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Isolation and Characterization of the Commercial and MPKV Probiotics

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ABSTRACT
Use of probiotics for the control of post-harvest diseases has opened new avenue in the management of post-harvest plant pathogens and organic farming. Such probiotics has never been used in the crop protection programme in agriculture. The post-harvest pathogens particularly Rhizopus, Aspergillus, Penicillium and Alternaria can be checked as a post-harvest pathogen by sprays of probiotics under natural field conditions when the loads of inoculums of these post-harvest pathogens are less in fruits. Application of probiotic as pre-harvest field spray helps to manage the post-harvest disease. These Probiotics showing positive effects in controlling the post harvest diseases and increase the retention quality of fruits. So that, here we are providing the information about the isolation and preparation of Probiotics solutions to spray in the orchards and their morphological and biochemical characteristics of the Probiotics. The yeast cultures from these material showed round, fluppy, raised colonies with dull creamy grey coloured or suppressed colonies with dull creamy grey coloured or pale yellowish colour. The Lactobacillus as probiotic was also obtained from the curd samples. The MPKV probiotic yeast showed oblong elongated yeast cells with budding habits, and could utilize the sugar (sucrose) up to 50 per cent concentration and tolerate the salt concentration of 10 per cent.
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INTRODUCTION

A fruit forms an important component of human diet as these are rich sources of minerals, vitamins, aminoacids, anthocyanidins and immune system boosters. Among various fruits, grape berries are a high priced fruits in India. In India, grape is cultivated over an area of 1.1 lakh ha with a productivity of 11.1 MT/ha [1] and total production of 12.35 million MT. Maharashtra state occupies 90,000 ha area with production of one million MT and ranks first in production and productivity with 62.7 per cent of countries total production.

Besides occurrence of regular diseases in grape orchards, post-harvest pathogens also infect the grape bunches in the gardens during humid climatic conditions, and in transit and storage. The post-harvest losses of grapes in developing countries have been estimated upto 27 per cent [8] and possibly the greatest single cause of post-harvest losses is caused by microorganisms. This usually occurs from initial infection by one or more specific pathogens during cultivation followed by secondary infection by a broad spectrum of saprophytes on the dead tissues during transit and storage. During storage, a number of fungi are known to cause spoilage of fruits. Under tropical conditions considerable losses are occurred due to rots caused by *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Botrytis cinerea*, *Botryodiplodia* spp., *Bipolaris* spp., *Curvularia* spp., *Fusarium* spp., and are becoming limiting factor for successful fruit harvest management [9]. The total estimated losses of fruits and vegetables in India due to inadequate post-harvest handling, transportation and storage are reported to the extent of 20-25 per cent [7]. These losses in terms of monetary value are more than 1.5 billion dollars annually.

Post-harvest losses caused by microorganism accounts for millions of dollars in perishable produce every year [4]. To minimize these losses and the produce, it is very necessary to control the post-harvest diseases. Post-harvest diseases can be controlled by biological, chemical and physical treatments [3]. These includes the use of bioagents and botanicals as biological treatments while chemical treatments includes use of antibiotics, fungicides, oils and as physical treatments vapor emitting compounds.

The post-harvest diseases minimized by application of therapeutically fungicides as pre-harvest treatment under field conditions, posses a serious threat of residues of these fungicidal chemicals on consumable product and therefore their use is being restricted worldwide. Therefore a novel method of biocontrol of these post-harvest diseases is a need of the day to save our produce. These biocontrol agents should be safe for human, as in case of Probiotics.

Probiotics are defined as the micro organisms which are beneficial in the human health [5]. These are orally administered or given in food supplements and are present in the gastrointestinal tract of human beings. These are also used for the health benefits of animals, birds and fishes [6] and [2]. Probiotics occur naturally in the fermented food product such as yoghurt, kefir, sauerkraut, cabbage kimchee and soybean-based miso and natto. Numerous health benefits have been attributed to probiotics including effects on gastrointestinal tract functions and diseases, immune functions, hyperlipidemia, hypertension and allergic conditions. These also enhance the recovery from fatigue and improved the immune function [5].

