Food Sci. Biotechnol. Vol. 14, No. 4, pp. 487 ~ 492 (2005)

Food Science and Biotechnology

Effect of *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 Dipping on Storage Property and Sensory Quality of Strawberry

Eun Kyoung Mo[1] and Chang Keun Sung[2,*]

[1]*Research and Development Center, DBIO Incorporation, Daejon 305-764, Korea*

[2]*Department of Food Science and Biotechnology, Chungnam National University, Daejon 305-764, Korea*

Abstract Simple competition plate bioassays of *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 were conducted to evaluate their potential as biological control agents that inhibit growth of *Botrytis cinerea* during post-harvest storage of strawberries (*Fragaria* × *ananassa* Duche, *Red-Pearl*). Occurrence rates of fungi on the surface of yeast-treated strawberries were evaluated during storage at 4°C. *P. anomala* SKM-T and *G. geotrichum* SJM-59 showed antifungal activities on agar plate, and *P. anomala* SKM-T maintained its desirable antifungal activity on surface of strawberries and its physicochemical properties during storage. Sensory evaluation was based on kinesthetics and consumer acceptability. Due to its potential antifungal activity, *P. anomala* SKM-T could function as biological control agent against spoilage fungi during post-harvest storage of strawberries.

Keywords: *Pichia anomala* SKM-T, *Galactomyces geotrichum* SJM-59, *Botrytis cinerea*, strawberries, biological control agent

Introduction

Yeast is a useful biological control agent for undesirable yeasts and fungi in post-harvest storage of foods, especially fruits, vegetables, and cereals. Yeast secretes killer toxins that have antimycotic agents with the potential to treat various fungal infections in humans, animals, and plants. The use of yeast as a biological control agent has, therefore, been adopted and developed worldwide.

The gray mold caused by *B. cinerea*is is a well-known disease that causes heavy losses of yields in fruits and cereals worldwide. The world market for chemical control of *Botrytis* spp. is estimated to be 200~300 million euros per year (1), suggesting that complete abolishment of chemical fungicides is unlikely. However, there is a renewed concern over environmental issues and a growing awareness that upsetting the natural microbial balance can lead to severe outbreaks of diseases, bringing about greater interest in biological control agents (1-2).

Strawberries are perishable and vulnerable to tissue damage during harvest and storage, and can also be infected with *B. cinerea* during storage. Reports have, therefore, highlighted physical and chemical methods of inhibiting *B. cinerea* infection during the post-harvest period (3-4). A modified atmosphere with an elevated level of carbon dioxide and low temperature effectively reduced the incidence of *Botrytis* infection; however, when strawberries are subjected to prolonged exposure to a high concentration of carbon dioxide, they develop off-flavor. In such circumstance, low temperature alone is ineffective against *Botrytis* infection (4-8). Another effective method of controlling post-harvest fungal infections involves prophylactic field sprays with benzimidazoles (9), but there is an increased concern on the potentially harmful health effects of chemical residues and the development of chemical tolerance in post-harvest pathogens (10-12). Thus, the demand for alternatives has increased immensely.

We report here the potential of yeasts as a practical biological control agent for the post-harvest storage of strawberries. This study also aimed at prolonging the shelf-life, and evaluating the physicochemical properties and sensory quality of strawberries treated with *P. anomala* SKM-T and *G. geotrichum* SJM-59.

Materials and Methods

Organisms *P. anomala* SKM-T and *G. geotrichum*SJM-59 were isolated from Korean feces (13), and *B. cinerea* was purchased from ATCC (American Type Cell Culture, Manassas, VA, USA). Yeasts and fungi were maintained by regular culturing on potato dextrose agar (PDA; Difco, Detroit, MI, USA) and/or potato dextrose broth (PDB; Difco) at 30°C for 24 hr, and 28°C for 5 days, respectively.

