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# PRODUCTION OF EXOPOLYSACCHARIDES BY *Halomonas eurihalina* $F_{2-7}$ AND *Xanthomonas campestris pv. Campestris* FROM SALTED WHEY

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## ABSTRACT

Salted whey produced from the Egyptian Domiati cheese (soft chees) after supplementing with carbon and nitrogen sources was used for production of exopolysaccharide (EPS) by *Halomonas eurihalina* $F_{2-7}$ and *Xanthomonas campestris pv. campestris*. The effect of whey pH, salt percent, sugars and non-protein nitrogen contents and the microorganism inoculum size and fermentation temperature and period on microorganism growth and EPS yield and their dry mutter and sugars content were studied.

Fermentation medium components which produces maximum cells growth and EPS yield varied between both microorganisms. *H. eurihalina* $F_{2-7}$ (3.0% inoculum) produced maximum EPS yield (2.8 g/L) when the whey contained 7.0% salt, supplemented with mannitol (10 g/L) and yeast extract (6 g/L) and the pH was 7.2 at 32°C for 10 days. While *X. campestris pv. campestris* (3.%inoculum) resulted in 13.6 g/L EPS (~ 5 times of Halomonas EPS) when grown in whey which its protein was hydrolyzed (≥67.15% hydrolysis ratio), salted with 3.0% NaCl, and supplemented with 3.0% sucrose at pH 7.5 for 5 days under shaking (200 rpm/min) at 25°C. Sugars content of the EPS was also affected by the growth medium and the produced organism. Glucose and mannose appeared to be the principal sugars but with varying ratios.

The EPS yield followed the trend of its cells growth and the yield reached the maximum at the microorganism stationary phase. Both microorganisms differed in their EPS yield and their effect on fermentation pH. While *H. eurihalina* $F_{2-7}$ increased the pH from 7.2 to 7.95, *X. campestris pv. campestris* reduced the pH from 7.5 to 6.65.

**Keywords:** Exopolysaccharides (EPS), H. eurihalina $F_{2-7}$, *X. campestris pv. campestris*, Salted whey, Carbon & Nitrogen sources, Fermentation conditions, EPS yield and composition

## INTRODUCTION

For many years, interest has concentrated on polysaccharides produced by numerous microorganisms such as *Xanthomonas campestris*, *Halomonas eurihalina* and *Alcaligenes viscosus* as well as certain strains of lactic acid bacteria for their economic importance accompanied with increasing their applications in food, pharmaceutical, agricultural, and chemical industries (Franz, 1989, Ooi and Liu, 2000 and Cohen *et al.,* 2002). Microbial exopolysaccharides (EPSs) are extra-cellular polysaccharides that are either associated with, and often covalently bound to, the cell surface in the form of capsules or secreted into the environment in the form of slime (Cerning *et al.* 1994).The amount and composition of EPS produced by bacterial cultures are often linked to biomass production (De Vuyst & Degest, 1999). Attempts have been made to improve such cultures yield by

manipulating fermentation conditions such as, medium composition (Briezinski& Roberts, 2002 and Chi & zheo, 2003), initial pH (shu & Lung, 2004), incubation temperature (Degeest *et al.* 2002) and period (Abd El-Gawad *et al.*, 2001).

In Egypt, enormous quantities of salted whey are produced from Domiati cheese making as a by-product. This whey has high organic components having high chemical oxygen demand (COD) of ~ 70g/L, which often causes disposal problems for cheese manufacturere (Marshal, 1982). In addition, the high salt is strong obstacle for its us in the preparation of other dairy and food products. Therefore, a new high value-added products and technologies are necessary for the dairy industry to decrease the expenses of waste disposal (Yang and Silva, 1995). Thus, utilizing salted whey as a fermentation medium for production of EPS by salt tolerant bacterial strains is an attractive approach.

The objective of this study was to adjust the salted whey composition and the growth conditions for maximum production of EPS by *Halomonas eurihalina* $F_{2-7}$ which are reported to be salt tolerant (Bejar *et al.* 1998) and *Xanthomonas campestris pv. campestris*.

## MATERIALS AND METHODS

**1. Exopolysaccharides (EPS) producing cultures:**

The EPS-producing *Halomonas eurihalina* $F_{2-7}$, (a moderately halophilic strain as reported by Bejar *et al.* 1998) was obtained from EPS Res. Group, Dept. of Microbiology, Faculty of pharmacy, Univ. of Granada, Spain. *Xanthomonas campestris pv. campestris,* was obtained from Agricultural Biotechnology Lab., National Cung Husing Univ., Taichung, Taiwan.

