Journal of Microbiology, Biotechnology and Food Sciences

Osman et al. 2012 : 1 (5) 1305-1318

REGULAR ARTICLE

POLYSACCHARIDES PRODUCTION FROM SOME *PSEUDOMONAS SYRINGAE* PATHOVARS AS AFFECTED BY DIFFERENT TYPES OF CULTURE MEDIA

Mohammed El-Anwar Osman[1], Wagih El-Shouny[1], Ragdah Talat[2], Heba El-Zahaby[1]*

Address: [1]Botany Department, Faculty of Science, Tanta 31527, Egypt
[2]Microbiology Department, Faculty of Medicine, Tanta 31527, Egypt

*Corresponding author: elshounyw@yahoo.com

ABSTRACT

Exopolysaccharides (EPS) produced from *Pseudomonas syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 were studied as affected by different types of culture media. The I.R. spectroscopy data of purified polysaccharides from these organisms were compared with authentic algal alginate sample. The results show presence of many functional groups in the obtained EPS which are: H- bonded hydroxyl group typical for polysaccharide (1050- 1100 and 3200-3600 nm) as well as C-H (at 1380-1450 cm and 2930 cm). The presence of bands at 2000-2800 nm region indicated the presence of free carboxyl groups. The spectrum showed a band at 948.5 nm, which assign to the C–O stretching vibration of uronic acid residues, and one at 888.3 nm assigned to the C1–H deformation vibration of β-mannuronic acid residues and also a band at 820.0 nm was detected. The obtained results indicate that purified EPSs from the tested organisms have great similarity with the authentic algal alginate sample. Thus, EPSs produced by *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 could be identified as alginate.

Keywords: Exopolysaccharides, *Pseudomonas syringae* pathovars, alginate

INTRODUCTION

Polysaccharide molecules may be more intimately associated with the cell surface either through linkage to a lipid-moiety, as in the case of lipopolysaccharide (LPS) molecules in Gram-negative bacteria **(Lam et al., 1992)** or linked to cell-wall techoic acid as in Gram-positive bacteria **(Roberts, 1995).** Bacterial exopolysaccharides are extensively used as thickening and gelling agents in a wide range of industrial products and processes due to their structural and physical properties diversity **(Copetti et al., 1997; Rinaudo, 2001).** Pneumococcal polysaccharide vaccine was effective for the prevention of pneumonia, which caused by *Streptococcus pneumoniae*, among old adults **(Jackson et al., 2003).** Many strains of *Streptococcus thermophilus* synthesize extracellular polysaccharides, these molecules produced as capsules that are tightly associated with the cell, or they may be liberated into the medium as a loose slime (i.e., "ropy" polysaccharide). The presence of exopolysaccharide does not confer any obvious advantage to growth **(Broadbent et al., 2003).** The maximum amount of EPS produced by *Streptococcus thermophilus* 1275 was 406 $mg.L^{-1}$ in 10 % reconstituted skim milk (RSM) in a Biostat B fermentor at 37 °C after 24 h of fermentation, the EPS production increased when the organism was grown in 10% reconstituted skim milk (RSM) for 24 h and supplementation with whey protein concentrate, the level of EPS increased to 1029 mg/L **(Zisu and Shah 2003).** The exopolysaccharide (EPS) production in shake flask cultures of *Phellinus linteus* KCTC 6190 at 30 °C was achieved in a medium contained 50 $g.L^{-1}$ of sucrose as carbon source **(Hwang et al., 2003)**. EPS production from *Lactobacillus* L191 increased when grown in the presence of sucrose **(Dykes et al., 1995)**. Polysaccharide production by *Agaricus blazi* AB 2003 was enhanced in medium containing yeast extract as the best nitrogen source for both biomass and polysaccharide biosynthesis **(Liu and Wang, 2009)**. *Staphylococcus epidermidis* polysaccharide production is increased during growth in iron-limited medium and under conditions of low oxygen availability. Additionally, stress-inducing stimuli such as heat, ethanol, and high concentrations of salt increase the production of polysaccharide. The same environmental conditions are known to repress tricarboxylic acid (TCA) cycle activity, leading to the hypothesis that altering TCA cycle activity would affect polysaccharide production **(Vuong et al., 2005)**. The purified EPS non-ropy strain of *Leuconostoc* sp. CFR 2181 contained 84% of total carbohydrates, 11.2% of reducing sugars, 2% of moisture, 0.8% of proteins and 0.6% uronic acid. The EPS consisted mainly of glucose (91%) with minor quantities of rhamnose and arabinose (1.8%) **(Vijayendra et al., 2008)**. The mucoid phenotype of *Pseudomonas* spp. is due to

