Growth Characteristics and Enzyme Activities of Halophilic Bacteria Isolated from Fish Sauce

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Growth Characteristics and Enzyme Activities of Halophilic Bacteria Isolated from Fish Sauce

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Abstract

Growth characteristics of three strains of halophilic bacteria isolated from Chinese fish sauce and Burmese fish sauce were investigated. Bacteria were cultured in SEHGAL and GIBBONS Complex (SGC) medium and nutrient broth (NB) medium with NaCl concentration of 3 M and 4 M, pH of each medium ranging from 4.5 to 6.5. Tests for lipase, gelatinase and caseinase activities were carried out in basic agar medium in plates. It was observed that pH between 5.0 and 5.5 was critical for growth of bacteria and this fact was more pronounced in medium with 4 M NaCl than in medium with 3 M NaCl. All bacteria showed positive results in lipase and gelatinase tests but bacteria isolated from Burmese fish sauce alone showed positiveness in caseinase test. Utilization of these bacteria in accelerated production of fish sauce was discussed.

Little is known about the halophilic bacteria of fish sauce origin. SAISITHI *et al.*[1] isolated many halophiles from a ninemonth old Thai fish sauce in medium with 10% salt. Most of the halophiles were identified as Bacillus sp. and were detected as having capability of producing volatile acids from cultures in a medium prepared by hydrolyzing rock fish. FUJII *et al.*[2] isolated many Bacillus strains from Philippines fish paste of which some could grow well in the medium containing more than 20% NaCl.

However, there is no such report yet which deals with the growth nature and enzyme activities of halophilic bacteria isolated from fish sauce. The main purpose of this work was to select the appropriate bacteria which can be utilized in accelerated fish sauce production. In this aspect, the growth characteristics of bacteria were investigated in various medium of high NaCl concentration and with different pH which resemble the conditions of fish sauce. Moreover, this work dealt with the preliminary tests of such enzyme activities as protease and lipase, the former, of course, has direct effect on proteolysis of fish during fermentation, and the latter, in some or other way, is regarded as a factor in evolution of fish sauce-like aroma[3].

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Materials and Methods

Medium

SEHGAL and GIBBONS Complex[4] (SGC) and nutrient broth (NB) (katsuo fish extract, 1.0%; polypeptone, 1.0%, both in w/v), both medium with various molar concentration of NaCl and various pH were used.

Bacteria

Bacteria C_1 and C_6, both were isolated from Chinese fish sauce in SGC, 3 M NaCl medium (pH 6.6–6.8) and bacteria B_3, isolated from Burmese fish sauce in NB, 2 M NaCl (pH 7.0) were used.

Determination of growth characteristics

Two-day old bacteria were inoculated into SGC and NB medium, in Klett-tubes, with NaCl concentration of 3 M and 4 M, and each medium with different pH ranging from 4.5 to 6.5. The tubes were incubated at 30°C under reciprocal shaking. The turbidity was measured every day by Klett-Summerson meter in Klett-Summerson unit against the uninoculated blank. The duplicates were carried out for each and every medium and the values were averaged.

Lipase test

Medium for lipase test was prepared as follows:-

For bacteria grown in SGC medium,

KCl	0.2%(w/v)
$MgSO_4$	2.0
$CaCl_2$	0.02
NaCl	3 M
pH	7.6–7.7

For bacteria grown in NB medium,

$CaCl_2$	0.02%(w/v)
NaCl	2 M
pH	7.6–7.7

Both media were autoclaved at 121°C for 20 min. After autoclaving, 0.5% (v/v) each of Tween 20, 40, 60 and 80 were added into the flask containing the sterilized medium while the medium was still hot.

The flask was thoroughly shaked until Tween was completely dissolved. Then the following ingredients were added to corresponding medium.

For bacteria grown in SGC medium,

Casamino acid	0.75% (w/v)
Yeast extract	1.0
Sodium citrate	0.3

Agar	2.0

For bacteria grown in NB medium,

Katsuo fish extract	1.0% (w/v)
Polypeptone	1.0
Agar	2.0

Then the flasks were autoclaved at 115°C for 15 min. After autoclaving, about 30 ml each of medium was poured into each of the plates and left overnight upside-down. Next day, the isolated bacteria were inoculated into the medium and incubated at 30°C for two weeks.

The positiveness of test was indicated by the cloudiness around the colony. Colony size and the size of cloudy zone were measured. Duplicates were done for each bacteria and the values were averaged.

Gelatinase test

Gelatin solution (20% w/v) was autoclaved in test tube (121°C, 15 min) together with basic agar (2%) medium (SGC or NB). Gelatin solution was added to basic agar solution in such a way that final concentration of gelatin was 0.4%. The medium was poured into the plate (approx. 20 ml per plate) and left overnight upside-down.

Next day, the plates were inoculated with bacteria and placed at 30°C for one week. The hydrolysis activity of gelatin was detected by pouring 3–4ml of $HgCl_2$ solution ($HgCl_2$, 15 g; conc. HCl, 20ml; volume made to 100ml with distilled water). The translucent zone around the colony contrast to white opaqueness of other area showed the positiveness of the test. Colony size and size of translucent zone were measured. Duplicates were done for each bacteria and the values were averaged.

