Potential of Yeast Isolates from Fruits and Vegetables for Biological Control of Chilli Anthracnose (*Colletotrichum capsici*)

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Abstract

Antagonistic yeasts against *Colletotrichum capsici* were isolated from Thai fruits and vegetables. Four antagonists (R13, R6, ER1, and L2) were found to inhibit *C. capsici* growth with biocontrol efficacies of 93.3 %, 83.1%, 76.6%, and 66.4%, respectively. These four antagonists were identified to be *Pichia guilliermondii*, *Candida musae*, *Issatchenkia orientalis*, and *Candida quercitrusa*, respectively, by using DNA sequencing of 26S rDNA, ITS regions, and these results were corroborated by physiological and morphological characteristics. Among the four strains, *P. guilliermondii* showed the highest efficacy in reducing disease incidence of chilli fruit. The optimum concentration of yeast cells to control the disease was found to be 10^8 cells/ml and the maximum disease incidence was observed at 33 ^{O}C. *C. capsici* at concentrations of 10^2-10^6 spores/ml caused lesion diameters between 0.67-1.58 cm on chilli fruits after 5 days. Postharvest treatment of chilli fruits by spraying with *P. guilliermondii* at concentration of 10^8 cells/ml and kept at 10 ^{O}C for 45 days showed a biocontrol efficacy of 80-90%. This is more effective than conventional preservation of chilli fruits with chlorinated water and distilled water.

Keywords: Biocontrol; Yeast; *Colletotrichum capsici*; Chilli fruits; Postharvest

Introduction

The general strategy of biological control is to be using one living organism to control another and the control agents may be antagonistic microorganisms or even natural plant- and animal- derived compounds. Recently, biological control has been developed as an alternative to synthetic fungicide treatment, and considerable success has been achieved upon utilizing

antagonistic microorganisms to control both preharvest and postharvest diseases (Janisiewicz and Korsten, 2002). A variety of microbial antagonists have been reported to control several different pathogens on various fruits and vegetables (Mari and Guizzardi, 1998; Fravel, 2005).

Among these antagonistic organisms, natural yeasts have been used remarkably efficacious as biological control agents. Yeasts possess many properties that make them useful for control purposes, i.e., yeasts generally do not produce allergenic spores or mycotoxins as many mycelial fungi do or antibiotic metabolites as may be produced by bacterial antagonists. Yeasts generally have simple nutritional requirements and are able to colonize dry surfaces for long periods of time, as well as withstand many pesticides used in the post-harvest environment. In addition, yeasts can grow rapidly on cheap substrates in fermenters and are therefore easy to produce in large quantities (Druvefors, 2004). Past studies and suggested modes of action of biocontrol yeasts indicate less likelihood of any hazard for the consumer. Furthermore, yeast cells contain high amounts of vitamins, minerals and essential amino acids and there are several reports on the beneficial effects of yeast in foods and feeds (Hussein et al., 1996).

In tropical vegetables such as chilli (*Capsicum annuum* L. var. *acuminatum* Fingerh.), one of the major diseases that attack them is anthracnose caused by *Colletotrichum capsici*. This disease appears as ripe rot and die-black. Ripe fruit rot is more conspicuous as it causes severe damage to mature fruits in the field as well as during transit and storage. Die-back usually appears after rain and a prolonged deposition of dew on the plants. Partially affected plants bear fewer fruits with of low quality. Under conditions favorable to disease development, up to 50% of the damage has been reported in pre-and post-harvest fruits (Smith and Crasson, 1959).

Recently, the demand for chillies in the world is increasing (Food and Agricultural Organization of the United Nations, 2004) and good quality chillies, i.e., absence of diseased appearance or fungal toxins are prerequisites for import and export (The Chile Pepper Institute, 2004). Thus it is very important to investigate the possibility of using a relatively harmless yeast biocontrol agent(s) of chilli anthracnose in order to reduce the use of chemical agents that may be harmful to humans and the environment. In this study, epiphytic yeast strains from fruits and vegetables were isolated and identified. Their capabilities to control anthracnose disease caused by *C. capsici* were investigated.

