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Short Communication

Emulsifying behavior of an exopolysaccharide produced by *Enterobacter cloacae*

Azam Abbasi[1] and Sedigheh Amiri[2]*

[1]Fars Engineering Research Center, Shiraz, Iran.
[2]Department of Food Science and Technology, Besat Education and Research Center, Shiraz, Iran.

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A major concern for industrial production of microbial emulsifiers is expensive substrates. The aim of this work was to produce a microbial exopolysaccharide (EPS) using sea broth. Seawater, seawater + distilled water and nutrient broth were inoculated with *Enterobacter cloacea* and incubated in a rotary shaker at 37°C for 80 h. The microorganism produced different amount of exopolysaccharide in different ratios of distilled water and seawater but there was no any production in the nutrient broth. The best result was obtained in the sea water broth without addition of distilled water. Stability of emulsions formed with corn oil and toluene was studied at different concentration of EPS (15 - 75) and in the presence of sodium chloride (5 – 40 mg ml^{-1}). The exopolysaccharide efficiently emulsified corn oil and toluene with water and salty solutions. There was a linear correlation between the concentration of EPS 71a and emulsification indices. The presents of salt up to 40 mg/ml did not show remarkable decrease in emulsion indices. This result suggests that the produced exopolysaccharide has a good potential to be used in the food industry.

Key words: *Enterobacter cloacae*, emulsification index, exopolysaccharide, osmotic pressure.

INTRODUCTION

Some microorganisms (mainly strains of bacteria and yeast) are able to produce biosurfactants, and may enhance hydrophobic substrate utilization or/and detoxification (Banat et al., 2000). These microbial products are divided into low-mass molecules that lower the surface and interfacial tensions and high-molecular-mass polymers (e.g. polysaccharide–protein surfactants), which are more effective at stabilizing oil-in-water emulsions (Rosenberg and Ron, 1999).

A number of bioemulsifiers contain a polysaccharide moiety attached to lipid and/or protein. Emulsan is the only commercialized bacterial emulsifier obtained from *Acinetobacter Colcoaceticus* (Rosenberg et al., 2002). Such exopolysaccharide having five times greater native viscosity than that of xanthan gum isolated from *Sphingomonas pausimobilis* GS- 1, which emulsified various hydrocarbons and oils (Ashtaputre et al., 1995). Calvo et al. (1998) reported an exopolysaccharide produced by a marine bacterium, *Halomonas eurihalina*, which could increase the viscosity of acidic solutions and ability to emulsify hydrocarbons.

Producing EPS 71a by *Enterobacter cloaceae* is bacterial reaction to hard conditions of the environment (e.g. osmotic pressure) and contains 45% total sugar, 18.75% protein, 7.0% sulfate and 9.23% uronic acid (Matsuda et al., 1992; Iyer et al., 2005). Structure of EPS 71a indicates that it can be a good emulsifier for food application.

The objective of this work was to use a cheap substrate for producing microbial emulsifier which can be used in industrial scales. Moreover, the effect of osmotic pressure on amount of produced exopolysaccharide by *E. cloaceae* was also investigated.

MATERIALS AND METHODS

Materials

Nutrient broth, sodium chloride, hexane and toluene were from Merck (Darmstadt, Germany), sea water was obtained from Persion Gulf. Other reagent were of reagent grade and commercially available.

*Corresponding author. E-mail : s_amiri@myway.com.

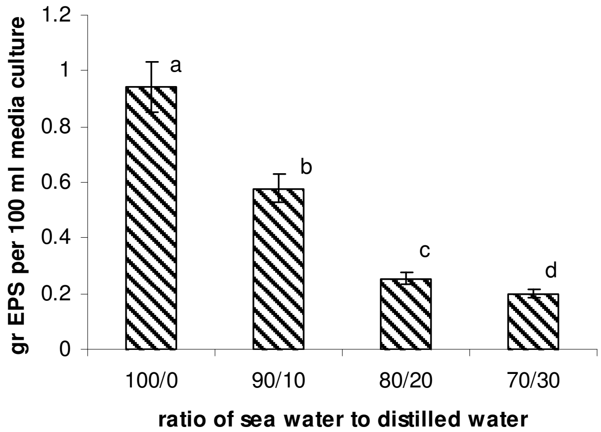

Figure 1. The produced exopolysaccharide per 100 ml of different ratio of distilled water and seawater. Results are average of three replicates.

