Food Sci. Biotechnol. Vol. 16, No. 2, pp. 193 ~ 197 (2007)

Food Science and Biotechnology

Effect of *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59 on Extending the Shelf Life of *Kkakdugi*

Eun Kyoung Mo[1], Sun Yung Ly[2], and Chang Keun Sung[3]*

[1]*Research and Development Center, DBIO Incorporation, Daejeon 305-764, Korea*
[2]*Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Korea*
[3]*Department of Food Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea*

Abstract In order to investigate the effects of *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, *Galactomyces geotrichum* SJM-59, and *Saccharomyces cerevisiae* on extending the shelf life of *kkakdugi*, 4 kinds of lyophilized yeasts adding *kkakdugis* were prepared and stored at 4°C for 30 days. Except *S. cerevisiae* adding group, 3 kinds of yeast adding groups were maintained their desirable levels (ca. pH 4.2 and 0.6% acidity) during the fermentation. The hardness of yeast adding groups was higher than those of control during the experiments. The number of yeast and the ratio of lactic acid against to total bacteria in *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups were lower than that of control and/or *S. cerevisiae* adding group. Based on acidity, *kkakdugi* made with *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 remained edible about 10 days longer than the control product.

Key words: *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, *Galactomyces geotrichum* SJM-59, *kkakdugi*, shelf-life

Introduction

Kkakdugi, a kind of *kimchi*, is a traditional Korean fermented food prepared with radishes, green onions, and red pepper as the major materials. The quality of *kkakdugi* can be effectively controlled by starter culture as well as fermentation condition.

According to earlier reports, the microorganisms involved in *kimchi* fermentation include about 200 species of bacteria and yeasts (1, 2). Proliferation of lactic acid bacteria during *kkakdugi* fermentation results in the over-ripening (acidifying and/or softening). Thus the control of the microbial proliferation is required without reducing the quality of *kkakdugi*. Many studies have focused on extension of *kkakdugi* shelf life using calcium acetate (3), eggshell powder (4), and anti-microbial agent (1).

The halotolerant yeast *Pichia farinosa* produces sodium mediated killer toxin that is a heterodimer consisting of α and β subunits (5). And *P. farinosa* is known to have resistance to cadmium ion and formation of cadmium binding complexes via synthesized cadmium binding protein that was similar to the cadmium metallothionein produced by *Saccharomyces cerevisiae* (6). *P. farinosa* was isolated from *shoyu*, Japanese fermented soy sauce, during the first stage of fermentation (7).

Pichia anomala, referred to as *Candida pelliculosa*, is known to have antagonistic property against a range of spoilage mold *Penicillium roquefortii* (8). Its other properties include alternative oxidase activity (9), D-ribose secretion (10), invertase production (11), and degradation of anthraquinone (12). *P. anomala* was isolated from cocoa fermentation during the first stage of fermenting process. It was reported that this strain contribute to the flavor production (13, 14). This strain was also isolated as an ordinary fermenting strain from a variety of Asian fermented foods: *tape* (Indonesian cassava root) (15), *ragi* (Indonesian leaven)(16) and *ruou can* (Vietnamese wine) (17). Besides Asian fermented foods, *P. anomala* was also isolated from yogurt and table olive (18, 19). In addition to *P. anomala* killer toxin has been demonstrated to have a specific inhibitory effect on the *in vitro* attachment of *Pneumocystis carinii* that is responsible for pneumonia in immunocompromised individuals (20).

Galactomyces geotrichum, often referred to as a fungi *Geotrichum candidum*, produces fatty acid esters and specific fruit aroma. Its lipolytic and proteolytic activities may form flavor compounds and have been partly characterized (21). This strain was isolated from Danish feta cheese, Munster cheese, and Raclette cheese during the ripening process (22, 23). According to the above reports, this strain has proteolytic and lipolytic activities that directly contribute to the cheese ripening.

The QPS (qualified presumption of safety of micro-organism in food and feed) approach is a system similar in concept and purpose to the GRAS (generally recognized as safe) definition used in the USA. However, it has been modified to take into account the different regulatory practices in Europe. It represents a possible route to harmonization of approaches for the safety assessment of micro-organisms used in feed/food production. It may also ensure a better use of assessment resources by focusing on those organisms which represent the greatest risks or uncertainties (24). According to QPS, *P. anomala* and *G. geotrichum* have a history of safe usage, thus these strains and their products were declared safe for human consumption.

