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On the acidostability of an acidophilic thermophilic bacterium

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SUMMARY

When an acidophilic thermophilic bacterium, *Bacillus* sp., is exposed to acid environment (pH 4.0) without glucose, the cell suspension consumes H^+. When the cells respire glucose, no consumption of H^+ by the cell suspension is observed. On addition of respiratory inhibitor or uncoupler to the cell suspension containing glucose, however, the suspension consumes H^+. It may be then suggested that the bacterium may have some energy-dependent H^+ exclusion mechanism.

Many cultures of acidophilic thermophilic bacteria related to the genus *Bacillus* have recently been isolated by Uchino and Doi[1] and Darland and Brock[2]. The bacteria have been characterized by their ability to grow at pH values from 2.0 to 6.0 (optimum 3 to 4) and at temperatures from 45 to 70 °C (optimum 60–65 °C). The acidophilicity (why the bacteria prefer such acid environment for their optimum growth) and acidostability (how they can tolerate acid environment) at such temperatures attracted our interests, since most bacteria exhibit no growth or cannot survive under such conditions. In our laboratory, an acidophilic thermophilic bacterium, which grows at pH values from 2.0 to 5.5 (optimum 4.0 to 4.5) and at temperatures from 35 to 65 °C (optimum 55 °C) and belongs to the genus *Bacillus*, has been isolated (Yamazaki, Y. and Nosoh, Y., unpublished). The present study was then attempted to reveal the mechanism of acidostability of the acidophilic thermophilic bacteria, using our isolate.

The organism was grown aerobically at 55 °C in a medium of the following composition per liter: 10 g glucose, 20 g polypeptone and 2 g yeast extracts; the pH was adjusted to 4.0 with HCl. The cells in a logarithmic phase were collected, washed with 0.9% NaCl solution and suspended in 20 mM phosphate buffer containing 0.9% NaCl and 5 mM $MgSO_4$ or in 0.9% NaCl solution of pH 4.0 or 7.0. The cell suspension thus prepared (absorbance at 650 nm = 6) was incubated aerobically. During the incubation, its pH value was kept constant at an appropriate value, using a Toa pH stat, and the volume of 0.1 M

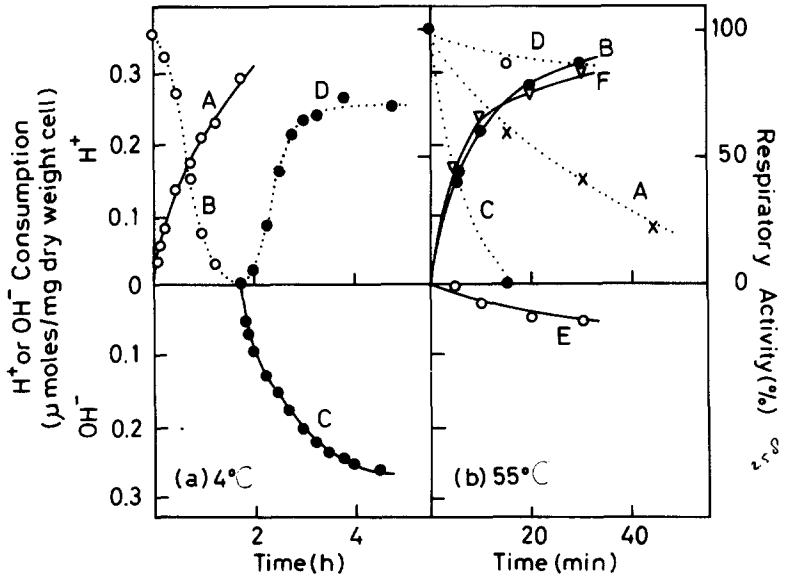

Fig. 1. Consumption of H^+ (0.1 M HCl) or OH^- (0.1 M NaOH) by the cell suspension and change in respiratory activity of cells, on incubation at 4 °C (a) and 55 °C (b). (a) A and B, consumption of H^+ and respiratory change at pH 4.0, respectively; C and D, consumption of OH^- and respiratory change at pH 7.0, after 2 h of incubation of the cell suspension at pH 4.0, respectively. (b) A, respiratory change at pH 7.0; B and C, consumption of H^+ and respiratory change at pH 4.0, respectively; D and E, respiratory change and consumption of OH^- at pH 4.0 in the presence of 20 mM glucose, respectively; F, consumption of H^+ at pH 4.0 with 20 mM glucose in the presence of $1 \cdot 10^{-4}$ M 2,4-dinitrophenol.

HCl or NaOH added to the suspension was recorded. The respiratory activity of the cells was measured at 55 °C in 20 mM phosphate buffer (pH 4.0) containing 20 mM glucose and 10 mM $MgSO_4$, using a Clark-type oxygen electrode.