Treatment of strawberries Strawberries (*Fragaria* × *ananassa* Duche, *Red-Pearl*) were purchased from six farmhouses (Nonsan, Korea). One kilogram of strawberries were lightly dipped in *P. anomala* SKM-T or *G. geotrichum* SJM-59 culture broth (1×10^4 cfu/mL) and immediately taken out. Remaining moisture was removed from the surfaces of strawberries by drying on sieve at 20 °C for 2 hr. The control group received no treatment. Strawberries were put in PVC boxes and stored at 4°C for 15 days prior to physicochemical assays and sensory evaluations. All six batches were assayed three times.

Assessment of antifungal activity Simple competition plate bioassay was performed to assess the antifungal

*Corresponding author: Tel: 82-42-821-6722; Fax: 82-42-822-2287
E-mail: kchsung@cnu.ac.kr
Received April 7, 2005; accepted June 27, 2005

activities of *P. anomala* SKM-T and *G. geotrichum* SJM-59 on the growth of *B. cinerea*. Paper disks containing active fungal mycelia were placed on both sides of a PDA plate streaked with yeasts (Fig. 1). All plates were incubated at 25 ± 3°C for 8 days. The formation of clear zones indicates antifungal activity of yeast, and the effectiveness of antifungal activity was confirmed when the clear zone was maintained for 7 days.

Occurrence rate of fungi *B. cinerea* inoculation was performed after yeast treatments. Spore suspension (20 μL, 1×10^7cfu/mL) was sprayed over the surface of strawberries, and the inoculated fruits were left standing for 2 hr at ambient temperature to dry the spore suspension. The yeast-treated strawberries and the control group (1 kg, 200 ea, Wo) were stored at 4°C for 15 days. The number of strawberries covered with fungi (Wf) was evaluated, and the occurrence rate was obtained using the following equation: Occurrence rate of fungi (%) = 100 - (Wo - Wf/Wo × 1/ 00).

Physical properties Weights of strawberries treated with *P. anomala* SKM-T and *G. geotrichum* SJM-59, and the control group were determined during storage at 4°C for 15 days, and their moisture contents were evaluated using the air-oven method. The hardness and fracturability of the strawberries were obtained from texture profile analysis (TPA) using Texture Analyser (TA-XT2, Stable Micro Systems, Godalming, Surrey, England). Operational conditions of texture analyzer were as follow: pretest speed, 10.0 mm/s; test speed, 5.0 mm/s; post test speed, 10.0 mm/s; sample area, 3.0 mm^2; distance (strain), 90.0%; force threshold, 20.0 g; contact force, 5.0 g; and probe, 2 (?) × 7 mm. External skin color was measured using a color meter (JX 777, Minolta, Tyoko, Japan) and expressed as L^*, a^*, and b^* color values. Hue angle (H) was calculated as H = arctan b^*/a^* (deg).

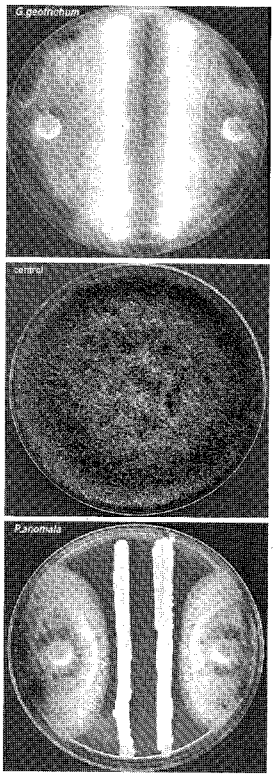

Fig. 1. Inhibition effect of *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 against the plant pathogene, *Botrytis cinerea*. Upper plate shows the competition between the *G. geotrichum* SJM-59 and *B. cinerea*. Middle plate shows control (only *B. cinerea*), bottom plate shows the competition between the *P. anomala* SKM-T and *B. cinerea*. All plates were incubated at 25 ± 3°C for 8 days.

Chemical Properties Acidity, pH, and contents of sugar, ascorbic acid, and anthocyanin were analyzed at regular intervals of 3 days for 15 days. Ten grams of strawberries were homogenized with 10 mL distilled water prior to pH measurement using a pH meter (Metrohm-654, Metrohm Ltd, Herisau, Switzerland). Titratable acidity was determined according to the method of AOAC (14). The sugar content was measured with a saccharometer (PR-101, ATAGO, Tyoko, Japan) using the homogenized solution described above. Ascorbic acid content was determined using 2,6-dichlorophenol-indophenol (DCPIP) titrimetric redox reaction method as described by the Association of Vitamin Chemists (15). Anthocyanin content was estimated using the optical density of pelargonidin-3-glucoside at 510 nm (16).

