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Short Communication

Role of L-Glutamate in the Tolerance of Osmotic Stress by *Escherichia coli*

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Studies on the cellular response to conditions of physical or chemical stress have played a very significant role in diverse areas of biology. The present investigation aims at determining concentrations of NaCl that are stressful for environmental and clinical strains of *Escherichia coli* and to investigate the effect of L-glutamate to counteract such stressful conditions. It was observed that growth of environmental, clinical and reference strains could be stressed by a NaCl concentration of 0.9 *M*. Growth decreased under conditions of stress as opposed to that without added NaCl. Clinical isolates showed much higher resistance than environmental strains to osmotic stress. Glutamate had a significant effect in overcoming osmotic stress under laboratory conditions. This was indicated by increased growth in the presence of glutamate (15 m*M*) compared to that occurred at 0.9 *M* NaCl without added glutamate. Distinct protein bands were produced under stressful conditions, which indicate that these proteins might be stress proteins that aid the isolates to counteract osmotic stress.

Keywords: Osmotic stress, L-Glutamate, *Escherichia coli*

Extremes of medium osmolarity are generally deleterious to bacterial cell growth. The ability of organism to adapt to osmotic stress is a fundamental biological process that protects them against fluctuations in water activity and solute content in their environment[1]. Many organisms including bacteria, yeast, plants and animals can adapt to various hyperosmotic stress by accumulating low-molecular-mass organic compounds known collectively as osmolytes[2]. Intracellular accumulation of osmolytes either by *de novo* synthesis or by transport from the growth medium confers tolerance to hyperosmotic stress[1,3-4]. It is well established that the intracellular accumulation of these solutes prevents water loss and maintains the turgor pressure of the cell essential for the cell growth. These molecules also stabilize the native state of various globular proteins against denaturing stresses and favour formation of protein assemblies[2,5]. By themselves, these solutes do not perturb the functional activity of macromolecules and hence are compatible with protein function[1,6-8].

While L-isomers of amino acids are predominantly found in all living organisms, D-amino acids in general[9] are also not uncommon in nature[10]. Cell walls of Gram-negative bacteria contain D-alanine, antibiotic peptides contain D-amino acids[11]. Many invertebrates have free D-amino acids in their body fluids that are known to participate actively in metabolism[12-14]. D-Enantiomers of amino acids have also been detected in human physiological fluids[15]. However, under stressed conditions, living organisms are known to accumulate only L-isomers of amino acids while no D-amino acid has been found to accumulate till date. Why living organisms have evolved to select only L-amino acids, as naturally occurring osmolytes is a matter of debate. The purpose of this present study is to examine the effects of physiological stress on the pattern of proteins synthesized by *E. coli* and to investigate the effect of L-glutamate on counteracting osmotic stress.

The four environmental isolates (E1, E2, E3 and E4), four clinical isolates (C1, C2, C3 and C4) and one ATCC strains of *E. coli* were used in this study. A minimal medium was used to support the growth of the organisms so that the medium ingredients would not interfere by contributing any protein to the cell extract. The minimal media was prepared mixing two separate solutions. Solution A contains 7.0 g K_2HPO_4, 3.0 g KH_2PO_4, 0.1 g Na-citrate.$2H_2O$, 0.1 g Mg SO_4. $7H_2O$, 1.0 g $(NH_4)_2SO_4$ and 900 ml water. Solution B contains 2.0 g glucose and 100 ml water. Stock solution of L-glutamate (solution C) was prepared by mixing 2.205 g of L-glutamate in 20 ml and water. The glutamate solution was freshly prepared and sterilized with syringe filter device of 0.45 µm pore size.

In order to impose salt stress, organisms were grown overnight at 37°C in absence and presence of NaCl salt of different molar concentration (0.6, 0.8 and 0.9 *M*). To counteract the stress, L-glutamate at a concentration of 15 m*M* was added to each medium. Cell growth was monitored by taking absorbance in a spectrophotometer at 600 nm total viable bacterial count was measured by drop-plate method[16]. Soluble protein in the culture supernatant was estimated according to the Bradford method[17]. SDS-PAGE analysis was carried out as described elsewhere[18].

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The growth pattern of the environmental and clinical isolates of *E. coli* at different NaCl concentrations is shown in Figure 1. The growth pattern of all the isolates investigated was similar; growth decreased with the increase of osmotic stress. In the presence of 0.9 *M* NaCl growth of all isolates was very poor. The osmotic stress was partly released in the presence of L-glutamate (15 m*M*) in the culture medium containing high concentration of NaCl (0.9 *M*). Although the growth of clinical isolates reduced more than environmental ones at 0.6 *M* NaCl concentration, it rose at higher concentrations. In case of the ATCC strain, growth reduced with higher salt concentration. For all samples, however, growth increased in the presence of L-glutamate. This was most remarkable for the ATCC strain, which counteracted the salt stress most extensively. A similar finding has previously been observed by other investigators[19].

