BIOLOGICAL CONTROL OF POST HARVEST FRUIT DISEASES USING ANTAGONISTIC YEASTS IN INDIA

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SUMMARY

Significant losses in harvested fruits are directly attributable to decaying fungi. Biological control using microbial agents including yeasts has been reported among several alternatives to the use of synthetic chemical fungicides for managing postharvest fruit decay. Twenty nine yeasts were isolated from different sources and among the isolates, YZ1, YZ7 and YZ27 showed broad spectrum of antagonistic activity (mycelial growth inhibition) against the test pathogens *in vitro* which were identified by molecular methods as *Candida tropicalis* YZ1 (CtYZ1), *Saccharomyces cerevisiae* YZ7 (ScYZ7) and *C. tropicalis* YZ27 (CtYZ27). Application of CtYZ1, ScYZ7 and CtYZ27 ($1\text{-}4\times10^8$ CFU ml^{-1}) significantly reduced the mean lesion diameter of wounds on banana artificially inoculated with *C. musae* by 85.5%, 88.7% and 91.9% respectively as compared to 75.8% in fungicide (Carbendazim 1.0 g l^{-1}) treated fruits over control at 4 days after storage at ambient condition. All the three yeasts also significantly reduced the latent natural decays caused by fungal pathogens on banana, litchi and strawberry fruits. The findings of this study could be elaborated and explored in management of post harvest diseases of fruits as an alternative to synthetic fungicides.

Keywords: Biological control, antagonistic yeasts, post harvest diseases, fruits.

INTRODUCTION

Cultivation of fruit crops is an important segment of agri-horticultural economic enterprise for the farmers in India and the country is the second largest producer of fruits in the world with an annual production 88.977 million tons during 2013-14, contributing 12.6% of the total world production (Anonymous, 2015). Tropical and subtropical fruits contribute a major share, and the most important fruits are mango, banana, papaya, citrus, guava, pineapple, litchi, sapota, apple and pomegranate. Increased consumer awareness on diet and health has resulted in a greater consumption of fruits. Losses due to pests and diseases in the field, storage, as well as in transit and market can amount up to 25% of the total production in industrialized countries and in developing countries damage is often higher, exceeding 50%, because of the lack of adequate storage facilities (Nunes, 2012). There are two principal factors which make plant products more susceptible to spoiling: the high water content in fruit which allows pathogen attack (Harvey, 1978) and the wounds present on the plant organs during storage, often as a result of harvesting and transportation. Synthetic fungicides are primarily used to control postharvest diseases (Sharma *et al.*, 2009). However, the use of postharvest fungicides is being increasingly limited because of environmental and toxicological risks. Moreover, the global trend appears to be shifting towards reduced use of fungicides on produce and hence, there is a strong public and scientific desire to seek safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities (Mari *et al.*, 2007). In addition, the repeated and continuous use of fungicides has led to the development of fungal resistant strains (Brent and Hollomon, 2007).

In the last few years, biological control of postharvest diseases of fruits has been developed as a promising alternative to chemical control. In the last two decades, several scientific studies have demonstrated the efficiency of yeasts as biocontrol agents against a wide variety of post harvest fruit pathogens (Droby *et al.*, 1993; Lima *et al.*, 1997; Wisniewski *et al.*, 2007). Yeasts stand out among microorganisms as potential agents to control plant diseases due to their ability to colonize the surface of leaves and fruit high reproductive rate and low nutritional requirement (Wisniewski *et al.*, 2007). Furthermore, yeasts do not produce spores or mycotoxins, unlike other potential biocontrol microorganisms such as filamentous fungi (Fan and Tian, 2000). Yeast based technologies and products (e.g Aspire® containing *Candida oleophila*, Yield Plus® containing *C. albidus*) for post harvest disease management of fruits are already practiced and developed in Europe. However, most studies have focused on the search for biocontrol agents that act on the pathogens of temperate fruits, for example, apples, pears, strawberries and grapes, but there remains only limited research on biocontrol agents toward tropical fruit pathogens. The objectives of this work were:

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Table 1. Yeast isolates obtained from phylloplanes of different plant species.