There is heightened concern about the fermented food using yeast. Because yeast related fermented food has high protein and vitamin B complex (25, 26). It was considered

*Corresponding author: Tel: 82-42-821-6722; Fax: 82-42-822-2287
E-mail: kchsung@cnu.ac.kr
Received July 21, 2006; accepted November 19, 2006

that slow acidification and maintainable sensory quality are derived from the addition of yeast in early stage of *kkakdugi* fermentation. The objective of this study was to investigate the feasibility of using *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 as a *kkakdugi* starter culture to obtain *kkakdugi* with an extended shelf life.

Materials and Methods

Microorganisms *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59 were isolated and identified by our research team (27). *Saccharomyces cerevisiae* was purchased from KACC (Korean Agricultural Culture Collection, Suwon, Korea). Yeasts were maintained by regular culturing on potato dextrose agar and/or broth (PDA/PDB; Difco Laboratories, Detroit, MI, USA). When the yeasts reached the logarithmic phase, they were harvested. The yeast culture broth was centrifuged at 1,400×g (4°C) for 20 min. The precipitate was frozen at -70°C for 24 hr, and lyophilized at -40°C (0.1 Torr) for 3 days with a vacuum freeze dryer (VFD0030-5085; Hanil Sci. Ind., Co., Incheon, Korea). After lyophilization, the viabilities of yeasts were determined using PDA. Based on the colony forming unit, the same concentration (1×10^4 CFU/mL) of lyophilized yeasts were mixed well with *kkakdugi* individually.

Preparation of *kkakdugi* In order to make *kakdugi*, radishes 50 g, (2×2×2 cm), red pepper powder 1.17 g, spring onion 1.67 g, garlic 0.84 g, ginger 0.25 g, sugar 1.17 g, salt 1.17 g, and lyophilized yeast (1×10^4 CFU/mL) were mixed well. *Kkakdugi* was put into vinyl bag (PET/CPP) and stored at 4°C (Low Temp. Incubator, LTI-1000SD; Eyela, Tokyo, Japan) for 30 days.

Titratable acidity and pH Samples (50 g of solid radish and *kkakdugi* juice) were homogenized together and centrifuged at 1,400×g for 10 min (4°C). Titratable acidity and pH of the supernatant were measured by a pH-metric method and pH meter, respectively. Titratable acidity was calculated as the lactic acid percentage. All samples were evaluated three times.

Determination of hardness Hardness of *kkakdugi* was obtained from texture profile analysis (TPA) using Texture analyser (TA-XT2; Stable Micro Systems, Ltd., Surey, England). Operational conditions of texture analyzer were as follow: pretest speed, 10.0 mm/sec; test speed, 5.0 mm/sec; post test speed, 10.0 mm/sec; sample area, 3.0 mm^2; distance (strain), 90.0%; force threshold, 20.0 g; contact force, 5.0 g; and probe, 2 (ϕ) × 7 mm. Hardness was performed in triplicated.

Microbial analysis The number of total bacteria was determined using plate count agar at regular intervals. Lactic acid bacteria numbers were enumerated on MRS agar in a CO_2 jar fermenter. All plates were cultured in triplicate and incubated at 37°C for 48 hr. PDA was used to determine yeast viable counts. VRBL was prepared according to the manufacturer's instructions, using ready-to-use VRBL powder medium (Merck, Germany). The pH was adjusted to 7.4±0.1. The medium was boiled for no more than 2 min until it was completely dissolved and then was cooled and stored at 47±2°C for no more than 3 hr before being poured into plates. Microbial assessments were replicated three times.

Statistical analysis All experiments were carried out in triplicate and the mean ± standard deviation (SD) was indicated. Statistical analyses were performed using one-way ANOVA with 95% confidence intervals. The ANOVA analyses were determined by Fisher's protected least-significant differences using SPSS program (ver. 11.0).

Results and Discussion

Titratable acidity and pH Changes in the pH and acidity of *kkakdugi* with *P. farinosa* SKM-1, *P. anomala* SKM-T, *G. geotrichum* SJM-59, and *S. cerevisiae* during fermentation at 4°C are shown in Fig. 1. A decrease in pH and an increase in titratable acidity were observed along with the increasing of experimental period in all groups. Control and yeasts adding groups maintained their initial pH levels (6.453±0.023) for 10 days. On the 15th day of storage, pHs of all groups were sharply dropped to around pH 4-4.2. *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups sustained their desirable pH levels (ca. 4.1-4.2) throughout the experimental period. On the contrary, pHs of control and *S. cerevisiae* adding group were decreased unceasingly, and no significant difference was detected between two groups.

