15 Yeasts and soy products

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15.1 Introduction

Soy products were invented in China more than 2500 years ago and were later developed in many Asian countries. Soy sauce can be divided into fermented soy sauce and chemical soy sauce, which is made by hydrolysis of vegetable protein (Figure 15.1-1) [9]. Fermented soy sauce can be divided into a Japanese-type, which uses soybeans and wheat, and a Chinese-type, which mostly uses soybeans. Japanese-type soy sauce is based on fermentation by yeasts. Soy sauce has long been used as an all-purpose seasoning in Asia. Nowadays it is also widely used in Western countries.

Fig. 15.1-1 Classification of soy products

15.1.1 Production of Japanese-type soy sauce

The procedure for producing Japanese-type soy sauce is shown in Figure 15.1-2 [18].

Soybeans and wheat are cooked to break down the proteins and starches, which are subsequently easily degraded by mold enzymes. After heat treatment, koji mold (Aspergillus oryzae or A. sojae) is inoculated and cultivated for a few days. During growth, the koji mold produces many kinds of enzymes, including protease, amylase, and peptidase. Next, brine is added to produce moromi mash, and the fermentation starts. The NaCl concentration of the moromi is 16 to 20%. During fermentation, proteins and starches are dissolved by the koji enzymes and converted into sugars, amino acids, and precursors of flavour compounds.
Fig. 15.1-2 Production procedure of Japanese-type soy sauce.
It takes about 6 months for a moromi fermentation and aging. At the beginning of the fermentation, lactic acid bacteria (*Tetragenococcus halophilus*) are added. Lactic acid is produced, which lowers the pH. This produces an optimum environment for fermentation by yeast. In the next phase, soy yeast (*Zygosaccharomyces rouxii*) is added and alcoholic fermentation starts. In some cases *Candida versatilis* or *C. etchellsii* is added to enhance the production of phenolic compounds. During the fermentation many flavour compounds are produced that are important to the quality of the soy sauce. After aging, the moromi is pressed and raw soy sauce is obtained. The raw sauce is heat-treated for pasteurization and enhancing the flavours.

### 15.2 Yeast biodiversity

Because the production of soy sauce generally occurs in the open air, microbial contamination is difficult to prevent. However, because of the high NaCl concentration, only salt-tolerant microorganisms can grow. Figure 15.2-1 lists the yeast species isolated from koji and moromi [22]. Because most contaminating yeasts in koji are not salt-tolerant, their numbers decrease rapidly in the moromi mash.

The yeast flora of moromi varies according to the phase of fermentation. In the early stage, the pH of the moromi is high (about 6.5), and *Pichia, Debaryomyces*, and some *Candida* spp. predominate. As the pH lowers due to the fermentation by lactic acid bacteria, *Z. rouxii* multiplies and produces ethanol. However, growth of *Z. rouxii* decreases as the alcohol fer-

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**Fig. 15.2-1** Yeasts isolated from soy sauce koji and moromi.

Beneficial aspects of yeasts in fermented soy products

mentation progresses, because the species is sensitive to alcohol at a concentration of about 4%. In matured moromi, *C. versatilis* and *C. etchellsii* predominate and these yeasts produce phenolic compounds.

### 15.3 Beneficial aspects of yeasts in fermented soy products

About 300 kinds of flavour compounds have so far been found in Japanese-type soy sauce [24]. They are classified according to their origin, e.g., materials (soybeans and wheat), koji molds, lactic acid bacteria, yeasts, and chemical reaction products. The flavour compounds produced by fermentation include alcohols, esters, phenols, and furanones. Table 15.3-1 lists the most important flavour compounds produced by yeasts.