The probiotic supplements includes bacteria like *Lactobacillus rhamnosus*, *Bifidobacterium longum*, *L. salivarius, L. plantarum, L. paracasei, B. lactis type* and *Streptococcus facealis, Clostridium butyricum* and *Bacillus masentericus* and some edible yeast species. As the probiotics are consumed orally and beneficial to the human health their presence on the consumable fruits will not have any harmful affect on the human. So that, isolation and preparation of these Probiotics and their morphological and biochemical characteristics were studied.

MATERIALS AND METHODS

Isolation of causal organism

The collected disease specimens from grape berries were surface sterilized with 1% sodium hypochloride solution for 2-3 minutes and partly diseased portion with some partly healthy portion was cut with the help of sterilized scissors under aseptic conditions in laminar air flow and kept on potato dextrose media plates. The inoculated petriplates were then incubated at 25±2°C for 3 days. The fungal growth obtained was purified by mycelial tip isolation method and further identified upto genus level based on their characteristics.

Commercial Probiotics

Sacro (contains Lyophilized *saccharomyces boulardi), Darolac (*contains *L. acidophilus, L. rhamnosus,B. longum and S. boulardi) and Sporocheck (*contains *Streptomyces faecalis, Clostridium butyricum, Bacillus mesentaricus and Lactic acid bacillus* (*Lactobacillus sporogenes*) available in the market .

Isolation and culturing of commercial probiotics

The commercial sachets of Sacro, Darolac and Sporocheck probiotics acquired from market was used for isolation and culturing of probiotics. The content of sachet was suspended in 10 ml sterile distilled water under aseptic condition and allowed to stand for 30 minutes. A loopful of suspension was streaked on malt extract agar medium for isolation of yeast and on nutrient sucrose agar medium for isolation of bacteria. The plates were incubated at 28±2ºC for two days and growth of the probiotic microorganisms were observed. The growth of probiotic isolates contained yeast cultures and bacterial growth.

MPKV probiotics

Probiotics were isolated from respective probiotic sample (Probiotic I, a edile yeast culture isolated from dhokla material; probiotic II, a edible yeast culture isolated from dosa material; probiotic III, a edible yeast culture isolated from bajra flour; probiotic IV, a edible yeast culture isolated from jowar flour and probiotic lactobacillus culture isolated from curd.

Isolation and formulation of MPKV Probiotics

Isolation of edible yeast

Fermented samples of raw dosa and dhokla material were subjected from isolation of edible yeast on malt extract medium by streak plate method. A loopful of material was streaked from isolation samples on yeast isolation medium in petriplates and inoculated plates were incubated in BOD at 28 ± 2ºC for 2 days for appearance of yeast colonies.

For isolation of edible yeast from cereal grain flours, the flour of jowar grain /bajra grain was suspended in distilled water (25g flour in 100 ml distilled water) and incubated at 28±2ºC in BOD incubator for three days to start the fermentation due to grain yeast. A loopful of suspension from this incubated and fermented flour sample was streaked on yeast medium and the plates were incubated for two days at 28±2ºC for appearance of yeast colonies. The isolated yeast colonies in the plates were further purified by streak plate method and single colonies thus obtained were maintained as pure cultures of the yeast as probiotics.

Isolation of *Lactobacillus*

One loopful of curd taken from curd sample was streaked on the sterilized Nutrient agar sucrose (NAS) medium in petriplates. The inoculated plates were incubated in BOD at 28 ± 2°C for 2 days for

observation of bacterial colonies. The isolated bacterial colonies in the plates were further purified by streak plate method and single colonies thus obtained were maintained as pure cultures of the bacteria (*Lactobacillus*) as Probiotic.

Morphological characterization of MPKV yeast

The edible yeast isolated from dosa material, dhokla material, bajra flour and jowar flour was characterized for differences among them, if any, on the basis of cultural growth, microscopic observations, NaCl tolerance test and utilization of sugar concentrations.

Cultural growth

The growth of the yeast culture viz., fluppy/raised/ suppressed with the colour of yeast colonies viz., dull creamy grey/ pale yellowish was recorded for the respective yeast isolate.

Microscopic studies

The cells shape of yeast isolates viz., oblong/ cylindrical/elongated with budding habits and their size was recorded.