**2. Media**

**2. a. Growth and preservation media:**

Malt yeast glucose proteos peptone (MY) medium (Marain and Rogovin, 1966) as modified by Rodriguzi-Valera *et al.* (1981) for adjusting its salt content at 7.5% using sea salt mixture, and yeast malt glucose peptone (YM) medium (Jeans *et al.* 1976) were used for growth and maintenance of *Halomonas eurihalina* $F_{2-7}$ and *Xanthomonas campestris pv. campestris,* respectively.

**2. b. Fermentation media:**

Defated whey powder (Lactalis industrie (BBA) Bourgbarre, France) was reconstituted to 7.5% TS in warm water. Normal, defated whey (by-product of Domiati cheese) with 7.33% TS was prepared. Protein of reconstituted whey was hydrolyzed to 67.15, 87.02 & 95.1 % hydrolysis degree, (H.D) using 0.02% (w/v) trypsine enzyme (BDH Chemical Ltd. Pool, England) according to Chobert, *et al.*(1988). All whey preparations were salted with 3.0% NaCl (EL-Nasr Co., Alex., Egypt) then were sterilized at 121°C/5 min. Synthetic selective media; MY and mineral medium (MM) prepared according to Jeans, *et al.* (1976) were adjusted at pH 7.2 & 7.5, respectively, then sterilized at 121°C/20 min.

**3. Optimization of fermentation conditions:**

The following points were studied:

1) Initial pH of the medium (6.5, 7.0, 7.2, 7.5 and 8.0) which was adjusted by HCl or NaOH.
2) Carbon source (1, 2& 3% of glucose, fructose, mannitol and sucrose) from Oxid, UK.
3) Whey protein concentration (0.2, 0.4 & 0.6%) by the fortification with whey protein powder (34% protein) from DeMelkind-Ustrie Co., Veghel, Neth. D.
4) Yeast extract (0.2, 0.4 & 0.6%) from Oxiod, UK, or protein hydrolyzed whey (Lab. preparation).
5) Fermentation temperature (25, 30, 32, 35, & 40°C).
6) Fermentation period (1-12 days).
7) Microorganism inoculation level (1,2 & 3%).
8) Salted level (3-9 % NaCl) with the best obtained supplementation and fermentation conditions

**4. Culture growth for EPS production:**

A 250 ml flasks containing 100 ml of sterilized MY broth medium were inoculated with 3.0 ml *H. eurihalina* $F_{2-7}$ and incubated at 32ºC for 8 days. Similar volume of sterilized MM broth medium were inoculated with 3.0 ml of *X. campestris pv. campestris* and incubated at 30ºC for 4 days on a 200 rpm/min. shacker ( Innova 4335 refrigerated incubation shaker, N.Br Sci.Co. Inc., Edison, NJ., USA.). Likewise, unhydrolyzed and hydrolyzed wheys with different carbon or/and nitrogen supplementations were inoculated with various experimental inoculum volumes of either *H. eurihalina* $F_{2-7}$ or *X. campestris pv. campestris* and incubated at different experimental temperatures and periods of fermentation.

**4. a. Extraction of Halomonas EPS:**

Cell-free supernatant fluids were obtained by adding 4% TCA and centrifuged at 20,000 rpm for 15 min under cooling (Sorvall RC. 28S. refrigerator centrifuge) then filtred through filter paper No. 42. The Halomonas EPS was precipitated with three volumes of cold ethanol, collected by centrifugation at 22,000 rpm for 15 min. under cooling, then dried at 55ºC for 4 hrs.

**4. b. Extraction of Xanthomonas EPS:**

Cell-free supernatant fluids were obtained by adding of 4% T.C.A and centrifuged at 3.000 rpm for 15 min. under cooling. Xanthan was precipitated by adding 1 ml saturated KCl and 300 ml cold ethanol to the cell-free supernatant fluids. The precipitated crude xanthan was collected by centrifugation under cooling at 6,000 rpm for10 min., then dried at 55ºC for 4 hrs.

**Analytical Methods:**

Total reduced sugars of dried EPS were determined by phenol sulphuric acid method according to Johan and Abd El-Twab (1957). TN and NPN of whey were determined by the micro-Kjeldahl procedure (AOAC, 1990), then used to calculate the hydrolyzed degree (HD %) of whey

proteins. For determining the content and the relative percentages of EPS sugars, samples were prepared as described by Sebstiani and Zelger (1998), then EPS samples were injected into HPLC (Hewllet Packared, series 050, with HEWLETT-PACKARD R.I. detector HP 1047 A and Biorad aminox HPX-87C column 300 x 6.5 mm.) at 85°C column temperature with deionized water (100%) as a mobile phase at flow rate of 0.8ml/min with standard sugars (sucrose, raffinose, glucose, mannose, and fructose). Retention time and peak area were used for calculations using Hewllet packared data analysis software.