Materials and Methods

Fruits and vegetables

Sources of yeast isolates were fruits and vegetatbles, i.e., banana (*Musa sapientum* L.), mango (*Mangifera indica* L.), longan (*Dimocarpus longan* Lour.), pineapple (*Ananus comosus* Merr.), rambutan (*Nephelium lappaceum* L.), rose apple (*Eugenia javanica* Lamk.), and sapodilla (*Achras sapota* L.), bird pepper (*Capsicum frutescens* L.), chilli (*C. annuum* L.

var. *acuminatum* Fingerh.), sweetbell pepper (*C. annuum* L. var. *grossum*), egg plant (*Solanum melongena* L.), plate brush (*S. torvum* Swartz) harvested from untreated fields in Singhburi Province, in the central region of Thailand. Red chilli fruits (*C. annuum* L. var. *acuminatum* Fingerh.), which had not been treated with any fungicides were used for a biocontrol assay.

The fungal pathogen

C. capsici DOAC 1511 was obtained from the Mycological Laboratory of The Department of Agriculture (DOA), Thailand. The fungal pathogen was maintained on potato dextrose agar (PDA) slants at 4 ^{O}C.

Yeast isolation

Antagonistic yeasts were isolated from the surface of Thai fruits and vegetables according to the method of Assis et al. (1999) with modification. Serial dilution was made in sterile distilled water and 0.1 ml of each dilution was plated on yeast malt extract agar (YM agar) and adjusted to pH 3.5 by sterile tartaric acid. Isolated colonies were picked based on their differences in color and morphology. Pure cultures were obtained by the streak plate technique and were maintained on nutrient yeast dextrose agar (NYDA) slants containing 8 g/L nutrient broth, 5 g/L yeast extract, 10 g/L glucose, and 20 g/L agar. The cultures were kept at 4 ^{O}C.

Biocontrol assay by *in vitro* test

Antagonistic efficacies in biocontrol of anthracnose disease in chilli fruits were investigated in all yeast isolates. Preliminary screening was done by using an *in vitro* test according to the method of He et al.(2003).Yeast isolates were cultured in liquid medium on a rotary shaker for 48 h and yeast cells were collected by centrifugation at 3,000 rpm for 20 min. 20 μl of spore suspension of *C. capsici* ($5x10^4$ spores/ml) was injected into a hole in the center of a plate containing PDA plus 15% chilli juice, then 20 μl of the washed yeast cell suspension ($5x10^9$ cells/ml) was applied to the hole. All plates were incubated at 28 ^{O}C and diameters of fungal colonies were measured after 5 days of incubation. Each experiment was done in triplicate.

Biocontrol assay by *in vivo* test

Screening of yeast isolates for biocontrol efficacy was also done by using *in vivo* tests (He et al., 2003). Chilli fruits (no wound or scar on the surface) from untreated orchards were selected for the experiments. They were surface-sterilized with 0.5% NaCl for 5 min and then washed with tap water. After air-drying, chilli fruits were treated with 70% ethanol. Each fruit was wounded by using a sterile cork-borer (0.6 mm in diameter and 1mm in depth), one wound per fruit.

Yeast isolates with high biocontrol efficacies, selected based on data from the *in vitro* tests, were

cultured in yeast dextrose broth (NYDB: NYDA without agar) and cells were collected by centrifugation at 3,000 rpm for 20 min. Yeast cells were washed twice with sterile distilled water (DIW) and resuspended in DIW. Then 20 µl of cell suspension of each strain of yeast at concentration of $5x10^9$ cells/ml was added to the wound of 30 treated chilli fruits. After air drying, 20 µl of *C. capsici*, $5x10^4$ cells/ml was added to the wound. Chilli fruits were put on a plastic tray and stored at 28 ^{O}C. Disease severity, as indicated by increased wound diameter, was counted after 5 days of inoculation. The ability to reduce disease incidence of each yeast strain was observed and compared. The most effective yeast strain was selected for further studies.

The severity of disease caused by *C. capsici*

The severity of anthracnose disease on chilli fruits caused by *C. capsici* was studied by using 30 wounded chilli fruits inoculated with *C. capsici* at $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$, and $5x10^6$ spores/ml. Chilli fruits were put on plastic trays and stored at 28 ^{O}C. Lesion diameter and percentage of disease incidence were measured after 5 days of inoculation.

Effects of yeast cell concentration and storage temperature on biocontrol efficacy

To study the effects of various concentrations of different yeasts and the fungal pathogen, each experiment was done with 30 wounded chilli fruits. The wounds were inoculated with 20 µl of antagonistic yeast isolates selected by in *vitro test* at concentrations of $5x10^6$, $5x10^7$, $5x10^8$, and $5x10^9$ cells/ml as counted by a hemocytometer. After 12 h, 20 µl of *C. capsici* at concentrations of $5x10^4$, $5x10^5$, and $5x10^6$ spores/ml were added to each wound. Chilli fruits were placed on plastic trays and stored at 28 ^{O}C. Disease incidence was recorded after days.

