 30°C, 1.377 ± 0.01 mg/100ml at 37°C and 0.60 ± 0.06 mg/100 ml at 40°C. The amount of Carbohydrate was higher at pH 8 and 37°C than other pH (6 & 7) and temperatures (30°C & 40°C). One noteworthy result was that the amount of protein was the same (0.32 ± 0.02 mg/100ml) for different pH and temperatures. The temperature and pH have no effect on the protein amount produced by *E. aurantiacum* while the carbohydrate amount varied.

Figure 2 shows the Infrared (I.R.) spectroscopy of the isolated EPSs produced from E. *aurantiacum* at pH (6, 7 & 8) and temperature (30°C, 37°C & 40°C). The results demonstrate shows that there is no significant difference in I.R. spectra of the EPSs produced at different culture conditions. Many functional groups were observed: H-bonded hydroxyl groups which are typical for exopolysaccharides were found (3600-3200 and 1050-1100 cm $^{-1}$). The list of the bands at 500-950 cm $^{-1}$ is present. Polysaccharides C-O-C and C-O-P was at 1100 cm $^{-1}$, absorption at 1000 cm $^{-1}$ was typical for glucose in pyranose form.

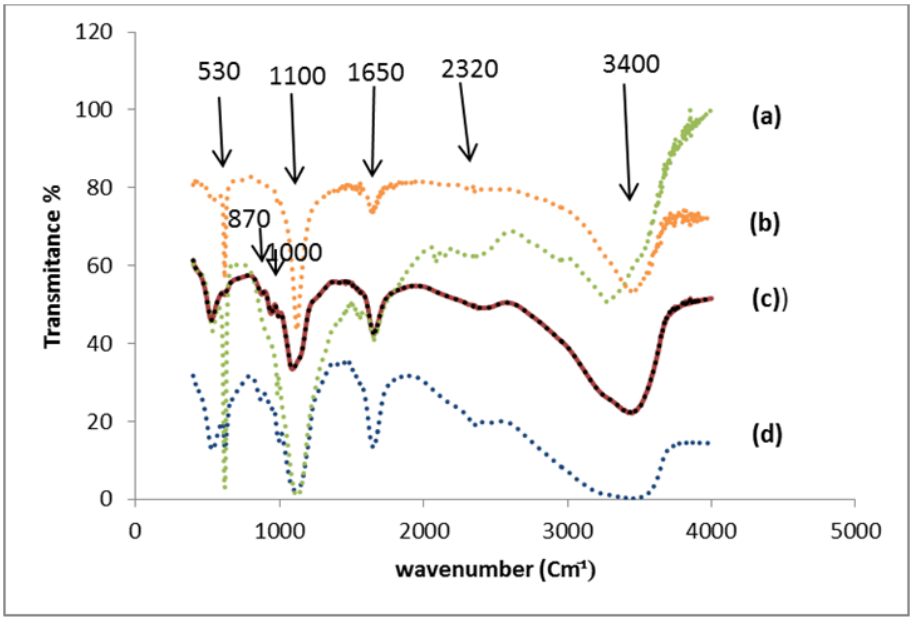


**Figure 2**. Comparative FT-IR spectra of polysaccharides: EPS 30°C **(a),** EPS 40°C **(b);** EPS 37°C & EPS pH 7 **(c)** , EPS pH 8 & EPS pH 6 **(d)**