The viable cells of *X.campestris* and *H. eurihalina* were counted on YM and MY agar plates at 30 & 32 °C, respectively for 72 hrs.

**Statistical Analysis:**

The two-way analysis of variance (ANOVA) was performed by running the MSTAT-C (ver.2.10, MSU, USA) Package on a personal computer. The same program was used to analyze two and three Factors Randomized Complete Block Design. The statistical significance of the data was determined by using *P* value at α = 0.001. Linear regressions were used to correlate the measured parameters.

## RESULTS AND DISCUSSION

**Initial pH of fermentation medium:**

The effect of medium pH on EPS production was studied by adjusting the fermentation medium (RSW, 3% NaCl) at pHs from 6.5 to 8.0 and results are illustrate in Fig. (1). EPS production increased with pH increase to reach maximum at pH's 7.2 and 7.5 for *H. eurihalina* $F_{2-7}$ and *X. campestris*, respectively, then declined. Least significant test showed no significant differences at α=0.05 within EPS yields obtained from reconstituted or normal salted whey at pH 6.5 by both cultures . On the other hand, Xanthan yield was highly greater ($p$<0.001) than that of Halomonas EPS at all initial pH wheys (Table, 3a).

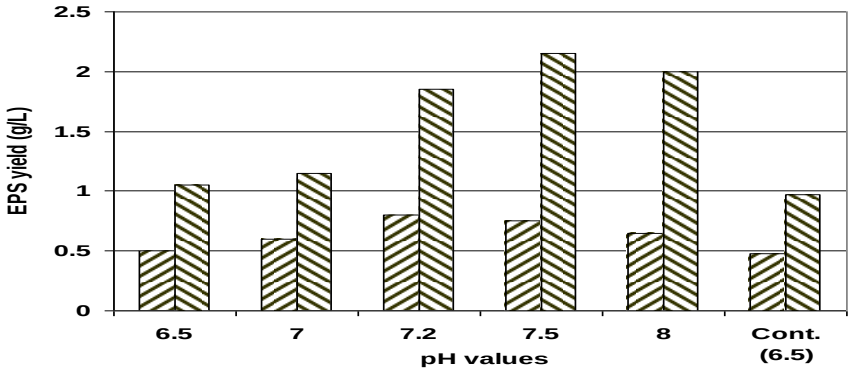



**Fig (1): Effect of initial pH of RSW and NSW (Control) on the production of EPS by *H. eurihalina* $F_{2-7}$ ▨ and *X. campestris* ▨**

At optimum pH's, (7.2 & 7.5) Halomonas and Xanthomonas EPSs yield were increased by 40.0 and 54.9 %, respectively over that of control. The optimal initial pH of EPS production medium ranging from 6.5 to 8.0 was also reported by Pham *et al.* (2000), Lee *et al.* (1999), Esgalhado *et al.* (1995) and Roserio *et al.*(1992) who ascribed that range to the type of bacterial strains and composition of fermentation medium. Throughout this research a pH of 7.2 for *H. eurihalina* F2-7 and 7.5 for *X. campestris* were used.

**Carbon and nitrogen sources:**

Reconstituted salted whey was supplemented with different carbon or / and nitrogen sources at various concentrations. As shown in Table (1), maximum EPS yield of Halomonas and Xanthomonas was 1.57g/L and 3.78 g/L, when mannitol (1.0%) or sucrose (3.0%) were used, respectively. This supplementation enhanced the EPS yield of Halomonas and Xanthomonas by 89.7 & 77.5% over the control, respectively. Likewise, addition of 3.0% sucrose or 2.0% fructose to fermentation medium of Halomonas and Xanthomonas enhanced EPS yield by 81.2 and 73.7%, respectively. Analysis of variance (Table, 3-b) proved that there were significant difference ($p$<0.001) among the EPS yield produced with different types and concentrations of supplemented sugars by both cultures. This may be due to the ability of the organism to use these sugars under fermentation conditions. The obtained results are in agreement with those of Kawahra and Obata (1998); Cerning *et al.* (1994) and Souw and Demain (1979). Therefore mannitol (1.0%) and sucrose (3.0%) were selected for *H. Eurihalina and* and *X.campestris*, respectively.

