

How alkaliphiles produce energy to sustain their life under extremely low concentration of proton

Alkaliphiles are defined as microorganisms with optimum growth pH of above 9 (10^{-9} mol dm⁻³ H⁺ concentration). This means that these microorganisms can grow in environment with one-hundredth times lower proton (H⁺) concentration than that of neutral environments (pH 7; 10^{-7} mol dm⁻³ H⁺ concentration). Alkaliphiles are thriving not only alkali soda lakes but also in ordinary environments such as garden soil and manure, and they live not only in the natural environments but also in artificial environments such as indigo fermentation used for dyeing or various mold type cheeses. Although the ecological function of alkaliphiles are not fully understood, their enzymes are utilized for laundry detergents and for the production of cyclodextrin, owing to their merits of maintaining stability and efficiency under a wide range of pH. Although alkaliphilic *Bacillus* spp. are very popular in research, a variety of genera within the phylum Firmicutes are known to be alkaliphilic bacteria.

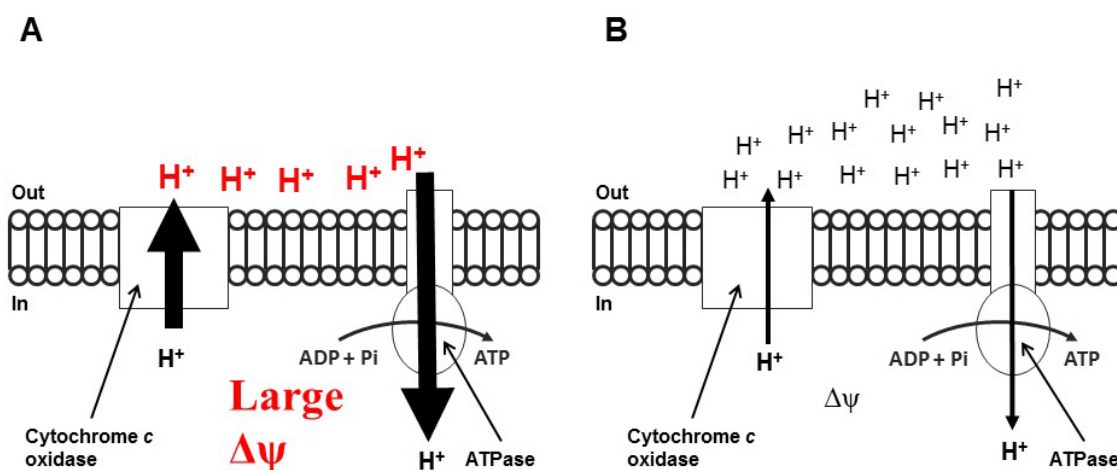


Fig. 1. High efficiency of H⁺ locating outer surface membrane. Although background H⁺ and rate of respiratory translocating H⁺ are lower in alkaliphile (A), efficiency of per H⁺ for production of ATP is higher in the alkaliphile compared with that of the neutralophile (B). This difference is attributed by the larger electrical potential (Δψ) in alkaliphile.

Most organisms utilize H⁺ for ATP production through ATPase. ATP is produced by passing through H⁺ in ATPase by afflux of H⁺ from the extracellular side and efflux into the intracellular side. Therefore, H⁺ motive force that drive ATPase is the forces to accelerate translocation of H⁺ from the surface of extracellular membrane to intracellular side across the membrane. Presently, two factors are known to contribute the H⁺ motive force that drives ATPase. One of the H⁺ motive force for ATPase is the difference in H⁺ concentration across the membrane (ΔpH; higher H⁺ concentration in the extracellular side). The other is the negatively charged electrical difference across the membrane (Δψ: intracellular higher in negative charge, which attracts extracellular H⁺ to

the intracellular side). One problem in considering the factors on extracellular H^+ is that we do not know the precise difference of pH across the membrane, because we do not know the exact pH at the surface of the extracellular side of the membrane is known. When only considering the bulk based pH, we cannot explain the production of ATP by the reported formula for the calculation of H^+ motive force. Therefore, the reconsideration of the two above-mentioned factors is essential in understanding ATP production in alkaliphiles.