Lee and Yang (28) reported that lactic acid content of 0.4-0.75% is the most desirable acidity for *kimchi*, 0.75-1% means the final stage of aging, and over 1% lactic acid is inedible. As experimental period increased, the acidity of control group was increased continuously. The desirable acidities were observed from the 10th- to the 20th-day of storage. The acidity of *S. cerevisiae* adding group increased more rapidly than that of control. Considering the acidity on the 30th day of storage over 1%, control and *S. cerevisiae* adding groups were proved to be inedible

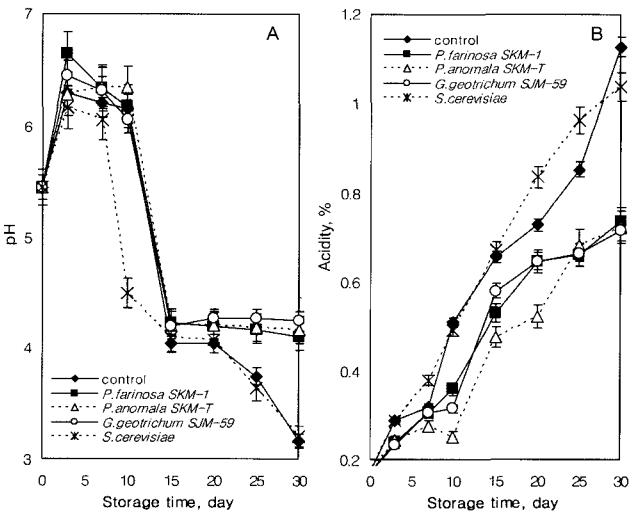

Fig. 1. Changes in pH and acidity during *kkakdugi* fermentation at 4°C. A, pH; B, acidity; p=0.05.

after 30 days. In contrast, acidities of *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups were increased slowly, and these groups retained their desirable acidity throughout the experimental period. Higher pH and lower acidity than control (not adding yeast) were also observed in vegetable fermentation with lactic acid bacteria and/or *S. cerevisiae* (29, 30), which are in agreement with our results.

Hardness Changes of hardness in control and yeast adding groups were determined at the regular interval using Texture analyzer (Fig. 2). Radishes used in this study were not soaked in brine. As *kkakdugi* fermentation developed, radishes had lower water content. Thus hardness was increased during the initial period of fermentation. As shown in Fig. 2, hardness was decreased as storage time increased. This phenomenon was similar to that observed by Rhee and Lee (31). Hardness was rapidly dropped after 7 days then slightly decreased throughout the experimental period in control and *S. cerevisiae* adding group. No significant differences were observed between these two groups. On the other hand, hardnesses of *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups were higher than that of control during the experimental period, significantly. However, no significant difference was detected between *P. farinosa* SKM-1 and *G. geotrichum* SJM-59 adding groups. Remarkably, *P. anomala* SKM-T adding group maintained its initial level until the end of experiment.

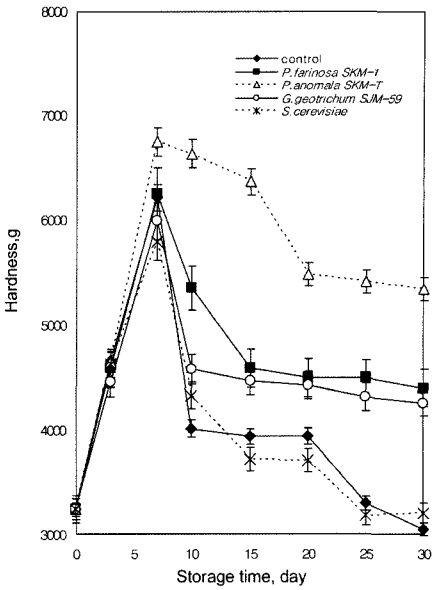

Fig. 2. Changes in hardness during *kkakdugi* fermentation at 4°C (p=0.05).

Change of microbes Total viable lactic acid bacteria counts were determined for all groups at 4°C for 30 days (Fig. 3). Total number of lactic acid bacteria in control increased up to 20 days then decreased. The number of viable lactic acid bacteria in yeast adding groups lower than those in control throughout the experimental period. However, no significant differences were observed in *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups. Kim and Kim (32) previously reported that increases in lactic acid bacteria were consistent with a decrease in pH, which is in agreement with our results.