Not only nitrogen source but also its concentrations significantly ($p$<0.001) increased EPS yield of both cultures (Table, 3-g). Supplementation of RSW with YE or WPC (0.2-0.6 %) as a nitrogen source (Table,1) showed that the highest yield of Halomoans EPS (1.29 g/L) and Xanthomonas EPS (6.55 g/L) was attained with 0.6 % YE or 0.6 % WPC, respectively, which coincided with that stated by Amran and Prigent (1993). They reported that whey often requires nitrogen source supplementation to compensate its lackage of sufficient low M.W nitrogen. YE provides the growth medium with vitamins and nuclic acids base components which make it superior over many purified peptones as a nitrogen source. For example, the amino acid arginin is believed to increase the conversion of α-D-glucose-6-phosphate to α-D-glucose-1-phosphate in E D pathways (Kevel *et al.,* 1984) and can further generate metabolic energy (Konings *et al.*1997). It was also reported that, the growth and EPS yield of *Lb. ramonses* RW-9595 M was stimulated by supplemented whey permeate with YE and some salts (Macedo *et al,* 2002). However, others preferred WPC over YE for its high amino acids content such as glutamic acid (Kalogiannis et al, 2003), this agrees with our results in case of supplementation of the *X. campestris* growth medium with 0.6% WPC ( Table, 1).

According to EPS yield, it is then recommended to supplemented whey with 1.0% mannitol and 0.6% YE for *H. eurihalina* or 3.0% source and ≥67.15% H.W.P. for *X. campestris* as a carbon and nitrogen sources for

inexpensive (Table, 1 & 3-c,d,e). Hydrolysis of WP is a method for supplementation, and providing the medium with important amino acids, reducing its pH fluctuation (has buffering capacity) in addition to its high solubility which, in turn, lead to an increase of xanthan gum production (Stredensky and Canti, 1999). These results agree with those reported by Brizinski and Roberts (2002), Kimeel *et al.* (1998) and Toba *et al.* (1992).

**Table (1): Effect of supplementation of RSW with different carbon & Nitrogen sources on EPS yield produced by *H. eurihalina* & *X. campestris*.**

| Supplementation | (%) | EPS (g/L) | |
|---|---|---|---|
| | | ***H.eurihalina*** | ***X. campestris*** |
| Carbon (C) source | | | |
| Control | 0 | $0.825^{M}$ | $2.13^{G}$ |
| Glucose | 1 | $0.87^{LM}$ | $2.50^{F}$ |
| | 2 | $0.975^{KL}$ | $3.15^{D}$ |
| | 3 | $1.025^{JK}$ | $3.40^{C}$ |
| Fructose | 1 | $0.945^{KLM}$ | $3.60^{B}$ |
| | 2 | $1.20^{I}$ | $3.70^{AB}$ |
| | 3 | $1.205^{I}$ | $3.70^{AB}$ |
| Mannitol | 1 | $1.565^{H}$ | $2.45^{F}$ |
| | 2 | $1.55^{H}$ | $2.70^{E}$ |
| | 3 | $1.44^{H}$ | $3.25^{CD}$ |
| Sucrose | 1 | $1.01^{JK}$ | $3.20^{D}$ |
| | 2 | $1.125^{IJ}$ | $3.35^{C}$ |
| | 3 | $1.495^{H}$ | $3.78^{A}$ |
| Nitrogen (N) Source | | | |
| control | 0 | $0.825^{KL}$ | $2.13^{G}$ |
| YE[a] | 0.2 | $0.83^{KL}$ | $2.80^{F}$ |
| | 0.4 | $0.97^{JK}$ | $3.45^{E}$ |
| | 0.6 | $1.29^{H}$ | $4.55^{D}$ |
| W.P.C[b] | 0.2 | $0.82^{L}$ | $5.65^{C}$ |
| | 0.4 | $0.86^{KL}$ | $5.85^{B}$ |
| | 0.6 | $0.90^{KL}$ | $6.55^{A}$ |
| H.W.P.[c] | 67.15 | $1.10^{IJ}$ | $2.81^{C}$ |
| | 87.02 | $1.14^{I}$ | $2.84^{C}$ |
| | 95.18 | $1.14^{I}$ | $2.89^{C}$ |
| * Best C& N sources | | | |
| control | 0 | $1.495^{C}$ | $3.69^{D}$ |
| [d]C &Y.E. | 0.2 | $1.65\ F^{B}$ | - |
| | 0.4 | $1.725^{B}$ | - |
| | 0.6 | $1.825^{A}$ | - |
| [d]C &W.P.C. | 0.2 | - | $6.73^{C}$ |
| | 0.4 | - | $6.95^{B}$ |
| | 0.6 | - | $7.35^{A}$ |
| [d]C& H.W.P | 0 | $1.495^{B}$ | $3.69^{B}$ |
| | 67.15 | $1.6^{AB}$ | $8.585^{A}$ |
| | 87.02 | $1.63^{A}$ | $8.77^{A}$ |
| | 95.18 | $1.66^{A}$ | $8.685^{A}$ |

