Stress Adaptation of Bacteria : Role of Proline

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Protective effects of proline on different bacterial strains against high and low temperature and high osmolarity are discussed. Role of proline in cryopreservation of microbial cells is mentioned and potential of this amino acid as a therapeutic agent is highlighted.

Keywords: Proline, Bacteria, Temperature, Osmotic stress, Cryopreservation.

Bacterial adaptation to different types of stress conditions (high and low temperatures, high salt, high pressure, oxidative stress) offers a challenging area of investigation. Studies on different aspects of bacterial stress adaptation not only provide insight into the intricacies of the cellular machinery but also gift us molecules of biotechnological importance. During the past few decades, a number of useful enzymes have been isolated from bacteria living in extreme environments[1].

Stress protectors

Environmental stress inflicts a number of deleterious effects on the cellular machinery. Proteins tend to denature and cell membrane loses optimum fluidity that is required for its functions. Some stress conditions (desiccation, ionizing radiation) even lead to the damage of DNA. Quite reasonably compounds that stabilize these biomolecules, help the cell to overcome stress. Some of them have protective effects against more than one type of stress conditions. For example Htp G, a homolog of the heat shock protein Hsp 90, was found to be essential for growth of the cyanobacterial strain *Synechococcus* PCC 7942 at high temperature and also for acclimation of the strain to low temperature[2] and increased oxidative stress[3]. During the past few years, a number of low-molecular weight compounds called chemical chaperones, which help to stabilize the native conformation of cellular proteins, are found to assume the role of stress protectors in bacteria. The role of trehalose in desiccation-tolerance of bacteria is well-documented[4]. Glycine betaine, an osmoprotectant, was shown to be thermoprotective in *Escherichia coli* [5] and to have growth-enhancing effects on *Listeria monocytogenes* at low temperature [6]. Recently, the stress protective potential of proline is also coming to the limelight. This article highlights some of the reports in brief.

Effect of proline at low temperature

When a strain of *E coli* (DH5a) was grown in presence and absence of various L-amino acids at different temperatures, proline was found to confer highest cryotolerance to the bacterium. Assuming growth obtained at 37°C without amino acids as 100%, growth at 15°C in a medium containing L-Proline was found to be 60% while growth at 15 °C without proline was 40% [7].

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Effect of Proline at high osmotic stress

The ability of proline to relieve growth inhibition of some lactic acid bacteria under osmotic stress is well-documented. However, similar phenomenon could not be demonstrated in another lactic acid bacterium *Gonococcus oeni.* The inability of proline to promote growth of this bacterium under osmotic stress was attributed to the absence of transporters which accumulate proline in other lactic acid bacteria viz *Lactococcus lactis and L. plantarum* [8].

Effect of proline at high temperature

High intracellular pool of proline in a *dna K* deletion mutant of *E coli* was achieved by transformation with a plasmid carrying a gene which encoded a defective version of g-glutamyl kinase, the first enzyme of proline biosynthetic pathway. The enzyme encoded by the mutated gene was resistant to feed back inhibition by proline. Thus the strain was proline overproducer. Due to absence of Dna K protein, the strain was prone to accumulate aggregated proteins when heat-shocked at 42 ^{o}C for 60 minutes. The accumulation was substantially less in the strain with high intracellular pool of proline, as revealed by two-dimensional gel electrophoresis. The viability of the *dna K* deletion mutant was adversely affected at 42^{o}C but was significantly improved in the proline-overproducer at the same temperature. The ability of proline to inhibit heat –induced denaturation of citrate synthase (a model enzyme used to test chaperoning activity) and to promote refolding of urea-denatured citrate synthase, was also demonstrated. Thus the role of proline as a chemical chaperone was evidenced[9].

Conclusion

Emerging evidences indicate that mechanisms involved in bacterial adaptation to different types of stress conditions are interlinked in some cases[10]. The role of proline as a link between different types of adaptive response in bacteria is yet to be confirmed. Proline has been found to be useful as an additive in cryopreservation of cyanobacterial and algal cells [11]. The ability of proline to act as a chemical chaperone offers scope for further studies on its role in the management of diseases caused by misfolding of cellular proteins.

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