Coliforms were increased in company with fermentation time of all groups until 20 days. After that, coliforms in control rapidly diminished. This phenomenon was also detected in *S. cerevisiae* adding group. Because coliforms are sensitive to acids, the main cause of their decline could be attributed to an excessive acid accumulation with pH as low as 4.0 (4). As contrasted with control, numbers of coliforms in *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups were lower than those of control for a period of 25 days.

As shown in Fig. 3C, the changes in the ratio of lactic acid bacteria against the total number of microbe (LA/TM) during *kkakdugi* fermentation. The number of total microbe increases because the growth of *Enterobacter* sp., *Pseudomonas* sp., and *Staphylococcus* sp. increase in the beginning of fermentation (35). Therefore, the ratio of LA/TM in the initial period of fermentation was lower than that in final period of all groups. The ratio of LA/TM was higher in *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups than control during the experiments. To the contrary, *S. cerevisiae* adding group exhibited low LA/TM ratios during the fermentation, which is consist with the findings of Kim *et al.* (30). Considering that the numbers of lactic acid bacteria and/or coliform in *S. cerevisiae* adding group were similar to that of control and yeast adding groups, we assumed that the increases of total microbe numbers had been coupled with a decline in the ratio of LA/TM in *S. cerevisiae* adding group. Therefore, it was considered that *S. cervisiae* has no ability to obstruct the growth of other microbes during *kkakdugi* fermentation.

As shown in Fig. 3D, yeast had a lag phase in the early stages of experiment because fermentation was performed at low temperature (4°C), and the number of total yeast increased rapidly as the aging process proceeded in control. It was reported that 85 kinds of yeasts were isolated and identified at the terminal period of *kimchi* fermentation including *Brettanomyces claussenii, Candida bogoriensis, Candida cacaoi, Candida guilliermondii, Citeromyces matritensis, Kluyveromyces vaeronae, Pichia membranaefacience, Rhodtorula glutinis, Saccharomyces bayanus, S. cerevisiae, Saccharomyces pretoriensis, Saccharomyces italicus,* and *Torulopsis salmanticensis* (36, 37). Therefore, a rapid increase in yeast levels was observed at terminal period of fermentation in control. The film formation and softening induced by yeast during the final stage of *kimchi* fermentation is the reason that rapid increases in yeast content can be interpreted as quality depreciation (36).

The total number of yeasts increased more rapidly in all yeast adding groups than in the control from initial to middle period of experiment. Total number of yeast was higher than that of control throughout the experimental period in *S. cerevisiae* adding group, which is in consistent with the observations of Kim *et al.* (37). Numbers of yeasts in *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 adding groups higher than that of others on the 3th day of fermentation, and these groups