overproduction of alginate and is a considerable virulence factor contributing to the intractability of infections most notably in cystic fibrosis (CF) lung, but also in pathogenic infections of plants **(Scanlan and Buckling 2011).**

The production of polysaccharide by specific organisms could be affected by different environmental factors surrounding the organisms and / or the growth medium. Thus, the aim of this study was to isolate the polysaccharides produced by some Gram-negative bacteria in pure form and studying the influence of different growth media on their production, and determination of their structure and protein content.

MATERIAL AND METHODS

In a preliminary experiment, 24 strains of Gram–negative bacteria were studied for their EPS production by using different culture media. The strains were belonged to *Pseudomonas aeruginosa, Eschericha coli, Klebsiella pneumoniae, Azotobacter vinelandii, Erwinia carotovora* and *Pseudomonas syringae.* The obtained data showed that *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 showed high values of the produced EPS and relative viscosity. Therefore, the polysaccharides production by these strains was intensively studied.

Bacterial isolates

Pseudomonas syringae strains (*P. syringae* pv. *tomato*, *P. syringae* pv. *coriandericola* 908, *P. syringae* Davson 973, *P. syringae* 1914, *P. syringae* 1223 and *P. syringae* 307) were provided from bacteriology lab, Faculty of Science, Tanta University, Egypt.

Screening for EPS production

The following media were used for cultivation of the tested cultures and for production of ESP.

1- Medium 1 (M1) : Nutrient agar medium **(Difco Manual, 1974).**

2- Medium 2 (M2): D-glucose mineral medium as suggested by **Larsen and Haug (1970).**

3- Medium 3 (M3): King'B medium **(King et al., 1954).**

4- Medium4 (M4): Starch-nitrate medium **(Difco Manual, 1974).**

Production of EPS

Pseudomonas syringae strains were grown in liquid nutrient medium (M1) separately and incubated for 24 hours at 30 ˚C**.** Sterilized Petri plates containing the designated media for each strain were inoculated with 1 ml of cultures of the tested strains having optical density of 1 at 660 n.m. The plates were incubated at 30 ˚C for 3 days, the mucoid surface growth were scraped off onto 25 ml of sterilized saline and centrifuged at 5000 g for 20 min. The precipitated bacterial cells were subjected to lyophilization in order to determine their dry weight. Protein was precipitated by addition of 10% TCA to the supernatant. The clear supernatant was subjected to ethanol precipitation by adding 3 volume of cold 95% ethanol with stirring as described by **Evans and Linker (1973)**. Crud EPS were recovered by centrifugation at 4500 g for 15 min., then the precipitated EPS were dried at 40 °C to estimate their dry weight.

Effect of different culture media on EPS production

The three selected strains of *Pseudomonas syringae* pv *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 which showed the highest growth and EPS production were tested separately on different solid media (Nutrient, Mineral, King`B and Starch-nitrate) to select the most suitable media for growth and EPS production.

Purification of EPS

The free-cells supernatant were obtained from the bacterial culture and subjected to purification step to obtain the purified EPS as follow:

Ethanol precipitation

To precipitate proteins from supernatant, 20 ml tricloroacetic acid 10 % were added to the sample **(Van den Berg et al., 1995)** and the mixture was allowed to stand overnight. The precipitated proteins were removed by centrifugation at 5000 g for 30 min. The clear supernatant was collected and the EPS was precipitated by adding 3 volumes of cold 95 % ethanol and allowed to stand overnight at 2˚C. To remove the remaining undissolved materials, the EPS was redissloved in distilled water and precipitated by ethanol for at least

three times. Finally the resulting precipitate was dissolved in distilled water and subjected to a fractional precipitation with ethanol. The EPS precipitates were collected by centrifugation at 4500g for 30 min, then dried to obtain dry weight. The total carbohydrate, uronic acid and protein contents were determined.

