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# Effect of culture conditions on lactic acid production of *Tetragenococcus* species

**T. Kobayashi[1], M. Kajiwara[1], M. Wahyuni[2], N. Hamada-Sato[1], C. Imada[1] and E. Watanabe[1]**

*[1]Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo, Japan, and [2]Department of Fish Processing Technology, Bogor Agricultural University, Bogor, Indonesia*

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## ABSTRACT

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**Aims:** To investigate the effects of the salt concentration, incubation temperature and initial pH of the medium on the fermentative ability of the halophilic lactic acid bacteria, *Tetragenococcus muriaticus* and *T. halophilus*.

**Method and Results:** The growth, lactic acid production and pH reduction ability of five strains of *T. muriaticus* and *T. halophilus* in MRS broth medium under various culture conditions such as salt concentration (3, 7, 15 and 23% NaCl), temperature (20, 30 and 40°C), and initial medium pH (5·8, 6·5 and 7·5) were investigated. Those of *T. halophilus* were seriously affected by a high salinity (23% NaCl); in contrast, those of *T. muriaticus* were affected by a low initial pH (5·8).

**Conclusions:** The results indicate that high saline concentrations and low pH values have significant impact on the growth, lactic acid production and pH reduction ability of *T. halophilus* and *T. muriaticus*, respectively.

**Significance and Impact of the Study:** This study appears to be important in biopreservation during the manufacture of fermented food products. Both *T. muriaticus* and *T. halophilus* may support each other in reducing pH in hypersaline or low pH environment. To our knowledge, this is the first report on the fermentation ability of *T. muriaticus*.

**Keywords:** biopreservation, fermentation ability, lactic acid, *T. halophilus*, *T. muriaticus*, *Tetragenococcus*.

## INTRODUCTION

Lactic acid bacteria (LAB) are the most commonly used micro-organisms in food preservation techniques such as fermentation. Lactic acid produced by LAB is a useful compound for food preservation because it maintains the acidic conditions of the fermented products, and is lethal to food spoilage and food poisoning bacteria. Among halophilic lactic acid cocci, *Tetragenococcus halophilus* is the most common in high-salt fermented food products. This bacterium has been widely recognized in various food products, such as anchovy pickles (Orla-Jensen 1919), salted anchovies (Villar *et al.* 1985), Japanese soya sauce (Sakaguchi 1958; Iizuka and Yamasato 1959; Nakagawa and Kitahara 1959), and Indonesian soya sauce (Röling *et al.* 1994; Röling and Verseveld 1996). Recently, a new species of *Tetragenococcus*, *T. muriaticus*, has been isolated from a Japanese traditional fermented fish sauce (squid liver sauce) (Satomi *et al.* 1997). Strains of this new species are clearly distinguished from *T. halophilus* in terms of their 16S rRNA gene sequences, total genomic homology and some phenotypic characteristics. Recently, *T. halophilus* and *T. muriaticus* have been isolated from Japanese-fermented puffer fish ovaries (Kobayashi *et al.* 2000), Thai 'nam-pla' fish sauce (Thongsant *et al.* 2002) and Indonesian 'terasi' shrimp paste (Kobayashi *et al.* 2003). These results confirm the widespread distribution of these two species in fermented food and their significant contribution to pH reduction, which is responsible for the extended shelf life of these products. However, *T. muriaticus* previously received considerable

*Correspondence to: T. Kobayashi, Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 104-8477, Japan (e-mail: takeshik@s.kaiyodai.ac.jp).*

attention as a histamine-producing bacterium and it was characterized in terms of its histamine production ability and histidine decarboxylase property (Kimura *et al.* 2002; Konagaya *et al.* 2002). To date, there is no information on the difference in fermentation ability between *T. muriaticus* and *T. halophilus*. To determine the contribution of *T. muriaticus* to biopreservations during the manufacture of fermented food products, it would be of importance and interest to clarify the effects of culture conditions on the fermentation ability of *T. muriaticus* when compared with that of *T. halophilus* under the same experimental conditions.