*** the Best C& N sources experiment was statistically analyzed separately.**
**[a]Yeast extract** **[b] Whey protein concentrate**
**[c]RSW with partial hydrolyzed protein**
**[d]1.0% mannitol for Halomonas & 3.0% sucrose for Xanthomonas**

**Fermentation conditions:**

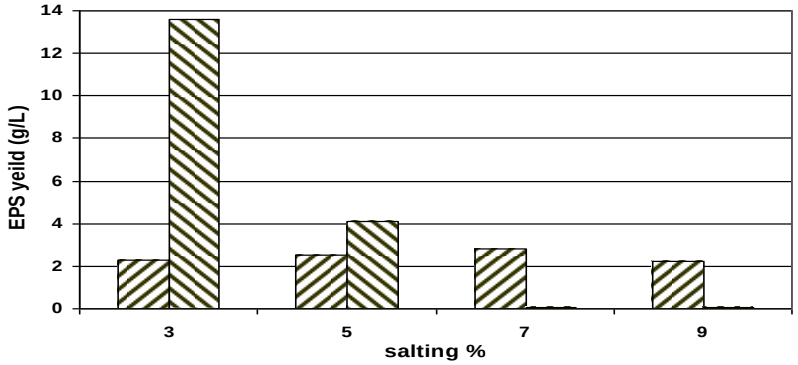
Table (2) showed that the maximum yield of Xanthomonas EPS was attained at half fermentation period required for that of Halomonas EPS (5 vs. 10 days). Beyond that optimum period, EPS yield tend to decrease significantly at 0.05 α level (Table, 3-i) which may be due to an enzymatic degradation for the product (Pham *et al.* 2000). Similar fermentation periods for producing a good yield of Xanthomonas EPS (4 days) and Halomonas EPS (8 days) were also reported (Abd El-Gawad *et al.* 2001; Bejar *et al.* 1998&1996).

Increasing fermentation temperature from 25°C to 40°C greatly decreased ($p$<0.001) the Xanthomonas EPS yield whereas, Halomonas EPS yield was significantly ($p$<0.001) increased (Table, 3-j). This decrease may ascribed to the activity of biosynthesis enzymes included or/and enzymatic degradation under these temperatures (Mata *et al.* 2006; Pham *et al.* 2000). The maximum EPS yield of Xanthomonas and Halomonas was 12.4 and 1.83g/L when fermentation temperature was 25°C & 32°C, respectively (Table, 2). Abd El-Gawad *et al.* (2001) observed that xanthan yield produced in permeate by *X. campestris* decreased at fermentation temperature over 30°C. This may suggested that the optimum temperature for the greatest xanthan yield depends also on the composition of the culture medium.

The EPS yield produced by both cultures was significantly affected ($p$<0.001) by the inoculum volume (Table, 3-h). Xanthomonas EPS yield was maximum at 3.0 % inoculum volume then decreased. However, non significant increase (α=0.05) for Halomonas EPS yield was observed (Table, 2). These results are in agreement with those of Lee *et al.* (2004) who observed that the EPS yield of *Grifala frondosa* was decreased from 2.0g/L to 1.3 g/L (35%) by increasing the inoculum volume from 3 to 6% (v/v). Therefore, 3.0% inoculum volume for both organisms were the optimum.

**Effect of NaCl:**

Both microorganisms differed ($p$<0.001) in their salt tolerance (Table 3-f). while *H. eurihalina* $F_{2-7}$ produced maximum EPS yield at 7.0% salt, *X. campestris pv. campestris* produced the maximum at 3.0% salt and was greatly lowered at 5% salt (Fig, 2).