Chemical characterization of EPS

The partially purified polysaccharide (EPS) samples were chemically analyzed. Uronic acid content was determined by carbazol assay as described by **Bitter and Muir (1962)**. Some alternative methods were applied for uronic acid quantification (**Blumenkrantz and Asboe-Hansen, 1973; Filisetti-Cozzi and Carpita, 1991)**. The glucose content was determined by phenol-sulfuric acid method as described by **Dubois et al. (1956).** Total sugars of EPS were quantified by anthrone method (**Helbert and Brown, 1955)**. The protein content of the produced EPS was estimated according to the method described by **Lowry et al. (1951)**. Other methods can be used for protein determination depending on the laboratory facilities. The measurement of microgram quantities of protein was acheived by **Bradford (1976)**. Quantification of protein using bicinchoninic acid was described by **Smith et al. (1985)**.

Infra-red spectroscopy (I.R.)

Samples of purified EPS were prepared for I.R. analysis according to the method described by **Sherborock-cox et al. (1984)**. One mg of the purified EPS was used in I.R. analysis by using salt discs. A mixture made by adding 1mg of EPS samples to 300 mg of pure dried KBr followed by pressing into disc, the whole I.R. spectrum (1000 – 4200 nm) was compared with a known polysaccharide pressed also together with KBr into disc. The spectra were recorded using Perkin-Elmer-1430 spectrophotometer.

Viscosity determination

The Viscosity of the purified polysaccharides was determined by viscometer using distelled water as standard **(Hardling, 1998)**.

Statistical analysis

One way and two way analyses of variance (ANOVA) were used according to **SPSS, (1999)**. In the present work, each value presented in tables is mean of three reading ± the standard deviatin (SD). The values are highly significant (**) at p≤ 0.001. The least significant difference is abbreviated as LSD.

RESULTS

Effect of different solid media on EPS production

The three selected strains *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 were cultivated on nutrient medium (M1), glucose-mineral medium (M2), King′ B medium (M3) and starch-nitrate medium (M4), separately, at 30˚C for 3 days.

Table 1 shows that the most suitable medium for exopolysaccharide production by *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* was King′B medium (M3).

Table 1 Effect of different solid media on EPS production by *Pseudomonas syringae* strains grown at 30 ˚C for 3 days of incubation

Media	***Pseudomonas syringae* strains**					
	P. syringae* pv. *tomato		***P. syringae* Davson 973**		***P. syringae* pv. *coriandricola* 908**	
	EPS $g.g^{-1}$ D.wt	Relative viscosity	EPS $g.g^{-1}$ D.wt	Relative viscosity	EPS $g.g^{-1}$ D.wt	Relative viscosity
M1	0.2 ± 0.1	1.1 ± 0.1	0.1 ± 0.0	1.0 ± 0.0	0.1 ± 0.1	1.0 ± 0.1
M2	0.3 ± 0.1	1.3 ± 0.1	0.1 ± 0.1	1.1 ± 0.1	0.1 ± 0.1	1.0 ± 0.1
M3	1.4 ± 0.1	2.5 ± 0.1	1.2 ± 0.1	2.4 ± 0.1	1.5 ± 0.1	2.6 ± 0.1
M4	1.1 ± 0.1	1.6 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	1.1 ± 0.1	2.1 ± 0.1
F-value	121.896**	133.6**	153.771**	187.664**	142.030**	278.792
LSD	0.178	0.168	0.168	0.177	0.188	0.158

Legend: EPS- Exopolysaccharide, M1- Nutrient agar medium, M2- D-glucose mineral medium, M3- King'B medium, M4- Starch-nitrate medium
The values are highly significant (**) at p≤ 0.001
The least significant difference is abbreviated as LSD

EPS Characterization

Table 2 shows that the highest content of protein (15%) and uronic acid (59%) were recorded in *P. syringae* pv. *coriandricola* 908. On the other hand, the contents of the total carbohydrate and total hexose showed more or less similar values in the three tested strains.

