BIOLOGIJA. 2006. Nr. 4. P. 46–48

Effect of glycine betaine on osmoadaptation of *Arthrobacter* strains

V. Časaitė*,

S. Bružytė,

R. Meškys

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Mokslininkų 12, Vilnius LT-08662, Lithuania

The role of glycine betaine osmoprotection of various *Arthrobacter* strains which display a variation in salt tolerance was investigated. When externally provided, this compound enhanced the growth of a half of the strains studied, demonstrating its utilization as an osmo-protectant. However, glycine betaine was inefficient on the other half of the strains studied. With the exception of *A. ramosus* and *A. sulfureus*, all strains were able to use glycine betaine as a sole carbon source.

Key words: glycine betaine, *Arthrobacter* spp.

INTRODUCTION

Most microorganisms have to cope with a range of abiotic stresses caused by fluctuations in their surroundings. Bacterial cells have developed powerful strategies to proliferate and survive under stressful conditions [1, 2]. Because the osmolarity of the environmental medium is one of the most variable parameters, more attention has been paid in recent years to understanding the mechanism of bacterial adaptation to increased osmolarity. Under osmotic stress, cells induce the processes that regulate the osmotic adjustment and maintain a sufficient cell turnover for the growth to proceed. Bacteria accumulate organic compounds, termed osmolytes, compatible solutes, or osmoprotectants. These solutes are nontoxic low molecular mass molecules that raise osmotic pressure and protect some macromolecular structures against denaturation. The main osmoprotectants include polyols and their derivatives, various sugars and zwitterions such as amino acids and betaines. Glycine betaine is reported to serve as the major effective osmoprotectant in gram-negative and gram-positive bacteria as well as in members of the family Archaea [3, 4].

Bacteria of the genus *Arthrobacter* have been found in many different terrestrial habitats. These aerobic chemoheterotrophs can metabolize a wide range of organic compounds, including herbicides, chlorinated phenols and alkanes, and complex aromatic compounds [5].

Arthrobacteria are resistant to desiccation and longterm starvation, they may be particularly useful for bioremediation in dry desert soils. The osmoadaptive responses of *Arthrobacter* species have not been thoroughly studied, it is not known which solute acts as a osmoprotectant. The objective of our study was to establish whether the selected bacteria could use glycine betaine as an osmoprotectant and (or) a sole carbon source. Therefore, we have analyzed some strains from our collection together with typical strains of the genus *Arthrobacter*.

* Corresponding author. E-mail: vidac@bchi.lt

MATERIALS AND METHODS

Bacterial strains and media *A. citreus* NRRL B-1258 (X80737), *A. atrocyaneus* NRRL B-2883 (X80746), *A. globiformis* NRRL B-2979 (X80736), *A. ramosus* NRRL B-3159 (X80742), *A. sulfureus* NRRL B-14730 (X83409), *A. crystallopoietes* NRRL B-14903 (X80738) were from ARS culture collection (NRRL, National Center for Agricultural Utilization Research, Illinois, USA), arthrobacterial strains PRH1 (AM236153), PY22 (AJ271410), VP3 (AM236152), PY21 (AJ271409), 96 (AJ879126), 94 (AJ879125), 85 (AJ879124), 68M (AJ853464), 83B (AJ879123), 68B (AJ879122), BL-3, 1-IN, KA3, P3, KA2V2, KA2, GAZ21, P2G, KA4, KA2V3, GAZ3, VM22, VP23, RD1, VM02, VP22, VPW7 and VPS4 were from our collection [6–8]; database accession numbers of 16S RNA genes used for phylogenetic reconstruction are in brackets. Mineral medium (5 g NaCl, 1 g $NH_4H_2PO_4$, 1 g K_2HPO_4, 0.4 g $MgSO_4 \times 7\ H_2O$ in 1 L of water) supplemented with 0.2% succinate or 0.2% glucose was used for analysis of the osmotolerance of all strains. Glycine betaine was prepared as 1 M solution and sterilized before incorporation into the medium. The final concentrations were 5 mM for osmoprotection assays and 20 mM when used as a carbon source in mineral medium.

