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Evaluation of Sorbitol as Osmoprotectant in Yeast Based Biocontrol Formulation against Anthracnose of Banana

Jasmin Thomas, Deewakar Baral, Jayanta Saha

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ABSTRACT

The yeast isolate *Saccharomyces cerevisiae* (*Sc*YZ7) was used as antagonistic yeast for the development of molasses urea-based biocontrol formulation to study the efficiency of sorbitol as osmoprotectant. The formulations containing 5,10 and 15% molasses concentration were prepared and tested with 2.5% and 5% sorbitol as additive. The formulation containing molasses (15%) + sorbitol (5%) and molasses (10%) + sorbitol (5%) maintained highest population (93.59% of initial population) whereas, in control the population was 80% of the initial population after 120 days of storage at 28°C. It has been observed that the viability of yeast cells increased in the formulations with sorbitol at different concentrations compared to the control. Sorbitol (5%) had protective role on yeast survival. The biocontrol efficiency of the formulation containing sorbitol was tested against anthracnose of banana cv Martaman caused by *Colletotrichum musae* under osmotic stressed condition. The lesion diameter was found less in yeast based formulations containing sorbitol (5%) compared to untreated control.

Keywords Antagonistic yeast, *Saccharomyces cerevisiae*, Molasses urea, Sorbitol, Biocontrol formulation, *Colletotrichum musae*.

Jasmin Thomas[1]*, Jayanta Saha[3]
[3]Associate Professor
[1, 3]Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, Nadia, West Bengal, India

Deewakar Baral[2]
[2]Assistant Professor
Division of Plant Pathology, Lovely Professional University, Punjab, India

Email: jasminthomas64@gmail.com
*Corresponding author

INTRODUCTION

In industrialized countries, Postharvest losses of fruit and vegetables can amount to up to 25% of total production, whereas in developing countries, losses are often higher, exceeding 50%, due to a lack of sufficient storage facilities (Nunes 2012). High water content, which permits pathogen attack and wounds during storage, which commonly occur as a result of harvesting and transportation, give microorganisms free access and induce rotting, are two major factors that make fruits more prone to spoilage. The role of yeast in the biological control of post-harvest diseases has received more attention recently, as safe and eco-friendly alternatives to synthetic chemicals (Mari *et al.* 2007). Large volumes of cell biomass must be produced, stabilized, packaged, stored and finally transported to their final destination in order to use the yeast in large-scale research and as a commercial product. Commercial biocontrol yeast formulations should have a long shelf life and maintain biocontrol activity. Biocontrol agents are subject to significant abiotic stressors throughout the manufacturing process and also application process which might affect their viability and future efficacy. Osmotic stress is prominent when they are applying on the host surface. When yeast cells are exposed to osmotic stress, it causes rapid outflow and cell death. Hence,

the ability to adapt to the osmolarity of the external environment should be considered when selecting and developing a potential biocontrol strain (Sui *et al.* 2015). The cells are simply preserved in water or a buffer, with or without additional protectants, such as sugars, in liquid formulations (Melin *et al*. 2011). Sugars, polyols and skim milk are commonly used in formulations of biocontrol agents to help maintain viability during the production process, including liquid culture (Liu *et al.* 2009).

The yeast cells adopted modified stress physiologically by enhancing the level of trehalose and primarily glycerol (polyols) as major compatible osmolytes, suggesting their role in defense mechanism against osmotic stress (Sharma and Sharma 2017). A study with halotolerant black yeast *Hortaea werneckii* has been found that the total amount of polyols correlated well with osmotic stress induced by high salinity (Kogej *et al.* 2007).

In India, several lab-based studies have been conducted but no formulations are available for commercial uses. Despite being one of the leading countries in the total production of fruits and vegetables in the world, India is still in the backseat when it comes to the development of yeast based biocontrol formulation to prevent post-harvest spoilage of fruits and vegetables. Polyols have been shown in several investigations to be the primary osmoprotectant in osmotic stress environment. Based on the above background the present research work was conducted to evaluate osmotic stress tolerance of antagonistic yeast strain and the efficiency of Sorbitol as osmoprotectant in Yeast based biocontrol formulation and its efficiency against Anthracnose of Banana.

MATERIALS AND METHODS

Biocontrol yeast: The yeast isolate *Saccharomyces cerevisiae* (*Sc*YZ7, NCBI accession no. KT459474) was obtained from Departmental Culture collection of Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya with known biocontrol activity.