To study the effects of storage temperature on biocontrol efficacy of the most effective yeast strain, wounds of 30 chilli fruits were inoculated with 20 µl of the most effective yeast cell suspension at the most appropriate concentration (data from previous results). After 12 h, 20 µl of spore suspension of *C. capsici* ($5x10^4$ spores/ml) was added to each wound. Chilli fruits were placed on plastic trays and stored at various temperatures, i.e., 18, 23, 28, and 33 ^{O}C. Disease incidence was counted after 5 days.

For the control groups of the *in vivo* test, either 20 µl of sterile distilled water or a spore suspension of *C. capsici* was added to the wound. These experiments were done in triplicates. The data were transformed into a percentage of biocontrol efficacy (BC) and disease incidence (DI) according to the formula: %BC = [(T - A)/T] x 100, where T is the number of infected wounds in the test (*C. capsici* only), and A is the number of infected wounds innoculated with the antagonist(s) and the pathogen. The values of BC ranged from 0 (no biocontrol efficacy) to 100%. The percentage of disease incidence, %DI = (A/T) x 100.

Identification of yeast antagonists by the rDNA sequence technique

Identification of antagonistic yeasts was done by using the rDNA sequence comparison technique as described by White et al. (1990) and Mitchell et al. (1992). The reaction mixture contained specific primers for D1/D2 region of the large subunit, 26S rDNA, that were NL-1 (5'-GCATATCAATAAG CGGAGGAAAAG-3') and NL-4 (5'-GGTCCG TGTTTCAAGACGG-3'). Moreover, primers of ITS region (Internal Transcribed Spacer region), ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATG-3'), were also used to amplify the intervening 5.8S gene for achieving a high separation value. The 26S and 5.8S genes were amplified by the PCR technique, which yielded about 600 bp in each DNA fragment. Aliquots of 10 μl of amplified products were separated electrophoretically on 0.8% (w/v) agarose gel in 1xTris-borate1-EDTA (TBE) buffer at a constant voltage of 100 V for 35 min, the bands were stained with ethidium bromide and photographed under tranilluminated UV light. Then the products were purified by using QIA quick (QIAGEN). Sequencing was carried out using the Automate DNA sequencer (3100-Avant Genetic Analyzer). The sequences were aligned and compared with the NCBI database by the Internet using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997).

Physiological and morphological characteristics

The methods used for evaluating morphology and for performing the fermentation and assimilation tests were as described by Kurtzman and Fell (1992). Physiological tests were repeated three times for each test strain. The physiological and morphological characteristics were used in the confirmation of yeast identification by the rDNA sequencing technique.

Postharvest disease control

Chilli fruits (no wound or scar on the surface) from untreated orchards were selected for the experiments. Three groups of fruits each were tested for preservation by the most effective yeast strain compared to that by water treatment and chemical treatment. Thus the first group of samples was sprayed with the most effective concentration of yeast cell suspensions in distilled water, based on results from the *in vivo* test of biocontrol efficacy. The second group was sprayed with distilled water, and the third group was washed with tap water and then soaked in chlorinated water (200 ppm). All samples were kept at 28 ^{O}C for 12 h. followed by storage at 10 ^{O}C for 45 days. The percentage of disease incidence in each group was calculated at different time intervals. Each experiment was performed in triplicate.

Statistical analysis

Data analysis for yeast biocontrol efficacies by *in vitro* assay, *in vivo* assay and postharvest disease control was done by using an analysis of variance (ANOVA) with SPSS 10.0 for window software (SPSS Inc., Chicago, IL, USA). Mean separations were performed by Duncan's multiple range tests, differences at $P < 0.05$ are considered significant.

Results

Primary screening of yeast

Fifty-four yeast strains *in toto* were isolated from fruits and vegetables. All yeast strains including *Saccharomyces cereviseae* (Baker's yeast) were screened for biocontrol efficacy using the *in vitro* test. In primary screening, only four yeast strains showed effective inhibition of *C. capsici* growth, i.e., no mycelial growing in the PDA plates (Figure 1). In these four yeast strains, two were isolated from rambutan and called R13 and R6. The other two strains were isolated from red egg plant and longan, they were defined as ER1 and L2, respectively. These four yeast strains were tested further for confirmation of their potential in controlling chilli anthracnose caused by *C. capsici*.