In the present study, we compared the growth, lactic acid production and pH reduction *in vitro* between *T. muriaticus* and *T. halophilus* under various saline concentrations, incubation temperatures and initial pH of several media. We also discussed the fermentation abilities of the two species during the manufacture of fermented food products.

## MATERIALS AND METHODS

### Strains of LAB

*Tetragenococcus halophilus* (*halophila*) IAM 1673 and IAM 1676$^T$ were obtained from the IAM culture collection, University of Tokyo, and *T. muriaticus* JCM 10006$^T$ and JCM 10007 from the JCM culture collection (Riken, Wako-shi, Japan). *T. muriaticus* a-9 is part of our laboratory collection and was isolated from the Indonesian traditional fermented seafood 'terasi' shrimp paste.

### Lactic acid production under various culture conditions

Each strain was precultured for 48 h at 30°C in MRS broth medium (MERCK, Darmstadt, Germany) with 7% NaCl. Cells were suspended in 7% NaCl-peptone water at a density of $10^8$ cells ml$^{-1}$. Viable bacterial cells in precultures were confirmed by the plate count enumeration using MRS medium supplemented with 7% NaCl and 1·2% agar. The cell suspension (0·1 ml) was inoculated into 10 ml of the test broth medium to prepare the various culture conditions. Unless stated otherwise, a cultivation temperature of 30°C and 7% NaCl-MRS broth (pH 7·5) were used in the experiments. The cultures were incubated for 6 days, and aliquots were taken after 2, 3 and 6 days, and used for the measurements of growth (O.D.) and the pHs of the media. Lactic acid concentration was also determined after 3 and 6 days of culture. To study the behaviour of the cultures under different saline environments, MRS broths (pH 7·5) with 3, 7, 15 and 23% (w/v) NaCl were used. To study the behaviour under different incubation temperatures, the test strains were incubated in 7% NaCl-MRS broth (pH 7·5) at 20, 30 and 40°C. For comparison at different pH, 7% NaCl-MRS broths with initial pH of 5·8, 6·5, and 7·5 were used. The pH was adjusted to 5·8 and 6·5 using MES buffer (Wako, Osaka, Japan) or to 7·5 using MOPS buffer (Wako) at a final concentration of 100 mM. Each buffer was filtered through a 0·22-$\mu$m sterilized cellulose acetate membrane and then added to the autoclaved MRS broth. Moreover, under the conditions of 23% NaCl at an initial pH of 7·5 and 7% NaCl at an initial pH of 5·8, growth, lactic acid concentration and the pH of the media were determined after 9, 18 and 24 days of incubation. All experiments were performed in duplicate and data are expressed as mean ± S.D. of them.

### Lactic acid analysis

Lactic acid concentration in the broth cultures was determined by high-performance liquid chromatography (HPLC, Shimadzu Organic Acid Analysis System; Shimadzu, Kyoto, Japan) using a Shim-pack SPR-H column (250 mm × 7·8 mm of internal diameter; Shimadzu) and an electroconductivity detector. Before HPLC, all the samples were diluted to 1/40 with distilled water and then filtered through 0·22-$\mu$m cellulose acetate membranes.

### Measurement of growth and pH

Growth was assayed by measuring optical density (O.D.) at 630 nm using a plate reader (Model 550; Bio-Rad, Hercules, CA, USA). The pH was directly measured using a pH meter (F-12; Horiba, Kyoto, Japan).