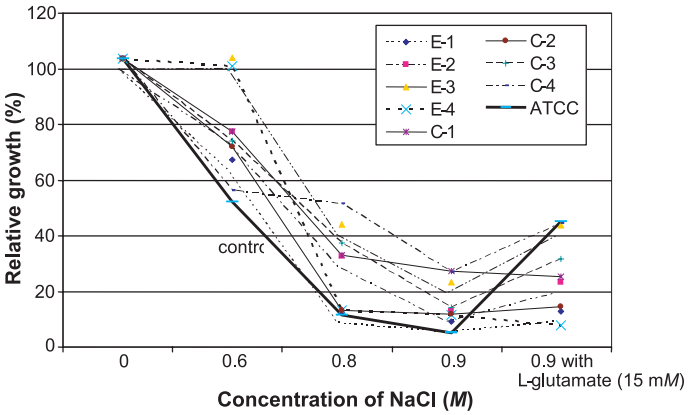

Figure 1. *Growth pattern of different Escherichia coli isolates at different salt concentration and effect of L-glutamate (15 mM) on osmotic stress.*

Soluble protein content in the culture broth decreased with the increase of NaCl concentration. This implies that the mechanism of cellular response at elevated osmotic stress is incomplete or that it impairs the efficiency of metabolic processes. However, in the presence of L-glutamate (15 m*M*), the osmotic stress was partially released. An exogenous supply of osmoprotectant amino acids, such as L-proline, glycine-betaine etc., have been shown to enhance the ability of the cell to grow in a hyper-osmotic medium[1,3-4,20].

Protein profile of different environmental and clinical *E. coli* isolates grown at stress condition (0.9 *M* NaCl) was analyzed by SDS-PAGE (Figure 2). In most cases, polypeptides had been observed to be separated into clear bands. Several distinct protein bands were observed in case of growth in the presence of 0.9 *M* NaCl plus 15 m*M* L-glutamate (Figure 3). These protein bands could not be resolved in case of the isolates grown in salt-free condition. It is, therefore, reasonable to assume that these proteins were induced by the stressful condition and might well be stress proteins.

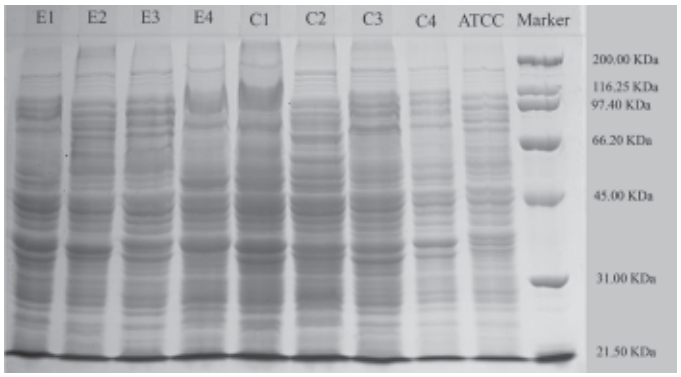

Figure 2. *SDS-PAGE analysis of protein profile of the culture filtrates of Escherichia coli isolates grown in the medium without NaCl or L-glutamate.*

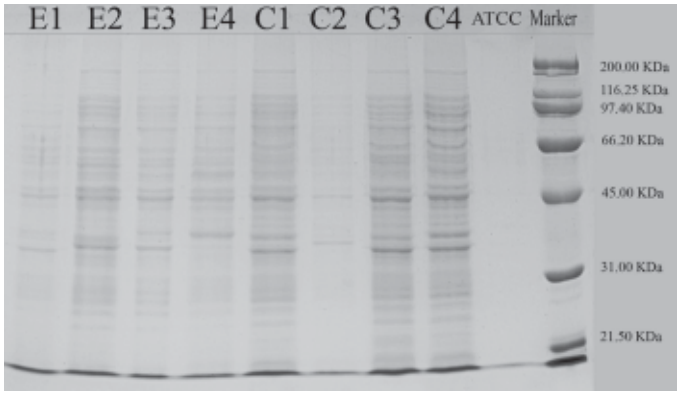

Figure 3. *SDS-PAGE analysis of protein profile of the culture filtrates of Escherichia coli isolates grown in the medium containing 0.9 M NaCl salt and 15 mM L-glutamate.*

In conclusion, analysis of the protein profile of the test organisms after resolution by SDS-PAGE demonstrated several distinct bands. Distinct protein bands were found for all the *E. coli* isolates grown at 0.9 *M* NaCl with 15 m*M* L-glutamate. These bands were not evident when growth occurred in the presence of the same salt concentration but without L-glutamate. It is suspected that these proteins might be induced by the osmotic stress and were therefore stress proteins. It is also reasonable to assume that L-glutamate aided the organisms to overcome stress induced by NaCl.

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