Comparison of biocontrol efficacy of the four selected yeast strains

The biocontrol efficacy of the four selected yeast strains was confirmed by using the *in vivo* test, or by their demonstrated ability to reduce disease incidence in chilli fruits. The results in Table 1 show that strain R13 had the highest biocontrol efficacy of 93.3%, or disease incidence of only 6.7%. Yeast strains R6, ER1, and L2 showed biocontrol efficacies of 83.1%, 76.6%, and 66.4%, respectively. Therefore, R13 strain was selected for further studies on the effects of temperature, yeast concentration, and storage time on control anthracnose.

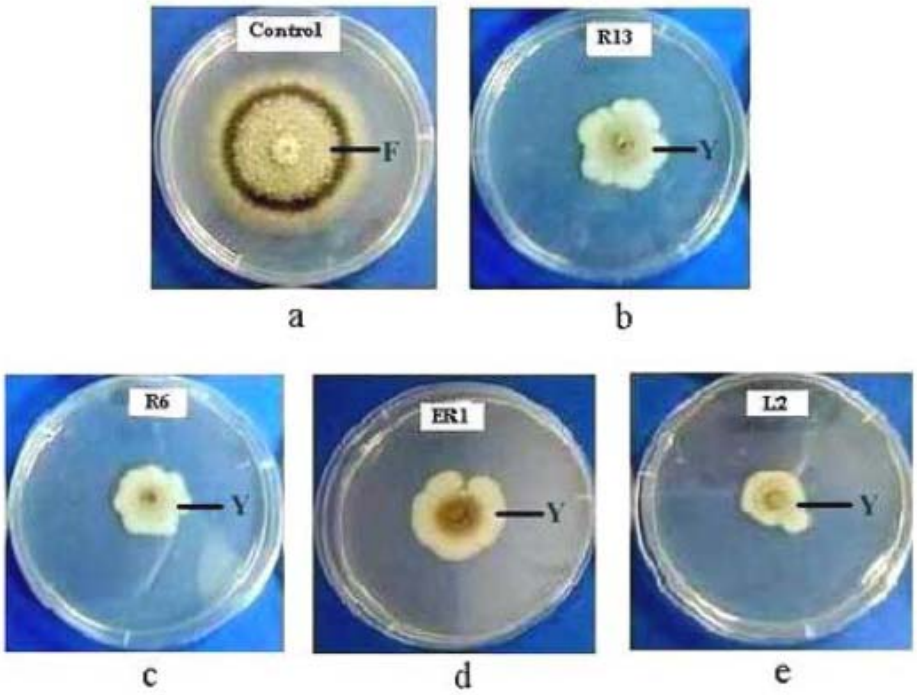

Figure 1. Biocotrol assay by using *in vitro* test of yeast isolates (R13, R6, ER1 and L2) showing colonny of *C. capsici* in control group without yeast inoculation (a) and inhibition of *C. capsici* growth by yeast strain R13 (b), R6 (c), ER1 (d) and L2 (e) on PDA plates. (F, fugal mycelia; Y, yeast cell mass)

Table 1. Biocontrol efficacies of the four selected yeast strains in reduction of disease incidence in chilli fruits

Yeast isolate	Disease incidence[z] (%)	Biocontrol efficacy[z] (%)
R13	6.7a	93.3a
R6	16.9b	83.1b
ER1	23.4c	76.6c
L2	33.6d	66.4d
Control[y]	100.0e	0.0e

[y]Control group was inoculated with sterile distilled water.

[z]Means in the same column followed by a different letter indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

The severity of disease in chilli fruits

The correlation between severity of disease and amount of *C. capsici* was investigated by increasing *C. capsici* from 10^2 to 10^6 spores/ml. The results in Table 2 and Figure 2 show an increase in lesion diameters from 0.67 to 1.58 cm. However, spore concentrations at 10^4 to 10^6 spores/ml show no significant effects on lesion expansion. The results suggest that *C. capsici* at 10^4 spores/ml can give maximum severity of disease on chilli fruits.