## RESULTS

### Optimal culture conditions for growth

The effects of culture conditions on the growth of *T. muriaticus* and *T. halophilus* are shown in Fig. 1. All the test strains grew in the media with 3–15% NaCl, and the optimal conditions for growth differed between the two species. Comparing the O.D. of the medium after 2-day incubation, the highest growth of *T. halophilus* was observed in 3 and 7% NaCl-MRS broths. In contrast, excellent growth of *T. muriaticus* was observed in 7% NaCl-MRS broth. In the other experiments, the optimal conditions for growth were similar between both *Tetragenococcus* species. Generally, it was observed that the ability to grow increases with incubation temperature. Only one strain of *T. muriaticus* grew better at 30°C than at 40°C. The growth of all the *Tetragenococcus* strains was enhanced in the media adjusted to pH 6·5 and 7·5 but was still slower than that in the media adjusted to pH 5·8.

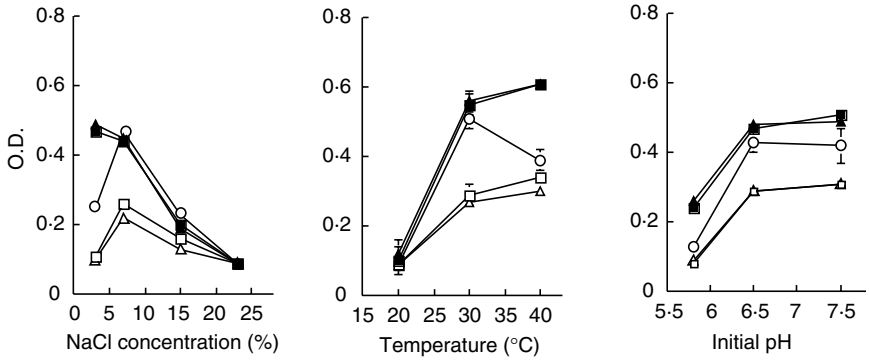


**Fig. 1** Effect of culture conditions on the growth of *Tetragenococcus muriaticus* and *T. halophilus*. (□) *T. muriaticus* JCM 10006$^T$; (△) *T. muriaticus* JCM 10007; (○) *T. muriaticus* a-9; (■) *T. halophilus* IAM 1676$^T$; (▲) *T. halophilus* IAM 1673. All of the data were obtained after the 2 days incubation

## Difference in fermentation ability at various saline concentrations

The lactic acid production at various saline concentrations (3, 7, 15 and 23%) in the culture media were measured and are shown in Table 1 and Fig. 2, as only lactic acid was produced as organic acid in the culture broths by HPLC analysis. The results of the lactic acid production are linked to the reduction in the pH of the media. For 6-day incubation of *T. muriaticus* and *T. halophilus*, the highest lactic acid concentration and highest pH reduction were observed in 15% NaCl-MRS broth.

The significant difference in fermentation ability between the above two species was observed in the media with 23% NaCl (Fig. 2). After the 6-day incubation, all the *T. muriaticus* strains showed vigorous growth: however, all the *T. halophilus* strains did not show growth. In 23% NaCl-MRS broth, lactic acid production by the *T. halophilus* strains was not detected after the 6-day incubation.

The growth of *T. halophilus* strains was delayed, but after 18 and 24 days of incubation, the lactic acid concentration in the broth media increased. Consequently, the final lactic acid concentrations in 23% NaCl-MRS broth reached 7210 ± 850 and 8110 ± 740 $\mu$g ml$^{-1}$ for the two strains of *T. halophilus*, and ranged from 6530 ± 360 to 7260 ± 740 $\mu$g ml$^{-1}$ for the three strains of *T. muriaticus*. In the same medium, the pH was reduced to 4·9 ± 0·2 and 4·9 ± 0·1 for *T. halophilus*, and 4·7 ± 0·0 to 5·0 ± 0·0 for *T. muriaticus*.

