BioNanoSci. (2017) 7:623–626
https://doi.org/10.1007/s12668-017-0460-9

Pleiotropic Effect of Salt Stress on Motility and Synthesis of Secreted Ribonucleases by *Bacillus pumilus*

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Published online: 19 October 2017

Abstract Members of the genus *Bacillus* can successfully counteract a sudden increase in salinity. In addition to the accumulation of osmolytes, saline stress also affects other aspects of bacterial physiology such as exoenzymes synthesis and motility. Here, we have shown that increase of salinity in growth medium leads to elevated biosynthesis level of low-molecular weight ribonuclease (RNase) binase I from *Bacillus pumilus*. The same effect was established previously for high-molecular weight binase II. Transmission electron microscopy revealed the absence of flagella and some other changes in salt-stressed cells of *B. pumilis*. We also detected the gene sequences homologous to the recognition sites of response regulator DegU in the binase I and binase II promoters. Using the *B. subtilis* strains with various mutations in DegU gene, we found that the two-component signal transduction system DegS-DegU which regulates the motility under salt stress participates in the control of biosynthesis for both secreted RNase of *B. pumilis* (binase I and binase II).

Keywords Ribonuclease · Binase I · Binase II · *Bacillus pumilus* · Salt stress · Motility · Flagella

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1 Introduction

Members of the genus *Bacillus* are often exposed to significant fluctuations in osmotic pressure as a result of drying or flooding of habitats. Like other bacteria, they resist the negative influences of salt stress primarily by regulating the concentration of soluble ions and organic compounds to support the turgor. It is also known that high salinity exerts pleiotropic effect on the physiology of the bacilli. In particular, the salt stress is an environmental signal affecting exoenzymes (alkaline protease, levansucrase) synthesis *in Bacillus subtilis* [1]. At the same time, salt stress causes severe impairment of the motility of *B. subtilis* [2]. We have shown that increase of salinity in growth medium leads to elevated biosynthesis level of secreted ribonuclease (RNase) of *B. pumilus* 7P binase II [3]. Binase II is the second secreted RNase, found in *B. pumilus* 7P. It differs from binase I by the absence of substrate specificity, the mode of RNA cleavage, and more than twice the molecular weight [4, 5]. Binase I is widely known as a promising antitumor therapeutic agent [6, 7] and despite the fact that biosynthesis of binase I has been thoroughly studied [8], there is no information on synthesis under salt stress. It is also interesting to find a correlation between the level of synthesis of both enzymes and the behavior of *B. pumilus* associated with motility.

2 Materials and Methods

Bacterial strains and plasmid are *B.subtilis* 168 trpC2, (*Bacillus* Genetic Stock Center), *B. pumilus* 7P (All-Russian Collection of Microorganisms—VKM), *B.subtilis* 8G5 (*trpC2*; *tyr*; *his*; *nic*; *ura*; *rib*; *met*; *ade*; lacks the *sipP* genes),