When the cell suspension in the phosphate buffer or in 0.9% NaCl solution, pH 7.0, was incubated at 4 °C, no change in the pH value of the suspension nor in the respiratory activity of cells was observed, even after 4 h of incubation. When the cell suspension of pH 4.0 was incubated, however, the pH value of the suspension increased gradually, and H^+ (0.1 M HCl) had to be added to the suspension to keep its pH value constant at pH 4.0 (Curve A in Fig. 1a). On incubation at pH 4.0, the respiratory activity of cells decreased and was completely lost after about 2 h of incubation (Curve B). When the activity was lost, the pH value of the suspension was changed from 4.0 to 7.0 and the incubation was continued at pH 7.0. It was observed, when the incubation was carried out in 0.9% NaCl solution, that the suspension consumed OH^- (0.1 M NaOH) to keep its pH value constant at pH 7.0 (Curve C). The respiratory activity of cells, both in the phosphate buffer and in

NaCl solution, was restored to about 70% of the original activity after 2 h of incubation (Curve D).

Intact cells or protoplast of the bacterium exhibited maximum respiratory activity at pH 4.0 and little activity at pH 7.0, while the cell-free extracts exhibited optimum activity at pH 7.0 and no activity at pH 4.0 (unpublished results). The facts suggest that the intercellular pH of the organism is about neutral, as suggested for other acidophilic mesophilic microorganisms such as *Ferrobacillus ferrooxidans*[3] and *Thiobacillus thiooxidans*[4,5], and that the acidophilicity and acidostability may depend on the membrane function of the organism. On addition of HCl to a suspension of mitochondria[6] or *Streptococcus faecalis*[7], the pH fell abruptly and then rose slowly. The rise in the pH value was considered to be due to the entrance of H^+ into the mitochondria or the cells. The consumption of H^+ or OH^- from the cell suspension of the acidophilic thermophilic bacterium may therefore be ascribable to the influx or efflux of H^+ into or from the cells, respectively, which is probably due to the concentration gradient of H^+ across the membrane, although the possibility of adsorption of H^+ onto the membrane surface could not be completely excluded.

When the cells were incubated at 55 °C in the phosphate buffer, pH 7.0, the respiratory activity of cells decreased rapidly (Curve A in Fig. 1b). This may be due to heat denaturation of the respiratory system of the bacterium. When the incubation was carried out at pH 4.0, the suspension consumed H^+ rapidly (Curve B), and the respiratory activity of cells decreased much more rapidly (Curve C) than at pH 7.0. When the pH value of the suspension was changed from 4.0 to 7.0 after 15 min of incubation, the restoration in respiratory activity, as observed with the cells incubated at 4 °C, was not observed. The respiratory system of the bacterium may have been irreversibly denaturated at 55 °C and at pH 4.0. When the bacterium was incubated with 20 mM glucose, the respiratory activity decreased only slightly, even at 55 °C and at pH 4.0 (Curve D), and slight production of H^+, which is probably due to the acid production by glucose oxidation, was observed (Curve E). On incubation of the cells with glucose in the presence of $1 \cdot 10^{-3}$ M NaN_3 or $1 \cdot 10^{-4}$ M 2,4-dinitrophenol, H^+ was rapidly consumed from the cell suspension (Curve F), as observed with the cells incubated in the absence of glucose. The results suggest that the membrane of this organism may have some energy-dependent mechanism by which H^+ is pumped out or inhibited from entering the cells, on exposure of the cells to acid environment. The cells exhibit no respiratory activity at pH 4.0 and at 4 °C. Consumption of H^+ from the cell suspension at pH 4.0 and at 4 °C was then not affected by the addition of glucose and NaN_3 or 2,4-dinitrophenol.

Staphylococcus aureus grows best in a neutral medium, but also grows in acid medium (pH 5.0). Haest *et al.*[8] have reported that the phospholipid composition of the membrane of the organism changed according to the pH change of the incubation medium, and they have suggested that the acid tolerance of the organism may be due to the positively charged membrane which will repel the entrance of positive ions such as H^+ into cells. Brock[9] has isolated a sulfur-oxidizing bacterium which grows best at pH 2–3 and at 70–75 °C. The cell wall of the bacterium was reported not to contain peptidoglycan, which

is the basic cell wall component of bacteria, and he considered that this peculiar composition of the cell wall may be related to the acidostability of the organism. The possibility that such a unique chemical composition of the cell membrane or cell wall may partly contribute to the acidostability of the present organism could not be excluded. It may be suggested, however, from the results obtained in the present study that the acid tolerance of the present acidophilic thermophilic bacterium may be due to the energy-dependent mechanism for the H^+ exclusion mechanism functioning on the membrane.

REFERENCES

1 Uchino, F. and Doi, S. (1967) *Agric. Biol. Chem.* 31, 817
2 Darland, G. and Brock, T.D. (1971) *J. Gen. Microbiol.* 67, 9
3 Din, G.A., Suzuki, I. and Lees, H. (1967) *Can. J. Biochem.* 45, 1523
4 Suzuki, I. (1965) *Biochim. Biophys. Acta* 104, 359
5 Iwatsuka, H., Kuno, M. and Maruyama, M. (1962) *Plant Cell Physiol.* 3, 157
6 Mitchell, P. and Moyle, J. (1967) *Biochem. J.* 104, 588
7 Harold, F.M. and Baarda, J.R. (1968) *J. Bacteriol.* 96, 2025
8 Haest, C.W.M., Degier, J., Op Den Kamp, J.A.F., Bartels, P. and Van Deenen, L.L.M. (1972) *Biochim. Biophys. Acta* 255, 720
9 Brock, T.D. (1972) *Arch. Mikrobiol.* 84, 54