

Adaptation Mechanisms of the Polyextremophile Natranaerobius Thermophilus to Saline-Alkaline-Hermal Environments

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Abstract : The first true anaerobic, halophilic alkali thermophile, *Natranaerobius thermophilus* DSM 18059T, serves as a valuable model for studying cellular adaptations to saline, alkaline and thermal extremes. To uncover the adaptive strategies employed by *N. thermophilus* in coping with these challenges, we conducted a comprehensive iTRAQ-based quantitative proteomic analysis under different conditions of salinity (3.5 M vs. 2.5 M Na⁺), pH (pH 9.6 vs. pH 8.6), and temperature (52°C vs. 42°C). The increased intracellular accumulation of glycine betaine, through both synthesis and transport, plays a critical role in *N. thermophilus*' adaptation to these combined stresses. Under all three stress conditions, the up-regulation of Trk family proteins responsible for K⁺ transport is observed. Intracellular K⁺ concentration rises in response to salt and pH levels. Multiple types of Na⁺/H⁺ antiporter (NhaC family, Mrp family and CPA family) and a diverse range of FOF1-ATP synthase are identified as vital components for maintaining ionic balance under different stress conditions. Importantly, proteins involved in amino acid metabolism, carbohydrate metabolism, ABC transporters, signaling and chemotaxis, as well as biological macromolecule repair and protection, exhibited significant up-regulation in response to these extreme conditions. These metabolic pathways emerge as critical factors in *N. thermophilus*' adaptation mechanisms under extreme environmental stress. To validate the proteomic data, ddPCR analysis confirmed changes in mRNA expression, thereby corroborating the up-regulation and down-regulation patterns of 19 co-up-regulated and 36 key proteins under saline, alkaline and thermal stresses. This research enriches our understanding of the complex regulatory systems that enable polyextremophiles to survive in combined extreme conditions.

Keywords : polyextremophiles, natranaerobius thermophilus, saline- alkaline- thermal stresses, combined extremes

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