Sensory evaluation In the first sensory evaluation, the control group and the yeast-treated strawberries were stored at 4°C for 15 days, and the preference for the control and yeast-treated groups was evaluated using a ranking test at regular intervals. For second sensory evaluation, fresh and yeast-treated strawberries were stored at 4°C for 7 days, then at room temperature (28 ± 3°C) for 24 hr prior to the preference test. A panel of 12 members (6 males and 6 females, ages 20's-30's) evaluated the appearance, color, and freshness of yeast-treated strawberries, and the level of preference from the customer viewpoint. Samples were presented randomly using the table of sampling digits, and the results of the sensory evaluation were analyzed based on the statistical table of Kahan *et al.* (17).

Statistical analysis Data were analyzed using ANOVA, and the means were compared by the least significant difference (LSD) test and/or Sheffe's test at a significance level of 0.05 and/or 0.01. All statistical methods were analyzed by SPSS program (ver. 11.0).

Results and Discussion

Assay of antifungal activity We investigated the inhibitory effects of *P. anomala* SKM-T and *G. geotrichum* SJM-59 on the growth of *B. cinerea* (Fig. 1). The clear zone was maintained up to 15 days at 25 ± 3°C in the group treated with *P. anomala* SKM-T.

Occurrence rate of grey molds To investigate whether *P. anomala* SKM-T and *G. geotrichum*SJM-59 could be biological control agents, we determined the content of grey mold on the surface of strawberries dipped in *P. anomala* SKM-T and *G. geotrichum* SJM-59 during

storage. Significant differences were observed on 10th day of storage. No grey mold was detected on the surface of strawberries treated with *P. anomala* SKM-T, whereas significant amount of fungi were detected on the surface of the control and *G. geotrichum* SJM-59 treated group (40%). On the 15th day of storage, the relative detection rates of fungi occurrence were 0.3 and 49% on the surface of strawberries treated with *P. anomala* SKM-T and *G. geotrichum* SJM-59, respectively.

Physicochemical properties of strawberries treated with yeasts In the control and *G. geotrichum* SJM-59 treated groups, the weight and moisture content decreased as the storage time increased (Fig. 2); however, the group treated with *P. anomala* SKM-T maintained its initial weight during the experimental period.

Figure 3 shows the changes in texture, hardness, and fracturability. The textures of the control and *G. geotrichum* SJM-59-treated groups decreased during storage. On the other hand, no difference was observed in the group treated with *P. anomala* SKM-T, and its hardness and fracturability improved significantly.

Immediately after yeast dipping treatment, no difference in hue angel was observed between the yeast-treated and control groups. *G. geotrichum* SJM-59-treated and control groups showed a decrease in the hue angle after the 5th day of storage, whereas *P. anomala* SKM-T maintained its initial value throughout the experimental period (Fig. 4). The group treated with *P. anomala* SKM-T maintained its initial *L** value, whereas the value was increased in the *G. geotrichum* SJM-59 treated group and decreased in the control group during storage.

The pH values of the strawberries treated with *P. anomala* SKM-T and/or *G. geotrichum*SJM-59 were close