<table>
<thead>
<tr>
<th>Flavour compounds</th>
<th>Yeast</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td><em>Z. rouxii</em></td>
<td>30 000</td>
</tr>
<tr>
<td>glycerol</td>
<td><em>Z. rouxii</em></td>
<td>10 000</td>
</tr>
<tr>
<td>4-hydroxy-5-methyl-3(2H)-furanone (HMMF)</td>
<td><em>Z. rouxii</em></td>
<td>256</td>
</tr>
<tr>
<td>4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone (HEMF)</td>
<td><em>Z. rouxii, C. versatilis</em></td>
<td>200</td>
</tr>
<tr>
<td>isobutyl alcohol</td>
<td><em>Z. rouxii</em></td>
<td>12</td>
</tr>
<tr>
<td>4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF)</td>
<td><em>Z. rouxii</em></td>
<td>10</td>
</tr>
<tr>
<td>isoamyl alcohol</td>
<td><em>Z. rouxii</em></td>
<td>4</td>
</tr>
<tr>
<td>2-phenylethanol</td>
<td><em>Z. rouxii</em></td>
<td>3.65</td>
</tr>
<tr>
<td>methionol</td>
<td><em>Z. rouxii</em></td>
<td>3</td>
</tr>
<tr>
<td>4-ethylguaiacol (4-EG)</td>
<td><em>C. versatilis</em></td>
<td>3</td>
</tr>
<tr>
<td>methionol</td>
<td><em>Z. rouxii</em></td>
<td>2</td>
</tr>
<tr>
<td>f-Abutyllactone</td>
<td><em>Z. rouxii</em></td>
<td>0.3</td>
</tr>
<tr>
<td>4-ethylphenol (4-EP)</td>
<td><em>C. versatilis</em></td>
<td></td>
</tr>
</tbody>
</table>

*Z: Zygosaccharomyce C: Candida*

### 15.3.1 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3-furanone (HEMF)

Among the furanones, HEMF gives Japanese-type soy sauce its characteristic flavour. The compound is produced by *Z. rouxii* and *Candida* spp. The concentration of HEMF in soy sauce is contained around 200 ppm, as the threshold value of HEMF is low (0.04 ppb). HEMF is a characteristic impact flavour compound of Japanese-type soy sauce. HEMF has antioxidative and antitumor activities as well [23, 17, 19].
The biosynthetic pathway of HEMF production in yeasts is unknown. SASAKI and coworkers suggested that sugar phosphates, such as d-ribulose 5-phosphate and d-sedoheptulose 7-phosphate, are suitable precursors for HEMF and that the pentose-phosphate cycle is essential for the production of HEMF by yeasts [30]. However, the low permeability of the cell membrane makes it difficult for yeasts to take up sugar phosphates directly. Moreover, HEMF is found in heat reaction mixtures of sugars and amino acids without any fermentation [5]. Based on these observations, some researchers proposed that HEMF is biosynthesized by yeasts from intermediates of the amino-carbonyl reaction. HAYASHIDA and coworkers reported that aging increased the concentration of HEMF in the moromi [11]. They inoculated Z. rouxii into YPD (yeast extract, peptone and dextrose) medium and added intermediates of the amino-carbonyl reaction (a heat-treated solution containing d-ribose and sodium l-glutamate), and measured the production of HEMF [10]. Their results suggested that intermediates of the amino-carbonyl reaction are precursors of HEMF. The same result was found in miso fermentation [34].

15.3.2 Phenolic compounds

4-Ethylguaiacol (4-EG) and 4-ethylphenol (4-EP) are phenolic compounds produced by C. versatilis [33]. Ferulic acid, a precursor of 4-EG, is released from plant cell wall polysaccharides by feruloyl esterase (EC 3.1.1.73.), produced by the koji molds. Cinnamate decarboxylase converts ferulic acid into 4-vinylguaiacol (4-VG), which is converted into 4-EG by vinylphenol reductase. In the same way 4-EP is produced from p-coumaric acid as a precursor. Candida etchellsii does not produce these compounds, because it lacks vinylphenol reductase [32].

15.3.3 Higher alcohols (fusel alcohols)

Higher alcohols, such as isobutyl alcohol, isoamyl alcohol, and 2-phenyl ethanol, produced by Z. rouxii are important flavour compounds. It is assumed that S. cerevisiae produces these higher alcohols from corresponding alpha-keto acids by decarboxylation and reduction [28]. These alpha-keto acids are derived from branched-chain amino acids that result from deamination of extracellular amino acids or from an amino acid biosynthetic pathway (Fig. 15.3-1).