	Types of plants	Plant parts	Locations of collection	Designation of isolates
Fruits	Mango (*Mangifera indica* L.)	Ripe fruit surface	Horticultural Farm, BCKV, Mohanpur	YZ 7
		Ripe fruit surface	Vegetable market, Mohanpur	YZ 6
	Banana (*Musa paradisica* L.)	Mature leaf surface	Horticultural Farm, BCKV, Mohanpur	YZ 15
		Mature leaf surface		YZ 16
	Litchi (*Litchi chinensis* Sonn.)	Ripe fruit surface	Horticultural Farm, BCKV, Mohanpur	YZ 3
		Ripe fruit surface		YZ 4
		Ripe fruit surface		YZ 5
	Manarin orange (*Citrus reticulata* Blanco)	Ripe fruit surface	Vegetable market, Mohanpur	YZ 1
		Ripe fruit surface		YZ 13
	Sapota (*Achras zapota* L.)	Ripe fruit surface	Vegetable market, Mohanpur	YZ 12
	Papaya (*Carica papaya* L.)	Young leaf surface	Horticultural Farm, BCKV, Mohanpur	YZ 10
		Young leaf surface		YZ 14
		Mature leaf surface		YZ 29
	Ber (*Zizyphus mauritiana* Lamk)	Mature leaf surface	Horticultural Farm, BCKV, Mohanpur	YZ 9
	Guava (*Psidium guajava* L.)	Mature fruit surface	Horticultural Farm, BCKV, Mohanpur	YZ 17
	Lemon (*Citrus lemon* L.)	Mature fruit surface	Horticultural Farm, BCKV, Mohanpur	YZ 19
	Jackfruit (*Artocarpus heterophyllus* Lamk.)	Young leaf surface	Horticultural Farm, BCKV, Mohanpur	YZ 18
Vegetables	Brinjal (*Solanum melongena* L.)	Young leaf surface	Instructional Farm, BCKV, Mohanpur	YZ 26
	Tomato (*Solanum lycopersicon* L.)	Mature fruit surface	Instructional Farm, BCKV, Mohanpur	YZ 24
	Bitter gourd (*Momordica charantia* L.)	Ripe fruit surface	Vegetable market, Mohanpur	YZ 27
	Radish (*Raphanus sativus* L.)	Young leaf surface	Instructional Farm, BCKV, Mohanpur	YZ 25
	Cauliflower (*Brassica olereacea* L.)	Young leaf surface	Instructional Farm, BCKV, Mohanpur	YZ 23
	Cucumber (*Cucumis sativus* L.)	Mature fruit surface	Instructional Farm, BCKV, Mohanpur	YZ 2
	Onion (*Allium cepa* L.)	Young leaf surface	Instructional Farm, BCKV, Mohanpur	YZ 28
		Young leaf surface		YZ 21
	Cabbage (*Brassica olereacea* L.)	Young leaf surface	Instructional Farm, BCKV, Mohanpur	YZ 22
	Broccoli (*Brassica olereacea* L.)	Young leaf surface	Horticultural Farm, BCKV, Mohanpur	YZ 8
Flowers	Tuberose (*Polianthes tuberosa* L.)	Flower surface	Horticultural Farm, BCKV, Mohanpur	YZ 20
	Gladiolus (*Gladiolus grandiflorus* L.)	Flower surface	Horticultural Farm, BCKV, Mohanpur	YZ 11

i) to isolate antagonistic yeasts from the phyllosphere of common cultivated plants in India; ii) to evaluate their efficiency for the control of post harvest fruit pathogens and identify the most effective antagonistic strains, and iii) to evaluate their bioefficacy on fruits.

MATERIALS AND METHODS

Isolation of yeasts and test pathogens. Fresh leaf, fruit and flower samples of tropical plant species for yeast isolation were collected during October 2012 either from experimental fields of Horticultural Research Station, Mondauri and Jaguli Instructional Farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India and some from the market at Mohanpur. The experimental fields are located at 22°43' N latitude and 88°34' S longitude at an elevation of 9.75 m above mean sea level. The samples were collected randomly from the selected plants in sterile paper packets, brought to the laboratory and were used for isolation within 2 hours after the collection. The leaf samples were cut into 2 cm × 2 cm discs, washed with sterile distilled water (SDW), air dried and both adaxial and abaxial leaf surfaces were placed aseptically on Petri plates with solidified Yeast extract Peptone Dextrose Agar (Yeast extract 5 g l^{-1}; Peptone 10 g l^{-1}; Dextrose 10 g l^{-1}; Agar 20 g l^{-1}; YPDA) medium, supplemented with chloramphenicol (100 mg l^{-1}) and streptomycin sulphate (50 mg l^{-1}). The plates were kept for 6 hours allowing the microorganisms to dislodge from the surface of the discs. Subsequently, the leaf discs were removed aseptically and the Petri plates were incubated at 28 ± 1°C for 48-72 h till distinguishing yeast colonies developed. Fruit and flower samples were washed separately in sterile distilled water in flasks for 5 minutes with vigorous shaking to allow the yeast cells to dislodge from the surfaces of the samples. Tenfold serial dilution were prepared from wash water, and 0.1 ml of each dilution was plated in three replicates on molten YPDA media and allowed to solidify till distinguishing yeast colonies developed (Chalutz and Wilson, 1990). After 48 hours incubation at 28 ± 1°C, single yeast colonies with different morphological appearances

were isolated, purified and stored in refrigerator on YPDA slants following periodic sub culturing for further study. Plant sources and its parts used for isolation, locations of collection and designations of the yeast isolates are mentioned in Table 1.