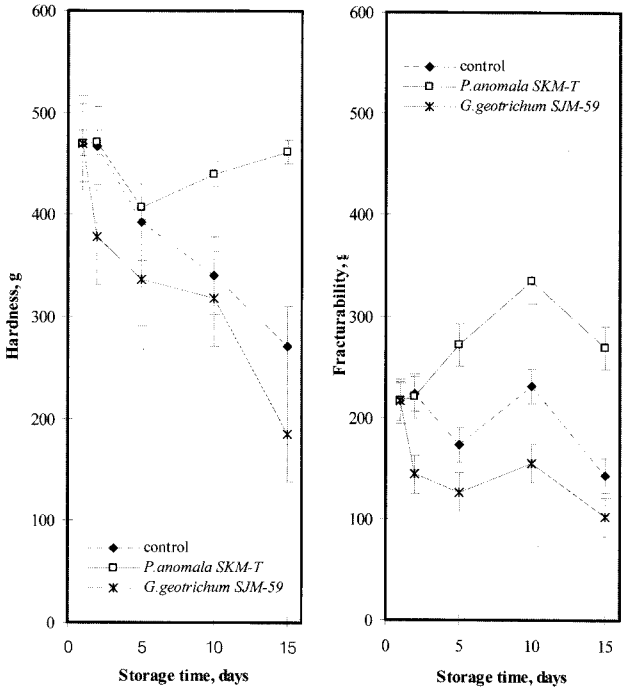

Fig. 3. The changes of texture properties of strawberries dipped into *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 culture broths during storage at 4°C. Left; hardness, p=0.001 on 15th day, Right; fracturability, p=0.041, p=0.026, and p=0.000 on 5th, 10th, and 15th day, respectively.

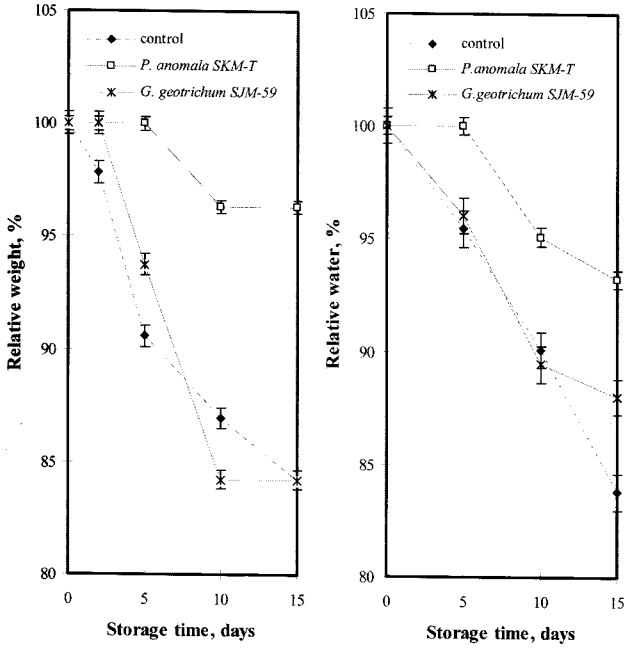

Fig. 2. Changes of relative weight and relative moisture content of strawberries treated with *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 during storage at 4°C. Left; relative weight, p=0.05, p=0.023, p=0.05 on 5th, 10th, and 15th day, right; relative moisture content, p=0.032, p=0.049 and p=0.01 on 5th, 10th, and 15th day, respectively.

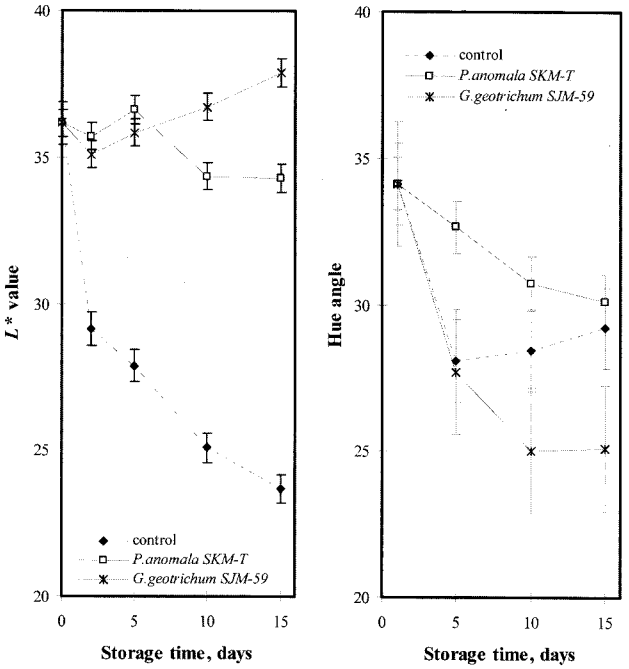

Fig. 4. Changes of the *L value and the hue angle of strawberries treated with *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 culture broths during storage at 4.** *L** values were different between the yeast treated groups and control group throughout the experiments, p=0.01. The hue angle was different between the *Pichia anomala* SKM-T treated group and control group (p=0.037).

to 3.5, and no significant pH variations were detected during storage; however, the pH of the control group increased. The yeast-treated strawberries showed higher titratable acidity than the control throughout the storage (Fig. 5).