Table 2. Effects of various *C. capsici* concentrations on the lesion diameter in chilli fruits

C. capsici concentration (spores/ml)	Lesion diameter[y] (cm)
5×10^2	0.67a
5×10^3	1.19b
5×10^4	1.54c
5×10^5	1.55c
5×10^6	1.58c

[y]Means followed by different letters indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

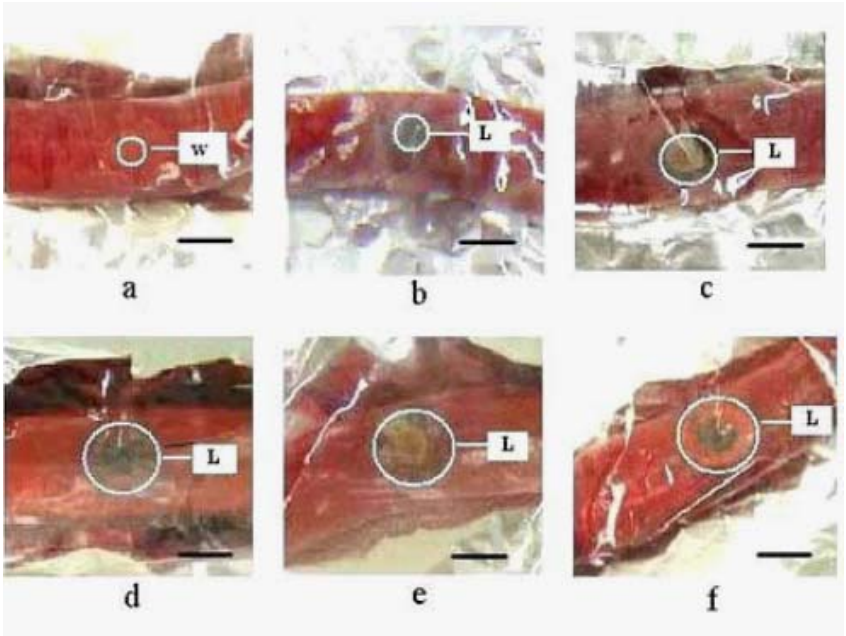

Figure 2. Effects of various *C. capsici* concentrations on lesion diameter in chilli fruits (a) no spore, (b) 5×10^2, (c) 5×10^3, (d) 5×10^4, (e) 5×10^5 and (f) 5×10^6 spores/ml. (W, wound; L, lesion area; bar = 1 cm)

Effects of R13 concentration on disease control

To investigate for the minimal yeast concentration required for disease reduction, yeast strain R13, at concentrations of 10^6-10^9 cells/ml was tested against *C. capsici* at 10^4, 10^5, and 10^6 spores/ml. As shown in Figure 3 upon infection with 10^6 spores/ml of *C. capsici*, the disease incidence was reduced from 23.6% to 10.3% as concentrations of R13 cells increased from 10^6 to 10^8 cells/ml. The control group of chilli fruits with only *C. capsici* inoculation showed disease incidence of 100% (data not shown).

At *C. capsici* concentration 10^5 spores/ml, disease incidence decreased from 20% to 6.7% as R13 increased from 10^6 to 10^8 cells/ml. Similar results were observed at 10^4 spores/ml of *C. capsici,* the disease incidence was reduced from 13.3% to 6.5%. Increased concentration of the yeast strain R13 from 10^8 to 10^9 cells/ml did not result in great reduction in disease incidence caused by 10^6 - 10^9 spores/ml of *C. capsici*. The results suggest that yeast strain R13 at a concentration of 10^8 cells/ml should be used for effective control.

