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Effect of Osmotic Downshock Treatment on the Yield of Ectoine Synthesized by *Halomonas* sp. EG6

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The halophilic bacterium strain EG6 was isolated from the Burg el Arab solar saltern lake in Egypt. Strain EG6 can grow in a wide range of NaCl concentrations up to 4 M. Its phylogenetic position was established by 16S rRNA gene sequencing as a member of the genus *Halomonas*, showing with 94, 95 and 97% similarity to *Halomonas ventosae* DSM 15911, *H. elongata* ATCC 33173 and *H. eurihalina* ATCC 49336, respectively. Morphological, physiological and biochemical tests were performed to characterize strain EG6. It produced ectoine as the main compatible solute for osmotic stress adaptation. Our aim was to establish the best conditions of osmotic downshock treatment required for high yield of ectoine production, comparing strain EG6 with the type strain *H. elongata* IFO (NBRC) 15536. We subjected strain EG6 to osmotic downshock treatment with different NaCl concentrations from 2 M to 0-0.7 M. HPLC and NMR analyses were carried out to quantify ectoine concentration and confirm its purity. Cells of strain EG6 had a great flexibility to withstand sudden changes in NaCl concentration, even when cells were subjected to osmotic downshock treatment from 2 M to 0.3 M NaCl. A total yield of ectoine of about 3.7 g/l was released after 7 days of osmotic downshock. This yield was the highest obtained when using a range of NaCl concentrations (0-0.7 M) for osmotic downshock

(Figure 1). On the other hand, *H. elongata* IFO15536 only released 2.89 g/l ectoine under the same conditions. For comparison, *Brevibacterium* sp. JCM 6894 released 2.46 mg/l as the highest yield of ectoine during 7 days of osmotic downshock treatment from 2 M to deionized water. These results indicate that strain EG6 can compensate the loss of ectoine with high efficiency. The percentage of release was calculated during osmotic downshock under the above-described conditions. The highest released rates were observed for a downshock from 2 to 0-0.2 M NaCl (95%–83%), but the concentrations of ectoine synthesized were low, while a moderate release rate (72%) was obtained for cells subjected to osmotic downshock from 2 to 0.3 M NaCl. This confirmed that the maximum yield of ectoine released not only depends on the amount of ectoine synthesized by the cells but also on the release rate. Growth of strain EG6 was highly increased during downshock treatment as compared to *H. elongata* IFO 15536. Thus, we concluded that 0.3 M NaCl was the optimum condition to obtain the highest yield of ectoine from strain EG6. The ectoine was found to be of high purity, suitable to be used for large scale ectoine production. *Halomonas* sp. EG6 may represent a new species that might be of interest for future studies.

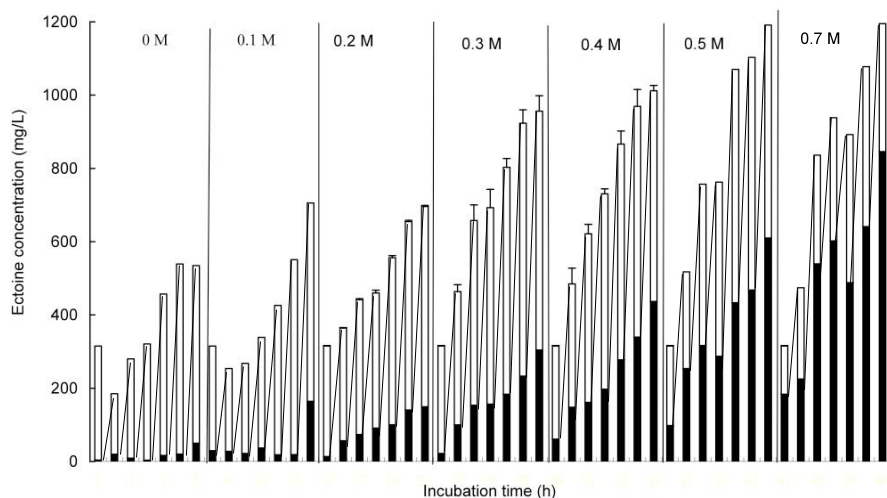


Figure 1—Synthesis and release of ectoine from strain EG6. Cells were grown in LB medium containing 2 M NaCl at 30°C for 24 h. After centrifugation, cells were subjected to osmotic downshock in the presence of different NaCl concentrations (0-0.7 M) for 10 minutes at 30°C. Subsequently the downshocked cells were incubated for further 24 hours in fresh medium with the same NaCl concentrations prior to shock. Open and shaded parts in each column indicate the amounts of ectoine released and remaining in the cells, respectively. Each culture was sampled 24, 48, 72, 96, 120, 144 and 168 hours after downshock. The values are the means \pm SD from two independent experiments.