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PHB and its Role in Salt Acclimatization in Halotolerant Azotobacter Salinestris Species

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Abstract

Poly β-hydroxybutyrate (PHB) are important storage compounds of carbon and energy. Bacteria usually produce PHB apparently in response to the limitation of an essential nutrient and conditions of physiological stress. Therefore, it is assumed that the function of PHB is to serve as an available carbon repository when bacteria face carbon limitation, supporting their survival. Recent findings indicate that PHB plays a protective role against stress-like conditions such as thermal and oxidative shock. The objective of the review is to look at the role of PHB in the salt acclimation process. Salinity is considered to be the most serious abiotic stress affecting crop productivity. It is an active process, spreading globally and a reason for land degradation. Several strategies have been assumed to be used in order to deal with soil salinity. Salt tolerant plant growth promoting rhizobacteria (ST- PGPR) used as a biofertilizer has known to increase the crop yield under stressed condition. *Azotobacter salinestris* species is known to tolerate 8% salt concentration and can be studied as a model organism to understand the role of PHB in salt acclimatization. Thus, a sustainable approach under saline condition.

Keywords: PHB, Azotobacter Salinestris Species, Salinity, Acclimatization, Sustainable Approach.

1. Introduction

Changing climatic conditions are the evident cause of increased stress levels on agricultural productivity (Bellard et al., 2012), and the limitation of crop production intensifies with the increasing human population. Therefore, Crop production will become more difficult due to climate change, resource scarcity (e.g. land, water, energy, and nutrients), and environmental degradation (e.g. declining soil quality, increased greenhouse gas emission, etc.).

Agricultural crops are exposed to many stresses that are induced by both biotic and abiotic factors. These stresses decrease the yield by affecting plant growth. Abiotic stress factors include high and low temperatures, salinity, drought, flooding, ultraviolet light, pollution, and heavy metals. Among all, salt stress is most commonly widespread. Salinity reduces the ability of plants to absorb water and nutrients and induces many metabolic changes causing rapid reduction in growth rate. (Jamil et al 2007)

It has been observed in the recent past that due to the increasing degree of salinity in some areas and expansion of salt-affected areas as a result of further intrusion of saline water, normal crop production becomes more restricted. (Haque S., 2006). Globally, more than 900 million hectares (M ha) of land, accounting for nearly 6% of the world's total land area and approximately 20% of the total cultivated land and 33% of irrigated agricultural lands are affected by high salinity (Shrivastava and Kumar, 2015). Presently, the total degraded land due to salinity is estimated to be 2.95 M ha in India. (Chinchmalatpure, 2017). Salinity in the country had received very little attention in the past. Thus, it has become increasingly important to explore the possibilities of increasing the potential of these (saline) lands for increased production of crops.

Several strategies have been assumed to be used in order to deal with soil salinity. A conventional method like scraping, flushing, leaching, adding amendments (gypsum, $CaCl_2$), etc. has had limited success as it affects the agro-ecosystem. The sub-surface drainage network consists of concrete or PVC pipes along with filters installed manually or mechanically at a particular spacing and depth below the soil surface and works by draining out the excess water containing soluble salts (Singh, 2009). The rapid adoption of this technology is hindered by the higher initial establishment costs, operational difficulties, lack of community participation, and the problems encountered in the disposal of drainage effluents (Sharma and Singh, 2015). Efforts have been made to identify salt-tolerant trees, shrubs, and grasses for cost-effective phytoremediation of these soils without any incurring expenditure in an environmentally friendly manner (Sharma and Singh, 2015). It is a slow process, limited to soil depths that are in the rooting zone and the selection of salt-tolerant plants would be difficult.

