

腐敗しょつるから分離した細菌の耐熱性

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Heat Resistance of Bacteria Isolated from Spoiled *Shotturu**¹

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In the previous paper¹⁾ it was suggested that bacteria belonging to the genus *Halobacterium* were most responsible for the spoilage of *shotturu* (fish sauce) and other groups of bacteria like the genus *Streptococcus* able to grow in high salt concentrations were secondarily concerned with the spoilage. So in this paper we studied the heat resistance of those causative bacteria for the heat treatment preventing the spoilage of *shotturu*.

The bacteria tested were six strains [XA55 (*Halobacterium*), XA360 (*Halobacterium*), XC56 (*Halococcus*), RB13 (*Micrococcus*), ZB9 (*Streptococcus*), and LC6 (*Staphylococcus*)] which were isolated from spoiled *shotturu* and *moromi* (semimanufactured goods of *shotturu*)¹⁾ and 2 authentic strains of extremely halophilic bacteria which are *Halococcus morrhuae* (IAM 1711) and *Halobacterium salinarum* (IAM 12045) obtained from Institute of Applied Microbiology, University of Tokyo.

The culture medium (20% NaCl-BP agar) for XA55, XA360, XC56, IAM1711, and IAM12045 was a modified 20% NaCl-BPG agar²⁾ in which 0.1% glucose was omitted. The culture medium for LC6, RB13, and ZB9 was 2.5% NaCl-BPG agar.²⁾ The strains were incubated at 35°C for 7 days, except that ZB9 was incubated for 14 days. The cells of XA55, XA360, XC56, IAM1711, and IAM12045 were suspended in 20% NaCl-BP broth which was 20% NaCl-BP agar without agar. The cells of LC6, RB9 were suspended in 2.5% NaCl-BPG broth which was 2.5% NaCl-BPG agar without agar. All the cells of the strains incubated by the same method were also suspended in *shotturu* filtrated by STERIVEX-GS (SVG501015) (Millipore Co.). These cell suspensions were stored in a refrigerator.

One ml aliquots of the cell suspensions were introduced into small test tubes (7×105 mm), which were sealed by heat immediately. The sealed test tubes were immersed in each water bath at 50°C, 55°C, 60°C, and 70°C and were removed after each of the heating periods. These tubes were cooled in cold water, broken

aseptically and the number of survivors were determined by the pour-plate method with the media corresponding to those on which the strains were cultured for the preparation of the cell suspensions. The media were incubated at 35°C for 20 days, except that those of LC6 and RB13 were incubated for 10 days and 14 days, respectively.

Viable counts of all the strains after heating at 50°C, 60°C, and 70°C for 10 min are shown in Table 1. In the two broths, the viable counts of XA55, XA360, LC6, and RB13 after heating at 60°C were below 10 cells/ml and those of XC56, ZB9, IAM1711, and IAM 12045 after heating at 70°C were below 10 cell/ml. From the data of Table 1, it is estimated that the D values at 60°C (D₆₀) of the former group are 1.7 to 2.5, and that the D values at 70°C (D₇₀) of the latter one are 1.7 to 2.5. The heat resistance of LC6, RB13, and ZB9 in *shotturu* is higher than that in the broth. The reason why the strains that require low concentration of salt (2.5–5.0%) for the growth¹⁾ show higher heat resistance in *shotturu* (approximately 27% NaCl) than in 2.5% NaCl-BPG broth is obscure. NaCl or other ingredients of *shotturu* may have some protective effects to heat.

The cells of XC56 and ZB9, which showed higher heat resistance than others in Table 1, were heated at 60°C, 65°C, and 70°C in *shotturu*. From the survival curves at these temperature D₆₀, D₆₅, and D₇₀ of the strains were determined and the z values were estimated from the D values. D₆₀, D₆₅, D₇₀ and z values of XC56 and ZB9 were 136, 10, 1.5 and 5.1°C (9.2°F), and 15.2, 2.7, 0.4 and 6.7°C (12.1°F), respectively.

According to Stumbo,³⁾ D₁₅₀ and z values of spoilage microorganisms of pasteurized foods were 0.50 to 3.00 and 8 to 12(°F), respectively. As 150°F of the D₁₅₀ is 65.56°C, D₆₅ of ZB9 is in the range of D₁₅₀ of the spoilage microorganisms. But D₆₅ of XC56 is about three times bigger than these figures. The z values of XC56 and ZB9, which are 5.1°C (9.2°F) and 6.7°C (12.1°F) respectively, fall in the ranges described above.

References

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Table 1. Heat resistance of the bacteria in 20% NaCl-BP broth, 2.5% NaCl-BPG broth, and *shotturu*

Strain No.	Heating medium* ¹	Viable counts (cells/ml)			
		No heating	Heating for 10 min		
			at 50°C	at 60°C	at 70°C
XA55	A	3.3×10 ⁹	2.6×10 ⁸	<10	<10
	C	1.9×10 ⁹	1.9×10 ⁸	<10	<10
XC56	A	5.6×10 ³	4.5×10 ³	2.5×10 ³	<10
	C	6.0×10 ³	5.4×10 ³	4.0×10 ³	<10
XA360	A	2.4×10 ⁶	1.8×10 ⁶	<10	<10
	C	2.7×10 ⁶	2.7×10 ⁶	<10	<10
LC6	B	7.2×10 ⁵	3.2×10 ⁵	<10	<10
	C	7.1×10 ⁵	6.5×10 ⁵	9.5×10 ⁴	<10
RB13	B	5.3×10 ⁴	2.9×10 ⁴	<10	<10
	C	2.3×10 ⁵	1.5×10 ⁵	4.3×10 ⁴	<10
ZB9	B	3.7×10 ⁴	3.9×10 ⁴	0.2×10 ⁴	<10
	C	4.7×10 ⁴	5.1×10 ⁴	4.2×10 ⁴	<10
IAM1711**	A	1.7×10 ⁸	1.7×10 ⁸	1.6×10 ⁸	<10
	C	1.6×10 ⁸	1.2×10 ⁸	1.4×10 ⁸	<10
IAM12045**	A	1.5×10 ⁸	1.5×10 ⁸	2.5×10 ⁸	<10
	C	1.0×10 ⁸	1.1×10 ⁸	1.1×10 ⁸	<10

*¹ A: 20% NaCl-BP broth, B: 2.5% NaCl-BPG broth, and C: *shotturu*.

*² The strains were obtained from Institute of Applied Microbiology, University of Tokyo.

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