Growth rate determination. Mineral medium precultures (50 µl) of the bacteria in late stationary phase were used to inoculate 5 ml of a desired medium. The cells were grown aerobically (agitation at 250 rpm) at 30 °C. Bacterial growth was monitored spectrophotometrically at 600 nm.

The phylogenetic tree of 16S RNA genes (~1.47 kb) was constructed by the unweighted pair-group method

using an arithmetic average (UPGMA) and the MEGA3 software package version 3.0 [9]. Genetic distance was calculated by the Kimura two-parameter method. The confidence level of branching in the phylogenetic tree was evaluated with the bootstrap test based on 250 resampling.

RESULTS AND DISCUSSION

To characterize the intrinsic osmotolerance of the different arthrobacterial species, we examined their growth rate in a mineral medium with NaCl concentration from 0.5 to 1.5 M. Some strains like *A. citreus*, GAZ21, PRH1, VM02, VP23, VP22, 85 were most sensitive to salt, they could poorly grow in the medium with 1 M NaCl. The KA2 and GAZ3 strains were able to withstand 1 M concentration, but their growth parameters were affected in the presence of 1.5 M NaCl. For all other strains, 1 M NaCl concentration caused a 40 to 80% reduction in the yield. In all cases, the first effect of the increased salt concentration was reduction of the growth rate, followed by a reduction in the biomass yield.

The osmoprotective effect of glycine betaine was tested at a salt concentration which reduced the growth significantly. For all strains except *A. citreus*, KA2, GAZ3, GAZ21, PRH1, VM02, VP23, VP22 and 85 the salt concentration was 1 M, the strains KA2 and GAZ3 were grown in the medium containing 1.5 M NaCl, and strains *A. citreus*, GAZ21, PRH1, VM02, VP23, VP22, 85 were grown in the presence of 0.5 M NaCl. The influence of glycine betaine on the growth of the strains was examined by cultivating the cells at elevated NaCl concentrations with and without glycine betaine. It turned out, that *A. globiformis, A. ramosus, A. sulfureus,* KA2V2, KA4, KA2V3, VM22, VPS4, PY21, 96, 94, RD1, GAZ3, 68B and 68M adapted faster to elevated NaCl concentrations when glycine betaine was present in the medium. Glycine betaine had no influence on growth yield in the presence of elevated NaCl concentration for the rest strains studied (Fig. 1).

Unlike most bacteria that use glycine betaine as an osmoprotectant, *Arthrobacter pascens* can catabolize glycine betaine and use this compound as a sole source of carbon and nitrogen for growth [10]. Among soil bacteria, the capacity to use glycine betaine as a growth substrate has been reported only for a few species, including rhizobial strains [11], *Azospirillum lipoferum* [12] and *Pseudomonas aeruginosa* [13]. To test whether all the selected *Arthrobacter* strains can catabolise glycine betaine and use this compound as a sole source of carbon, their growth was monitored in the presence of 20 mM glycine betaine which was added to the carbon-free mineral medium. All species, with the exception of *A. ramosus* and *A. sulfurous,* were found to be able to grow in that medium.

It has been concluded from these experiments that glycine betaine is a good energy substrate for the growth of arthrobacterial species, but its-omoprotective property seems to be restricted to a few strains. From 34 *Arthrobacter* strains, a half use glycine betaine as an osmoprotector and 89 % as a sole source of carbon.

The phylogenetic reconstruction of the *Ar-throbacter* strains, based on the 16S rDNA sequence data (Fig. 2), provided additional information for clarifying relationships among the ability to use glycine betaine as an osmoprotectant. It turned out that this ability did not co-rrelate with the bacterial phylogenetic relationship.