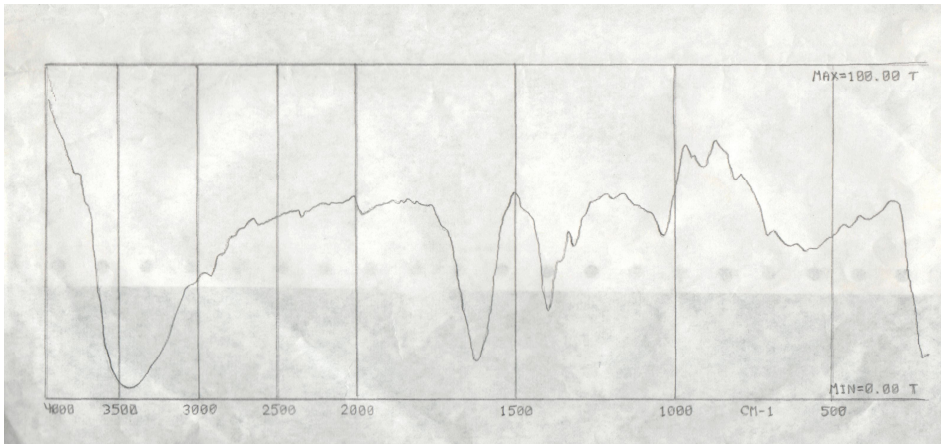
Table 2 Chemical analysis of EPS preparations produced by *Pseudomonas syringae* pathovars grown on king'B medium at 30 ˚C for 3 days of incubation

Bacterial EPS	Protein (%)	Total carbohydrates (%)	Uronic acid (%)	Total hexoses (%)
P. syringae **pv. *tomato*-EPS**	7.0	15.0	29.0	24.0
P. syringae **Davson 973-EPS**	12.0	17.0	45.0	27.5
P. syringae **pv. *coriandericola* 908-EPS**	15.0	16.5	59.0	27.2

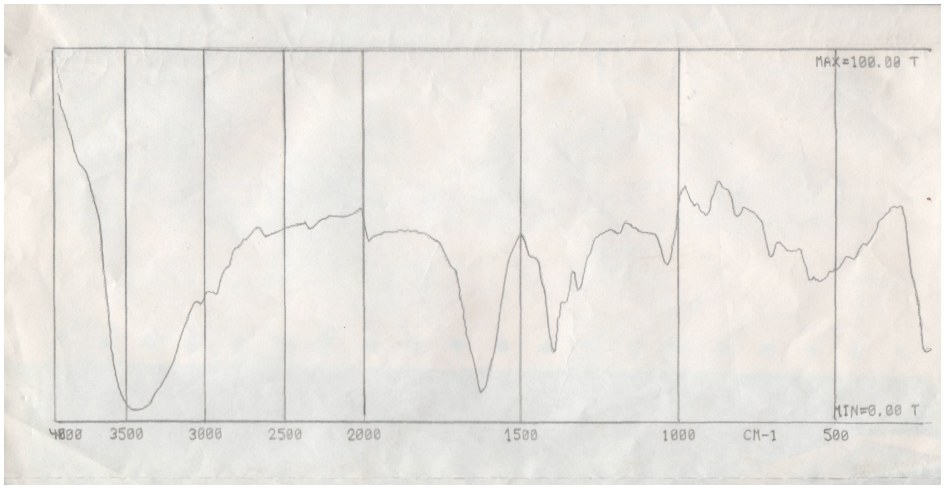
Legend: EPS- Exopolysaccharide

Fig.1 shows the Infrared (I.R.) spectroscopy of the isolated EPSs produced from *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 as compared with algal alginate as authentic sample. The results demonstrate that there is no significant difference existed could be observed in I.R. spectra of the EPSs produced by the three different strains and the authentic algal alginate sample. Many functional groups were observed: H-bonded hydroxyl groups which are typical for exopolysacchrides were found (3600-3200 and 1050-1100 nm) as well as C-H groups (at 2930 and 1380-1450 nm). The presence of band at 2000-2800 nm region indicates the presence of free carboxylic groups. The peaks at about 1400 and 1610 nm are related to the presence of carboxylate function as expected for uronic acid salts. The strong bands at 1735 and 1250 nm are indicative for ester groups. The band at 820 nm seems to be characteristic of mannuronic acid residues. According to the obtained data, the exopolysacchrides produced by *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 could be defined as alginate.