AOKI and coworkers isolated mutants of Z. rouxii that were deficient in amino acid uptake and measured the mutants’ ability to produce higher alcohols [9]. They found that higher alcohols in soy sauce are derived from extracellular amino acids, and that decreasing the uptake of leucine and phenylalanine resulted in a decreased production of 2-phenyl ethanol and isoamyl alcohol, respectively.
15.3.3.1 2-Phenyl ethanol

In *S. cerevisiae*, 2-phenyl ethanol is produced from glucose or from phenylalanine [28]. AOKI and coworkers isolated a mutant of *Z. rouxii* resistant to p-fluoro-phenylalanine and studied the production of higher alcohols by this isolate [2]. This mutant, which could not metabolize phenylalanine, produced a large amount of 2-phenyl ethanol in moromi, but no isoamyl alcohol, isobutyl alcohol, or methionol. The authors suggested that 2-phenyl ethanol is produced from glucose through the phenylalanine biosynthetic pathway, and not from extracellular precursors as in *Z. rouxii*. The activity of prephenate dehydrogenase (EC 4.2.1.51) was decreased in this mutant, and this decrease may reduce the intracellular concentration of tyrosine and derepress the biosynthesis of prephenate. As a result of the conversion of prephenate to phenylpyruvate, which is a precursor of 2-phenyl ethanol, 2-phenyl ethanol was over produced.

15.3.3.2 Isoamylalcohol

To produce a large amount of isoamyl alcohol, YOSHIKAWA and coworkers isolated mutants resistant to 5,5,5-trifluoro-DL-leucine, an analogue of L-leucine [48]. One of these mutants
produced about three times as much isoamyl alcohol as the parental strain. The activity of alpha-isopropylmalate synthase (EC 4.1.3.12) was not inhibited by L-leucine, a feedback inhibitor. This result suggested that the accumulation of alpha-isopropylmalate, a precursor of isoamyl alcohol, is due to the overproduction of isoamyl alcohol in the mutant.

15.3.3.3 3-(Methylthio)-1-propanol (Methionol)

Methionol is usually produced from methionine in yeasts. However, a mutant of *Z. rouxii* produced a large amount of methionol in a medium without methionine [3]. This mutant was derived from a mutant resistant to L-ethionine, an analogue of methionine. The authors observed a decrease in the concentration of S-adenosylmethionine due to a reduction of S-adenosylmethionine synthase (EC 2.5.1.6), and an intracellular accumulation of methionine. It was assumed that overproduction of methionol in this strain resulted in the accumulation of methionine.

15.3.3.4 Polyol

Polyols, such as glycerol and arabitol, are produced by *Z. rouxii*. These compounds are involved in the salt-tolerance of *Z. rouxii*. Polyol may contribute to the mild taste of soy sauce. The mechanism of polyol production is discussed under “Salt-tolerance”, below.

15.4 Detrimental aspects of yeasts in fermented soy products

Sometimes moromi is spoiled by film-forming yeasts, mainly belonging to the genera *Zygosaccharomyces*, *Hansenula*, and *Pichia*. Film forming yeasts cover the surface of the moromi and produce undesirable flavours, such as n-butyric acid. The use of sanitary fermentation tanks decreased spoilage by film-forming yeasts.

15.5 Salt tolerance of yeasts in soy fermentation

Soy yeasts can grow in the presence of a high concentration of NaCl (~ 4 M). The mechanisms of salt tolerance have been investigated in *Z. rouxii*. In a medium with a high salt concentration, polyols accumulate [7], and the sterol-ester and free fatty acid content of membrane lipids increase [44, 43]. Based on these observations, several approaches have been used to elucidate the mechanisms of salt tolerance.
15.5.1 Accumulation of polyols

Zygosaccharomyces rouxii accumulates intracellular glycerol in response to an increased concentration of NaCl [7]. Glycerol regulates and protects the cells against osmotic pressure. The production and accumulation of glycerol starts immediately after initiation of salt stress, and the intracellular osmotic pressure is maintained similarly to the external osmotic pressure [47]. In the non salt-tolerant species S. cerevisiae glycerol accumulation is induced by osmotic shocks [7]. Recently, the osmosensing signal transduction pathway, named the high-osmolarity glycerol response (HOG) pathway, has been elucidated in S. cerevisiae [6, 29, 13]. Under highly osmotic conditions, osmotic stress is sensed by SLN1 and SHO1, sensor proteins located in the membrane. SLN1 forms a phosphorelay system with YPD1 and SSK1. This system transmits the signal to a pair of SSK2 and SSK22 (MAP kinase kinase kinase) and further to PBS2 (MAP kinase kinase). SHO1 transmits the signal to STE11 (MAP kinase kinase kinase) and further to the PBS2 (MAP kinase kinase). PBS2 phosphorylates HOG1 (MAP kinase) and HOG1 enhances the transcription of the GPD1 gene, which encodes for glycerol-3-phosphate dehydrogenase (EC 1.1.99.5). At the same time, glucose influx increases, and the glucose is metabolized to dihydroxyacetone phosphate. This is converted to glycerol-3-phosphate, which subsequently is altered into glycerol (Fig. 15.5-1).

