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Novel haloarchaeon *Natrinema thermophila* having the highest growth temperature among haloarchaea with a large genome size

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Environmental temperature is one of the most important factors for the growth and survival of microorganisms. Here we describe a novel extremely halophilic archaeon (haloarchaea) designated as strain CBA1119^T isolated from solar salt. Strain CBA1119^T had the highest maximum and optimal growth temperatures (66 °C and 55 °C, respectively) and one of the largest genome sizes among haloarchaea (5.1 Mb). It also had the largest number of strain-specific pan-genome orthologous groups and unique pathways among members of the genus *Natrinema* in the class *Halobacteria*. A dendrogram based on the presence/absence of genes and a phylogenetic tree constructed based on OrthoANI values highlighted the particularities of strain CBA1119^T as compared to other *Natrinema* species and other haloarchaea members. The large genome of strain CBA1119^T may provide information on genes that confer tolerance to extreme environmental conditions, which may lead to the discovery of other thermophilic strains with potential applications in industrial biotechnology.

The growth of most microorganisms is influenced by physical factors such as temperature, water activity, pH, pressure, salinity, and oxygen concentration as well as chemical factors such as availability of nutrients (e.g., carbon and nitrogen)[1–4]. Microorganisms are usually classified based on optimal growth temperature-i.e., as psychrophiles, mesophiles, thermophiles, and hyperthermophiles, which grow best at temperatures of ≤15 °C, 15 °C–45 °C, >45 °C, and 80 °C, respectively[5]. These classes also differ in terms of the amino acid composition, structure, and thermostability of proteins[6]. Growth temperature seems to be related to genomic features; one study showed that the average length of proteins is shorter in thermophiles (growing best at 45 °C) compared to their homolog in mesophiles (15 °C–45 °C), whereas the proportion of purine bases in the coding strand is higher in the former than in the latter[7]. Other environmental factors besides temperature affect genome size: for example, the small genomes of prokaryotes are thought to reflect adaptation to strong selective pressures in large microbial populations, while the genome size in geophytes was found to be positively correlated with early flowering and growth tendency under humid conditions[8,9].

Extremely halophilic archaea (haloarchaea) belonging to the domain Archaea are usually found in hypersaline environments such as salt lakes and crystallizer ponds from artificial marine solar salterns and in salty fermented foods and salted hides[10,11], as well as in avian feather[12]. The growth temperature of haloarchaeal type strains ranges from −1 °C to 62 °C, with few growing at temperatures >60 °C (see Supplementary information). Genus *Natrinema* in the family Natrialbaceae includes eight known species of haloarchaea: *Natrinema altunense*, *Nnm. ejinorense*, *Nnm. gari*, *Nnm. pallidum*, *Nnm. pellirubrum*, *Nnm. salaciae*, *Nnm. soli*, and *Nnm. versiforme*[13–19]. In

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