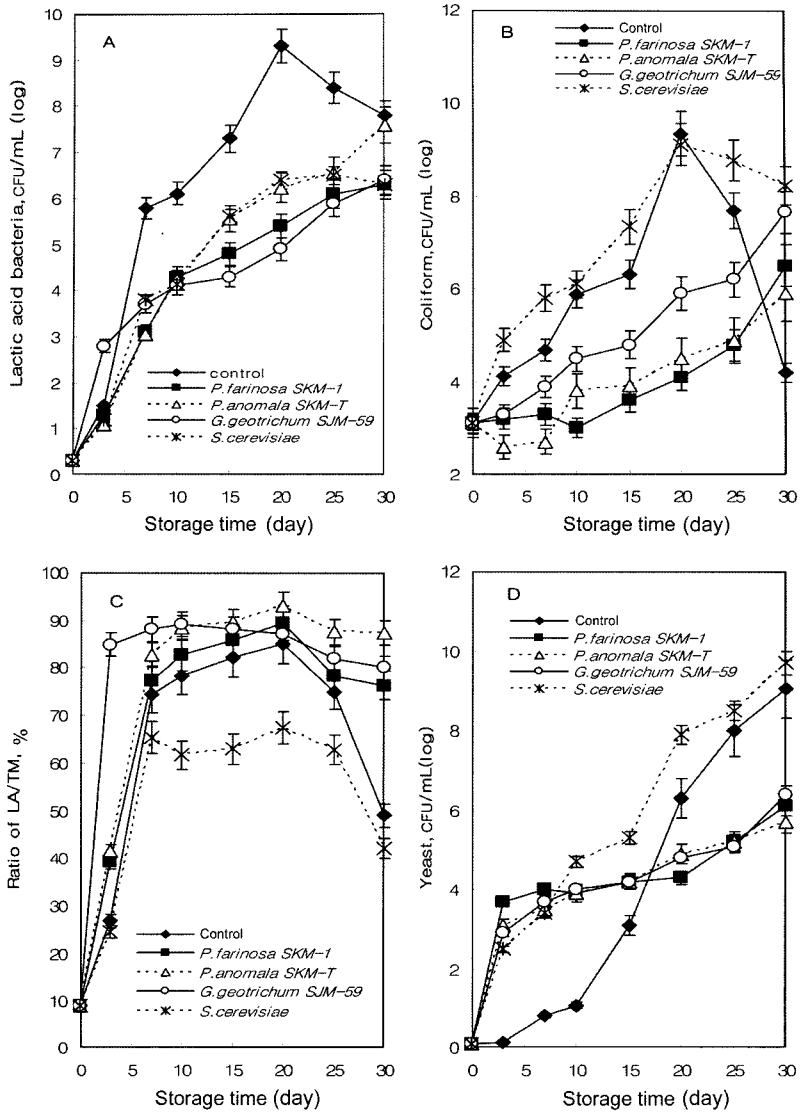

Fig. 3. Microfloral changes during *kkakdugi* fermentation at 4°C. A, changes in lactic acid bacteria population; B, changes in coliform numbers; C, ratio of lactic acid bacteria against to total microbes; D, changes of yeast; p=0.05.

extended their initial levels until the 20th day, significantly. Slow increasing of yeasts was detected at the end of the fermentation period for these three groups. However, numbers of total yeasts of these groups lower than that of control from the middle to final stages of fermentation, significantly.

In conclusion, this study shows that addtion of *P. farinosa* SKM-1, *P. anomala* SKM-T, *G. geotrichum* SJM-59, and *S. cereivisiae* extended the shelf life of *kkakdugi*. *Kkakdugi* fermented with these three yeasts maintained their desirable pH and acidity levels throughout the fermentation process, while control group was gradually acidified and softened by overproduction of lactic acid bacteria and other microbes. Furthermore, LA/TM ratios of these groups higher than that of control and/or *S. cerevisiae* adding groups throughout the experiment. A higher LA/TM ratio indicates a more hygienic fermented food product of higher quality (3, 36). The obtained results demonstrate that *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 supplements are remarkable in extending the shelf life of *kkakdugi*. Contrary to our expectations, extending effect was not detected in *S. cerevisiae*. Authors considered that the addition of *S. cerevisiae* leads the over-production of total yeast resulting in film formation at the end of the experimental period. However, we could not explain the mechanism and/or reason exactly, thus related study was necessarily.

Especially, *P. anomala* belongs to biosafety class 1 category, i.e., the handling of the organism dose not requires specific precautions (38). Organisms within biosafety class 1 have been defined as saprobes or plant pathogens occupying non-vertebrate ecological niches, or commensals. Within the literature there are reports that *P. anomala* can act as an opportunistic pathogens (39), as can indeed *S. cerevisiae* (40). Therefore this strain has no safety problem when it applied to *kkakdugi*.

Kimchi starter should have the ability to grow and/or survive in environments of low temperature, acidic, and anaerobic conditions. Moreover it should produce flavor components which can screen out the moldy odor of

kimchi (37). *P. farinosa* SKM-1, *P. anomala* SKM-T, and *G. geotrichum* SJM-59 have the ability to grow under the *kimchi* as well as *kkakdugi* fermentable condition, and they produce a lot of flavor volatile compounds (27, 41). Therefore, *P. anomala* SKM-T could be utilized to extend shelf life of *kkakdugi* for commercial purpose without safety problem. However, further studies are needed to extract the active compounds from *P. anomala* SKM-T and/or to elucidate its mechanism to extending the shelf life of *kkakdugi*.