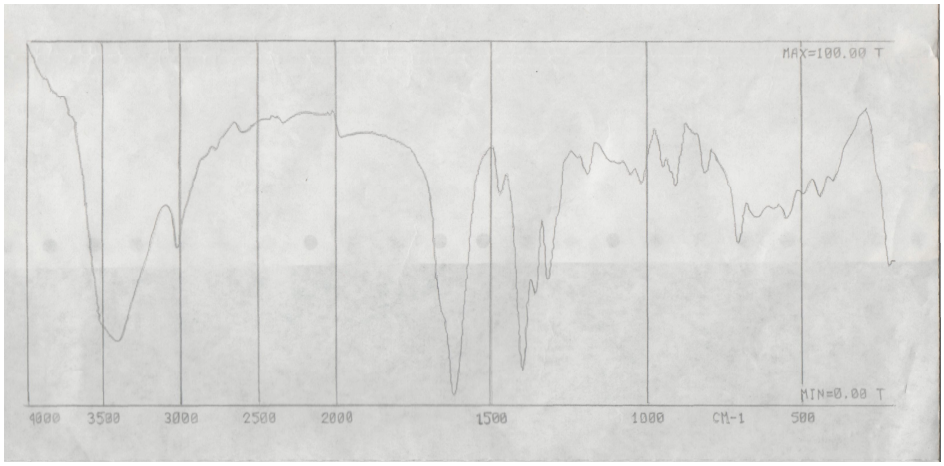
(A)


MAX=100.00 T
MIN=0.00 T
4000 3500 3000 2500 2000 1500 1000 CM-1 500

(B)


MAX=100.00 T
MIN=0.00 T
4000 3500 3000 2500 2000 1500 1000 CM-1 500

(C)


MAX=100.00 T
MIN=0.00 T
4000 3500 3000 2500 2000 1500 1000 CM-1 500

(D)

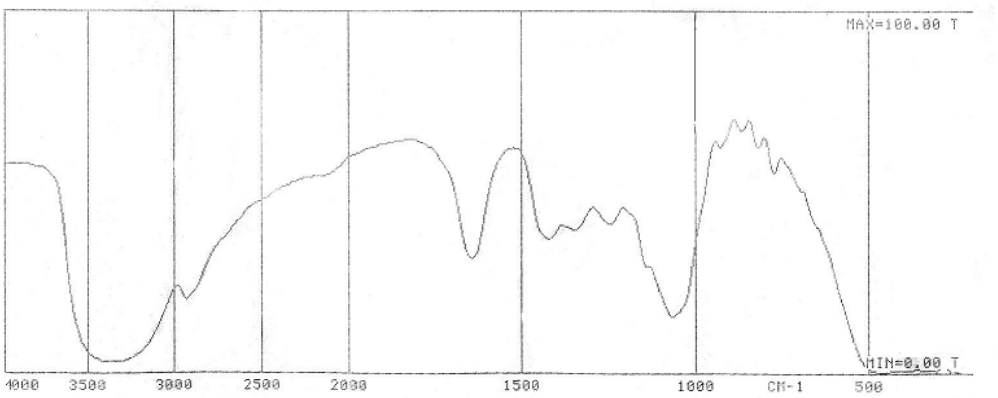


Figure 1 I.R. spectra of exopolysaccharides (EPS) of *P. syringae* pv. *tomato* (A), *P. syringae* Davson 973 (B), and *P. syringae* pv. *coriandricola* 908 (C) as compared with algal alginate (D) as authentic sample