Fig. 15.5-1 The osmosensing signal transduction pathway in S. cerevisiae. FBP: fructose-1,6-biphosphate; G3P: glyceraldehydes-3-phosphate; DHAP: dihydroxyacetone lactoylglutathione; GPD1: glyceraldehydes-3-phosphate dehydrogenase; SHO1: osmosensor; SLN1: histidine kinase; YPD1: inter-mediate protein between SLN and SSK1; SSK1: enzyme activator; STE11: MAP kinase kinase kinase; SSK2: MAP kinase kinase kinase; SSK22: MAP kinase kinase kinase; PD32: MAP kinase kinase; HOG1: MAP kinase; MAPK: mitogen-activated protein kinase.
Two putative mitogen-activated protein (MAP) kinase genes have been cloned from *Z. rouxii*, namely ZrHOG1 and ZrHOG2, which are homologous to the *S. cerevisiae* HOG1 gene [15]. The deduced amino acid sequences of these genes show a high homology to the sequence of HOG1 and contain a TGY motif for phosphorylation by MAP kinase kinase. These genes can complement the hog1-delta-null mutant of *S. cerevisiae*. In disruption experiments, disruptants of ZrHOG1 or ZrHOG2 in *Z. rouxii* could grow only at a concentration of 2.5 M NaCl, even though the parental strain could grow at 3 M NaCl. Consequently, the authors suggested that the HOG pathway of *Z. rouxii* is similar to that of *S. cerevisiae*.

**15.5.2 Alteration of membrane lipid composition**

Alterations in membrane lipid composition have been investigated in media with various NaCl concentrations [44, 43]. Sterol-ester, free fatty acids, and oleic acid increased, and triacylglycerol and linoleic acid decreased as the NaCl concentration increased. Polyene antibiotics, such as nystatin, amphotericin B, and filipin, bind to ergosterol in the cell membrane and change the membrane permeability. USHIO and coworkers studied the relationship between sterol and salt tolerance in nystatin-resistant mutants of *Z. rouxii* [38]. A mutant with a decreased ergosterol content, showed delayed growth at a high NaCl concentration. A second mutant with an altered sterol composition, could not grow in medium containing more than 8 % NaCl. The accumulation of glycerol in these mutants was not changed, but they leaked extracellularly glycerol. Moreover, the fluidity of the membrane lipid bilayer was increased. These results suggest that alterations in the fluidity of the membrane lipid bilayer and the permeability of the cell membrane are involved in salt tolerance.

**15.5.3 H⁺-ATPase and sodium-proton antiporter**

The intracellular concentration of Na⁺ remained low when *Z. rouxii* was cultivated in a medium with a high concentration of NaCl [26]. In *S. cerevisiae*, it has been suggested that the transfer of Na⁺ depends on the sodium-proton antiport mechanism, which uses a proton gradient as driving force [31, 8]. This proton gradient is formed by H⁺-ATPase in the plasma membrane and allows the cell to take up amino acids and sugars.