When the test strains were incubated in 3, 7 and 15% NaCl-MRS broths, both *T. halophilus* strains and *T. muriaticus* a-9 showed more rapid growth than *T. muriaticus* JCM 10006$^T$ and JCM 10007. For example, after the 6-day incubation in 7% NaCl-MRS broths, the O.D. values of *T. halophilus* IAM 1676$^T$, IAM 1673 and *T. muriaticus* a-9 reached 0·61 ± 0·01, 0·58 ± 0·01 and 0·49 ± 0·05, respectively. However, the O.D. values of *T. muriaticus* JCM 10006$^T$ and JCM 10007 were 0·40 ± 0·06 and 0·42 ± 0·02, respectively.

**Table 1** The ability of lactic acid production of *Tetragenococcus* strains at various saline concentrations

| Strain | Saline concentrations (%) | Lactic acid ($\mu$g ml$^{-1}$) 3 days* | 6 days* |
|---|---|---|---|
| *T. halophilus* | | | |
| IAM 1676$^T$ | | 7150 ± 340 | 6590 ± 1250 |
| IAM 1673 | 3 | 7140 ± 110 | 8410 ± 250 |
| IAM 1676$^T$ | | 7220 ± 210 | 8880 ± 450 |
| IAM 1673 | 7 | 7420 ± 320 | 9180 ± 460 |
| IAM 1676$^T$ | | 8240 ± 1830 | 11 930 ± 240 |
| IAM 1673 | 15 | 8960 ± 2060 | 12 650 ± 840 |
| *T. muriaticus* | | | |
| JCM 10006$^T$ | 3 | 2690 ± 1490 | 6150 ± 680 |
| JCM 10007 | | 3050 ± 1140 | 6290 ± 380 |
| a-9 | | 9100 ± 1730 | 9900 ± 680 |
| JCM 10006$^T$ | 7 | 4450 ± 670 | 5880 ± 440 |
| JCM 10007 | | 5720 ± 720 | 6700 ± 490 |
| a-9 | | 8290 ± 140 | 10 910 ± 1120 |
| JCM 10006$^T$ | 15 | 4420 ± 700 | 9340 ± 680 |
| JCM 10007 | | 5660 ± 790 | 10 560 ± 270 |
| a-9 | | 6990 ± 1150 | 10 190 ± 350 |

*Incubation time.

## Difference in fermentation ability at various incubation temperatures

The lactic acid production at 20, 30 and 40°C are presented in Table 2. We observed clear differences in the results for 40°C incubation between the two *Tetragenococcus* species. *Tetragenococcus halophilus* grew more rapidly than all the *T. muriaticus* strains. After the 6-day incubation, the O.D. values of *T. halophilus* IAM 1676$^T$ and IAM 1673 reached 0·66 ± 0·05 and 0·65 ± 0·05, respectively. However, the O.D. values of *T. muriaticus* JCM 10006$^T$, JCM 10007 and a-9 reached 0·47 ± 0·05, 0·45 ± 0·04 and 0·40 ± 0·03, respectively. These results are linked to the pH reduction and increase in lactic acid production. Consequently, the pH