Saline environments also harbor diverse microbial flora, which exhibit modified physiological and structural characteristics under the prevailing saline conditions. A variety of salt-tolerant bacterial species have been isolated from saline soil (Abbas et al. 2018). These salt-tolerant plant growth-promoting rhizobacteria (PGPR) are promising as a bacterial inoculum for the improvement of plant growth under saline conditions by reducing the impact of salt stress on plant growth and its productivity (Liddycoat et al. 2009; Saharan and Nehra 2011; Upadhyay et al. 2011). Various salt-tolerant plant growth-promoting bacterial species of *Bacillus, Pseudomonas, Azotobacter*, and *Enterobacter* (Subramaniam et al. 2012; Nakbanpote et al. 2014) have been isolated. These microbes experience intense osmotic pressure and thus use the "compatible solute strategy" or the "salt-in strategy" to resist salt stress (Anwar and Chauhan; 2012; Shivanand P and Mugeraya G; 2011). Most bacteria accumulate compatible solutes such as choline, betaine, ectoine, proline, glycine, glutamic acid, and other amino acids at high concentrations without interfering with cellular processes (Brown 1976). However, the accumulation of organic solutes that are more compatible with cell physiology is preferred (Wood 2001).

2. Azotobacter

Among all these PGPRs, *Azotobacter* is of great interest because it has a high respiratory and metabolic rate. They fix atmospheric nitrogen and have also been known for their ability to produce different growth hormones like IAA, Gibberellins, etc besides 1- Aminocyclopropane-1-carboxylic acid (ACC) deaminase which helps in the regulation of ethylene (Glick et al.1999). Indole acetic acid (IAA) is one of the most biologically vital auxins. In plants, IAA plays a key role in both root and shoot development (Kumar A. et al 2014). It produces metabolites like siderophores (Narula et al. 1981, Nieto & Frankenberger 1989, Tindale et al 2000) which scavenge iron from the environment and make it available to plants and it is also known to produce hydrogen cyanide (HCN) which plays a role in biological control of pathogens. The phosphate solubilization ability of *Azotobacter* is considered one of the most important traits associated with plant phosphorus nutrition. (Patil et al. 2013). *Azotobacter* has the ability to form an unusual resting structure called a cyst which is necessary for surviving under adverse environmental conditions. (Gimmestad et al. 2009).

Azotobacter is a genus that consists of the members, which are free-living large, Gram-negative, aerobic rods capable of fixing nitrogen non symbiotically primarily found in neutral to alkaline soils. This organism was first isolated and described by Beijerinck in 1901. There are around six species in the genus

Azotobacter some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2–10μm long and 1–2μm wide. It also produces two polymers that have industrial importance, alginate and poly-β-hydroxybutyrate (PHB).

Azotobacter salinestris is a gram-negative coccobacillus (oval with pointed end), older cells are round, pleomorphic, and are often found in pairs and chains of six to eight cells. The organism is known to form cysts. Cells are motile by means of peritrichous flagella. Cells from old cultures are non-motile. Physiological characteristics include growth at room temperature (30^o to 36^o) and a neutral to slightly alkaline pH requirement. Most strains, except type strain, produce a capsule and synthesize poly β-hydroxybutyrate. Strains are urease-positive, amylase-positive, and catalase positive. Carbon sources include fructose, galactose, glucose, sucrose, mannitol, melibiose, 0.25% sodium benzoate, and starch. A brown-black to tan-brown pigment, allomelanin (a catechol melanin), is produced by most strains when grown at high aeration under N-fixing conditions.