Fruit samples: Fruit samples of banana were collected from the AICRP on fruits, Mandouri, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal, India, for biocontrol efficiency studies under laboratory storage conditions.

Test pathogen: *Colletotrichum musae* causing anthracnose of banana was used for studying the antagonistic potential of the yeast-based formulation.

Media selected for mass multiplication of yeast cells: Molasses urea media (Cane molasses, 50 g L^{-1}, Urea 1.2 g L^{-1}) were used for multiplication and formulating the yeast cells. CFU ml^{-1} was counted using hemocytometer, (CFU : Colony Forming Units).

Evaluation of osmotic stress tolerance of yeast Sc YZ-7 formulations with sorbitol as additive: The formulations containing upto 15% molasses concentration were used in the study. These formulations were tested with 2.5% and 5% sorbitol as additive. Sorbitol added to formulations containing different concentration of molasses and the cells were at the starting of their exponential phase. Control set received no stress (5% molasses). The osmotic stress tolerance of the yeast isolate grown in molasses urea media without any additives was also evaluated. The number of viable cells (CFU mL^{-1}) was determined by taking 10 µl (composite sample of replicated flasks) aliquots from each formulation and plating on YPDA (PDA media supplemented with yeast extract (1g L^{-1}) for profuse growth of yeast culture) plates after 4, 15, 30, 60 and 120 DAS (Thomas *et al.* 2015).

Modified serial dilution method

Media was prepared and poured in the plates (15–20 ml per 9 cm diameter pre- sterilized plates). Serial dilutions in sterilized distilled water upto 10^{-6} were prepared. The micro-tips were flushed 4-5 times and changed after each dilution. Reverse side of the petriplates were marked. 10 µl aliquots from the dilutions were applied as micro-drops in marked areas. Plating was started from the last dilution and using the same tip. The plates were allowed to dry in the LAF cabinet for 4-6 minutes. Plates were sealed and incubated at 28±1°C for 24 h. Colonies were counted in each sector and the CFU ml^{-1} of the sample was worked out by applying the formula: $n \times 10^{d+2}$ where, n = no. of colonies in 10 µl sample, d = dilution level

Table 1. Population of *Saccharomyces cerevisiae* YZ 7 in Molasses urea medium based liquid formulation with different concentration of sorbitol as osmoprotectant at different days after storage (DAS) at 28 ±1°C. *Values are means of three replications. ** 0 means day at which sorbitol added to formulations containing different concentration of molasses and the cells were at the starting of their exponential phase.

Treatments	Population of yeast cells (Log_{10} C fu/ml) at days after treatment					
	0**	4	15	30	60	120
5% molasses	8.17*	8.06	7.51	7.82	7.76	6.61
5% molasses + 2.5% sorbitiol	8.17	8.04	7.72	7.88	7.99	7.51
5% molasses + 5% sorbitol	8.17	8.11	8.03	8.03	7.87	7.64
10% molasses	8.21	8.17	7.80	7.87	7.91	7.61
10% molasses + 2.5% sorbitol	8.21	8.15	7.80	7.93	7.92	7.65
10% molasses + 5% sorbitol	8.21	7.96	8.05	8.03	7.96	7.69
15% molasses	8.23	8.07	7.82	7.87	7.84	7.45
15% molasses + 2.5% sorbitol	8.23	7.97	7.92	8.08	8.01	7.53
15% molasses + 5% sorbitol	8.23	7.92	7.96	8.16	8.03	7.61
SEM±	0.007	0.012	0.017	0.014	0.005	0.033
CD (5%)	0.022	0.036	0.050	0.041	0.016	0.100

yielding countable colonies.

***In vivo* studies on biocontrol efficiency of yeast formulation with sorbitol:** The efficacy of the formulations was tested against *Colletotrichum musae* causing anthracnose of banana. One day before the treatments, mature green fruits were harvested and transported to the laboratory, where they were ripened in a calcium carbide chamber for 24 hrs. The fruits were rinsed with running tap water, air dried and surface sterilized with 70% ethanol on the day of treatment to remove any superficial contamination that may have occurred during harvesting and post-harvest handling. The banana fruits were wounded in the experiment by pricking them on the surface three times with a sterile needle. The formulations of yeast isolate namely *Sc*YZ-7 in cane molasses (5 gL^{-1}, urea, 1.2 gL^{-1}) was tested against *C. musae* on banana cv. Martaman. The following treatments were selected for evaluation of bioefficacy of the yeast-based formulation: 5% molasses + 5% sorbitol, 15% molasses + 5% sorbitol, Treated control (only *Sc* YZ-7) and untreated control. The fruits were dipped in the yeast formulation with 1×10^7 CFU mL^{-1} concentration and dried for 15–20 minutes at room temperature. The fruits were injured by pin prick and artificially inoculated with the pathogen (3.1×10^6 spores/ml). After completion of all the treatments the bananas were kept in clean plastic trays at room temperature 28±1°C, 90 ± 1% RH for 3-4 days. The lesion diameters were recorded after 3 days.