Sugar content decreased slightly after 5 days in the control group and after 10 days in all other groups (Fig. 6A). No differences were observed between *P. anomala* SKM-T- and *G. geotrichum* SJM-59-treated groups during storage.

In spite of the differences related to the variability among treatments, the general responses of ascorbic acid losses during storage were the same (Fig. 6B). The amount of ascorbic acid decreased by 50% in the control group at day 5, decreased slightly over 25% in the yeast-treated groups throughout the storage period.

The anthocyanin content of the control group increased at day 5, then decreased after 5 days (Fig. 6C); however, the anthocyanin levels of the yeast-treated groups did not vary from their initial contents, and no differences were observed between *P. anomala* SKM-T and *G. geotrichum* SJM-59 treatments.

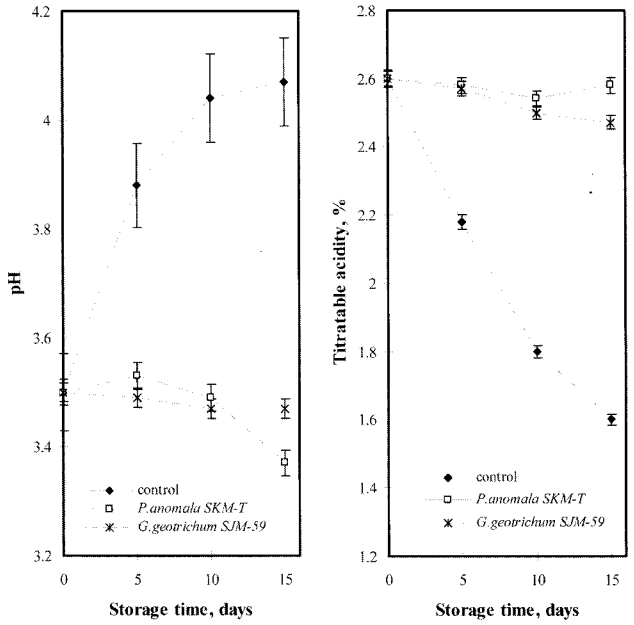

Fig. 5. Changes of pH and titratable acidity in the control group and yeast treated strawberries during preservation at 4°C. The pH and titratable acidity were different between the yeast treated groups and control group during the experiment, p= 0.05.

Sensory evaluation In the first sensory evaluation (Fig. 7), the group treated with *P. anomala* SKM-T was preferred significantly to the control and *G. geotrichum* SJM-59 treated groups throughout the entire experimental period (p = 0.01). On the other hand, in another sensory evaluation, the preference for the group treated with *P. anomala* SKM-T was significantly higher than those for the control and *G. geotrichum* SJM-59-treated groups. In addition, the 12 panel members could not discern between fresh strawberries and those treated with *P. anomala* SKM-T, which had been stored at 4°C for 7 days and at room temperature for 48 hr.

B. cinerea was strongly inhibited on the agar plate and the surface of strawberries treated with *P. anomala* SKM-T. These results could be explained as follows. Firstly, the yeast and fungi compete for nutrients (18), and the rapid growth of yeast can restrict the availability of nutrients and