The organism has been isolated from saline soil of different countries like Western Cananda (Page WJ and Shivprasad S; 1991), Bangladesh (Akhter et al; 2012), Egypt (Omer AM et al; 2016), Allahabad, India (Hafeez M. et al; 2018), West Java, Indonesia (Hindersah R et al; 2020) and Eastern Kenya (Wanjira P. et al ; 2022). William J. Page, 1991 had showed the dependency of *Azotobacter salinestris* on Na+ ion for growth. He incubated the cells in Burk's medium for 16 hours with different Na+ ions concentration. In this experiment he observed an inverse relation between the lag phase and the Na+ ion concentration i.e. that there was an increased lag phase with decreased Na+ ion. The organism had shown tolerance to NaCl upto 8% (Chennappa G. et al; 2016). Its tolerance to high salt concentration makes this organism as a sustainable approach under saline condition (Omer A.M. et al; 2016). Pot experiment carried by Omer A. M. et al too evaluate the effect of *Azotobacter salinestris* on the morphological and biochemical characteristics of sorghum gave significant result on the parameter studied

2.1 Mechanisms Of Salt Tolerant *Azotobacter Salinestris* Species Towards Salinity Tolerance

The adaptation mechanisms of bacteria to saline environment have been studied broadly by various researchers. Various salt-tolerant strains of *Azotobacter* has been screened had proved that some strains are able to colonize the rhizosphere successfully and promote plant growth in saline soils (Van Oosten et al; 2018.). Two salt tolerant strains were also reported to alleviate saline stress by improving sodium exclusion and potassium uptake. (Rojas-Tapias et al; 2012)

Habitually, the bacteria growing in non-saline conditions may exhibit a great modification in cell morphology when subject to high salt stress. Swelling, elongation and shrinkage (reduction in cell volume) are the characteristic features of sensitive bacteria towards salt stress. Salt-tolerant bacteria normally show a structural modification to cope up with salt stress.

Study carried out by Magdy et al (1990) has showed accumulation of osmolytes like amino acid glutamate and the disaccharide trehalose in the stressed *Azotobacter chroococcum* ZSM4, *species*. *Azotobacter salinestris* produces salicyclic acid, proline and exopolysaccharide as osmoprotectant (Omer AM et al; 2016)

3. Poly β- hydroxybutyrate (PHB)

Poly β-hydroxybutyrate (PHB) is a polyhydroxyalkanoate (PHA), a polymer belonging to the polyesters class that has desirable physical and chemical properties similar to plastics and is biodegradable. In many prokaryotes, PHB granules are important storage compounds of carbon and energy which permit survival of the cells in the absence of suitable carbon sources. Microbial biosynthesis of

PHB starts with the condensation of two molecules of acetyl CoA to give acetoacetyl CoA catalyzed by β-keto thiolase which is subsequently reduced to hydroxybutyrlCoA by aceto-acetyl (CoA) reductase. This latter compound is then used as a monomer to polymerize PHB which is catalyzed by PHB synthase.

PHB is produced by microorganisms evidently in response to a state of constraints of an essential nutrient and conditions of physiological stress. However, intracellular PHA accumulation and degradation capacity also enhances the tolerance of bacterial cells to various stress conditions including low temperatures and freezing (Stanislav et al; 2016) or oxidative stress (Goh et al; 2014; Iustman et.al 2015). There is lack of understanding of the mechanisms by which the PHB cycle favors stress alleviation. Anabolic and catabolic pathways of PHB appears to be indispensable in protecting cells against increased stressed conditions (Kadouri et al. 2005). Furthermore, it appears that, the main product of intracellular degradation of PHB i.e. 3-hydroxybutyrate (3HB) might also plays an additional role in the stress response of bacteria. Martin et al (2002) has reported the function of 3HB as a compatible solute of the deep-sea bacterium *Photobacterium produndum* and more recently, study carried out by Soto et al. (2012) reported that 3HB serves as a compatible solute in *Pseudomonas* sp. CT13, protecting the cells from protein aggregation under combined salt and thermal stress. Accumulation of poly-β-hydroxybutyrate in response to high salinity in rhizobia has been studied indicating its role in cell protection (Arora et al; 2006). One of the research study had proved the role of PHB in maintaining the redox balance during low temperature adaptation in *Pseudomonas* sp. 14-3 (Ayub et al; 2009). Experiments on stress tolerance in non PHB producing organisms like *Escherichia coli* strains showed that a complete PHB mobilization had aid in survival of bacterium as well as enhanced the tolerance to stressed condition (Qian Wang et al; 2009).