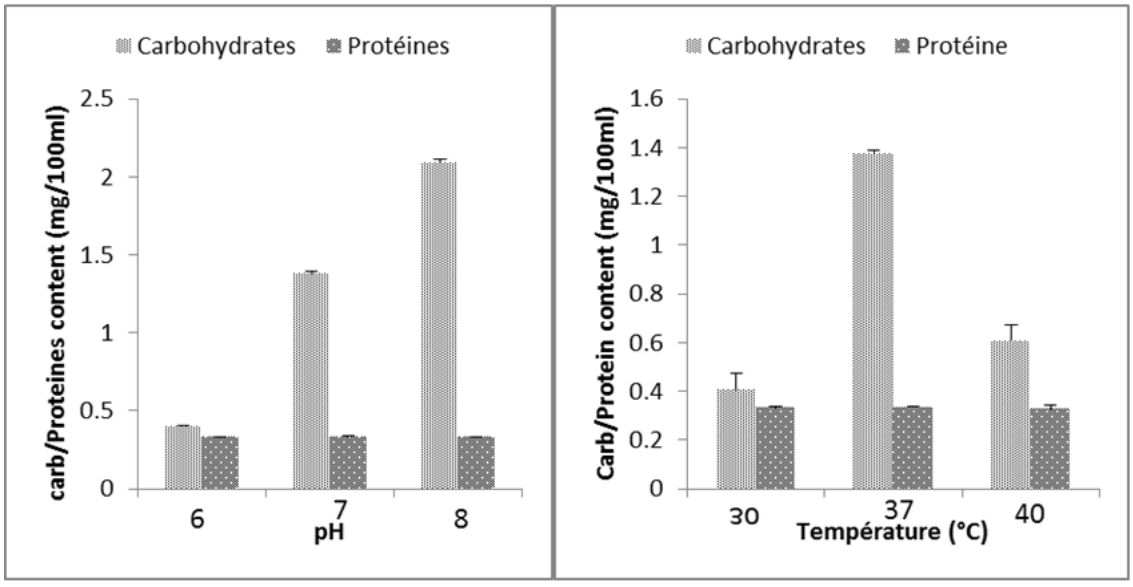


**Figure 3**. Estimation of carbohydrates and protein content of *E. aurantiacum* exopolysaccharide as a function of pH and temperature

## 4. Discussion

We report here for the first time the influence of the incubation temperature and pH on the growth and the EPS production by *E. aurantiacum* isolated from Marchica lagoon in morocco. *E. aurantiacum* reveals a mucoid character on the nutrient agar. In general, the nature of mucoid bacteria can be detected in different ways or by visual inspection on an agar medium, UV light [8], the electron microscopy or coloration [19]. The bacterium has a white color on the nutrient agar containing ruthenium red (pink color). In fact, the ruthenium red is a cationic colorant which gives a pink color to the culture medium. The polysaccharides (anionic) of the mucoid bacteria mask the pink color and it appears white on the pink medium. Non-mucoid colonies are pink. Bouzar et al selected strains of *Lb. delbrueckiissp. Bulgaricus* producing different levels of extracellular polysaccharides by the ruthenium coloration test [4].

The fermentation profile of *E. aurantiacum* strain is characterized by an increase in the EPS production during the exponential phase and a decrease during the stationary phase. The EPS production by *E. aurantiacum* strain exhibited a fermentation kinetic similar to that of Liamas [19]. Under optimal growth conditions, the production of EPS starts during the exponential phase and increased concomitantly with the rise in number of viable cells. The highest quantity of EPS was obtained at 48h. These results are similar to those obtained by Boukahil and Czuprynski [3], who reported that a strain of *M. haemolytica* forms a robust biofilm at 37 °C, with maximal biofilm formation at 48 h. The decrease of amount of polysaccharides during the stationary phase of growth could be due to the activation of a glycohydrolase, degrading the polymers as suggested by Pham *et al* [23].

Microorganisms belonging to *Bacilli* have been known to produce exopolysaccharides. *Exiguobacterium aurantiacum* is an alkaliphilic extremophile [10] belonging to the *Bacilli* class. Extremophiles can be used in various applications due to the presence of rare enzymatic activities which enable them to produce exopolysaccharides [18]. It has been reported that the production of EPS is a response to the nutrient composition of the growth medium. In the present study, High level of EPS production was achieved by *E. aurantiacum* in YPMG medium. In fact, the YPMG medium is a rich medium containing the yeast extract, peptone and glucose. The yield of EPS could be strongly associated with the yeast extract concentration [2]. These results agree with those of some other authors. Other bacterium, such as *Serratia* produced the EPS on mannitol glutamate medium with yeast extract [15]; *Serratia ficaria* are also capable to produce the EPS under optimized conditions of yeast glucose medium [14]. Other bacterial species are known for their high production of EPS such as *S. meliloti* and *Rhizobium tropici* strains, e.g., *S. meliloti* SU-47 exhibited a maximum EPS yield of 7.8 g.L−1 under optimized conditions of yeast mannitol medium [6] . The MUTZC3 mutant of *R. tropici* and Rhizobial isolate JAB6 exhibited the EPS productions (5.52 ± 0.36 and 5.06 ± 0.20 g.L−1 EPS, respectively) in liquid medium PSYL containing the yeast extract [8]. In general, the pH, the temperature and the carbon source influence the production of EPS [11,21]. In the present study, the best EPS production was obtained at pH 8 and 37°C under the conditions used.