DISCUSSION

The present study concentrated on EPS production from some Gram-negative bacteria as affected by the components of the growth medium. The results obtained show that the King'B medium designed by **King et al. (1954)** was the most suitable medium for the biosynthesis of viscous EPS by the tested organisms. The highest EPS production and relative viscosity (1.5 $g.g^{-1}$ D.wt and 2.6, respectively) were obtained by *Pseudomonas syringae* pv. *coriandricola* 908 grown on King'B medium. The relationship between the EPS production and culture media depend mainly on the type of producing microorganisms. In this concern, **Zisu and Shah (2003)** noted that, the maximum amount of EPS produced by *Streptococcus thermophilus* 1275 was 406 $mg.L^{-1}$ in 10 % reconstituted skim milk (RSM) in a Biostat B fermentor at 37°C after 24 h of fermentation. Also, **Vijayendra et al. (2008)** reported that the fermentation of non-ropy strain of *Leuconostoc* sp. in shake flask containing modified MRS broth for 72 h at 22°C, the EPS produced was 13.8 $g.L^{-1}$.

Obtained results (Table 2) showed different values of uronic acid, total carbohydrates, hexoses and proteins for the three tested *Pseudomonas* strains. This indicates that the production of specific EPS by a bacterium species is strain dependant. High uronic acid contents of EPSs produced by the three *P. syringae* strains refer to the presence of alginate in the obtained polymers.

Carboxylated sugars like uronic acid formed important fraction of bacterial EPS. The uronic acid content of *P. syringae* pv. *coriandricola* 908 EPS was 59% of the polymer by weight. This was relatively greater than EPS of fouling bacteria like *Halomonas* strains which contained 0.6-11.1% **(Bejar et al., 1998)**. Lower uronic acid content was observed in the purified EPS produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181. The polymer contained 84% of total carbohydrates, 11.2% of reducing sugars, 0.8% of proteins and 0.6% uronic acid (**Vijayendra et al., 2008)**.

The purified exopolysaccharides which obtained from *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 were identified using the I.R. spectroscopy which compared with authentic algal alginate sample. The results showed presence of many functional groups in the tested EPS (Fig 1 A, B, C and D) similar to that present in authentic algal alginate sample. Thus, I.R. spectra of the purified EPS samples proved the presence of carboxyl group, which may serve as binding sites for divalent cations. The carboxyl group may also work as functional moieties to generate new or modified polymer variants using different approaches like novel. Similar finding was also recorded by **Vijayabaskar et al. (2011).** Thus, EPSs produced by *P. syringae* pv. *tomato*, *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908 could be identified as alginate. Similar results were also obtained by **Bejar et al. (1998)** who found that the exopolymer of *Halomonas eurihalina* strain H 96 contained significant amounts of uronic acid. The band at 820.0 cm^{-1} seems to be characteristic of mannuronic acid residues as reported by **Chandıa et al. (2004)**. The mucoid phenotype of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* SBW25 is due to overproduction of alginate and is a considerable virulence factor contributing to the intractability of infections most notably in cystic fibrosis (CF) lung, but also in pathogenic infections of plants **(Scanlan and Buckling 2011).**

CONCLUSION

It could be concluded from the above mentioned data that King′B medium (M3) was the most suitable medium for EPS production from the three selected strains (*P. syringae* pv. *tomato***,** *P. syringae* Davson 973 and *P. syringae* pv. *coriandricola* 908). The produced EPSs have great similarity to the authentic algal alginate. This is particularly true of the results obtained from viscometry, uronic acid quantification and I.R. spectra. Therefore, the polymers could be industrially useful. This is, however, dependent on the cost, effectiveness of extraction and purification. These promising results could also be regarded as initiatory steps

towards the utilization and modification of EPSs as future cheap sources for the production of valuable drugs with antioxidant and anticancer properties.

Acknowledgments: For supplying the phytopathogenic bacterial strains, the authors gratefully acknowledge Prof. Wagih El-Shouny. This work was supported by the Bacteriology Laboratory, Faculty of Science at Tanta University, Egypt.