**Fig(2): Effect of NaCl concentration on the production of EPS by *H. eurihalina* $F_{2-7}$ ▨ and *X. campestris pv.campestris* ▧**

**Table (2): Effect of fermentation conditions on EPS yield produced by *H. eurihalina* & *X. campestris* in compositionally optimized RSW.**

| Fermentation conditions | EPS (g/L) | |
|---|---|---|
| | *H. eurihalina* | *X. campestris* |
| **Temperature (°C)** | | |
| 25 | 0.555 $^{I}$ | 12.4 $^{A}$ |
| 30 | 1.20 $^{G}$ | 8.80 $^{B}$ |
| 32 | 1.813 $^{E}$ | - |
| 35 | 1.645 $^{F}$ | 3.90 $^{C}$ |
| 40 | 0.955 $^{H}$ | 3.60 $^{D}$ |
| Period (day) | | |
| 2 | - | 4.90 $^{E}$ |
| 3 | - | 8.83 $^{D}$ |
| 4 | 0.85 $^{J}$ | 12.40 $^{BC}$ |
| 5 | - | 13.60 $^{A}$ |
| 6 | 1.10 $^{I}$ | 12.30 $^{C}$ |
| 7 | - | - |
| 8 | 1.825 $^{G}$ | - |
| 10 | 2.10 $^{F}$ | - |
| 12 | 1.45 $^{H}$ | - |
| Inoculum size (v/v %) | | |
| 1 | 1.15 $^{E}$ | 8.55 $^{D}$ |
| 3 | 2.10 $^{E}$ | 13.53 $^{A}$ |
| 6 | 2.15 $^{E}$ | 12.45 $^{B}$ |
| 9 | 2.20 $^{E}$ | 11.19 $^{C}$ |
| 12 | 2.30 $^{E}$ | 8.90 $^{D}$ |

**Relationship between cells counts, EPS yield and the pH of fermentation process:**

Fig (3) shows the development of fermentation pH, culture counts and EPS yield during fermentation period. EPS produced during all stages of microorganism growth, but maximum production was almost during the stationary phase. Maximum EPS yield was at 10$^{th}$ day and the 5$^{th}$ for *H. eurihalina* $F_{2\text{-}7}$ and *X. campestris pv. campestris*, respectively. The later microorganism produced EPS concentration 5 times the concentration produced by *H.eurihalina* $F_{2\text{-}7}$. These results are in line with those of Papagiani *et al.* (2001), Bejar *et al.*(1996) and Quesada *et al*.(1993)

Least significant differences test (Table, 3-c,d,e) showed that viable cells of Halomonas were significantly (at 0.05 α level) higher than that of Xanthomonas (16 x10$^{7}$ vs. 7.7 x 10$^{7}$ cfu/ml. at maximum). The type of organism highly correlated with cells count (0.938), EPS yield (0.89) and fermentation pH (0.871)

The fermentation pH increased gradually with *H. eurihalina* $F_{2\text{-}7}$ reaching a pH of 7.95, but decreased by *X. campestris* pv.campestris growth reaching pH 6.65. The optimum pH of harvested xanthan gum was higher than that of its maximum cells count (pH 7.5 vs. pH 6.85). While the optimum pH of maximum Halomonas EPS yield was lower than that of their maximum cells counts respectively, (Fig, 3). This change in the pH may be due to the effect of fermentation metabolites (Becker *et al*., 1998). these results were in accordance with those of Bejar *et al.*(1996) and Quesada *et al.*(1993).

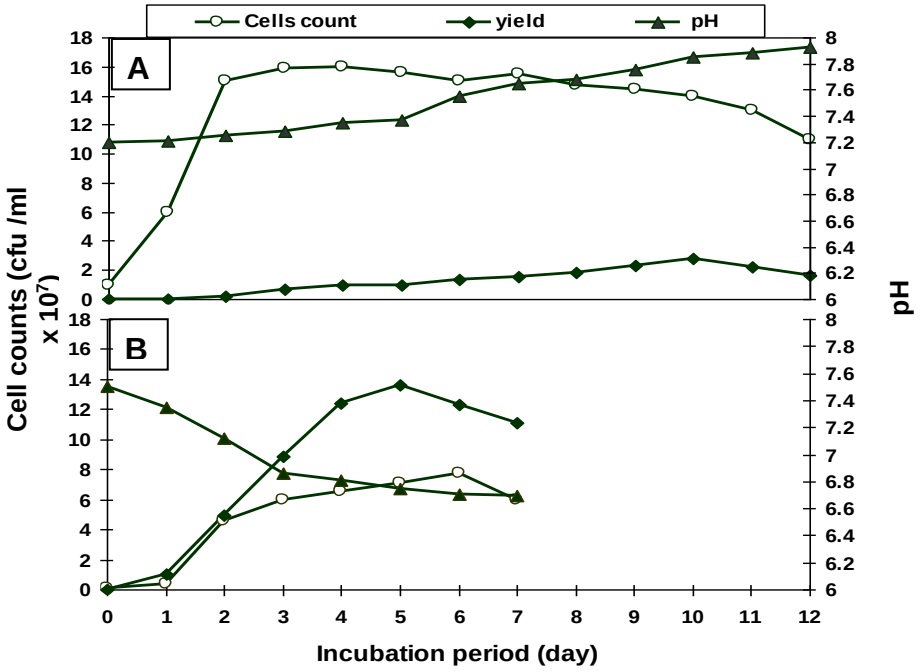



**Fig (3): Relationship between the number of cells, EPS yield and pH of fermentation medium for *H. eurihalina* (A) and *X. campestris* (B) during fermentation process**