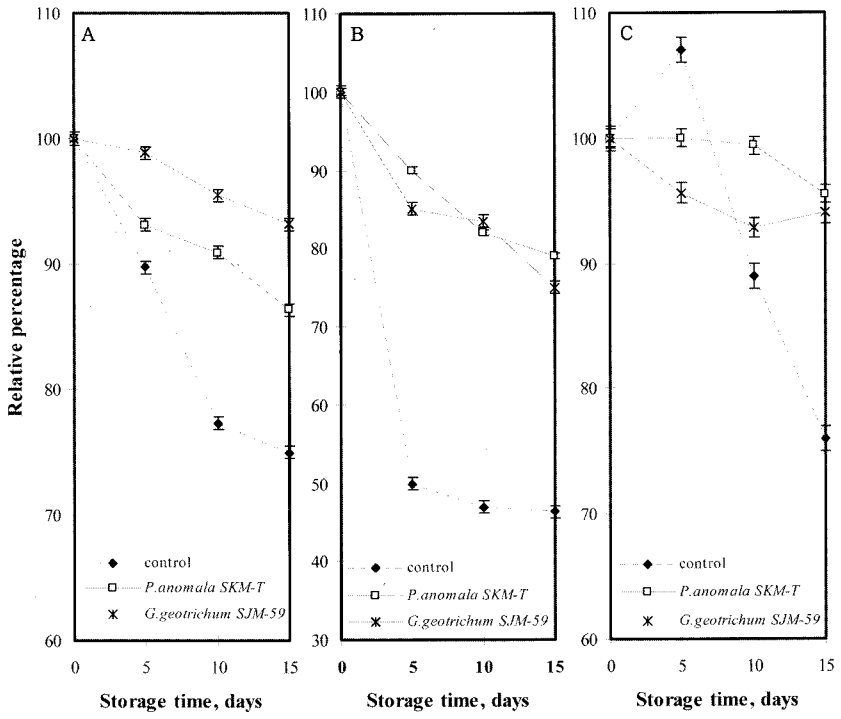

Fig. 6. Changes of sugar, ascorbic acid, and anthocyanin contents of strawberries treated with yeasts and the control group during stored at 4°C for 15 days. A; relative sugar content, B; relative ascorbic acid content, C; relative anthocyanin content.

sites for colonization of the fungi, which are essential for germinating the mold spores, and strengthening the inhibitory effect of the agar plate. Secondly, volatile metabolites are another source of antagonism in the agar plate. The tested strain, *P. anomala* SKM-T, produces many volatile flavor compounds (19). In particular, it produces a large amount of phenylethyl alcohol, which has a bactericidal effect when the concentration is higher than 0.5% in water (19-20). Furthermore, *P. anomala* is also a strong producer of ethyl acetate, which could have an antifungal effect (21). Thirdly, *P. anomala* SKM-T produces an exo-β-1,3-glucanase that can affect the degradation of the cell wall of *B. cinerea*; this hydrolytic enzyme may be involved in the disintegration of the hypha of *B. cinerea* (22). Although the mechanism that underlies the observed inhibition has been mentioned above, the major inhibitory reason has not yet been clarified.

Other noticeable effects of *P. anomala* SKM-T as a biological control agent are shown in Figs. 2~7, including the prevention of over-ripening by *P. anomala* SKM-T. In general, strawberry tissues soften, whereby the physico-chemical properties increase and/or decrease due to the over-ripening during storage (23). However, in the group treated with *P. anomala* SKM-T, fruits maintained their initial weight, moisture, hue angle, lightness, and anthocyanin content for 15 days. The pH value decreased and the titratable acidity value increased, although ascorbic acid content slightly decreased. These results were derived from volatile acids produced by *P. anomala* SKM-T (19). Biological control agents can, therefore, maintain the texture properties and sensory quality. Hardness and redness were sustained, and fracturability increased more during the initial period of storage for the group treated with *P. anomala* SKM-T. Fracturability is related to hardness and cohesiveness, and increases according to hardness and cohesiveness. Hence, the internal bonds were prevented from over-ripening in strawberries when dipped in *P. anomala* SKM-T. The inhibitory effect of over-ripening in strawberries appears to originate from 1H-indole-3-ethanol, which is produced by *P. anomala* SKM-T (19). Indole-3-ethanol can be converted into indole acetic acid (IAA), a major auxin. Auxin delays ripening and senescence, although the mechanisms remain elusive. Additional experiments are needed to determine the precise mechanism of inhibition observed in our study.