REFERENCES

BEJAR, V. – LLAMAS, I. – CALVO, C. – QUESADA, E. 1998. Characterization of exopolysaccharides produced by 19 halophilic strains of the species *Halomonas eurihalina. Journal of Biotechnology,* vol. 61, 1998, no. 2, p. 135-141.

BITTER, T. – MUIR, H.M. 2003. A modified uronic acid carbazole reaction. *Analytical Biochemistry,* vol. 4, 2003, p. 330-334.

BLUMENKRANTZ, N. – ASBOE-HANSEN, G. 1973. New method for quantitative determination of uronic acids. *Analytical Biochemistry,* vol. 54, 1973, p. 484–489.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Analytical Biochemistry,* vol. 72, 1976, no. 1–2, p. 248–54.

BROADBENT, J. R. – MCMAHON, D. J. – WELKER, D. L. – OBERG, C. J. – MOINEAU, S. 1962. Biochemistry, Genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*. *Journal of Dairy Sciences,* vol. 86, 1962, p. 407-423.

CHANDIA, N.P. – MATSUHIRO, B. – VASQUEZ, A. E. 2004. Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. *Applied Phycology,* vol. 6, 2004, p. 127-133.

COPETTI, G. – GRASSI, M. – LAPASIN, R. – PRICL, S. 1997. Synergic gelation of xanthan gum with locust bean gum: a rheological investigation. *Journal of Glycoconjugate*. vol. 14, 1997, no.8, p. 951-961.

DIFCO MANUAL. 1974. Difco manual of dehydrated culture media and reagent for microbial and laboratory producers 9th edition, 1974.

DUBOIS, M. – GILLES, K.A. – HAMILTON, J.K. – REBERS, P.A. – SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry,* vol. 28, 1956, p. 350-356.

DYKES, G. A. – GEORNARAS, A. – VON HOLY A. 1995. Advantages of sucrose-dependent extracellular polysaccharide production to lactic acid bacteria in sucrose-rich environments. *Letters in Applied Microbiology,* vol. 21, 1995, no. 5, p. 327 – 329.

EVANS, L. R. – LINKER, A. 1973. Production and characterization of the slime polysaccharide of *Pseudomonas aeurginosa*. *Journal of Bacteriology,* vol. 116, 1973, p. 914-923.

FILISETTI-COZZI, T.M.C. - CARPITA, N.C. 1991. Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry,* vol. 197, 1991, p. 157-162.

HARDLING, S.E. 1998. The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solutions. *Progress in Biophysics and Molecular Biology*, Amsterdam: Elsevier, vol. 68, 1998, p. 207-262.

HELBERT, J. R. – BROWN, K. D. 1955. Factors influencing quantitative determination of methyl pentose and ketohexoses with anthrone. *Analytical Chemistry,* vol. 27, 1955, p. 1791-1796.

HWANG, J. H. – KIM, S. W. – CHOI, J. W. – YUN, J. W. 2003. Production and characterization of exopolysaccharides from submerged culture of *Phellinus linteus* KCTC 6190. *Journal of Enzyme and Microbial Technology,* vol. 33, 2003, no. 2-3, p. 309-319.

JACKSON, L.A. – NEUZIL, K.M. – YU, O. – BENSON, P. – BARLOW, W. E. – ADAMS, A. L. – HANSON, C.A. – MAHONEY, L.D. – SHAY, D. K. – THOMPSON, W.W. 2003. Effectiveness of *Pneumococcal* polysaccharide vaccine in older adults. *Journal of Medicine,* vol. 348, 2003, no. 18, p. 1747-1755.

KING, E. O. – WARD, M. K. – RANEY, D. E. 1954. Two simple media for the demonstration of pyrocyanin and flurescein. *Journal of Laboratory and Clinical Medicine,* vol. 44, 1954, p. 301-313.

LAM, J. S. – HANDELSMAN, M. Y. – CHIVERS, T. R. – MACDONALD, L. A. 1992. Monoclonal antibodies as probes to exam serotype- specific and cross-reactive epitopes of lipopolysaccharides from serotypes O2, O5, and O16 of *Pesudomonas aeurginosa*. *Journal of Bacteriol*ogy, vol. 174, 1992, no. 7, p. 2178-2184.