Data analysis: The lab experiments were arranged in Completely Randomized Design (CRD). Data analysis was done using OPSTAT and Microsoft Excel Software.

RESULTS AND DISCUSSION

Evaluation of osmotic stress tolerance of yeast *Sc* YZ-7 formulations with sorbitol as additive: The viability of *Sc* YZ-7 in molasses urea based liquid formulation was evaluated with different concentrations of sorbitol such as 2.5% and 5% in the formulation as additive (Table 1). The effect of sorbitol was tested against the formulation containing upto 15% of molasses which has shown highest cell number throughout the experiment. The formulation containing molasses (15%) + sorbitol (5%) and molasses (10%) + sorbitol (5%) maintained highest count (93.59% of initial population) whereas, in control the count was 80% of the initial population after 120 days of storage at 28°C. After 60 days of storage, the formulation containing molasses (5%) + sorbitol (5%) and molasses (15%) + sorbitol (5%) maintained almost similar percentage of initial population about 97.1% and 97.5% respectively. It has been observed that the number of yeast cells increased in the formulations with sorbitol at different concentrations compared to the control. Sorbitol (5%) had protective role on yeast survival.

According to Wani *et al.* (2013), compatible

Fig. 1. *In vivo* antagonistic efficacy of yeast based liquid formulation against *Colletotrichum musae* on artificially infected banana fruits (A): Untreated control, (B): Molasses (5%) + sorbitol (5%), (C): Molasses (15%) + sorbitol (5%), (D): Treated control (only Sc YZ-7).

solutes help in survival under extreme osmotic conditions either by maintaining osmotic balance by increasing their concentration to avoid dehydration or by maintaining membrane fluidity and stabilizing cellular proteins by keeping them hydrated. *Zymomonas mobilis* ATCC 20191 produced the most sorbitol after 36 hrs on sucrose at 300 g/L (Barros and Celligoi 2006). A study on *Zymomonas mobilis* recorded that within a pre-determined range (200 to 300 g/L), increase in sugar concentration were accompanied by increases in sorbitol formation, which was most likely a response to the need for osmotic protection. On the other hand, across the full time period analyzed, cultivation time had a positive connection with sorbitol production (Vignoli *et al.* 2010).

Table 2. Anthracnose lesion diameters on artificially inoculated banana fruits treated with different *Sc*YZ 7 formulations. * Values are means of three relications.

Treatments	Average lesion diameter (mm) 7 days after treatment*
Molasses (5%) + sorbitol (5%)	1.00
Molasses (15%) + sorbitol (5%)	1.17
Treated control (only *Sc* YZ-7)	3.11
Untreated control	11.33
SEM±	0.341
CD (5%)	1.131

Viability of the postharvest biocontrol agent *Candida sake* CPA-1 stored as liquid formulation and

evaluated against *P. expansum* infection of apples. During the 210 days of storage, the number of *C. sake* cells grown in the sorbitol modified medium and stored at 4°C with the isotonic trehalose solution remained stable around 100% (Abadias *et al.* 2003). From the experiment, it was observed that the yeast isolate *Saccharomyces cerevisiae* (*Sc*YZ7, NCBI accession no. KT459474) showed viability under osmotic stress environment which was induced by higher molasses concentration (molasses (10%) and molasses (15%) compared to control formulation (molasses 5%) even after 4 months of storage. It shows that *Sc*YZ7 is potential biocontrol strain as it is adaptable to osmotic stressed condition. The formulation containing molasses (15%) + sorbitol (5%) and molasses (10%) + sorbitol (5%) maintained highest viability than control after 4 months storage. It indicates the protective role of sorbitol in the liquid formulation. From the study it has been found that sorbitol (5%) can be used as additive in the yeast biocontrol formulation.