As a result, from the present study it is evident that the *E. aurantiacum* strain can be considered as potential microbial cell factories for EPS production. In the present study, low EPS production is at the temperature of 40 ° C. According to Sutherland, reduction of the cultivation temperature by 10°C below optimal level inhibits the EPS biosynthesis by microbial cells. However, under low temperature of growth, environment profiles of the high productivity of exopolysaccharide occur by bacterial cells [25]. The synthesis of polysaccharides by the *E. aurantiacum* strain would also be associated with growth. The polymers are mainly synthesized during cell growth, and once the exponential phase of growth completed, the EPS production would stabilize and decrease. De Vuyst *et al.* [11] also observed that the biosynthesis of EPS associated with growth in *Sc. Thermophilus* result of a direct relationship between optimal growing conditions and yields higher EPS. Polymer production is inversely proportional to the bacterial growth index, in general, which suggests a regulatory relationship between the bacterial metabolism and catabolism in which, up to some point on the growth curve, the cells do not invest in carbon skeletons for growth, to the detriment of their metabolic activity [8].

With regard to the chemical composition of our EPS, the amount of total carbohydrates in the EPS varies depending on the culture conditions used. However, the amount of protein produced is the same. The amount of carbohydrates in the EPS is higher than that of proteins. In general, the EPS of most bacteria consists of a polysaccharide [24]. These results are similar to those who showed that the EPS of other members of the family Bacillus, including. *B. subtilis*, was found in large part to consist of polysaccharides with lesser amount of proteins [25]. Future experiments could be performed to identify the osidic composition and the molecular weight of these cell molecules.

The bacterial EPS extracts at different pH and temperature gave characteristics bands for EPS. Here, carbonyl (C=O) stretching peak and OH stretching peak was at broad and the maximum peak and the band at 1000-1500 cm $^{-1}$ showed the presence of polysaccharide. Similar finding was also recorded by Osman et al [22].

## 5. Conclusions

In conclusion, the pH and temperature influence the exopolysaccharide production by *Exiguobacterium aurantiacum* isolated from Marchica lagoon in morocco. Under optimum growth conditions, *E. aurantiacum* has the ability to produce significant quantities of exopolysaccharides. This hypothesis is supported by the results of the EPS quantification and FT-IR analyses of the studied EPS. This bacterium may prove to be an excellent model species for the development of biotechnology products. We are currently studying its chemical structure and therefore its biotechnological applications.

## Acknowledgments

This study is part of ULIXES Project (Number 266473) “Unravelling and exploiting Mediterranean Sea microbial diversity and ecology for Xenobiotics and polluants clean up”, funded by the seventh framework programme (FP7-KBBE-2010-4). We acknowledge the Marchica agency for their invaluable assistance during the sampling phase of the research project.

## References

[1] Aguilera, M., Quesada, M.T., del Aguila, V.G., Morillo, J.A., Rivadeneyra, M.A., Ramos-Cormenzana, A. and Monteoliva-Sa´nchez, M, “Characterization of *Paenibacillus jamilae* strains that produce exopolysaccharide during growth on and detoxification of olive mill wastewaters”, *Bioresource Technology* , 99. 5640-5644. January 2008.

[2] Bonet, R., Simon-Pujol, M.D., Congregado , F, “Effects of Nutrients on Exopolysaccharide Production and Surface Properties of *Aeromonas salmonicida”*, *Applied and environnemental microbiology*, 59 (8). 2437-2441. August 1993.

[3] Boukahil, I., Czuprynski, C.J, “Characterization of *Mannheimia haemolytica* biofilm formation in vitro”, *Veterinary Microbiology*, 175. 114-122. November 2014.

[4] Bouzar, F., Cerning, J., Desmazeaud, M, “Exopolysaccharide production in milk by *Lactobacillus delbrueckii* ssp. *bulgancus* CNRZ 1187 and by two colonial variants”, *Journal of Dairy Science*, 79. 205-211. October 1995.

[5] Bradford, M.M, “A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding”, *Anal. Biochem*, 72. 248-254. January 1976.

[6] Breedveld, M.W., Zevenhuizen, L.P.T.M., Zehnder, A.J.B, “Osmotically induced oligo- and polysaccharide synthesis by *Rhizobium meliloti* SU-47”, *Journal of General Microbiology*, 136. 2511-2519. August 1990.

[7] Bryan, B.A., Linhardt, R.J., Daniels, L, “Variation in Composition and Yield of Exopolysaccharides Produced by *Klebsiella* sp. Strain K32 and *Acinetobacter calcoaceticus* BD4”, *Applied and Environnemental Microbiology*, 51. 1304-1308. June 1986.

[8] Castellane, T.C.L., Lemos, M.V.C., Lemos, E.G.M, “Evaluation of the biotechnological potential of *Rhizobium tropici* strains for exopolysaccharide production”, *Carbohydrate Polymers*, 111. 191-197. April 2014.

