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Asian Applied Microbiology 2021: Lysine 265 is critical for NhaD-type Na+/H+ antiporter from *Halomonas hydrothermalis*- Omasan Eyinmisan Urhie, Department of Microbiology, Northeast Agricultural University Harbin China

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Abstract

The Nh-NhaD is an Na+/H+ antiporter from *Halomonas hydrothermalis* belonging to a new family of bacterial Na+/H+ antiporters of the NhaD family. In this present work, two amino acids Lys 265 and Asp 167 were substituted with alanine so as to identify amino acids vital for the antiporter activity. The deduced amino acid sequence of Hh-NhaD comprises 492 residues with calculated molecular weight of 54367.39Da and a PI of 8.82. KNabc/pUC-om-NhaD, KNabc/pUC 18, Knabc/pEasyT3 vector (negative controls), Knabc/pEasyT3-NhaD-His-tag, Knabc/pEasyT3-NhaDN167 and KNabc/pEasyT3-NhaDK265 were grown in LBK medium containing 0-0.8mol/L NaCl or 0-300mM LiCl in order to test the ability of Hh-NhaD to confer salt tolerance. KNabc/pUC-om-NhaD, Knabc/pEasy T3-NhaD-His-tag and Knabc/pEasyT3-NhaDN167 were able to grow in the presence of upto 0.6mol/L NaCl while KNabc/pEasyT3-NhaDK265 could not survive in the presence of LBK medium containing 0.2mol/L NaCl. The same trend was observed for LiCl test. The expression of Hh_nhaD conferred on E. coli KNabc cells the ability to grow under alkaline conditions in KNabc/pUC–om-NhaD and Knabc/pEasyT3-NhaD-His-tag. KNabc/pEasyT3-NhaDK265 could not survive under alkaline condition of pH 8.0 which indicates the importance of Lys 265 residue in pH regulation. The function of Hh_NhaD was identified in detail and the importance of Lys 265 in pH regulation and cation transport was proven in this study. Hh_NhaD a membrane protein was experimentally established to exhibit Na+(Li+)/H+ antiport activity with pH dependency. Hh_nhaD gene encode a novel NhaD-type Na+/H+ antiporter and Lys 265 is very essential for cation binding and pH regulation in NhaD from *H. hydrothermalis*.

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