The test pathogens *Colletotrichum musae* and *Fusarium oxysporum*, were isolated from diseased ripe fruits of banana (*Musa paradisiaca* cv. Martaman), *C. gloeosporioides* from diseased mango (*Mangifera indica* cv. Himsagar), *C. capsici* from diseased ripe chilli (*Capsicum annum* cv. Bullet) and *Aspergillus niger* from diseased citrus (*Citrus lemon* cv. Kagzi) on plates with Potato Dextrose Agar (Potato decoction, 200 ml l^{-1}; Dextrose, 20 g l^{-1}; Agar 20 g l^{-1}; PDA) supplemented with chloramphenicol (100 mg l^{-1}) and streptomycin sulphate (50 mg l^{-1}) and incubated at 28 ± 1°C. Pure cultures were obtained from hyphal tips and their pathogenicities were established following the methods given by Khan *et al.* (2001) for *C. musae*, Prabakar *et al.* (2008) for *C. gloeosporioides*, Chanchaichaovivat *et al.* (2007) for *C. capsici*, Alvindia *et al.* (2004) for *F. oxysporum* and Lima *et al.* (1998) for *A. niger* on the respective fruits. The fungal pathogens were identified following standard references- von Arx (1957), Sutton (1980, 1992), Bailey and Jeger (1992) and Freeman *et al.* (1998) for *Colletotrichum* species; Fravel *et al.* (2003) and Leslie *et al.* (2006) for *F. oxysporum* and Geiser *et al.* (2007) for *A. niger*. The pathogens were maintained on PDA slants at 4°C following periodic sub culturing.

***In vitro* evaluation of the yeast isolates against the test pathogens.** Antagonistic potential of the isolated yeasts were tested against the five test pathogens following dual culture technique. Six millimeter diameter disc of 7 days old test pathogens were inoculated on 9 mm Petri plates, containing PDA supplemented with yeast extract (1.0 g l^{-1}), at a distance of 2 cm from the periphery. On the other side of the Petri plate, a streak from a yeast cell suspension (1-1.6 × 10^8 CFU ml^{-1}) was made 2 cm from the periphery or sterile distilled water for control. The plates were incubated at 28 ± 1°C till such days when the test fungi has just reached the margin of the line where sterile distilled water was streaked in control Petri plates. The radial growth of the fungi was measured and the mycelial growth inhibition (MGI %) of the pathogens by the yeast isolates were calculated as: (radius of pathogen on control – radius of pathogen on treatment / radius of pathogen on control) × 100 (Skidmore and Dickinson, 1976). The experiment was laid out in a complete randomized design with three replications each. The fungal mycelium was microscopically observed to assess hyphal changes (if any) due to contact with the yeasts as described by Platania *et al.* (2012). Mycelium disks which were obtained from the Petri plates in which the yeast could inhibit the pathogen growth, were removed from an area proximate to the yeast cells and a thin layer was longitudinally cut from the culture medium and studied under phase contrast optical microscope (Carl Zeiss AxioScope, Germany) and their morphologies were compared with those of the control.

Identification of the most effective antagonistic yeast isolates. The yeast isolates showing a broad spectrum of antagonistic activity (mycelia growth inhibition) against the test pathogens were identified by sequencing D1/D2 domain of 26S rRNA amplified using primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') from Microbial Type Culture Collection and Gene Bank (Council of Scientific and Industrial Research-Institute of Microbial Technology, Chandigarh, India). The identification of the closest relatives of the sequences were obtained using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST/).

Determination of biological efficiency of the antagonistic yeast strains. The biological efficiency at different concentrations of yeast strains *Candida tropicalis* YZ1 (CtYZ1), *C. tropicalis* YZ27 (CtYZ27) and *Saccharomyces cerevisiae* YZ7 (ScYZ7) were determined by their *in vitro* ability to inhibit germination of spores of the anthracnose pathogen *C. musae* in potato dextrose broth (PDB). One ml aliquot of cell suspension of each yeast strains at different concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{10} CFU ml^{-1}) was added to 100 ml Erlenmeyer flasks each containing 20 ml PDB supplemented with yeast extract (1.0 g l^{-1}). At the same time, aliquots (1 ml) of spore suspensions (10^4 spores ml^{-1}) of *C. musae* were added to the respective flasks. SDW instead of yeast suspension served as control. The numbers of germinated spores were observed at 12 hours after incubation at 28 ± 1°C. Experiment was performed in triplicate and the extent of spore germination was assessed by looking for the presence of germ tubes. Results were expressed in terms of percentage of spores germinated as compared to the control. Percentage of spore germination inhibition was calculated according to the formulae: % spore germination inhibition = (SC – ST) × 100/SC, where SC- average number of spores germinated in control set and ST- average number of spores germinated in test set.