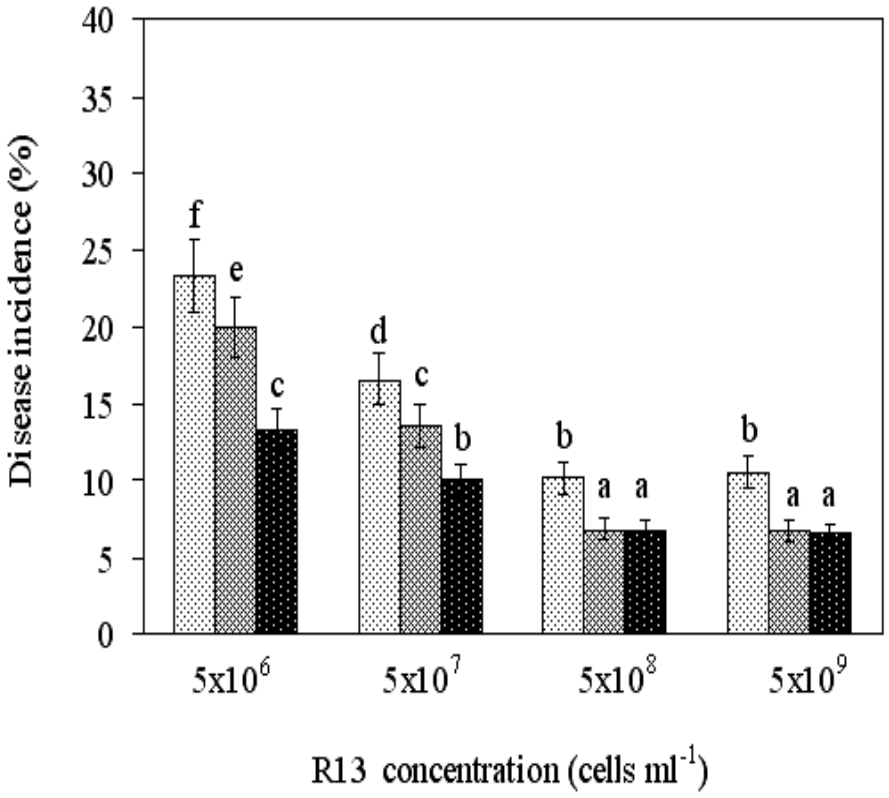

Figure 3. Effects of various concentrations of yeast strain R13 ($5x10^6$-$5x10^9$ cells/ml) on reduction of disease incidence in chilli fruits inoculated with various concentrations of *C. capsici*: $5x10^6$ spores/ml (▒), 5 x 10^5 spores/ml (▓), and $5x10^4$ spores/ml (■). Means in the bar graph indicate significant differences ($P < 0.05$) according to Duncan's multiple range test.

Effects of temperature on disease control by R13

Chilli fruit wounds inoculated with 10^4 spores/ml of *C. capsici* were treated with R13 cells (10^8 cells/ml) and stored at various temperatures (18-33 ^{O}C) for 5 days. As shown in Figure 4, the higher the storage temperature, the higher the disease incidence, i.e., an increase disease incidence from 1.1% to 10.0% as the temperature increased from 18 to 33 ^{O}C (Figure 4). The antagonistic effects of R13 were inversely related to the storage temperature; the biological control was better when the storage temperature was lower. Therefore, it is important to store chilli fruit at a low temperature.

Identification of the yeast antagonists

Yeast strains R13, R6, ER1, and L2 with the ability to control *C. capsici* growth in chilli fruits were studied for their colony characteristics, cell morphology and species identification. The results in Table 3 show colony characteristics and cell morphology. The identified species were used for DNA analysis of 26S and 5.8S rDNA regions. The sequence analysis of large subunit (26S) ribosomal DNA gene of yeast species R6, ER1, and L2 showed high identity with those of *C. musae*, *I. orientalis*, and *C. quercitrusa*, i.e., 97%, 99%, and 99%, respectively (Table 4). Identification of R13 was performed by using 5.8S rDNA region (TSI1/TSI4), which provided more effective classification than analysis of D1/D2 region. The percentage identity of R13 to *P. guilliermondii* was as high as 98% (Table 4). The results of physiological characteristics of yeast strains according to fermentation and assimilation tests (Table 5) agreed with the data of the rDNA analysis (Table 4). The different pattern of carbon fermentation and assimilation of R13, R6, ER1, and L2 as shown in Table 5 indicate that R13 could utilize more carbon sources than others, while ER1 used very few carbon sources in this system.

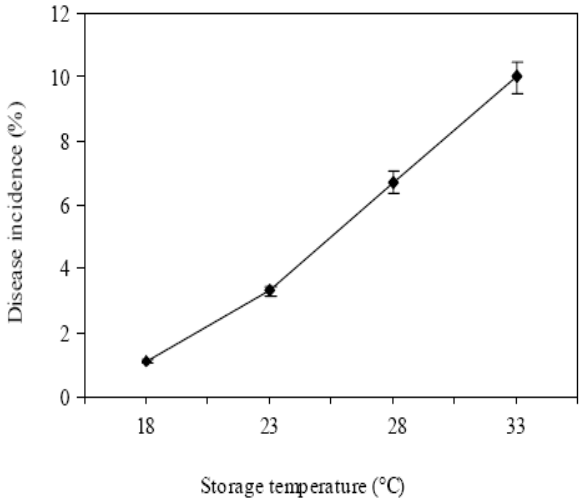

Figure 4. Effects of temperature (18-33 ^{o}C) on percentage of disease incidence. The *C. capsici* inoculated chilli fruits, were treated with yeast strain R13 (5 x 10^8 cells/ml) and incubated at various temperatures for 5 days