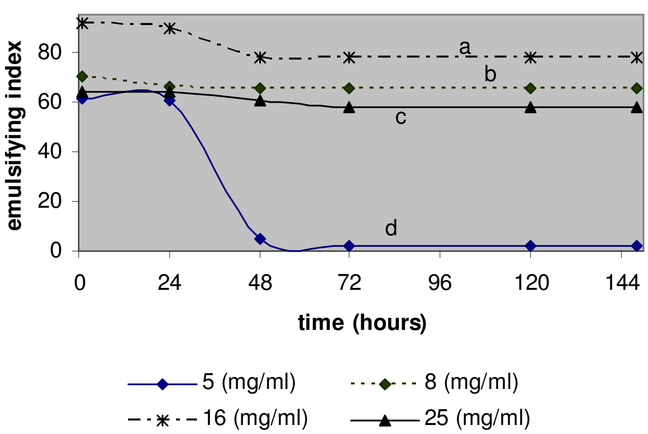

Figure 2. Stability of emulsion of EPS 71a with corn oil at different concentration of EPS. Results are average of three replicates.

Organism and exopolysaccharide production

E. cloaceae, an exopolysaccharide producing bacteria, was purchased from the Persian Type Culture Collection (PTCC). This culture was inoculated into A) seawater broth, B) seawater broth which was enriched with sodium phosphate (1%) and sodium nitrate (1%), C) nutrient broth and D) seawater broth and distilled water in a ratio of 90:10, 80:20 and 70:30. These media was incubated in a rotary shaker (125 rpm) at 37ºC for 80 h. The bacterial cells were pelleted out of the media by centrifuging the culture broth at 7000 rpm for 15 min. The exopolysaccharide was precipitated and recovered by isopropanol. The precipitate was dried in vacuum drier at 50℃ and its weight was determined

Emulsifying stability

Emulsifying stability was measured using a modified method of Cooper and Goldenberg (Cooper and Goldenberg, 1987). In this method, hydrocarbon or oil was added to aqueous phase containing the exopolysaccharide (hydrocarbon: exopolysaccharide in a ratio of 3:2, v/v) and agitated vigorously for 2 min on cyclo-mixer. The oil emulsion and aqueous layers were measured at every 24 h interval (1, 24, 48 and 72 h etc). An emulsification index (*E*) was calculated as the {(volume of the emulsion layer × total volume^{-1}) ×100}. Stability of emulsions produced by corn oil or hydrocarbon was studied with respect to concentration of EPS (5, 8, 16 and 25 mg/ml). After 24, 48 and 72 h, the emulsification index was measured and noted as *E*24, *E*48, *E*72, respectively. The emulsification index was also studied for corn oil which was emulsified with respect to concentration of EPS 71a (16 mg/ml) and presence of sodium chloride (5–40 mg/ml).

Statistical analysis

All data were analyzed by the ANOVA procedure of COSTAT, and comparison of means was performed using Duncan multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

E. cloaceae, identified as an exopolysaccharide producing bacteria based on API system, yielded fibrous exopolysaccharide after 72 h of incubation at 37˚C. The inoculation of bacteria to nutrient broth did not result in the production of desired exopolysaccharide. However, lack of osmotic pressure in the environment caused the bacteria not to produce the product. The microorganism produced different amount of exopolysaccharide per 100 ml of different ratio of distilled water and seawater (Figure 1) ($p<0.05$). The produced exopolysaccharide, EPS 71a, reduced with decreasing the ratio of seawater to distilled water. Therefore, it seems that the production of exopolysaccharide is the bacterial reaction to the hard conditions of the environment. The existence of salt in the seawater causes an osmotic pressure in the broth which makes the bacteria protect themselves from it. Production of exopolysaccharide is an option for the bacteria to keep themselves safe from the salt.