***In vivo* evaluation of biocontrol efficacy of yeasts.** Screening of antagonist yeasts CtYZ 1, ScYZ 7 and CtYZ 27 for biocontrol efficacy on banana (*Musa paradisiaca* cv. Martaman), litchi (*Litchi chinensis* Sonn. cv. Bombai) and strawberry (*Fragaria* × *ananassa* Duch. cv. Sweet Charlie) fruits were done following the *in vivo* test methods suggested by Wisniewski *et al.* (1991). Banana and litchi fruits were obtained from the orchard of Horticultural Farm, BCKV, Mohanpur, West Bengal, while strawberry fruits were obtained from an experimental green house in the Department of Fruits and Orchard Management, Faculty of Horticulture, BCKV, Mohanpur, Nadia, West Bengal. Mature fruits were harvested

Table 2. Percent mycelial growth inhibition (MGI) of different post harvest pathogens by different yeast isolates on dual culture plates.

Yeast isolate	Percent mycelial growth inhibition* (MGI %) of					
	Cm	Cc	Cg	An	Fo	Average
YZ 1	**31.3 b**	**21.3 c**	15.3 j	7.3 l	11.3 g	**17.3**
YZ 2	6.9 i	11.3 j	10.0 p	8.7 j	14.7 c	10.3
YZ 3	6.7 i	4.7 o	8.7 r	4.7o	9.3 i	6.8
YZ 4	6.8 i	14.7 h	**22.0 c**	11.3 f	5.3 m	12.0
YZ 5	8.8 h	8.0 l	16.0 i	12.0 e	8.0 k	10.6
YZ 6	**20.8 d**	14.7 h	14.0 l	9.3 i	**20.0 a**	**15.8**
YZ 7	**24.3 c**	15.3 g	16.0 i	5.3 n	12.0 f	14.6
YZ 8	4.3 j	7.3 m	6.7 s	7.3 l	16.0 b	8.3
YZ 9	15.7 e	**29.3 a**	17.3 g	4.7 o	4.7 n	14.3
YZ 10	4.4 j	9.3 k	14.0 l	12.0 e	10.0 h	9.9
YZ 11	4.6 d	12.7 i	9.3 q	9.3 i	14.7 c	10.1
YZ 12	11.3 g	11.3 j	**20.0 e**	14.0 d	11.3 g	13.6
YZ 13	12.0 g	8.0 l	**20.0 e**	15.3 b	14.0 d	13.9
YZ 14	8.9 h	18.0 f	10.7 o	14.7 c	12.0 f	12.8
YZ 15	6.8 i	4.0 p	6.7 s	12.0 e	5.3 m	7.0
YZ 16	8.9 h	**22.0 b**	12.0 n	8.7 j	16.0 b	13.5
YZ 17	4.4 j	**21.3 c**	24.7 a	12.0 e	12.0 f	14.9
YZ 18	6.8 i	**20.7 d**	16.7 h	14.7 c	14.7 c	14.7
YZ 19	8.9 h	**20.7 d**	**24.0 b**	8.0 k	9.3 i	14.2
YZ 20	11.9 g	2.0 q	8.7 r	3.3 p	7.3 l	6.6
YZ 21	8.6 h	4.0 p	4.7 t	8.7 j	12.7 e	7.7
YZ 22	4.4 j	15.3 g	14.7 k	11.3 f	12.0 f	11.5
YZ 23	8.9 h	9.3 k	9.3 q	10.7 g	14.0 d	10.4
YZ 24	6.8 i	5.3 n	6.7 s	6.7 m	11.3 g	7.4
YZ 25	2.2 k	1.7 e	18.7 f	14.7 c	11.3 g	13.1
YZ 26	4.4 j	21.3 c	**20.0 e**	14.0 d	10.0 h	13.9
YZ 27	**37.8 a**	**22.0 g**	**21.3 d**	15.3 b	14.7 c	**22.2**
YZ 28	8.5 h	18.0 f	8.7 r	10.0 h	9.3 i	10.9
YZ 29	13.7 f	187e	12.7 m	17.3 a	8.7 j	14.2
SEM	**0.314**	**0.114**	**0.398**	**0.104**	**0.071**	
C.D (0.05)	**0.890**	**0.324**	**0.14**	**0.297**	**0.201**	

*Values are averages of three replications. Data in columns with the different letters are significantly different according to Duncan's multiple range test at p = 0.05. Cm: *Colletotrichum musae*; Cc: *C. capsici*; Cg: *C. gloeosporioides*; An: *Aspergillus niger*; Fo: *Fusarium oxysporum*.