References

1. Ko SH, Kim HS, Jo SC, Cho SH, Park WS, Lee SC. Evaluation of pH-sensitive eudragit E100 microcapsules containing nisin for controlling the ripening of *kimchi*. Food Sci. Biotechnol. 14: 358-362 (2005)
2. Um SH, Shin WS, Lee JH. Real-time PCR monitoring of *Lactobacillus sake, Lactobacillus plantarum*, and *Lactobacillus paraplanratum* during *kimchi* fermentation. Food Sci. Biotechnol. 15: 595-598 (2006)
3. Lee MY, Lee YK, Kim SD. Additional effect of calcium acetates prepared from ash of black snail and vinegars on the quality of *mul-kimchi*. Food Sci. Biotechnol. 13: 289-296 (2004)
4. Kim JM, Song KY, Kim SY, Shin WC, Yoon SS. Effect of eggshell powder on extending the shelf-life of *mulkimchi*. Food Sci. Biotechnol. 13: 136-140 (2004)
5. Fabre CE, Blanc PJ, Goma G. Production of 2-phenylethyl alcohol by *Kluyveromyces marxianus*. Biotechnol. Progr. 14: 270-274 (1998)
6. Inouhe M, Sumiyoshi M, Tohoyama H, Joho M. Resistance to cadmium ions and formation of a cadmium-binding complex in various wild-type yeasts. Plant Cell Physiol. 37: 341-346 (1996)
7. Noda F, Hayashi K, Mizunuma T. Antagonism between osmophilic lactic acid bacteria and yeasts in brine fermentation of soy sauce. Appl. Environ. Microb. 40: 452-457 (1980)
8. Boysen ME, Björneholm S, Schnürer J. Effect of the biocontrol yeast *Pichia anomala* on interactions between *Penicillium roquefortii, Penicillium carneum*, and *Penicillium paneum* in moist grain under restricted air supply. Postharvest Biol. Tec. 19: 173-179 (2000)
9. Sakajo S, Minagawa N, Yoshimoto A. Structure and regulatory expression of a single copy alternative oxidase gene from the yeast *Pichia anomala*. Biosci. Biotech. Bioch. 63: 1889-1894 (1999)
10. Wulf PD, Soetaert W, Schwengers D, Vandamme EJ. Screening and mutational improvement of a D-ribose secreting *Candida pelliculosa* strain. J. Ferment. Bioeng. 82: 1-7 (1996)
11. Pérez JA, Rodríguez J, Rodríguez L, Ruiz T. Cloning and sequence analysis of the invertase gene INV 1 from the yeast *Pichia anomala*. Curr. Genet. 29: 234-240 (1996)
12. Itoh K, Kitade CY. A pathway for biodegradation of an anthraquinone dye, C. I. disperse red 15, by a yeast strain *Pichia anomala*. Bull. Environ. Contam. Tox. 56: 413-418 (1996)
13. Schwan RF, Wheals A. The microbiology of cocoa fermentation and its role in chocolate quality. Crit. Rev. Food Sci. 44: 205-221 (2004)
14. Schwan RF, Rose AH, Board RG. Microbial fermentation of cocoa beans, with emphasis on enzymatic degaradation of the pulp. J. Appl. Bacteriol. Symp. Supp. 79: 96S-107S (1995)
15. Djien KS. *Tape* fermentation. Appl. Microbiol. 23: 976-978 (1972)
16. Saono S, Karjadi D. Microflora of *ragi*, its compositions and as a source of industrial yeasts, pp. 241-250. In: Traditional Food Fermentation as Industrial Resources in ASCA Countries. Saono S, Winarmo FG, Karjadi D (eds). The Indonesian Institute of Sciences (LIPI), Jakarta, Indonesia (1982)
17. Thanh HP, Thouc TL, Ino H, Kosaki M. *Ruou can* (tube wine) in Vietnam. pp. 520-528. In: Proceeding of International Conference Asian Network on Microbial Research. November 28 - December 2, International Conference on Asian Network on Microbial Research, Chiiang Mai, Thialand (1999)
18. Caggia C, Restuccia C, Pulvirenti A, Giudici P. Identification of *Pichia anomala* isolated from yoghurt by RFLP of the ITS region. Int. J. Food Microbiol. 71: 71-73 (2001)
19. Quintana DMC, Garcia P, Fernandez GA. Characteristics of the growth of table olive yeasts at low temperature. Grasas Aceites 54: 264-271 (2003)
20. Séguy N, Cailliez JC, Polonelli L, Dei-Cas E, Camus D. Inhibitory effect of a *Pichia anomala* killer toxin on *Pneumocystis carnii* infectivity to the SCID mouse. Parasitol. Res. 82: 114-116 (1996)