3.1 Role Of Thiolase In PHB Synthesis Under Stress Condition.

Thiolase II (Acetoacetyl-CoA thiolase EC 2.3.1.9), is a condensing enzyme that catalyses the production of acetoacetyl-CoA from two molecules of acetyl-CoA. Most of these thiolases act in anabolic processes as the first step in the biosynthesis of polyhydroxybutyrate (PHB) via the thiolase II pathway. PHB synthesis is usually induced under stressed conditions and thiolase II absorbs an indispensable position in this production (Senior and Dawes, 1973; Kadouri *et al.*, 2005). In a research study carried on eukaryotic thiolase II catalyzing isoprenoid biosynthesis had played an important role in physiological adaptation of alfa-alfa (Medicago sativa) to low temperature and high salt concentration. (Soto et al; 2011). The hypothesis that thiolase II, an enzyme crucial in the biosynthesis of polyhydroxybutyrate (PHB) and isoprenoids, plays a conserved role in abiotic stress adaptation across diverse organisms. These molecules serve as vital antioxidants, protecting cells from damaging reactive oxygen species (ROS) generated under stress conditions. (Ayub et al., 2009; Soto et al.,2011).

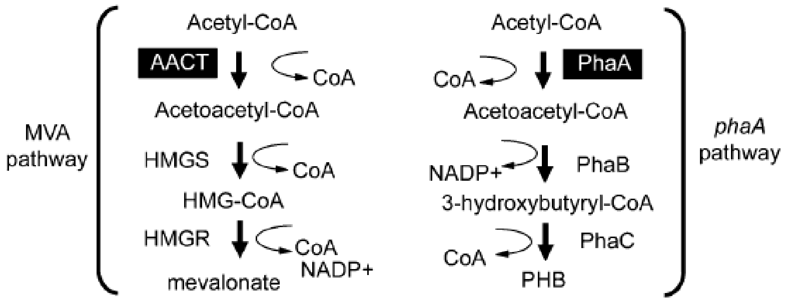

Figure 1. Biosynthesis of isoprenoids via mevalonate (MVA) and polyhydroxybutyrate (PHB) via the thiolase II pathway in eukarya and bacteria, respectively. Thiolase II products involved in these pathways are boxed.

Source: Soto et al; 2011

The activity of thiolase II is tightly regulated at both the transcriptional and post-transcriptional levels. Studies have shown that thiolase II expression is upregulated in response to various abiotic stresses, including drought, salinity, and heavy metal exposure. This suggests a coordinated response mechanism where the organism increases the production of thiolase II to cope with the increased abiotic stress. (Fox AR et al.; 2014). While the regulation of thiolase II by CoA provides a crucial mechanism for controlling PHB and isoprenoid biosynthesis, it does not fully explain why this enzyme was selected as a key player in abiotic stress adaptation across diverse organisms. To address this, we propose that thiolase II acts as a sensor of the TCA cycle, playing a central role in maintaining cellular redox balance during stress. (Baxter et al., 2007; Grant, 2008; Liu et al., 2005).

4. Conclusion:

Salinity is a hazard to agriculture as it affects greatly soil, microorganisms, and plants throughout their growth cycle, from germination to maturation. Salt tolerant – PGPR especially show a great capacity for saline stress alleviation through various mechanisms. Salt tolerant *Azotobacter* inoculants under salinity can reduce chemical fertilization. This review suggest the potential protective role of PHB in the stress tolerance of such agronomically important microorganisms as *Azotobacter*. Further studies of PHB accumulation in the salt tolerant *Azotobacter* species under the stress of different salt concentrations will help in understanding its salt acclimation process. The article concludes with a discussion of future perspectives and research avenues to further validate the role of thiolase II in abiotic stress adaptation.

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