Caseinase test

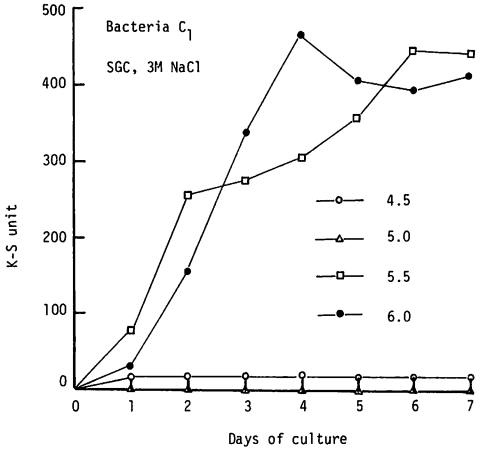
The same preparation of plates as gelatinase test was carried out using 2% Difco skim milk powder solution and the skim milk solution was added to the basic agar solution so that the final concentration of skim milk was 0.8%. The cloudiness around the colony showed the positiveness of the test. Colony size and the size of cloudy zone were measured. Duplicates were done for each bacteria and the values were averaged.

Results and Discussion

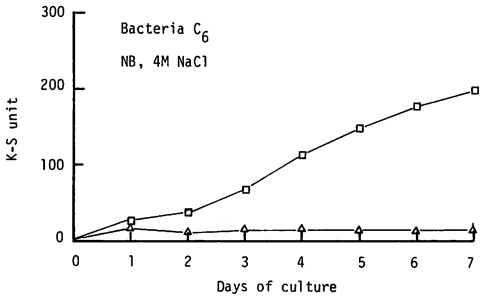
Growth characteristics of bacteria

All bacteria in this work grew well in both SGC and NB medium with pH 6.0 and above even though NaCl concentration in medium was 4 M (the data not shown). However, between pH 5.5, and 5.0, the growth became critical and this phenomenon pronounced in medium with 4 M NaCl than in medium with 3 M NaCl, as can be seen from in Fig. 1–3.

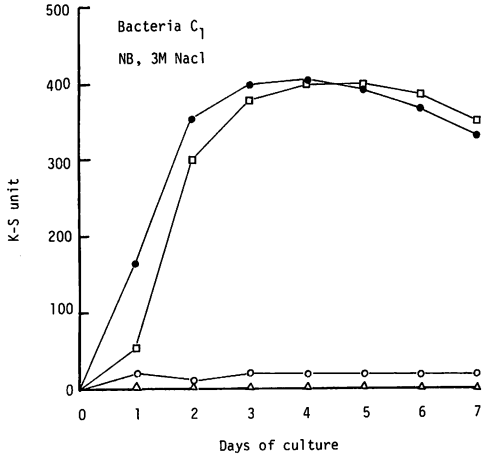
It is interesting that salt concentration of fish sauces from which these bacteria were isolated



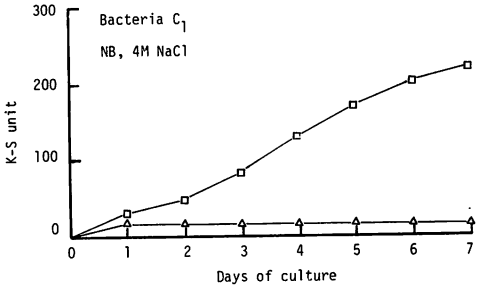
(a) in SGC, 3M NaCl
(the same symbols are used from Fig. 1. to Fig. 3. throughout)