Until now it has been supposed that in the arthrobacterial cells betaine plays the primary role in carbon

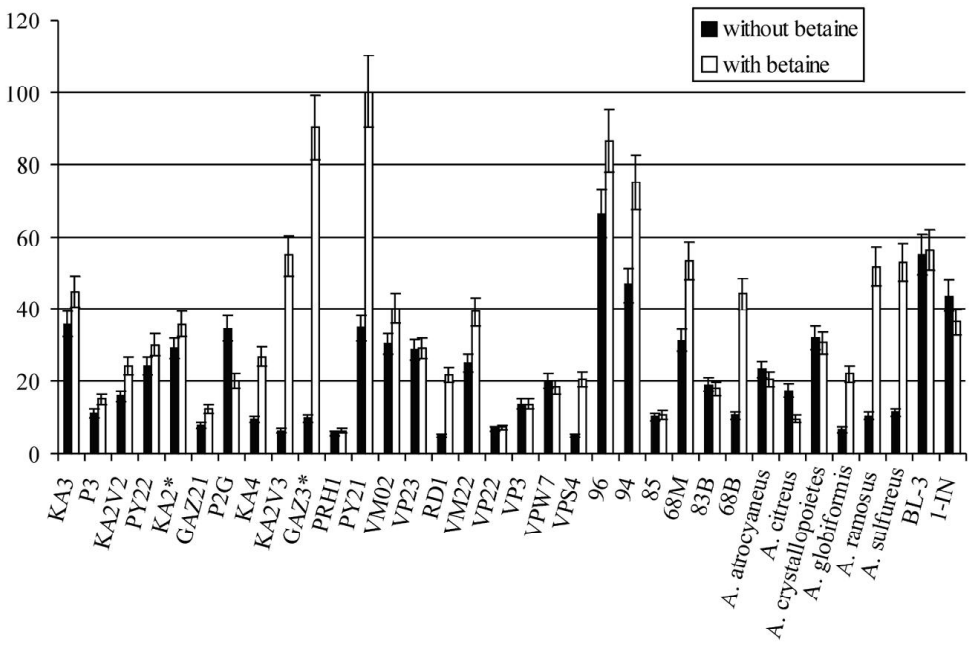

Fig. 1. Effects of glycine betaine on the growth yield of arthrobacterial species. Cultivation was carried out in mineral medium containing NaCl and glycine betaine (5 mM) (white bars), and in medium containing NaCl but without glycine betaine (black bars). Succinate 0.2% or glucose 0.2% was used as a carbon source; strains were grown for 24 h. A value of 100% corresponds to the optical density (at 600 nm) of culture in the absence of salt. For every growth experiment, the data are an average of at least three different repeats with a standard deviation of less than 12%. (*KA2 and GAZ3 were grown in the medium containing 1.5 M NaCl instead of 1 M or 0.5 M NaCl.)

metabolism and not in adaptation to osmotic stress [10, 14]. Our study has demonstrated that some *Arthrobacter* strains utilize this compound as a carbon source and as an osmoprotector in elevated osmolarities, too. In another soil bacterium, *Rhizobium meliloti* [15], the choline oxidation pathway permits a catabolism of choline and betaine as carbon and nitrogen sources but promotes betaine accumulation when the bacterium is subjected to osmotic stress. A similar situation could take place in *Arthrobacter* spp. A detailed study of betaine metabolism should contribute to the elucidation of the mechanism by which betaine is used for both, in osmoprotection and as an energy source.

ACKNOWLEDGEMENTS

We thank V. Dzekevičienė and A. Krutkis for their assistance. This work was partially supported by the Lithuanian State Science and Studies Foundation.