[9] Cerning, J., Renard, C.M.G.C., Thibault, J.F., Bouillanne, C., Landon, M., Desmazeaud, M, and Topisirovic L, “Carbon source requirements for exopolysaccharide production by *Lactobacillus casei CG11* and partial structure analysis of the polymer”, *Applied and Environnemental Microbiology* , 60. 3914–3919. November 1994.

[10] Collins, M.D., Lund, B.M., Farrow, J.A., Schleifer, K.H, “Chemotaxonomic study of an alkaliphilic bacterium *Exiguobacterium aurantiacum* gen-nov sp-nov”, *Journal of General Microbiology*, 129. 2037-2042. January 1993

[11] De Vuyst, L., Vanderveken, E., Van de Ven, S., Degeest, B, “Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis”, *Journal of Applied Microbiology*, 84. 1059-1068. November 1998

[12] Donot, F., Fontana, A., Baccou, J.C., and Schorr-Galindo, S, “Microbial exopolysaccharides: Main examples of synthesis, excretion genetics and extraction”, *Carbohydrate Polymers*, 87. 951-962. January 2012

[13] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F, “Colorimetric method for determination of sugars and related substances”, *Anal. Chem*, 28. 350-356. March 1956

[14] Gong, W.X., Wang, S.G., Sun , X.F., Liu, X.W.,, Yue, Q.Y., Gao, B.Y, (2008) “Bioflocculant production by culture of *Serratia ficaria* and its application in wastewater treatment”, *Bioresource Technology*, 99. 4668–4674. January 2008

[15] Ipper, N.S., Cho, S., Lee, S.H., Cho, J.M., Hur, J.H., and Lim, C.K., “Antiviral Activity of the Exopolysaccharide Produced by *Serratia* sp. Strain Gsm 01 against Cucumber Mosaic Virus”, *Journal of Microbiology and Biotechnology*, 18(1). 67-73. July 2008.

[16] Iyer, A., Mody, K., Jha, B, “Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*”, *Marine Pollution Bulletin*, 49. 974–977. December 2004.

[17] Jeswani, H., Mukherji, S, “Batch studies with *Exiguobacterium aurantiacum* degradind structurally diverse organic compounds and its potential for treatment of biomass gasification wastewater”, *International Biodeterioration & Biodegradation*, 80.1-9. March 2013.

[18] Kalin, M., wheeler, W.N., Meinrath, G, “The removal of uranium from mining waste water using algal/microbial biomass*”*, *Journal of Environmental radioactivity*, 78. 151-177. January 2005.

[19] Llamas, I., Mata, J.A., Tallon, R., Bressollier, P., Urdac, M.C, Quesada, E and Béjar, V, “Characterization of the Exopolysaccharide Produced by *Salipiger mucosus* A3T, a Halophilic Species Belonging to the *Alphaproteobacteria*, Isolated on the Spanish Mediterranean Seaboard”, *Marine Drugs*, 8. 2240-2251. July 2010

[20] Mozzi, F., Savoy de Giori, G., Oliver, G., Font De Valdez, G, “ Effect of culture pH on the growth characteristics and polysaccharide production of *Lactobacillus casei”*, *Milchwissenschaft*, 12 (49). 667-670. 1994.

[21] Mozzi, F., Savoy de Giori, G., Oliver, G., Font de Valdez, G, “Exopolysaccharide production by *Lactobacillus casei*. II. Influence of the carbon source”, *Milchwissenschaft*, 50. 307-309. 1995.

[22] Osman, M.E., El-Shouny, W., Talat, R., El-Zahaby, H, “Polysaccharides production from some Pseudomonas syringae pathovars as affected by different types of culture media”, *Journal of microbiology Biotechnology & food sciences*, 1(5). 1305-1318. 2012.

[23] Pham, P.L., Dupont, I., Roy, D., Lapointe, G., Cerning, J, “Production of exopolysaccharide by *Lactobacillus rhamnosus* and analysis of its enzymatic degradation during prolonged fermentation”, *Appl. Environ. Microbiol*, 66. 2302-2310. 2000

[24] Sandal, I., Inzana, T.J., Molinaro, A., De Castro, C., Shao, J.Q., Apicella, M.A., Cox, A.D., St Michael, F., Berg, G, “Identification, structure, and characterization of an exopolysaccharide produced by *Histophilus somni* during biofilm formation”, *BMC Microbiol*, 11. 186. August 2011

[25] Vijayabaskar, P., Babinastarlin, S., Shankar, T., Sivakumar, T., Anandapandian, K.T.K, “Quantification and Characterization of Exopolysaccharides from *Bacillus subtilis* (MTCC 121)”, *Advances in Biological Research*, 5(2). 71-76. January 2011.