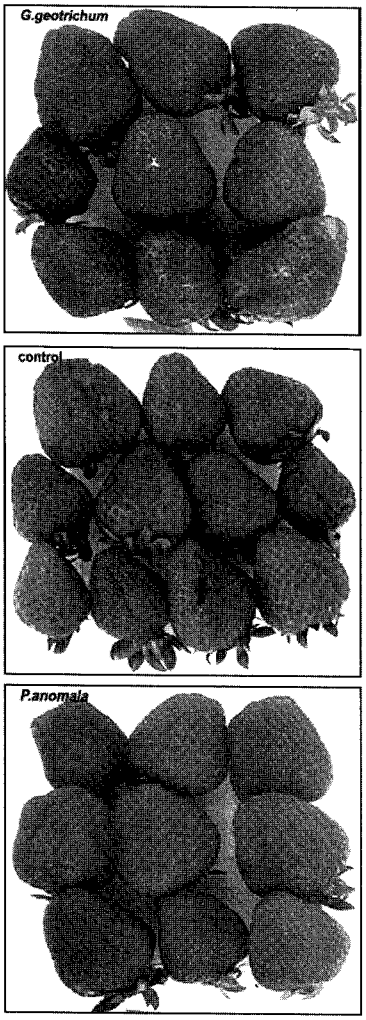

Fig. 7. The appearances of strawberries treated with *Pichia anomala* SKM-T, *Galactomyces geotrichum* SJM-59, and the control group. The control group, *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 were stored at 4 for 15 days. Microbial damage (black dots) was detected from the surface of the control group, but no microbial decay appeared in *P. anomala* SKM-T treated group. The white dots are the colony of *G. geotrichum* SJM-59 in upper.

The inhibitory effect of *G. geotrichum* SJM-59 on the growth of *B. cinerea* was indiscernible. While *G. geotrichum* SJM-59 showed strong inhibition on the agar plate, it had no inhibitory effect on the strawberries. From the surface of the group treated with *G. geotrichum* SJM-59, we gray mold could scarcely be detected, which is a symptom of the *B. cinerea* infection, possibly because the tested strain, *G. geotrichum* SJM-59, produces phenylethyl alcohol and ethyl acetate which are known as antifungal compounds (19, 21). The greatest disadvantage of the *G. geotrichum* SJM-59 treatment was the deterioration of the texture, whikch was observed only on the surfaces of the group treated with *G. geotrichum* SJM-59. The cohesiveness of the internal texture, however, was not significantly changed in this group during storage (data not shown); furthermore, *G. geotrichum* SJM-59 produced a large amount of pectate lyase and other cell wall hydrolyzing enzymes (data not shown). These results thus suggest the involvement of many kinds of enzymes that produced *G. geotrichum* SJM-59, although the mechanisms have yet to be clarified.

Considering *P. anomala* belongs to the biosafety class 1 (24), and the results of safety assessment on *P. anomala* SKM-T have been submited, *P. anomala* SKM-T is presented here as an interesting biological control agent during the post-harvest storage of strawberries with especially remarkable storage properties, as well as antifungal activity against *B. cinerea*. In addition, the group treated with *P. anomala* SKM-T showed desirable sensory qualities during cold preservation, and the sensory qualities of fresh strawberries resembled those of the *P. anomala* SKM-T-treated group when transferred from 4°C to ambient temperature within 48 hr of storage. In general, the quality of strawberries significantly decreases at room temperature within 2 days after cold preservation (CA/

MA); however, *P. anomala* SKM-T treatment did not change the sensory quality after cold storage. The efficacy of *P. anomala* SKM-T in reducing post-harvest decay was demonstrated. The treatment retained its effectiveness, and *B. cinerea* was not significantly inhibited during 15 days storage at 4°C. These may be attributed to good colonization and survival of the tested strain, enabling *P. anomala* SKM-T to compete with other plant pathogenic fungi on the surface of strawberry. Further studies are needed to characterize the anti-*B. cinerea* antagonistic factors associated with the *P. anomala* SKM-T and to evaluate their effectiveness at inhibiting *B. cinerea* in strawberries.