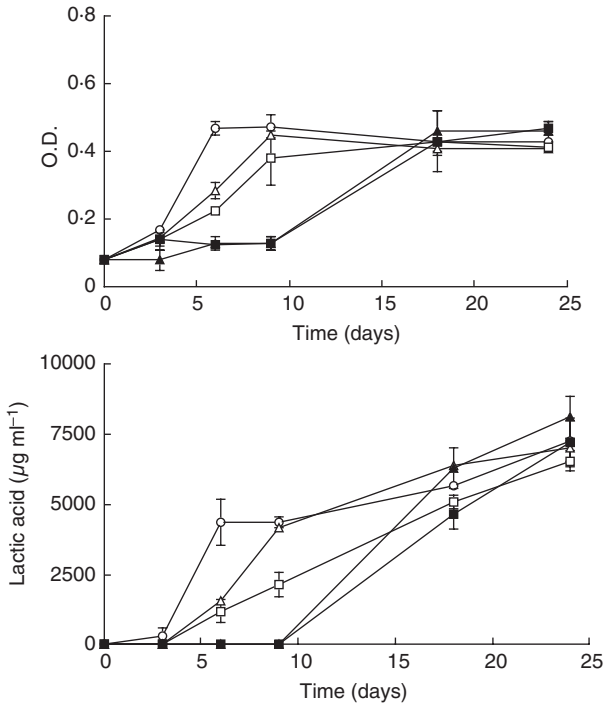


**Fig. 2** Changes in the growth and the ability of lactic acid production of *Tetragenococcus* strains in NaCl 23%-MRS broth (pH 7·5) at 30°C. (□) *T. muriaticus* JCM 10006$^T$; (△) *T. muriaticus* JCM 10007; (○) *T. muriaticus* a-9; (■) *T. halophilus* IAM 1676$^T$; (▲) *T. halophilus* IAM 1673

decreased to 4·7 ± 0·0 and 4·8 ± 0·0 for *T. halophilus*, and 5·0 ± 0·1 to 5·4 ± 0·1 for *T. muriaticus*.

When the test strains were incubated at lower temperatures, the fermentation ability was not different between the two species because *T. halophilus* and *T. muriaticus* a-9 grew more rapidly than *T. muriaticus* strains from the JCM culture collection; these results are linked to lactic acid production and pH reduction.

## Difference in fermentation ability at various pHs

The lactic acid productions in the broth media with initial pH of 5·8, 6·5 and 7·5 are presented in Table 3 and Fig. 3. All the test strains grew well in the media with initial pH of 6·5 and 7·5, which were found optimal for lactic acid production and pH reduction for all the *Tetragenococcus* species tested.

However, the growth of all the *T. muriaticus* strains was significantly affected in the medium with an initial pH of 5·8 (Fig. 3). After the 6-day incubation of *T. halophilus*, the O.D. values of IAM 1676$^T$ and IAM 1673 reached 0·50 ± 0·00 and 0·47 ± 0·03, respectively. However, the growth of the three *T. muriaticus* strains was limited and the O.D. values of JCM 10006$^T$, JCM 10007 and a-9 reached only 0·13 ± 0·02, 0·12 ± 0·01 and 0·24 ± 0·02, respectively.

**Table 2** The ability of lactic acid production of *Tetragenococcus* strains at various temperatures

| Strain | Temperature (°C) | Lactic acid (μg ml$^{-1}$) 3 days* | 6 days* |
|---|---|---|---|
| *T. halophilus* | | | |
| IAM 1676$^T$ | | 820 ± 400 | 6090 ± 570 |
| IAM 1673 | 20 | 1220 ± 330 | 6900 ± 560 |
| IAM 1676$^T$ | | 7220 ± 210 | 8890 ± 450 |
| IAM 1673 | 30 | 7420 ± 320 | 9180 ± 460 |
| IAM 1676$^T$ | | 12 150 ± 660 | 13 130 ± 70 |
| IAM 1673 | 40 | 12 530 ± 50 | 12 820 ± 190 |
| *T. muriaticus* | | | |
| JCM 10006$^T$ | | 120 ± 120 | 2120 ± 1400 |
| JCM 10007 | 20 | 300 ± 10 | 3260 ± 1380 |
| a-9 | | 500 ± 210 | 6790 ± 480 |
| JCM 10006$^T$ | | 4450 ± 670 | 5910 ± 420 |
| JCM 10007 | 30 | 5720 ± 720 | 6700 ± 490 |
| a-9 | | 8290 ± 140 | 10 910 ± 1120 |
| JCM 10006$^T$ | | 4410 ± 240 | 10 030 ± 530 |
| JCM 10007 | 40 | 5560 ± 140 | 8600 ± 610 |
| a-9 | | 8830 ± 230 | 9900 ± 240 |

Data at 30°C was quoted from Table 1.
*Incubation time.