Received 2006 03 04
Accepted 2006 08 22

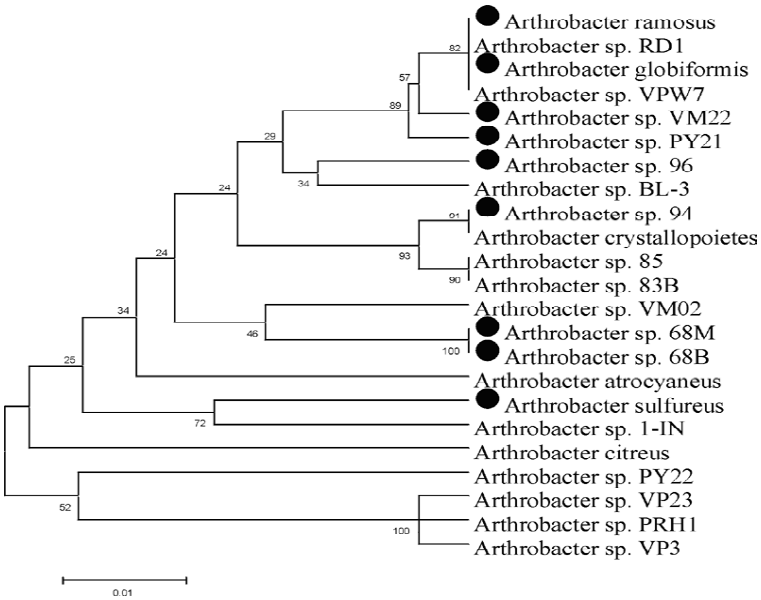

Fig. 2. Dendrogram depicting estimated phylogenetic relation-ships based on pairwise comparisons of partial 16S rDNA sequences of the *Arthrobacter* strains studied. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0. 0.01D means Kimura's two-parameter distance, UPGMA method. Strains able to use glycine betaine as osmoprotector are marked by circles

References

1. O'Byrne CP, Booth IR. Int J Food Microbiol 2002; 74: 203–16.
2. Wood JM. Microbiol Mol Biol Rev 1999; 63: 230–62.
3. Welsh DT. FEMS Microbiol Rev 2000; 24: 263–90.
4. Poolman B, Glaasker E. Mol Microbiol 1998; 29: 397–407.
5. Boylen CW. J Bacteriol 1973; 113: 33–7.
6. Meškys R. PhD thesis, Institute of Biochemistry and Vilnius University, Vilnius, 1998.
7. Semėnaitė R. PhD thesis, Institute of Biochemistry and Vilnius University, Vilnius, 2003.
8. Zujienė J. Master thesis, Vilnius University, Vilnius, 2005.
9. Kumar S, Tamura K, Nei M. Briefings in Bioinformatics 2004; 5: 150–63.
10. Rozwadowski KL, Khachatourians GG, Selvaraj G. J Bacteriol 1991; 173: 472–8.
11. Boncompagni E, Osteras M, Poggi MC et al. Appl Environ Microbiol 1999; 65: 2072–7.
12. Hartmann A. In: W. Klingmuller (ed.). Azospirillum. Vol. IV. Genetics–Physiology–Ecology. Springer-Verlag, New York, N.Y. 1988: 122–30.
13. Lisa TA, Casale CH, Domenech CE. Curr Microbiol 1994; 28: 71–6.
14. Deutch CE, Perera GS. J Appl Bacteriol 1992; 72: 493–9.
15. Smith LT, Pocard JA, Bernard T et al. J Bacteriol 1988; 170: 3142–9.

V. Časaitė, S. Bružytė, R. Meškys

GLICINO BETAINO POVEIKIS *ARTHROBACTER* GENTIES BAKTERIJŲ OSMOADAPTACIJAI

Santrauka

Buvo patikrinta, kaip *Arthrobacter* genčiai priklausančios bakterijos naudoja glicino betainą. Tyrimui buvo pasirinkti tipiniai *Arthrobacter* genties bakterijų kamienai *A. atrocyaneus, A. citreus, A. crystalopoietes, A. globiformis, A. ramosus, A. sulfureus,* bei *Arthrobacter* genčiai priklausantys kamienai KA3, P3, KA2V2, PY22, KA2, GAZ21, P2G, KA4, KA2V3, GAZ3, PRH1, PY21, VM22, VP23, RD1, VM22, VP22, VP3, VPW7, VPS4, 96, 94, 85, 68M, 83B 68B, BL-3 ir 1-IN. Nors glicino betainas daugelio mikroorganizmų yra naudojamas kaip osmoprotektorius, darbo metu paaiškėjo, kad šį junginį apsaugai nuo osmosinio streso naudoja tik dalis *Arthrobacter* genties bakterijų – 15 kamienų iš 34 tirtų pasižymėjo tokia savybe. Glicino betainas *Arthrobacter* dažniau naudojamas kaip anglies šaltinis, net 89 % tirtų kamienų galėjo skaidyti šį junginį.