LARSEN, B. – HAUG, A. 1970. Biosynthesis of alginate. Part I. Composition and structure of alginate produced by *Azotobacer vinelandii* (Lipman). *Carbohydrates,* vol. 17, 1970, p. 287-296.

LIU, G. Q. – WANG, X. L. 2009. Selection of a culture medium for reducing costs and enhancing biomass and polysaccharide production by *Agaricus blazei* AB 2003. *Food Technology and Biotechnology,* vol. 47, 2009, no. 2, p. 210-214.

LOWRY, O. H. – ROSEBROUGH, N. J. – GARR, A. L. – RANDALL, R. J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry,* vol. 193, 1951, p. 256-275.

RINAUDO, M. 2001. Relation between the molecular structure of some polysaccharides and original properties in sol and gel states. *Food Hydrocolloids,* vol. 15, 2001, no. (4-6) , p. 433-440.

ROBERTS, I. S. 1995. Bacterial polysaccharides in sickness and in health. *Journal of Microbiology,* vol. 141, 1995, p. 2023- 2031.

SCANLAN, P. D. – BUCKLING, A. 2011. Co-evolution with lytic phage selects for the mucoid phenotype of *Pseudomonas fluorescens* SBW25. *The International Society for Microbial Ecology Journal,* vol.174, 2011, Abstract.

SHERBOROCK-COX, V. S. – RUSSE, N. J. – GACESA, P. 1984. The purification and chemical characterization of the alginate present in extracellular material produced by mucoid strains of *Pseudomonas aeurginosa*. *Carbohydrate Research,* vol. 135, 1984, p. 147-154.

SMITH, P.K. – KROHN, R.I. – HERMANSON, G.T. – MALLIA, A.K. – GARTNER, F.H. – PROVENZANO, M.D. – FUJIMOTO, E.K. – GOEKE, N.M. – OLSON, B.J. – KLENK, D.C. 1985. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry,* vol. 150, 1985, no. 1, p. 76–85. Erratum in: *Analytical Biochemistry,* vol.163, 1987, no. 1, p. 279.

SPSS. 1999. SPSS Base of 10.0 users guide. *Spss inc,* 1999.

VAN DEN BERG, D.J.C. – ROIJN, G.W. – JANSEEN, A.C. – GIUSEPPIN, M.L.F. – VREEKER, R. – KAMERLING, J.P. – VLIEGENTHART, J.F.G. – IEDEBORE, A.M., – VERRIPS, C.T. 1995. Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of polysaccharide. *Applied and Environmental Microbiology,* vol. 61, 1995, no. 8, p. 2840-2844.

VIJAYABASKAR, P. – BABINASTARLIN, S. – SHANKAR, T. – SIVAKUMAR, T. – ANANDAPANDIAN, K.T.K. 2011. Quantification and characterization of exopolysaccharides from *Bacillus subtilis* (MTCC 121). *Advances in Biological Research*, vol. 5 ,2011, no. 2 , p. 71-76.

VIJAYENDRA, S. V. N. – PALANIVEL, G., – MAHADEVAMMA, S. – THARANATHAN, R.N. 2008. Physico-chemical characterization of an exopolysaccharide produced by a non-ropy strain of *Leuconostoc* sp. CFR 2181 isolated from *dahi*, an Indian traditional lactic fermented. *Carbohydrate Polymers,* vol. 72, 2008, no. 5, p. 300- 307.

VUONG, C. – KIDDER, J. P. – JACOBSON, E. R. – OTTO, M. – PROCTOR, R. A. – SOMERVILLE, G. A. 2005. *Staphylococcus epidermidis* polysaccharide intercellular adhesin

production significantly increases during tricarboxylic acid cycle stress. *Journal of Bacteriology,* vol. 187, 2005, no. 9, p. 2967–2973.

ZISU, S. P. – SHAH, M. J. 2003. Effects of exopolysaccharide producing strains of *Streptococcus thermophilus* on technological and rheological properties of set-type yoghurt. *International Dairy Journal*, vol. 13, 2003, no. 11, p. 1344-1352.