**Table 3** The ability of lactic acid production of *Tetragenococcus* strains at various initial pH

| Strain | Initial pH | Lactic acid (μg ml$^{-1}$) 3 days* | 6 days* |
|---|---|---|---|
| *T. halophilus* | | | |
| IAM 1676$^T$ | | 9970 ± 690 | 12 220 ± 330 |
| IAM 1673 | 6·5 | 10 130 ± 760 | 12 850 ± 1320 |
| IAM 1676$^T$ | | 7220 ± 210 | 8880 ± 450 |
| IAM 1673 | 7·5 | 7420 ± 320 | 9180 ± 460 |
| *T. muriaticus* | | | |
| JCM 10006$^T$ | | 4150 ± 380 | 6010 ± 480 |
| JCM 10007 | 6·5 | 5260 ± 10 | 7100 ± 480 |
| a-9 | | 8500 ± 560 | 10 980 ± 360 |
| JCM 10006$^T$ | | 4450 ± 670 | 5880 ± 440 |
| JCM 10007 | 7·5 | 5720 ± 720 | 6700 ± 490 |
| a-9 | | 8290 ± 140 | 10 910 ± 1120 |

Data at initial pH 7·5 was quoted from Table 1.
*Incubation time.

The cultures of *T. halophilus* had lactic acid concentrations from 5920 ± 50 to 6360 ± 730 μg ml$^{-1}$ and pH from 4·5 ± 0·0 to 4·6 ± 0·0, however, those of *T. muriaticus* had lower values ranging from 730 ± 150 to 1850 ±

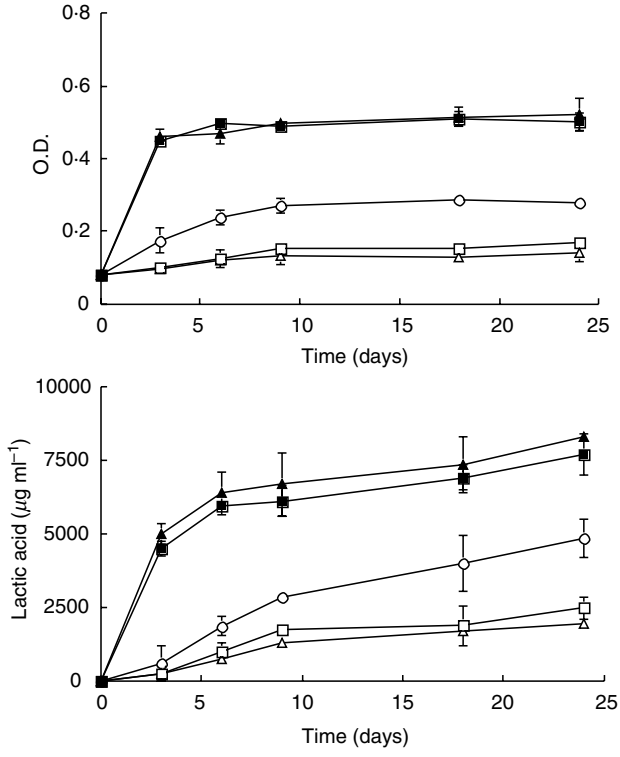


**Fig. 3** Changes in the growth and the ability of lactic acid production of *Tetragenococcus* strains in NaCl 7%-MRS broth (pH 5·8) at 30°C. (□) *T. muriaticus* JCM 10006$^T$; (△) *T. muriaticus* JCM 10007; (○) *T. muriaticus* a-9; (■) *T. halophilus* IAM 1676$^T$; (▲) *T. halophilus* IAM 1673

320 $\mu$g ml$^{-1}$ and from 5·4 ± 0·1 to 5·6 ± 0·0, respectively. Although the growth of *T. muriaticus* was more sensitive to low pHs than that of *T. halophilus* during the 6-day incubation, *T. muriaticus* showed gradual growth with longer incubation period. Finally, the cultures of *T. halophilus* had lactic acid concentrations from 7690 ± 690 and 8260 ± 100 $\mu$g ml$^{-1}$, and a pH of 4·5 ± 0·0; however, those of *T. muriaticus* had these parameters ranging from 1950 ± 190 to 4850 ± 650 $\mu$g ml$^{-1}$ and from 4·9 ± 0·1 to 5·1 ± 0·1, respectively.