and brought to the laboratory where they were washed with running tap water, air dried and surface sterilized with 70% ethanol. In case of banana, experiment was conducted with or without artificial pathogen inoculation. For artificially inoculated experiment, the banana fruits were wounded by pricking on the surface at three sites with a sterile needle. The cells of CtYZ 1, ScYZ 7 and CtYZ 27 grown in YPD broth for 48 h were harvested by centrifugation at 3000 rpm for 20 min, washed twice with SDW and re-suspended in SDW at a concentration of 1×10^8 CFU ml^{-1}. *C. musae* was grown on PDA for 7 days till enough sporulation was observed after which, the conidia were collected by flooding the plates with SDW and filtered through sterile muslin cloth. The artificially wounded banana fruits were dipped for 1 minutes into the yeast cell suspensions of 1×10^8 CFU ml^{-1} and kept for 30 minutes for air drying and then dipping in the conidial suspension of *C. musae* (1.2×10^4 spores ml^{-1}) for 1 minute. In case of bioassay on natural infections on banana, litchi and strawberry, only the antagonist yeasts were applied as above but without any artificial wounding and pathogen inoculation. Wounded fruits inoculated only with the pathogen served as positive control for artificially inoculated fruits while fruits dipped in SDW served as negative control for natural infection experiments. There were three replicates of 3 fruits each in case of banana and 30 fruits each in case of litchi and strawberry in each treatment. A standard fungicide Carbendazim 1.0 g l^{-1} was used to compare the treatments. The treated fruits were arranged separately from each other on plastic trays covered with transparent polythene sheet and stored in a ventilated cabinet in the dark at 28 ± 1°C. The disease severity was calculated as indicated by increased lesion diameter for artificially inoculated infection while for natural infection, the 0-4 point disease severity scale was followed (0 point = 0% decay, 1st point = 10% decay of the fruit surface, 2nd point = 11-25% decay of the fruit surface, 3rd point = 26-50% decay of the fruit surface and 4th point = over 50% of the fruit surface decayed). The diseases were recorded up to 5 days after storage (DAS) for artificially inoculated banana fruits, up to 6 DAS each for natural infection on banana and litchi and up to 3 DAS in strawberry. Per cent disease indices (PDI) were then calculated following the formula given by McKinney (1923): PDI = Sum of individual disease rating × 100 / Number of fruit samples × Maximum value of scale.

Data analysis. The experiments were arranged in a completely randomized design (CRD) and data on mycelial radial growth inhibition, per cent number of fruits infected (disease incidence) lesion diameters and percentage of infected fruits (disease severity) were subjected to analysis of variance (ANOVA) using DSAASTAT software (ver. 1.101) developed by Dipartmento di Scienze ed Ambientali (DSAA), Perugia, Italy. Statistical significance was judged at the level of p = 0.05. When the analysis was statistically significant, Duncan's multiple range test was used for separation of mean.

RESULTS AND DISCUSSION

***In vitro* evaluation of the yeast isolates against the test pathogens.** The mycelial growth of the five test pathogens in dual culture with yeast isolates on Petri plates were significantly and differentially reduced, by the yeast isolates (Table 2). Out of the 29 yeast isolates YZ 1, YZ 6, YZ 7 and YZ 27 showed appreciable inhibitory effects (more than 20% MGI) on *Colletotrichum musae*; YZ 1, YZ 9, YZ 16, YZ 17, YZ 18, YZ 19 and YZ 27 on *C. capsici*; YZ 4, YZ 12, YZ 13, YZ 19, YZ 26 and YZ 27 on *C. gloeosporioides*; YZ 6 on *Fusarium oxysporum* while none of the isolates showed more than 20% MGI on *Aspergillus niger*. Among all the isolates examined, YZ 1, YZ 7 and YZ 27 exhibited a broad spectrum of antagonistic activity against the

tested pathogens with an average MGI of 17.3%, 15.8% and 22.2% respectively on dual plates.

It was evident from the results that yeast isolates caused differential degrees of mycelial growth inhibition of post harvest pathogens. Such growth inhibition by the yeast isolates against fungal pathogens has been recorded by Suzzi *et al.* (1995). They screened 586 natural wine yeasts from grapeberries of different genera (*Saccharomyces cerevisiae*, *Candida*, *Pichia*, *Zygosaccharomyces*, *Saccharomycoides*) from different zones of Italy against the fungal pathogens like *Alternaria alternata*, *Aspergillus niger*, *Colletotrichum acutatum*, *Fusarium oxysporum* and some sclerotial fungi like *Rhizoctonia solani*, *Macrophomina* sp. *etc.* and found that only a very few strains of yeasts could completely inhibit the pathogen and reported that the biocontrol activity was found to be a strain characteristic and did not solely depend on species or genus. The production of antibiotics as a mechanism of antifungal activity by these isolates was ruled out as no clear and distinct inhibition zone or halo was produced between the colonies of the yeast isolates and the test pathogens on dual plates. Incidence of hyphal deformities of *C. musae* in dual culture plates were observed by removing the mycelial samples from the parts of the growth medium located close to the inhibition zone. Microscopic analysis (at 40×) revealed that the *C. musae* hyphae in dual plates appeared thin, deeply stained (cotton blue stain), irregularly septate with the yeast cells clustered around the hyphae of *C. musae* in clumps as compared to the thick, elongated, light stained mycelia and presence of spores in the control. Similar kind of hyphal abnormalities was observed in *Botrytis cinerea* against *Debaryomyces hansenii* in co-culture by Wisneiwski *et al.* (1991).