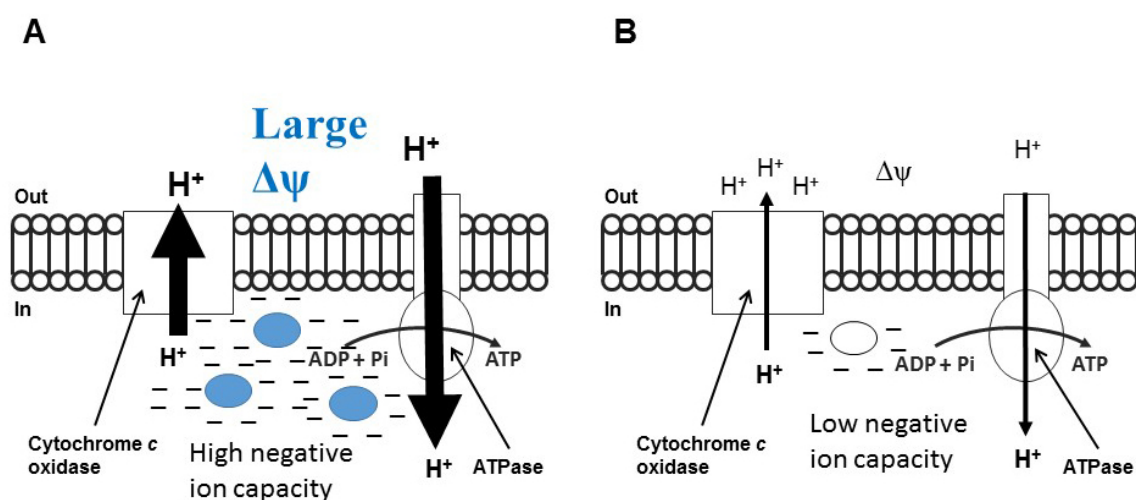


Fig. 2. Mechanism of the occurrence of high Donnan effect in alkaliphile

It is difficult to explain on the occurrence larger electrical potential ($\Delta\psi$) in alkaliphile (A) comparing with that neutralophile (B) because lower frequency of H^+ translocation in alkaliphile (due to the positive charge of H^+). Higher production of negatively charged molecules such as acidic protein in the intracellular space in alkaliphile produce the Donnan effect. This effect will contribute to the formation of higher $\Delta\psi$ in alkaliphile.

It has been known that the respiratory chain consisting of respiratory complex that transfers electrons from NADH to O_2 transfers H^+ from the intracellular side to the extracellular side during the electron transfer process. The translocated H^+ contribute to the formation of H^+ motive to drive ATPase. It is considered that there is no native background ΔpH in alkaliphiles. Therefore, it is expected that H^+ translocated by the respiratory chain will be the main source for the H^+ motive force to drive ATPase. To better understand the relationship between the rate of H^+ translocation by the respiratory chain and ATP production, we estimated H^+ translocation and ATP production rates by using two kinds of alkaliphiles and one neutralophile. Although the rates of H^+ translocation were lower in alkaliphiles than that of the neutralophile, their ATP production rates were much higher than that of the neutralophile. If ATP production per H^+ was always the same, we could not explain the higher ATP production rates of the alkaliphiles. The higher intensity of H^+ for driving ATPase in alkaliphiles would be attributed higher $\Delta\psi$ in alkaliphiles (Fig. 1). Although it is difficult to explain the high $\Delta\psi$ in alkaliphiles, contribution of Donnan effect (Donnan effect can be observed when charged particles unable to pass through a semipermeable membrane create an uneven

electrical charge) for the formation of high $\Delta\Psi$ is very likely, as the intracellular negative ion capacities in alkaliphiles are much higher than that of the neutralophile (Fig. 2).

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Publication

[Contribution of intracellular negative ion capacity to Donnan effect across the membrane in alkaliphilic *Bacillus* spp.](#)

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