21. Daigle P, Gélinas P, Leblanc D, Morin A. Production of aroma compounds by *Geotrichum candidum* on waste bread crumb. Food Microbiol. 16: 517-522 (1999)
22. Westall S, Filtenborg O. Yeast occurrence in Danish feta cheese. Food Micorbiol. 15: 215-222 (1998)
23. Wyder MT, Bachmann HP, Puhan Z. Role of selected yeasts in cheese ripening: An evaluation in foil wrapped Raclette cheese. Lebensm. -Wiss. Technol. 32: 333-343 (1999)
24. European Food Safety Authority (EFSA). EFSA scientific colloquium summary report (QPS; Qulified presumption of safety of micro-organisms in food and feed). pp. 95-96. In: EFSA Scientific Colloquium. December 13-14, European Food Safety Authority, Brussels, Belgium (2004)
25. Berry DR, Russel I, Stewart GG. Growth of yeast, product formation. pp. 157-345. In: Yeast Biotechnology. Labatt Brewing, London, Egland (1987)
26. Larry RG. Alcoholic beverages. p. 307. In: Food and Beverage Microbiology. Van Nostrand Reinhold, New York, NY, USA (1987)
27. Mo EK, Lee JH, Xu BJ, Sung CK. Identification of yeasts from Korean feces and prerequisite characterization for preparation of probiotics. Food Sci. Biotechnol. 13: 63-70 (2004)
28. Lee YH, Yang IW. Studies on the packaging and preservation of *kimchi*. J. Korean Soc. Agric. Chem. 13: 207-218 (1970)
29. Cheigh HS, Kim HY, Yeo KM, Kim BN. Fermentation aspects of fruit-vegetable juice by mixed cultures of lactic acid bacteria isolated from *kimchi* and yeast. J. Korean Soc. Food Sci. Nutr. 27: 1059-1064 (1998)
30. Kim SD, Kim KH, Oh YA. Effects of yeast addition during salting and preparation on fermentation of *kimchi*. J. Korean Soc. Food Sci. Nutr. 27: 1077-1085 (1999)
31. Rhee HS, Lee GJ. Changes in textural properties of Korean radish and relevant chemical, enzymatic activities during salting. Korean J. Diet. Culture 8: 267-274 (1993)
32. Kim SD, Kim MH. Calcium lactate affects shelf-life and firmness of *kimchi*. Food Sci. Biotechnol. 12: 497-503 (2003)
33. Ryu CS, Kim EK, Kim YB. Changes in the bacterial flora during *kkakdugi* fermentation and the physiological characterization of lactic coccal isolates. Korean J. Food Sci. Technol. 30: 650-654 (1998)
34. Oh JY, Han YS. Purification and characterization of L-galactono-γ-lactone oxidase in *Pichia* sp. isolated from *kimchi*. Korean J. Food Sci. Technol. 35: 1135-1142 (2003)
35. Choi KC. Studies on the yeasts isolated from *kimchi*. Korean J. Microbiol. 16: 1-10 (1978)
36. Lee SH, Park NY, Choi WJ. Changes of the lactic acid bacteria and selective inhibitory substances against homo and hetero lactic acid bacteria isolated from *kimchi*. Korean J. Appl. Microbiol. Biotechnol. 27: 410-414 (1999)
37. Kim HJ, Kang SM, Yang CB. Effects of yeast addition as starter on fermentation of *kimchi*. Korean J. Food Sci. Technol. 29: 790-799 (1997)
38. de Hoog GS. Risk assessment of fungi reported from humans and animals. Mycoses 39: 407-417 (1996)
39. Chakrabarti A, Singh K, Narang A, Singhi S, Batra R, Rao KLN, Ray P, Gopalan S, Das S, Gupta V, Gupta AK, Bose SM, Mcneil MM. Outbreak of *Pichia anomala* infection in the pediatric service of a tertiary-care center in northern India. J. Clin. Microbiol. 39: 1702-1706 (2001)
40. Murphy A, Kavanagh K. Emergence of *Saccharomyces cerevisiae* as a human pathogen: Implications for biotechnology. Enz. Microb. Tech. 25: 551-557 (1999)
41. Mo EK, Kang HJ, Lee CT, Xu BJ, Kim JH, Wang QJ, Kim JC, Sung CK. Identification of phenylethyl alcohol and other flavor volatile compounds from yeasts, *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59. J. Microbiol. Biotechn. 13: 800-808 (2003)