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Outline of Research Results

Analysis of a novel physiological function of the respiratory chain of *Zymomonas mobilis*

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Research aims

Zymomonas mobilis is an ethanol-fermenting bacterium that is responsible for the fermentation of tequila. In addition to the ethanol fermentation pathway, *Z. mobilis* also possesses a respiratory chain. I previously isolated eleven respiratory-deficient mutant (RDM) strains of *Z. mobilis*[1] and revealed that five of these strains (RDM-1, -2, -3, -6, and -7) were deficient in cytochrome *bd*-type ubiquinol oxidase (CydAB), and that five other strains (RDM-4, -5, -8, -9, and -11) were deficient in NADH dehydrogenase (NDH).[2] Compared to the wild-type (wt) strain, the RDM strains exhibited faster growth rates and higher levels of ethanol production at high temperatures, under aerobic conditions. These results indicated that the RDM strains were more efficient ethanol producers under these conditions. [2, 3]

The observed phenotypes of the RDM strains also indicate that the respiratory chain of *Z. mobilis* is an inhibitory factor for growth under aerobic conditions, at high temperatures. As the respiratory chain appears to be unnecessary in *Z. mobilis*, it is unclear why the respiratory chain components have been conserved in this bacterium. Recently, it was proposed that the respiratory chain of *Z. mobilis* has a novel physiological role.[3] To investigate this possibility, I analyzed the physiological function of the respiratory chain of *Z. mobilis*.

Methods

The wild type *Z. mobilis* strain ZM6 (ATCC 29191) was used to generate the various RDM strains, as previously described.[1] The *Z. mobilis* wt and RDM strains (RDM 1–9, and 11) were cultured in liquid medium containing 0.5% (w/v) yeast extract and 2% (w/v) glucose. Growth curves were performed in test tubes incubated at 30°C with shaking at 50 rpm, and growth was monitored every 1 h by measuring the absorbance at 600 nm (OD-Monitor C&T, Taitec, Koshigaya, Japan). The NDH (pZA22-*ndh*) and CydAB (pZA22-*cydAB*) expression vectors used for complementation experiments were constructed previously.[2] Intracellular sodium chloride concentrations were determined using Mohr's method. D-sorbitol levels were measured enzymatically using a F-kit with D-Sorbitol/Xylitol (Roche Diagnostics, Basel, Switzerland).

Results

I hypothesized that the respiratory chain of *Z. mobilis* plays a role in abiotic stress tolerance. To address this hypothesis, the growth rates of the RDM strains were compared with that of the wt strain in the presence of various stressors, including osmolarity, heat, cold, and organic solvents. Among the examined stressors, osmotic stress from exposure to sodium chloride and potassium chloride, but not ethylene glycol, impaired the growth of all RDM strains compared to the wt.

Complementation experiments were performed to examine the apparent association between the respiratory chain and salt tolerance. To complement respiratory defects, pZA22-*ndh* and pZA22-*cydAB* expression vectors were introduced into the corresponding RDM strains, and growth of the complemented strains was evaluated in the presence of salt-containing medium. The complemented RDM strains exhibited similar growth profiles as the wt strain in media containing either 0.25 M sodium chloride or 0.25 M potassium chloride (Fig. 1). In addition, the wt strains harboring either the *ndh* (wt-pZA22-*ndh*) or *cyd*AB (wt-pZA22-*cydAB*) vector exhibited a greater than 2-fold increase in cell density at 30 h compared to the wt-pZA22 control strain (Fig. 1). Interestingly, the cell morphology of

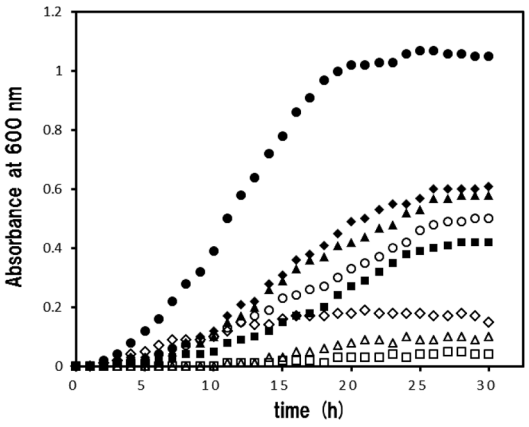

Fig. 1. Growth rates of RDM strains complemented with NDH, under salt stress conditions.
The wt (○), RDM-4 (△), RDM-5 (□), and RDM-11 (◇) strains were grown in liquid medium containing 0.25 M sodium chloride. Open and closed symbols denote strains possessing the pZA22 and pZA22-*ndh* vectors, respectively.

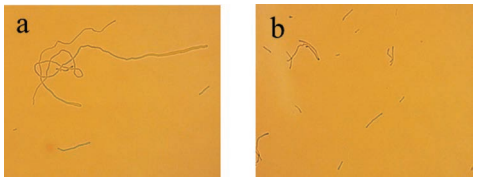

Fig. 2. Cell morphology of the RDM-4 strain complemented with NDH, under salt stress conditions. Strains RDM4-pZA22 (a) and RDM4-pZA22-*ndh* (b) were cultivated in liquid medium containing 1.8 M sodium chloride and observed by light microscopy.

the RDM strains was abnormally elongated when grown in salt-containing media (Fig. 2a). This morphology was restored to near wild type appearance, however, in the complemented strains (Fig. 2b). Together, these results suggest that the respiratory chain of *Z. mobilis* is involved in salt tolerance.[4)]

To elucidate the mechanism by which the respiratory chain of *Z. mobilis* mediates salt tolerance, the intracellular sodium chloride concentrations of the wt-pZA22 and wt-pZA22-*ndh* strains were compared after culturing in the presence of 1.8 M sodium chloride. The two strains exhibited similar intracellular sodium chloride concentrations, indicating that the respiratory chain of *Z. mobilis* is not involved in Na^+ transport. It was previously reported that *Z. mobilis* accumulates sorbitol as a compatible solute under osmotic stress conditions. Therefore, I compared the intracellular concentrations of D-sorbitol in the wt-pZA22 and wt-pZA22-*ndh* strains. After culturing in the high-salt medium, the wt-pZA22-*ndh* strain displayed a 1.25-fold increase in the amount of accumulated sorbitol compared to the wt-pZA22 strain.

Conclusion

The results of this study indicate the respiratory chain of *Z. mobilis* is involved in mediating salt tolerance, and that the regulation of sorbitol production may be an integral part of this process.[4)] My findings also revealed that salt tolerance is enhanced in *Z. mobilis* by the overexpression of respiratory chain enzymes such as NDH and CydAB.[4)]

References

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3) Takeshi Hayashi and Kensuke Furukawa. (2012) Respiratory-deficient mutants acquire high ethanol productivity under aerobic condition and thermotolerance. *Kagakutoseibutu*. 50: 780–781.
4) Takeshi Hayashi, Tsuyoshi Kato, Satoshi Watakabe, Wonjoon Song, Shizuho Aikawa, and Kensuke Furukawa. (2015) The respiratory chain provides salt stress tolerance by maintaining a low NADH/NAD^+ ratio in *Zymomonas mobilis*. *Microbiology*. 161: 2384–2394.