(b) in SGC, 4M NaCl

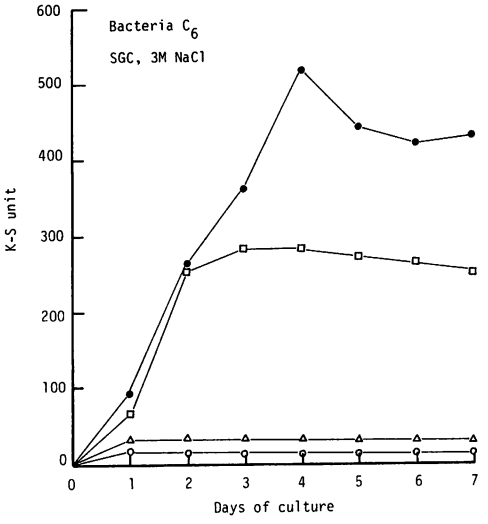


(c) in NB, 3M NaCl

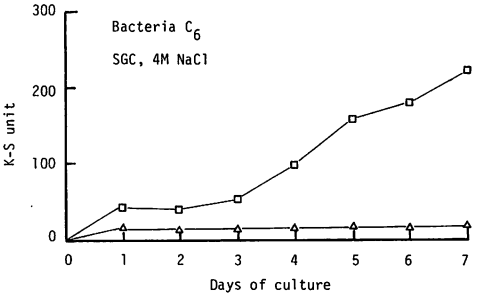


(d) in NB, 4NaCl

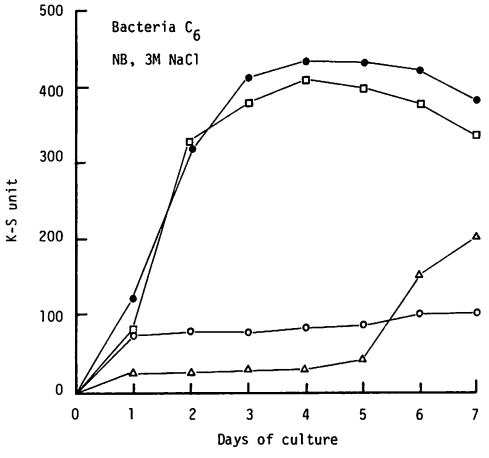
Fig. 1. Growth of bacteria C_1 in different medium with different NaCl concentration and of various pH.



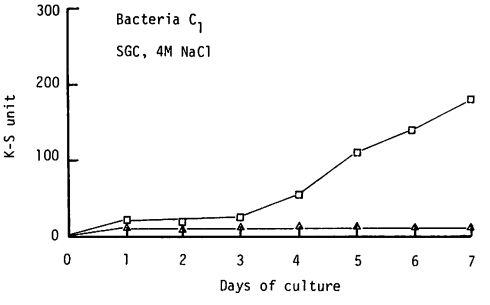
(a) in SGC, 3M NaCl



(b) in SGC, 4M NaCl

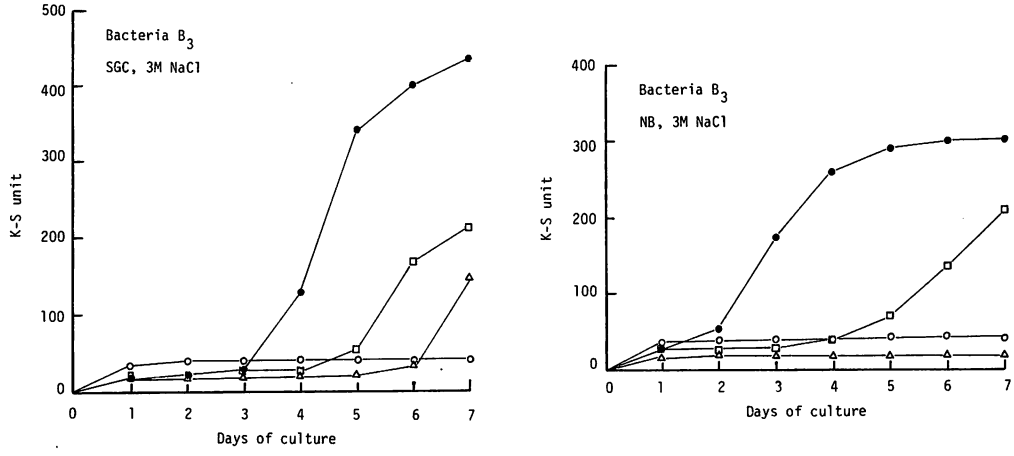


(c) in NB, 3M NaCl



(d) in NB, 4M NaCl

Fig. 2. Growth of bacteria C_6 in different medium with NaCl concetration and of various pH.



(a) in SGC, 3M NaCl (b) in NB, 3M NaCl

Fig. 3. Growth of bacteria B_3 in different medium with different NaCl concentration and of various pH.

lay between 3 M and 4 M. And pH value of Chinese fish sauce was about 6 and that of Burmese fish sauce was about 6.8. In this aspect, the bacteria would grow well in fish sauce if they were added in production of fish sauce. However, OOSHIRO *et al.*[5] stated that pH values of all fish sauce samples prepared from Japanese fish (sardines and mackerel species) lay between 5.0 and 5.5, which was the critical pH for bacteria investigated in this work. In this aspect, the utilization of these bacteria in production of fish sauce from Japanese fish might be critical also.

Table 1. Enzyme activities of bacteria

Bacteria	Lipase		Gelatinase		Casinase	
	colony (mm)	zone (mm)	colony (mm)	zone (mm)	colony (mm)	zone (mm)
C_1	5	26	11	14	11	—
C_6	6	19	10	17	10	—
B_3	7	27	9	41	9	17

Enzyme activity of bacteria

Enzyme activities of bacteria are described in Table 1. Bacteria C_1 and C_6 had lipase activity and gelatinase activity but lack in casinase activity. On the other hand, all three enzyme activities were detected for bacteria B_3. However, bacteria B_3 was tested in NB, 2 M NaCl

medium only and it is probable that the enzyme activities would be depressed in medium by higher salt concentration. One point to be regarded is that the tests were carried out in medium with pH 6.0 and above where the bacteria grew well. And one important fact is that there might be discrepencies if the bacteria were cultured in liquid medium.

Studies on identification of these bacteria, enzyme formation by bacteria in liquid medium with high salt concentration and utilization of these bacteria in accelerated production of fish sauce will be published elsewhere.

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