When the test strains were incubated in MRS broths with different initial pH of 6·5 and 7·5, lactic acid production varied depending on the strain but not on the species: the growth properties of *T. halophilus* strains and *T. muriaticus* a-9 were similar. Significant differences were observed among the four test strains. *Tetragenococcus halophilus* IAM 1673 and 1676$^T$ grew more rapidly than *T. muriaticus* JCM 10006$^T$ and JCM10007. For example, after 3 days of incubation in the media with an initial pH of 6·5, the O.D. values of *T. halophilus* IAM 1676$^T$ and IAM 1673 reached 0·55 ± 0·02 and 0·53 ± 0·04, respectively. The O.D. values of *T. muriaticus* JCM 10006$^T$ and JCM 10007 reached 0·38 ± 0·02 and 0·39 ± 0·03, respectively, while that of *T. muriaticus* a-9 was 0·50 ± 0·03.

## DISCUSSION

Prior to the present study, the microflora containing two *Tetragenococcus* species was reported for fermented food products such as Japanese-fermented puffer fish ovaries (Kobayashi *et al.* 2000), Thai nam-pla fish sauce (Thongsant *et al.* 2002) and Indonesian terasi shrimp paste (Kobayashi *et al.* 2003). However, why these two *Tetragenococcus* species coexist in these products and their actual role in the fermentation process of these products are as yet unknown. Taxonomically, there are some differences in phenotypic characteristics (e.g, fermentation ability) between *Tetragenococcus* species. The main differences between the two *Tetragenococcus* species isolated from Japanese-fermented seafood are their abilities to grow in 0% NaCl medium and to ferment L-arabinose, D-mannitol, maltose, D-mellezitose, maltotriose, glycerol and sucrose. On the basis of their observed abilities of sugar utilization, *T. halophilus* and *T. muriaticus* are suggested to play different roles in carbohydrate decomposition in fermented food products, one as an oligosaccharide fermenter and the other as a monosaccharide fermenter, respectively (Kobayashi *et al.* 2000).

The results of this study clearly showed that there are some differences in fermentation ability between the two *Tetragenococcus* species. The optimal salinities obtained for the growth of *T. halophilus* were 3 and 7% NaCl. However, *T. muriaticus* grew at high salt concentrations with the optimum being 7%, which was a little higher than the optimum salt concentration for *T. halophilus*. Recently, a similar finding that the growth of *T. halophilus* in MRS medium is maximal at 1 M NaCl has been reported (Hervé *et al.* 2000). Moreover, the results obtained here indicate that the fermentation ability of the two species is markedly affected by culture conditions such as high osmotic pressures and low pH. The growth of *T. halophilus* was influenced by high osmotic pressures. *Tetragenococcus halophilus* grew at low pH. Each specimen might be suitable for pH reduction during the manufacture of fermented food products with high salt concentration or low pH, respectively.

*Tetragenococcus muriaticus* and *T. halophilus* have been isolated from salted puffer fish ovaries and their exudate obtained during the manufacture of such fermented food in rice bran (Kobayashi *et al.* 2000). In the manufacture of this product, raw puffer fish ovaries are first salted for at least 6 months. The salted ovaries contain 17·7–18·8% NaCl, and the exudate of the salted ovaries contain 30·7–32·8% NaCl. Then the salted ovaries are picked up and pickled in rice bran for more than 2 years. The salt concentration of the pickled ovaries or rice bran around the pickled ovaries is then reduced to 11·5–14·3% (Kobayashi *et al.* 1995). In the

previous study (Kobayashi *et al.* 2000), a considerable number of strains of *T. muriaticus* were isolated from high-salt ovaries and exudates, but *T. halophilus* was not isolated, while in the pickled products and rice bran around the ovaries, both species were isolated. We speculate that these two species may be selected based on the difference in their sensitivity to changes in osmotic pressure in the manufacture of fermented puffer fish ovaries.

