Physiology and regulation of vacuolar sodium pump of *Enterococcus hirae*

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Our biochemical and molecular biological studies have revealed that a vacuolar sodium-translocating ATPase functions in *Enterococcus hirae*. The sodium ATPase is encoded by an 11-kb *ntp* operon: *ntpFIKECGABD(H)J*. Enterococci are extremely tolerant to high pH and high salt among streptococci. We here examined the significance of the *ntp* operon in homeostasis of Na^+ and K^+ ions of this bacterium under these severe environments. Potassium accumulation via the proton motive force-dependent potassium transport system KtrI was inhibited by a protonophore such as CCCP. The growth of this bacterium was also inhibited by CCCP in a K^+-limited medium. However, even in the presence of CCCP, the cell growth was recovered by addition of NaCl in media. Sodium-dependent growth was also observed by the F_0F_1, H^+-ATPase mutant AS25 in the absence of CCCP, and the internal K^+ level was recovered by increasing the external Na^+, suggesting that Na^+ circulation is important for K^+ accumulation of this bacterium at the limited proton motive force. Sodium-dependent growth was not observed by the mutants defective in the Na^+-ATPase subunits or the NtpJ K^+ transporter KtrII which is independent of the proton motive force. Finally, the promoter activity of the *ntp* operon was examined in various culture conditions with a plasmid harboring the fragment of the *ntp* promoter region linked with the CAT gene as reporter. The ntp promoter is specifically responsive to Na^+ but not Li^+ ions. The *ntp* operon is an important one, containing genes that encode transport systems of Na^+ and K^+ ions. Functional expression of the *ntp* operon is indispensable for physiology of this bacterium, especially coping with severe external environment.