Identification of the most effective antagonistic yeast isolates. The isolates YZ1, YZ7 and YZ27 showing a broad spectrum of antagonistic activity (mycelia growth inhibition) against the test pathogens were identified by sequencing D1/D2 domain of 26S rRNA from Microbial Type Culture Collection and Gene Bank (Institute of Microbial Technology, Chandigarh, India). The strains showing similarity scores higher than 99% were selected as type strain. The sequences of YZ1 and YZ27 were identical to that of *Candida tropicalis* strain OMY and YZ7 to *Saccharomyces cerevisiae* YJM451 and were assigned with GenBank Accession Nos. KT459475, KT459476, and KT459474 respectively.

Biological efficiency of the antagonistic yeasts. Each test concentrations of the three antagonist yeast strains showed notable inhibition of *C. musae* spore germination (Table 3). Absolute spore germination inhibition of *C. musae* was recorded by the yeast strain of CtYZ1 at a concentration of 1×10^9 CFU ml^{-1} while for the strains ScYZ7 and CtYZ27, it was recorded at a concentration of 1×10^8 CFU ml^{-1}. Based on the result, the concentration of 1×10^8 CFU ml^{-1} was selected for all the three strains in further *in vivo* studies.

Table 3. Percent of spore germination inhibition of anthracnose pathogen *C. musae* by antagonistic yeasts.

Yeast strains	Cell concentrations (CFU ml^{-1})					
	1×10^5	1×10^6	1×10^7	1×10^8	1×10^9	1×10^{10}
CtYZ 1	44.9b	48.5b	75.3b	99.2a	100a	100a
ScYZ 7	55.5a	65.5a	78.8ab	100.0a	100a	100a
CtYZ 27	37.5b	52.3b	82.2a	100.0a	100a	100a

*Values are averages of three replications. Data in columns with the different letters are significantly different according to Duncan's multiple range test at p = 0.05.

***In vivo* evaluation of biocontrol efficacy of yeast. On banana**. The mean lesion diameter of anthracnose lesions on artificially inoculated banana fruits was significantly reduced (p = 0.05) by yeast application as compared to those of the control fruits. The yeast CtYZ 27 caused maximum mean lesion diameter inhibition of 91.9% followed by ScYZ7 and CtYZ1 with 88.7% and 85.5% respectively (Fig. 1) while Carbendazim 1.0 g l^{-1} recorded an inhibition percentage of 75.8% as compared to control at 4 days after storage (DAS).

Application of all the three yeasts significantly reduced the latent natural infections of banana fruits as evident from the results presented in Fig. 2. At 5 DAS, fruits treated with CtYZ 27 exhibited the lowest Percent Disease Index of 16.7% followed by CtYZ 1 with 22.2% and ScYZ 7 with 25% as compared to 27.8% in Carbendazim 1.0 g l^{-1} and 75% in control fruits. *Colletotrichum musae* (Berk and Curt) causes anthracnose in banana fruit and is confined to mature fruits (Waller, 1992). The control of anthracnose disease of banana mainly relies on post-harvest fungicide treatments (Eckert and Ogawa, 1985; de Lapeyre de Bellaire and Nolin, 1994). However, synthetic chemicals currently used to manage this disease like the Benzimidazole fungicides are not satisfactory and has many shortcomings, including resistance build-up in pathogen population and fungicide residues which may cause health problems. Reported biocontrol yeasts against anthracnose of banana include *Debaryomyces hansenii* TISTR 5155, *Candida sake* TISTR 5143, *Aureobasidium pullulans* TISTR 3389 and *Candida utilis* (Tongsri and Sangchote, 2009) and *Pichia anomala* strain K and *Candida oleophila* strain O (Lassois *et al.*, 2008). This is the first record of *Saccharomyces cerevisiae* and *Candida tropicalis* as biocontrol agents against banana anthracnose.