**The yield and composition of EPS:**

As reported in Table (4), the EPS yield and the percentage of Dray matter (DM), proteins and carbohydrates were affected ($P$<0.001) by fermentation medium and/or the EPS producing organism. In contrast to Xanthomonas EPS, Halomonas EPS contained higher DM and proteins and lower carbohydrate percentages. In addition, the increase in Halomonas EPS proteins may be due to the link between some amino acids to the polymers (Bejar *et al*., 1998; Kalogiamis *et al*., 2003).

EPS sugars content depended upon the culture and the production medium. However, glucose and mannose seemed as principal natural sugars with different ratios. Halomonas EPS from MY and Xanthomonas EPS from ORSW contained the five sugars (Glucose, Mannose, Sucrose, Fructose and Raffinos) but with different relative percentages. Sugar contents of EPS varied according to the microorganism and the media. These differences, were reported by other workers (Bejar *et al.* (1998), Mata, *et al.*2006 and Calvo *et al.* 1998). Unlike Xanthomonas EPS produced from ORSW, Halomonas EPS of that medium didn't contain sucrose, fructose & raffinos.

**Table (3): Analysis of variance for optimization of RSW and their fermentation process conditions**

| # | Source of Variance | (*P*) | Corr. | $R^2$ | LSD | # | Source of Variance | (*P*) | Corr. | $R^2$ | LSD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (a) | M.O.(A) | *** | o.782 | 0.910 | 0.1410 | (g) | M.O.(A) | *** | 0.405 | 0.717 | 0.1077 |
| | pH (B) | *** | 0.465 | | | | Salt conc. (B) | *** | -0.59 | | |
| | (A x B) | *** | - | | | | (Ax B) | *** | | | |
| (b) | M.O.(A) | *** | 0.888 | 0.948 | 0.1365 | (h) | M.O.(A) | *** | 0.779 | 0.832 | 0.1470 |
| | C. source(B) | *** | - | | | | N. source(B) | *** | | | |
| | (A x B) | *** | - | | | | (A x B) | *** | | | |
| | C.Conc.(C) | *** | - | | | | N. conc.(C) | *** | | | |
| | (A x C) | *** | - | | | | (Ax C ) | *** | | | |
| | (B xC) | *** | - | | | | (B x C) | *** | | | |
| | (A x B x C) | *** | - | | | | (Ax B x C) | *** | | | |
| (c) | M.O.(A) | *** | 0.938 | 0.940 | 0.4603 | (i) | M.O.(A) | *** | 0.954 | 0.955 | 0.5055 |
| | Time(B) | *** | - | | | | volume (B) | *** | | | |
| | (A x B) | *** | | | | | (Ax B) | *** | | | |
| (d) | M.O.(A) | *** | 0.891 | 0.942 | 0.2365 | (j) | M.O.(A) | *** | 0.907 | 0.919 | 0.1599 |
| | Time(B) | *** | | | | | period (B) | *** | | | |
| | (A x B) | *** | | | | | (Ax B) | *** | | | |
| (e) | M.O.(A) | *** | 0.871 | 0.877 | 0.1085 | (k) | M.O.(A) | *** | 0.796 | 0.869 | 0.1616 |
| | Time(B) | *** | | | | | Temp. (B) | *** | -0.34 | | |
| | (A x B) | *** | | | | | (Ax B) | *** | | | |
| (f) | Con.(A) | *** | 0.947 | 0.950 | 0.0893 | (L) | Con.(A) | *** | 0.859 | 0.859 | 0.1413 |

**C.Conc.= Carbon concentration Corr.= Correlation conc.=Concentration Ferment.= Fermentation *** *P*<0.001 N.Conc.= Nitrogen concentration Temp= temperature LSD= Least significant differences C.Source= carbon source $R^2$= Multiple R**