**15.5.3.1 H⁺-ATPase**

WATANABE and coworkers compared the characteristics of membrane located H⁺-ATPase in membranes from *Z. rouxii* and *S. cerevisiae* [46]. Characteristics of H⁺-ATPase of *Z. rouxii*, such as optimal pH, Km values for ATP, and sensitivity toward ATPase inhibitors, did not depend on the concentration of NaCl. However, the activity of the H⁺-ATPase doubled because the addition of NaCl to the medium increased the amount of the enzyme. Fur-
thermore, the salt tolerance of *Z. rouxii* decreased in the presence of H+-ATPase inhibitors. The activity of H+-ATPase of this species was constitutively higher than that of *S. cerevisiae*, and the H+-ATPase was activated by the addition of glucose to the medium. Unlike in *S. cerevisiae*, the specific activity of the enzyme from *Z. rouxii* was found to be independent of the growth phase. On the other hand, it has been suggested that mitochondrial ATPase activity is not essential for salt-tolerance, because respiratory-deficient mutants that lacked mitochondrial ATPase activity were still salt tolerant [42]. From these observations, it appears that plasma membrane H+-ATPase is involved in salt tolerance in *Z. rouxii*. A cloned plasma membrane H+-ATPase gene from *Z. rouxii* showed a low homology of the amino acid sequences of the N-terminal region when compared with that of *S. cerevisiae* [41]. The relation between salt tolerance and amino acid sequences of the N-terminal region was not clear. Moreover, the plasma membrane H+-ATPase isolated from *C. versatilis* behaved like that of *Z. rouxii* [45]. As a result of these observations, WATANABE and coworkers assumed that these traits of plasma membrane H+-ATPase isolated from salt-tolerant yeasts are significant for supplying the energy to drive transport systems, such as sodium-proton antiporters.

### 15.5.3.2 Sodium-proton antiporter

To elucidate the relationship between sodium-proton antiporters and salt-tolerance, two sodium-proton antiporter genes, namely *Z-SOD2* and *Z-SOD22*, have been isolated from *Z. rouxii* [40, 14]. These genes are homologues of a sodium-proton antiporter gene of *Sch. pombe*. Cells in which *Z-SOD2* was disrupted could not grow in the presence of 3 M NaCl, but were able to grow in the presence of 50 % sorbitol. This proved that *Z-SOD2* is involved in salt-tolerance, but not in osmotolerance. The salt-tolerance and osmotolerance of cells in which *Z-SOD22* was disrupted were unchanged. Thus, *Z-SOD2* encodes a functional product as an antiporter.

### 15.5.3.3 Other genes

In a study of salt-sensitive mutants, USHIO and coworkers attempted to clone the gene involved in with salt-tolerance by using the gene complementing method [37]. The cloned gene, *STA1*, showed 53.3 % similarity to *UGA43* of *S. cerevisiae*, a negative regulator of the nitrogen assimilation pathway that contains a typical zinc-finger motif to bind DNA. Decreased salt-tolerance and intracellular accumulation of Na+ were observed in cells in which *STA1* was disrupted. The researchers assumed that *STA1* is essential for NaCl tolerance and that the protein regulates the transcription of some genes involved with the efflux of Na+.
15.6 Genetic improvement of soy yeasts

There are only few reports on the genetic improvement of Z. rouxii. In this section we discuss the genetic engineering and genetic improvement of the species.

15.6.1 Plasmids

Among soy yeasts, only Z. rouxii has plasmid DNA. A plasmid resembling the 2 µm plasmid of S. cerevisiae DNA was isolated from Z. rouxii IFO 1130 [20]. Characteristics of this plasmid, named pSR1, were investigated in detail [39]. The plasmid has the same gross structure as 2 µm plasmid DNA, and is a double-stranded circular DNA molecule of a 6251-bp nucleotide sequence with inverted repeats and two isomeric forms resulting from intramolecular recombination at the inverted repeat region. pSR1 shows no similarity to other circular DNA plasmids in yeasts. The pair of inverted repeats of pSR1 are able to function as autonomously replicating sequence (ARS) in Z. rouxii and S. cerevisiae. The plasmid encodes 3 open reading frames named P, R, and S loci. The R locus encodes a site-specific recombinase, and the P and R loci encode trans-acting products to stabilize pSR1 [20,6].

15.6.2 Construction of a host-vector system for *Zygosaccharomyces rouxii*

Using pSR1, researchers constructed a host-vector system for Z. rouxii. IMURA and coworkers cloned a glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) gene from Z. rouxii to obtain a promoter that is functional in the species [12]. The same promoter is well known as a high expression promoter in S. cerevisiae. They verified the ability to promote gene expression by using LacZ as a reporter gene and showed that the promoter has a comparable expression ability to that of S. cerevisiae. An Escherichia coli – Z. rouxii shuttle vector has been constructed with the ARS sequence of pSR1 or ARS1 of S. cerevisiae as a replicon in yeast and the LEU2 gene of S. cerevisiae or the Tn601 gene, encoding the G418 resistance gene of E. coli, as a selectable marker [39]. Several hundred to 2000 transformants were obtained per microgram of plasmid DNA.