On litchi. The application of yeast CtYZ1, ScYZ7 and CtYZ27 (1-4×10^8 CFU ml^{-1}) reduced the natural fruit decay incidence and severity caused by fungal pathogens in litchi significantly over different storage times under ambient condition and also at par with Carbendazim 1.0 g l^{-1} as compared to those of the control fruits. The CtYZ1,

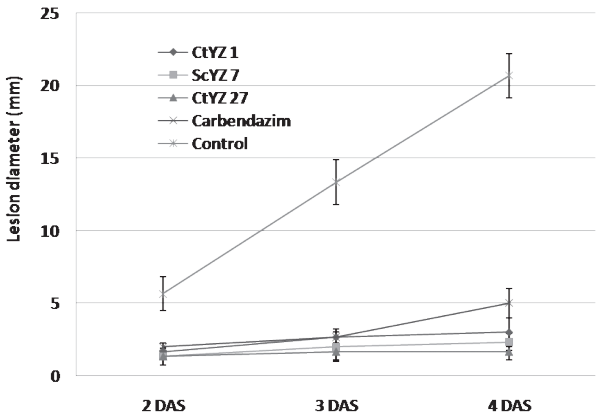

Fig. 1. Changes in anthracnose lesion diameter (mm) of artificially inoculated banana fruits (*C. musae* at 10^4 spores ml^{-1}) by CtYZ1, ScYZ7 and CtYZ27 application (1×10^8 CFU ml^{-1}) at different days of storage as compared to Carbendazim treated and untreated fruits (control) at 28 ± 1°C. Each value is the mean of three replications. Bars represent the standard deviation from the mean.

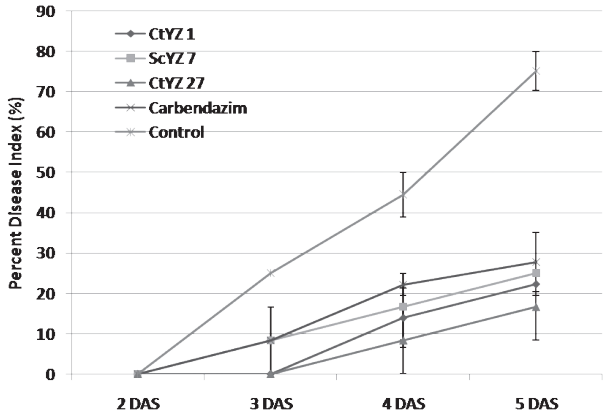

Fig. 2. Percent Disease Index (%) of latent infection of anthracnose on banana fruits after application of antagonistic yeast strains CtYZ1, ScYZ7 and CtYZ27 ($1\text{-}4\times10^8$ CFU ml^{-1}) after different days of storage (DAS) as compared to Carbendazim treated and untreated fruits (control) at 28 ± 1°C. Each value is the mean of three replications. Bars represent the standard error.

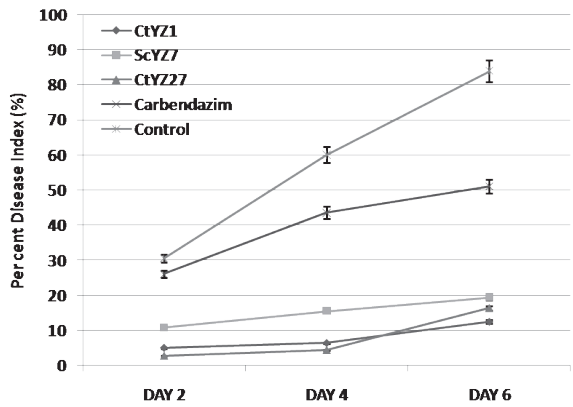

Fig. 3. Percent Disease Index (%) of natural decay in litchi fruits after application of antagonistic yeast strains CtYZ 1, ScYZ7 and CtYZ27 ($1\text{-}4\times10^8$ CFU ml^{-1}) At 2, 4 and 6 days of storage (DAS) as compared to Carbendazim treated and untreated fruits (control) at 28 ± 1°C. Each value is the mean of three replications. Bars represent the standard error.

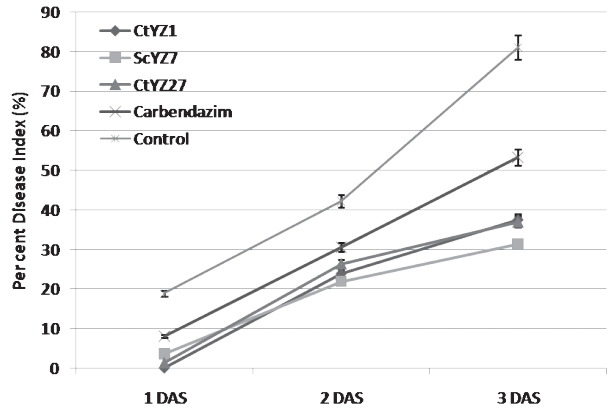

Fig. 4. Percent Disease Index (%) of natural decay in strawberry fruits after application of antagonistic yeast strains CtYZ1, ScYZ7 and CtYZ27 ($1\text{-}4\times10^8$ CFU ml^{-1}) at 2, 4 and 6 days of storage (DAS) as compared to Carbendazim treated and untreated fruits (control) at 28 ± 1°C. Each value is the mean of three replications. Bars represent the standard error.