***In vivo* studies on biocontrol efficiency of yeast formulation with sorbitol:** The study was conducted to evaluate the bio efficacy of yeast based biocontrol formulation on banana anthracnose disease. After 3 days of storage, the bananas treated with water (untreated control) showed more lesion diameter compared to other treatments (Table 2). The lesion diameter was least in the fruits treated with formulations containing molasses (5%) +sorbitol (5%) followed by molasses (15%) + sorbitol (5%) (Fig. 1). From the result, it has been found that sorbitol (5%) plays an important role on yeast survival and increase the efficiency of the formulation under osmotic stressed condition.

The antagonistic property of yeast is due to its competition for nutrients and space on the fruit surface. The mean lesion diameter of anthracnose lesions on artificially inoculated banana fruits was significantly reduced by yeast *Sc*YZ 7 application as compared to those of the control fruits (Zhimo *et al.* 2016). There are reports on 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). A study conducted by Xie *et al.* (2017) observed that yeast mannan treatment strongly suppressed ethylene synthesis and progress of ripening and softening of tomato fruit during storage. The solubilization and de-polymerization of cell wall polysaccharides were inhibited, and therefore the integrity of cell wall architecture was maintained by the yeast mannan treatment.

The findings suggests that the development of cheap Yeast based biocontrol formulation with high Osmotic stress tolerance capacity and longer shelf life for post-harvest disease management at commercial scale is possible in the presence of some protective additives.

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REFERENCES

Abadias MJ, Usall N, Teixido, Vinas I (2003) Liquid formulation of the postharvest biocontrol agent *Candida sake* CPA-1 in isotonic solutions. *Phytopath* 93: 436—442.

Barros M, Celligoi MAPC (2006) Synthesis of sorbitol by *Zymomonas mobilis* under high osmotic pressure. *Braz J Microbiol* 37 : 324—328.

Kogej T, Stein M, Volkmann M, Gorbushina AA, Galinski EA, Gunde-Cimerman N (2007) Osmotic adaptation of the halophilic fungus *Hortaea werneckii* : Role of osmolytes and melanization. *Microbiol* 153: 4261—4273.

Liu J, Tian SP, Li BQ, Qin GZ (2009) Enhancing viability of two biocontrol yeasts in liquid formulation by applying sugar protectant combined with antioxidant. *Biocontrol* 54 : 817—824.

Mari M, Neri F, Bertolini P (2007) Novel approaches to prevent and control postharvest diseases of fruit. *Stewart Postharvest Review* 3: 4.

Melin P, Schnurer J, Hakansson S (2011) Formulation and stabilization of the biocontrol yeast *Pichia anomala*. *Antonie Van Leeuwenhoek* 99: 107—112.

Nunes CA (2012) Biological control of postharvest diseases of fruit. *Eur J Pl Pathol* 133: 181–196.

Sharma A, Sharma SC (2017) Physiological basis for the tolerance of yeast *Zygosaccharomyces bisporus* to salt stress. *HAYATI J Biosci* 24 (4): 176—181.

Sui Y, Wisniewski M, Droby S, Liu J (2015) Responses of yeast biocontrol agents to environmental stress. *Appl Environ Microbiol* 81(9): 2968—2975.

Thomas P, Sekhar AC, Upreti R, Mujawar MM, Pasha SS (2015) Optimization of single plate-serial dilution spotting (SP-SDS) with sample anchoring as an assured method for bacterial and yeast cfu enumeration and single colony iso-

lation from diverse samples. *Biotechnol Rep* 8: 45—55.
Tongsri V, Sangchote S (2009) Yeast metabolites inhibit banana anthracnose fungus *Colletotrichum musae*. *Asian J Food Agro Industry* 2: 807—816.
Vignoli JA, Cazetta ML, Silva RSS, Celligoi MAPC (2010) Influence of high osmotic pressure on sorbitol production by *Zymomonas mobilis*. *Brazilian Arch Biol Technol* 53(5): 1169—1175.
Wani HS, Singh BN, Haribhushan A, Iqbal Mir J (2013) Compatible solute engineering in plants for abiotic stress tolerance role of glycine betaine. *Curr Genom* 14: 157—165.
Xie F, Yuan S, Pan H, Wang R, Cao J, Jiang W (2017) Effect of yeast mannan treatments on ripening progress and modification of cell wall polysaccharides in tomato fruit. *Food Chem* 218: 509—517.
Zhimo VY, Bhutia DD, Saha J (2016) Biological control of post harvest fruit diseases using antagonistic yeasts in India. *J Pl Pathol* 98(2): 275—283.