Table 3. Colony characteristics and cell morphologies of the four effective yeast strains

Yeast strain	Colony characteristics	Cell morphology	Culture medium	Incubation time (day)
R13	smooth, white	ovoidal to elongate	5 % malt extract agar	3
R6	smooth, white, creamy	subglobose to ovoidal	corn meal agar	14
ER1	smooth, cream	ovoidal to elongate	5 % malt extract agar	3
L2	smooth, white	cylindrical, presence of pseudohyphae	corn meal agar	7

Table 4. Identification of the four effective yeast strains by rDNA sequencing

Yeast isolate	Species	Identity[a] (%)	Score (Bit)	rDNA[b] region
R13	*Pichia guilliermondii*	98	1025	ITS1/ITS4
R6	*Candida musae*	97	902	D1/D2
ER1	*Issatchenkia orientalis*	99	1074	D1/D2
L2	*Candida quercitrusa*	99	1072	D1/D2

[a]The percentage identity among DNA fragments was calculated with BLAST program and the sequences were compared with those from NCBI data base

[b]region of rDNA gene used for identification

Table 5. Comparison of fermentation and assimilation tests (using biochemical reactions) of the four effective yeast strains (R13, R6, ER1, and L2) with *P. guilliermondii*, *C. musae*, *I. orientalis*, and *C. quercitrusa* as listed by Kurtzman and Fell (1998).

Test	Strain R13	*P. guilliermondii*	Strain R6	*C. musae*	Strain ER1	*I. orientalis*	Strain L2	*C. quercitrusa*
Fermentation:								
D-Glucose	+[a]	+	+	+	+	+	+	+
D-Galactose	-	v	-	-	-	-	-	v
Sucrose	+	+	-	-	-	-	-	-/s
Maltose	-	-	-	-	-	-	-	-/s
Lactose	-	-	-	-	-	-	-	-
Raffinose	+	+	-	-	-	-	-	-
Trehalose	+	+	+	s	-	-	-	-
Assimilation:								
D-Glucose	+	+	+	+	+	+	+	+
D-Galactose	+	+	-	-	-	-	+	+
L-Sorbose	+	v	+	+	-	-	+	+
D-Glucosamine	+	+	+	l	+	+	+	+
Actidione	+	NL	-	NL	-	NL	-	NL
Saccharose	+	+	+	+	-	-	+	+
N-acetyl-D-glucosamine	+	+	+	l	+	+	+	+
DL-Lactate	-	v	-	-	+	+	+	l
L-Arabinose	+	+	-	-	-	-	-	-
Cellobiose	+	+	-	-	-	-	-	-
Raffinose	+	+	-	-	-	-	-	-
Maltose	+	+	+	+	-	-	+	+
Trehalose	+	+	+	+	-	-	+	+
2-Keto-D-gluconate	+	+	+	+	-	-	+	+
α-Methyl-D-glucoside	+	+	-	l	-	-	+	+
D-Glucitol	+	+	+	+	-	-	+	l
D-Xylose	+	+	+	+	-	-	+	v
D-Ribose	+	+	-	-/l	-	-	+	l
Glycerol	+	+	+	+	+	+	+	+
L-Rhamnose	-	v	-	-	-	-	-	-
Palatinose	+	NL	+	NL	-	NL	+	NL
Erythritol	-	-	-	-	-	-	-	-
Melibiose	+	+	-	-	-	-	-	-
D-Glucuronate	-	NL	-	-	-	NL	-	-
Melezitose	+	+	+	+	-	-	+	+
D-Gluconate	+	v	+	l	-	-	+	+
Levulinate	-	NL	-	NL	-	NL	-	NL
D-Mannitol	+	+	+	+	-	-	+	l
Lactose	-	-	-	-	-	-	-	-
myo-Inositol	-	-	-	-	-	-	-	-

[a]+, positive reaction; -, negative reaction; v, variable reaction; s, positive but slow reaction; -/s, negative or positive but slow reaction; l, latent reaction (rapidly developing a positive reaction after a lag phase); -/l, negative or latent reaction; NL, test results not listed by Kurtzman and Fell (1998)

Postharvest disease control of R13

Fresh chilli fruits were treated with R13 before storage to determine the suitability of R13 for postharvest disease control. Three groups of washed chilli fruits were treated with distilled water, chlorinated water (200 ppm), and cell suspension of R13 (10^8 cell/ml) by spraying. The treated chilli fruits were kept at 10 ^{O}C and examined for disease incidence at 15, 30, and 45 days after storage. As shown in Table 6, the R13 cells appear to be more effective than chlorinated water in reducing disease incidence during storage. Although disease incidence gradually increased time in storage, significant differences among the treatment occurred after days of storage. After 45 days of storage, percentage of disease incidence in chilli fruits treated with R13 cell suspension, chlorinated water, and water were 16.7%, 20.0%, and 26.7%, respectively (Table 6).