Acknowledgments

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

Referneces

1. Khi, J. Biological control of *Botrytis* spp. by *Ulocladium atrum* through competitive colonisation of necrotic plant tissues, Plant Research International B.V., Wageningen, Netherlands, p. 2 (2004)
2. Walker GM, McLeod AH, Hodgson VJ. Interactions between killer yeasts and pathogenic fungi. FEMS Microbiol. Lett. 127: 213-222 (1995)
3. Sommer NF, Fortlage RJ, Mitchell FG, Maxie EC. Reduction of postharvest losses of strawberry fruits from gray mold. J. Am. Soc. Hort. Sci. 98: 285-288 (1973)
4. Mass LL. Post harvest disease of strawberry. In Chielders, N.F.(Ed.), The strawberry cultivars to marketing. Horticultural publications, Univ. Florida, Gainsville, FL, pp. 329-353 (1981)
5. Li C, Kader AA. Residual effects of controlled atmospheres on post harvest physiology and quality of strawberries. J. Am. Soc. Hortic. Sci. 114: 629-634 (1989)
6. Brown W. On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and carbon dioxide. Ann. Bot. 47: 257-283 (1992)
7. Ke D, Kader AA. Mode of oxygen and carbon dioxide action on strawberry ester biosynthesis. J. Am. Hortic. Sci. 119: 971-975 (1994)
8. Aguilar CN, Herrera RR, Saenz JCM, Vega, MDLLR, Esquivel JCC. Blanching at low temperatures: A thermal bioprocess applied to fruits and vegetables to improve textural quality. Food Sci. Biotechnol. 13: 104-108 (2004)
9. Dennis C. Effect of pre-harvest fungicides on the spoilage of soft fruit after harvest. Anal. Appl. Biol. 81: 227-237 (1975)
10. Spotts RA, Cervantes LA. Populations, pathogenicity, and benomyl resistance of *Botrytis* spp., *Penicillium* sp., and *Mucor piriformis* in packing houses. Plant Dis. 70: 106-108 (1986)
11. Klein JD, Lurie Z. Post harvest heat treatment and fruit quality. Post-harvest News Information 2: 15-19 (1991)
12. Yan TY, Chung HH, Rhee CO. The effects of vacuum packaging on the quality of embryo-retaining milled rice. Food Sci. Biotechnol. 13: 476-471 (2004)
13. 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)
14. AOAC. Official methods of analysis, 13th ed., Association of Analytical Chemists, Washington DC. p. 271 (1980)
15. Association of Vitamin Chemists. Methods of Vitamin Assays, New York Interscience Publishers. (1951)
16. Lara I, Garcia P, Vendrell M. Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria ananassa* Duch.) fruit. Postharv. Biol. Technol. 34: 331-339 (2004)
17. Kahan G, Cooper D, Papavasiliou A, Kramer A. Expanded for dertemining significance of differences for ranked data. Food Technol. 27: 60 (1973)
18. Janisiewicz W. Biological control of diseases in fruits. In: Biological of plant diseases. Vol. II, CRC Press Inc., Boca Raton, FL, USA. pp. 153-166 (1988)
19. Mo EK, Kang HJ, Lee CT, Xu BJ, Kim JH, Wang QJ, Kim JC, Sung CK. Identification of phenylethyl alcohol and other volatile flavor compounds from yeasts, *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59. J. Microbiol. Biotechnol. 13: 800-808 (2003)
20. Arctander, S. Perfume and flavor chemicals: Aroma chemicals. Montclair, NJ, USA, No. 2513 (1969)
21. Fredlund E, Druvefors UA, Olstrope MN, Passoth V, Schnurer J. Influence of ethyl acetate production and ploidy on the anti-molud activity of *Pichia anomala*. FEMS Microbiol. Lett. 38: 133-137 (2004)
22. Jijaki MH, Lepoivre P. Characterization of an exo-β-1,3-gluanase produced by *Pichia anomala* strain K, antagonist of *Botrytis cinerea*on apples. Phytophathology 88: 335-343 (1998)
23. Huber DJ. Strawberry fruit softening: The potential roles of polyuronides and hemicellulose. J. Food Sci. 49: 1310-1315 (1984)
24. De Hoog, GS. Risk assessment of fungi reported from humans and animals. Mycoses 39: 407-417 (1996)