**Table (4): Effect of growth medium and EPS producing culture on the yield and composition of EPS.**

| Growth medium / Yield & EPS Composition | Halomonas-EPS | | | Xanthomonas-EPS | | |
|---|---|---|---|---|---|---|
| | [a]MY | [c]NSW | [d]ORSW | [b]MM | [c]NSW | [d]ORSW |
| **Yield (g/L)** | 2.72 | 0.48 | 2.80 | 13.58 | 0.97 | 13.60 |
| **EPS Composition (%)** | | | | | | |
| **[e]DM (%)** | 97.30 | 93.19 | 94.80 | 91.13 | 88.37 | 90.10 |
| **Protein (%)** | 5.56 | 14.75 | 11.75 | 1.95 | 7.07 | 8.81 |
| **Carbohydrate (%)** | 32.72 | 21.89 | 25.92 | 56.61 | 31.53 | 29.20 |
| **Sugars (RP)** | | | | | | |
| **Glucose** | 7.27 | - | 7.88 | 8.57 | - | 19.63 |
| **Mannose** | 3.97 | - | 18.57 | 10.76 | - | 10.11 |
| **Sucrose** | 8.62 | - | ND | 3.58 | - | 17.9 |
| **Fructose** | 22.96 | - | ND | ND | - | 2.68 |
| **Raffinos** | 6.54 | - | ND | ND | - | 4.13 |

**[a] selective medium for Halomonas [c]Normal salted whey**
**[b] selective medium for Xanthomonas [d]Optimized reconstituted salted whey**
**RP= Relative percentage of total sugars [e]Dray matter**
**ND= not detected**

In conclusion, it can be recommended that salted whey produced from Domiati cheese making could be a useful fermentation medium for

producing EPS by *H. eurihalina* $F_{2-7}$ or *X. campestris pv. campestris* when its composition and the conditions of the fermentation process are well optimized.

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## إنتاج الاكسوبولى سكاريدات بواسطة *H. eurihalina,X. Cambestris* من الشرش المملح

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استخدم الشرش المملح الناتج من الجبن الدمياطي بعد تدعيمه بمصادر مختلفة للكربون والنيتروجين في إنتاج السكر العديد EPS بواسطة *Halomonas eurihalina*$F_{2-7}$ و *Xanthomonas campestris pv. campestris* . وتم دراسة تأثير كل من Initial pH للشرش , نسبة الملح ومصادر الكربون (جلوكوز- فركتوز- مانيتول- سكروز) والنيتروجين (مستخلص خميرة- بروتين شرش مركز- بروتين شرش محلل) بتركيزات مختلفة, وكذلك ظروف التخمر المختلفة (نسبة تلقيح – حرارة- مدة) على نمو الميكروبين وإنتاج الـEPS وتركيبه ونسبة السكريات به .

وقد أشارت النتائج إلى أن تركيب بيئة التخمر التي تعطي أقصى إنتاج من الـEPS تختلف من ميكروب لآخر, حيث كانت البيئة المكونة من الشرش المملح (٧%) والمدعمة بسكر المانيتول (١٠جم /لتر) ومستخلص الخميرة (٦جم/لتر) وذات pH= ٧,٢ وحرارة تخمر ٣٢°م لمدة ١٠ أيام مع نسبة تلقيح ٣% *H. eurihalin* هي التي أعطت أقصى إنتاج (٢,٨ جم/لتر) بينما أعطى الميكروب *X. campestris* أقصى إنتاج وهو ١٣,٦ جم/لتر (حوالي ٥ أضعاف إنتاج الميكروب الأول) عند تلقيحه بنسبة (٣%) في الشرش متحلل البروتين ( ٦٧,١٥ أو أكثر) والمملح بمعدل ٣ % والمدعم بالسكروز(٣%) على pH= ٥.٧ وحرارة تخمر ٢٥°م لمدة ٥ أيام مع الرج (٢٠٠ لفة / ق). وقد تأثر محتوى الـEPS الناتج من السكريات باختلاف بيئة النمو والميكروب المستخدم . إلا أن كل من الجلوكوز والمانوز وجدا كسكريات أساسية في جميع الـEPS المنتجة ولكن بنسب مختلفة.

وقد إتضح أن كمية الـEPS الناتجة تأخذ نفس اتجاه (Trend) النمو لخلايا الميكروب المنتج ويصل الإنتاج إلى أقصاه في مرحلة ثبات النمو للميكروبات مع الاختلاف بين الميكروبين في الكمية المنتجة من الـEPS وكذلك التغير في pH التخمر حيث انخفض الـpH من ٧,٥ إلى ٦,٦٥ في حالة الميكروب الأول بينما ارتفع من ٧,٢ إلى ٧,٩٥ في حالة الميكروب الثاني.

**1203 1204 1205 1206 1207 1208 1209 1210 1211 1213**

**1214 1215 1216**

**1203 1204 1205 1206 1207 1208 1209 1210 1211 1213**

**1214 1215 1216**