15.6.3 Improvement of *Zygosaccharomyces rouxii* using a host-vector system

The secretion of Aspergillus oryzae alkaline protease in Z. rouxii was investigated to increase the efficiency of soy sauce production [25]. An expression plasmid consisting of the glyceraldehyde-3-phosphate dehydrogenase promoter, the prepro-alkaline protease cDNA of A. oryzae, and the whole sequence of pSR1 and the G418 resistance gene has been con-
structured. The transformants obtained secreted about 300 mg/mL of *A. oryzae* alkaline protease (EC 3.4.24.40) into the medium.

### 15.6.4 Other reports of genetic engineering

Genetic engineering is a powerful tool to improve strains. However, the technique is not widely accepted in the food industry and its consumers. One of the reasons is that heterogeneous DNAs, which are used to improve the properties of strains and act as a selection marker of the transformants, remained in the host. *LacZ*, *LEU2*, and the *G418* resistance gene are very useful as selectable markers in transformants, but unfortunately, they do not occur in *Z. rouxii*. Food-grade vectors that are acceptable for the food industry and its consumers, need to be based on the use of homogenous selection marker genes. Therefore, a host-vector system using selection marker genes from *Z. rouxii* is needed. Recently, SYCHROVA and coworkers isolated *ADE2* [35] and *HIS3* [36] from *Z. rouxii*, which encode phosphoribosyl-aminoimidazole carboxylase and imidazoglycerolphosphate dehydratase (EC 4.2.1.19), respectively. These are very useful as transformant-selectable markers in *S. cerevisiae*. Therefore, the construction of a food-grade host-vector system in *Z. rouxii* is expected to occur in the near future.

The R locus of the plasmid pSR1 encodes a site-specific recombinase [20]. MATSUZAKI and coworkers developed methods to delete or invert a chromosome segment that uses the R gene of the plasmid [21]. At first, they inserted a DNA fragment, bearing a specific recombination site on the inverted repeats of pSR1, at the target site of *S. cerevisiae* using an integrative vector. Then the cells were transformed with a plasmid bearing the R gene of pSR1, which is placed downstream of the *GAL1* promoter. When the transformants were cultivated in galactose medium, the R gene was expressed. A similar method developed for DNA manipulation in plant cells [27] has not been adapted to soy yeasts yet.

### 15.7 Prospects and conclusions

In the brewing of soy sauce, yeasts play an important role, especially in the production of many flavour compounds. Because most flavour compounds, including higher alcohols and polyols, are derived from the fermentation by *Z. rouxii*, research has concentrated on this species. To enhance the production of higher alcohols, which are derived from branched-chain amino acids, researchers have isolated mutants that are resistant to amino acid analogues and have investigated the mechanisms of overproduction of these compounds. As the brewer’s yeast *S. cerevisiae* produces these flavour compounds, and because molecular genetics has been well developed, the mechanisms of higher alcohol production and polyol accumulation are best understood in this species. Comparative studies suggest that the mechanisms in *Z. rouxii* are similar to those of *S. cerevisiae*. To achieve further improvements in
the quality of soy sauce it will be necessary to establish methods to control the production of these flavour compounds by *Z. rouxii*.

A *Z. rouxii* host-vector system has been developed, but only few reports are available on the improvement of *Z. rouxii* by genetic engineering. The reason for this may be that it is not sufficient to elucidate just the genes involved in regulating the desirable characteristics in soy sauce brewing. Therefore, the development of a genomics approach to study soy yeasts will be important.

Sodium chloride plays an important role in soy sauce brewing. At high NaCl concentrations, *Z. rouxii* and *C. versatilis* are able to grow, but other microorganisms, which produce undesirable flavours, do not grow. This control of the microorganism flora in moromi determines the quality of soy sauce.

To explain the salt-tolerance of the important microorganisms, researchers have investigated the accumulation of polyols, the increase in sterol-esters and free fatty acids in the membranes, and characteristics of H+-ATPase and the sodium-proton antiporter in *Z. rouxii*. However, we believe it is important to elucidate the interactions between these mechanisms as well.

### 15.8 References


KIKKOMAN home page: www.kikkoman-usa.com


References