Table 6. Disease incidence in chilli fruits treated with distilled water, chlorinated water, and yeast strain R13 cells during storage at 10 ^{o}C for 45 days

Storage time (day)	Disease incidence[y] (%)		
	Distilled water	Chlorinated water	Yeast strain R13 cell
15	6.7a	3.3b	3.3b
30	23.3c	16.7d	13.3e
45	26.7f	20.0g	16.7h

[y]Percentages followed by a different letter indicate significant differences ($P < 0.05$) according to Duncan's multiple range tests.

Discussion

This studies have demonstrated that four epiphytic yeasts (*P. guilliermondii* strain R13, *C. musae* strain R6, *I. orientalis* strain ER1, and *C. quercitrusa* strain L2) isolated from fruits and vegetables reduce disease incidence in chilli fruit caused by *C. capsici* to verying degrees. *P. guilliermondii* strain R13 has the highest biocontrol efficacy both *in vivo* and *in vitro* tests. The antagonistic activity of *P. guilliermondii* strain R13 depends on its initial concentration and the storage temperature of the chilli fruit. The disease incidence in *C. capsici* infected chilli fruits can be reduced to allow as 6.5% at the yeast concentration of 10^8 cells at 28^{O}C. Less disease incidence was observed at lowest storage temperature, i.e., 1.1% at 18^{O}C compared to 10.0% at 33^{O}C. This is not surprising since the appropriate temperature range for *C. capsici* conidia germination and disease development have been shown to be at 28-33^{O}C (Misra and Mahmood, 1960). *P. guilliermondii* has been shown previously to have biocontrol efficacies against several diseases in postharvest fruits and vegetables (Droby et al., 1997). *P. guilliermondii* strain 5 A at a concentration of 10^8 cells/ml inhibited disease incidence up to 94-98% in orange (*Citrus sinensis* L. Osbeck) infected with 10^5 spores/ml of *Penicillium digitatum*, *P. italicum* and *Botrytis cinerea* at 20^{O}C for 5 days. Droby et al. (1997) has shown that *P. guilliermondii* strain US-7 at 10^7 cells/ml reduces disease incidence by

40% in grapefruit (*Citrus paradise* MacFad.) infected with *Penicillium digitatum* at 10^4 spores/ml (Lima et al., 1999). However, there is no report concerning the use of *P. guilliermondii* to control *C. capsici* in chilli fruits. This study presents the first evidence that *P. guilliermondii* strain R13 can reduce disease incidence caused by *C. capsici* by 93.3% at similar conditions to other investigations. Strain R13 helps prevention of postharvest decay of chilli fruits both with and without wounds on their surfaces. The percentage of biocontrol efficacy is comparable to results from all other studies, suggesting that *P. guilliermondii* strain R13 has a high potential to be used as a biocontrol agent against *C. capsici* infection in postharvest control of chilli fruits.

Postharvest chilli fruits are usually preserved by washing or spraying with chlorinated water at 75-400 ppm chlorine and stored at low temperature (7-10^{O}C) before shipment (Suslow, 1997; Danet, 2005). The present study, however, shows that *P. guilliermondii* strain R13 is more effective in preserving chilli fruits than chlorinated water (200 ppm) at 10^{O}C for 30 and 45 days. This suggests that *P. guilliermondii* strain R13 may replace the chlorine treatment, which may be harmful to humans especially in the chemical preparation step (Camelo, 2004). Moreover, the use of chlorine in fruits and vegetables is banned in some countries due to its reaction with organic matter leading to formation of chlorate compounds and trihalomethanes, substances thought to be carcinogenic (Carlsen, 2004; Link, et al., 1994). Accumulation of these substances also causes pollution of the environment (Smith, 2001; Stringer and Johnston, 2002). These findings provide substantial incentive for development of yeast biocontrol as an alternative to inhibit anthracnose disease by *C. capsici* in postharvest chilli fruit. Nevertheless, more studies including pilot trials need to be performed.

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