ScYZ7 and CtYZ27 treated fruits showed Percent Disease Index (%) of 12.5%, 19.4% and 16.4% respectively as compared to 51.1% in Carbendazim treated fruits and 83.9% in control fruits at 6 DAS (Fig. 3). The fungi associated with the decay of the fruits were isolated and identified by mycelia and spore characteristics as *Aspergillus* sp., *Rhizopus* sp., *Penicillium* sp. and *Colletotrichum gloeosporoides*.

The litchi is a tropical to subtropical fruit grown worldwide and suffers from postharvest losses, estimated to be as high as 50% prior to consumption, mainly due to the decay caused by infection of post harvest pathogens such as *Aspergillus*, sp., *Penicillium* sp. and *Rhizopus* sp. causing decay during and after harvest through skin injury, while *Colletotrichum* sp. and *Botryodiplodia* sp. infects fruits either in the field or through the cut stem end during harvest or handling (Scott *et al.*, 1982). To overcome these pathogens, the litchi industry commercially uses SO_2 fumigation (Swarts, 1985) which has the limitation of leaving undesirable residues (Kremer-Köhne, 1993). Alternative methods to manage post harvest diseases in litchi has mainly focused on use of strategies like modified atmosphere packaging (Kaiser, 1995), heat treatments (Lichter *et al.*, 2000) and biological control using *Bacillus subtilis* (Sivakumar *et al.*, 2007). The application of CtYZ1, ScYZ7 and CtYZ27 on mature litchi stored at ambient condition significantly reduced the natural decay incidence and severity at par with standard fungicide tested as shown

in the results. The uneven structure of the pericarp segments with micro-cracks could have provided ideal sites for these antagonistic yeasts to colonize and multiply on the fruit surface and thereby maintain a protective barrier which restricted filamentous fungal infection, without compromising fruit integrity. The use of antagonist yeasts to manage post harvest diseases reported here offers a promising approach taking into account the inherent properties of yeasts and the promising results as indicated in this experiment.

On strawberry. The early signs of mold development in strawberries (control) appeared after one day of storage. The disease severity of the fruits treated with antagonist yeasts were significantly reduced as evident from lower Percent Disease Index (%) recorded (37.5%, 31.4% and 36.9% in CtYZ1, ScYZ7 and CtYZ27 treated fruits respectively) as compared to 53.3% in Carbendazim 1.0 g l^{-1} treated and 81.1% in control fruits at the end of the experiment (3 DAS) (Fig. 4). Strawberries (*Fragaria × ananassa* Duch.) are highly susceptible to mechanical injury, physiological deterioration, water loss and microbiological decay during storage. They usually have short ripening and senescent periods that make marketing a challenge (García *et al*., 1998). The wounds, which may inevitably occur during harvest, transport, and handling, not only damage the harvested fruit, but also provide pathways for pathogen invasion, especially for the wound-invading necrotrophic fungi (Janisiewicz and Korsten, 2002). The nutritional environment at the wound site may be favourable to antagonistic yeasts which rapidly colonizes the fruit niche and will be competing with the pathogen for nutrients. There are several reports of use of yeasts like *Cryptococcus laurentii* (Fan *et al*., 2009), *Cryptococcus albidus* (Helbig, 2002), *Pichia guilermondii* (Guetskyl *et al*., 2002), *Rhodotorula glutinis* (Zhang *et al*., 2007), *Aureobasidium pullulans* (Adikaram *et al*., 2002) and *Candida oleophila* (Lima *et al*., 1997) as biocontrol agents against post harvest diseases in strawberry. Results of the present study indicate that *Candida tropicalis* YZ1, *Candida tropicalis* YZ27 and *Saccharomyces cerevisiae* YZ7 were also efficient enough to control post harvest moulds and can be used as biocontrol agents against postharvest strawberry diseases.

The yeast species identified in this study have already been reported and studied with the aim of exploiting their potentialities as biocontrol agents (Sangchote and Saoha, 1998; Santos *et al*., 2004; Abd-Alla *et al*., 2009; Nally *et al*., 2012; Sriram and Poornachanddra, 2013; Sukorini *et al*., 2013; Lopes *et al*., 2015) suggesting their probable innocuity for human health. However, this lack of pathogenicity will have to be carefully checked before using these strains (especially YZ1 and YZ27) as antagonists on post harvest fruits. Our results showed that selected antagonistic yeast strains could potentially be used as a non-chemical alternative treatment against green mold. Additional studies are necessary to elucidate the modes of actions of the promising strains of *S. cerevisiae* (YZ7) and *C. tropicalis* (YZ1 and YZ27). To determine whether these strains could be developed into commercial biocontrol agents, their shelf life studies and consistency in bioefficacy are being carried out in the author's laboratory so that they can be confirmed further in large-scale experiments under commercial storage conditions.

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