The changes in the population of both *Tetragenococcus* species may also be related to the pH of the product. The product has a pH range from 6·3 to 5·7 during the salting process, and the pH range of rice bran is from 5·8 to 5·1 during the pickling process. This pH change explains well the predominance of *T. halophilus* during the manufacture of the product based on the observations in the present study. Recently, we have isolated *Tetragenococcus* species during the manufacture of tearsi shrimp paste. The two *Tetragenococcus* species were distributed in an extreme ratio. Among the 39 strains randomly isolated from terasi shrimp paste, 38 strains were identified as *T. halophilus* and one strain as *T. muriaticus* (Kobayashi *et al.* 2003). In the manufacture of terasi shrimp paste, shrimps caught in the sea are first salted at *ca* 10% in the fishing boat. During the actual manufacture, salt is again added at 5%. Considering the chemical aspects of terasi shrimp paste manufacture, the amount of salt added to the products is *ca* 17% in total and a temporary pH decrease to 4·5 because of lactic acid production is observed (Surano and Hosono 1995). Although the final terasi product is supposed to contain a higher salt concentration of up to 23% (Putro 1993), *T. halophilus* is shown to be able to make growth worth for a relatively longer culture period (24 days) even at 23% salt condition (Fig. 2). Therefore, *T. halophilus* may be selected by this low pHs during terasi shrimp paste manufacture.

*Tetragenococcus muriaticus* was initially isolated from Japanese-fermented squid liver sauce as a histamine-producing lactic acid bacterium (Satomi *et al.* 1997). However, the microflora of lactic acid bacteria in Japanese-fermented squid liver sauce has not yet been reported. Recently, Thongsant *et al.* (2002) have isolated *T. muriaticus* strains from Thai nam-pla fish sauce and found wide variations in their phenotypic characteristics, e.g. sugar fermentation ability. To date, there is no information on the relationship between the chemical properties of nam-pla and the isolation ratio of the two *Tetragenococcus* species during the manufacture of this food. Therefore, in fish sauces such as Japanese-fermented squid liver sauce and nam-pla fish sauce, the ecological aspects of the existence of *Tetragenococcus* species should be investigated.

From a physiological viewpoint, a detailed study of the osmoprotectans of *T. halophilus* has been performed recently (Hervé *et al.* 2000). Glycine betaine, carnitine and choline, which are important in the osmoregulation of *T. halophilus*, were studied and these compounds were found to improve the growth parameters of this species; glycine betaine and carnitine were also found to accumulate in this bacterium. Furthermore, *T. halophilus* is reported to possess an ability to convert choline to glycine betaine. Hence, further study of the osmoprotectans of *T. muriaticus* should be carried out.

The study on the fermentation ability of both species in different growth media such as glucose-limited media is also in progress because of the high concentration of glucose (20 g $l^{-1}$) as carbon source in MRS medium. Different parameters (temperature, salt concentration and initial pH) affecting fermentation abilities were tested independently in this study. However, it could have been more informative to test growth parameters together with their interactions. Moreover, the lactic acid production by the two *Tetragenococcus* species during mixed culture remains to be determined. Therefore, the detailed fermentation ability of these species is presently under investigation from various viewpoints of culture conditions.

In conclusion, this study is the first to show the fermentation ability of *T. muriaticus*. Our results indicate that the growth, lactic acid production and pH reduction ability of the two *Tetragenococcus* species studied are seriously affected by the high salt concentrations or low initial pHs of the medium. Both *Tetragenococcus* species may support each other for biopreservation. This finding appears to be important in the manufacture of fermented food products with high salt concentration.

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