The exopolysaccharide efficiently emulsified corn oil and toluene. Emulsification indices of emulsions - produced by exopolysaccharide with toluene and corn oils- are shown in Figures 2 and 3. According to Figure 2, the emulsion of EPS 71a with corn oil at 16 mg ml–1 concentration showed the highest stability, while increasing concentration of EPS resulted in the lower emulsion indices.

For toluene, emulsion at 25 mg ml^{-1} concentration had the highest emulsification index. Both systems showed the lowest emulsification indices at 5 mg/ml EPS. The emulsions produced by corn oil were in two forms of soft or semi-solid gel and liquid emulsion. Corn oil-in-water stabilized by EPS 71a was steady in the presence of sodium chloride and there was no significant difference between various concentrations of sodium chloride (Figure 3).

The emulsifying ability of the exopolysaccharide, produced by *E. cloaceae* was superior or comparable with that of other gums such as tragacanth. Tragacanth stabi-

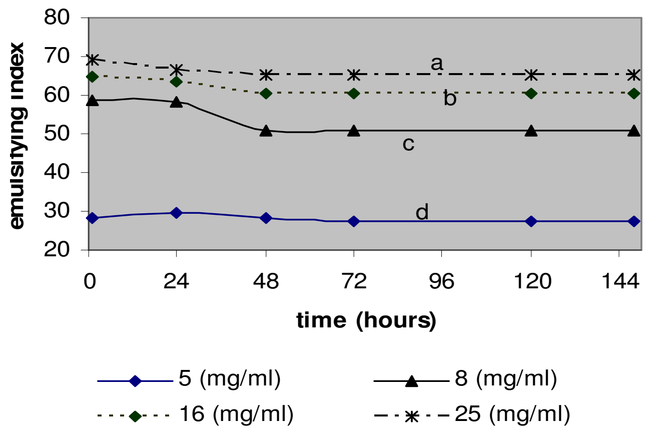

Figure 3. Stability of emulsion of EPS 71a with toluene at different concentration of EPS. Results are average of three replicates.

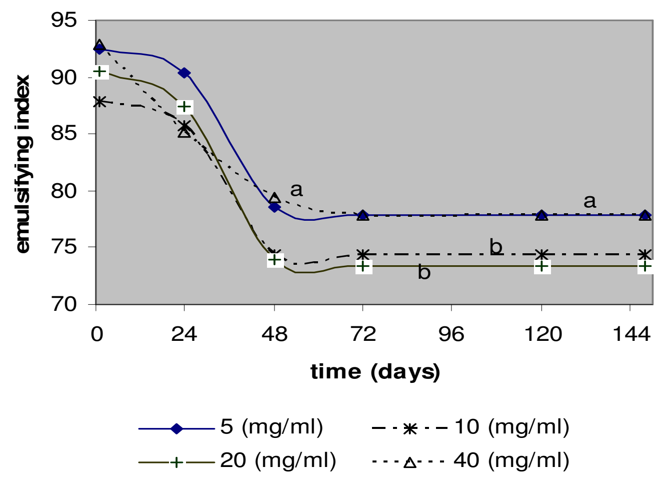

Figure 4. Emulsifying index of EPS 71a (16 mg ml−1) in the presence of salt. Results are average of three replicates.

lized emulsion with corn oil at a concentration of 40 mg/ml, which is two times greater than exopolysaccharide required for formation of a stable emulsion. The emulsions stabilized by EPS 71a had a satisfactory shelf life under usual condition of storage. These emulsions also were stable under harsh conditions such as high concentration of salts. Therefore, EPS 71a can be an appropriate option for using in the ionic formulation foods.

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