NaCl tolerance test

The yeast isolates were tested for their NaCl tolerance. The malt extract agar medium having different concentration of NaCl viz. 0, 5, 10, 15 and 20 per cent were used. A loopful of yeast culture was streaked on the respective NaCl concentration tubes and was incubated in BOD incubator at 28±2ºC for 3 days to observe the growth of yeast on particular NaCl concentration to determine the NaCl tolerance limit.

Utilization of sugar concentration

The malt extract agar medium having different concentration of sugar (sucrose) viz., 0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 per cent were used. A loopful of yeast culture was streaked on the respective sugar concentration tubes and were incubated in BOD incubator at 28±2ºC for 3 days to observe the growth of yeast on particular sugar (sucrose) concentration. The edible yeast isolated from dosa material, dhokla material, jowar grain flour and bajra grain flour were designated as yeast I, II, III and IV respectively while Lactobacillus was designated as isolate V. The MPKV probiotic formulation contained the microbial cultures of all above isolates.

RESULTS AND DISCUSSION

Growth characters of MPKV probiotics

The growth characters of MPKV yeast Probiotic (Table 1 and Plate1) indicated that the probiotic isolate I and II has round, fluppy raised yeast colonies with dull creamy grey colony colour whereas the probiotic isolate-III had round suppressed yeast colonies with dull creamy grey colony colour and probiotic isolate-IV had round suppressed yeast colonies with pale yellowish colony colour. It was evident that the yeast of fermented dosa and dhokla material formed round fluppy raised colonies while that of bajra and jowar flour formed round suppressed colonies indicating that the dosa and dhokla yeast was same while bajra and jowar yeast was same. However the jowar and bajra yeast differed in their colony colour and therefore seems to be different.

Table 1. Growth characteristic of MPKV yeast Probiotics

Probiotic isolate	Colony character	Colour of colony
I	Round floppy raised	Dull creamy grey
II	Round fluppy raised	Dull creamy grey
III	Round Suppressed	Dull creamy grey
IV	Round Suppressed	Pale yellowish

Morphology of yeast isolate

The morphological characters particularly yeast cell shape, size and budding habits were observed microscopically. The results (Table 2 and Plate1) were indicated that the shape of yeast cells of probiotic – I and II was oblong while that of probiotic – III and IV it was elongated. It was evident that the fermented dosa and dhokla material had oblong cell yeast while in bajra and jowar flour it had elongated shape yeast. Further all the yeast isolates were variable in their cell size. The cell size of probiotic isolate-

I was bigger (2.85 - 4.80 x 2.37 – 2.99) than other yeast isolates (1.05 – 1.15 x 0.91 – 0.92). All the yeast isolates had budding habits.

Table 2. Microscopic observation of MPKV yeast probiotic

Probiotic yeast isolate	Shape of yeast cell	Size (µm)	Budding habits
I	Oblong	2.85-4.80 × 2.37-2.99	Present
II	Oblong	1.39 × 0.95	Present
III	Elongated	1.13-1.56 × 0.91-0.92	Present
IV	Elongated	1.05-1.15 × 0.70-0.76	Present

NaCl tolerance limit of MPKV yeast isolates

The NaCl salt tolerance limit of all the four yeast isolate were studied by using salt concentration 0-20 per cent. The results (Table 3) indicated that all the 4 yeast isolates grew well on the medium having 10 per cent salt. At 15 and 20 per cent salt concentration there was no growth of yeast isolates.

Table 3. Efficiency of NaCl tolerance limit of MPKV probiotics

Probiotic yeast isolate	Growth on NaCl salt (at per cent concentration)				
	0	5	10	15	20
I	+ +	+ +	++	-	-
II	+ +	+ +	+ +	-	-
III	+ +	+ +	+ +	-	-
IV	+ +	+ +	+ +	-	-

+ + = Full growth of yeast, - = No growth of yeast, I,II,III,IV = Yeast

The results indicated that the probiotic yeast can tolerate the NaCl salt concentration upto 10 per cent. On the basis of salt tolerance all 4 yeast isolates seems to be the same.

Growth of MPKV yeast isolates at different sugar concentrations

The growth pattern of four MPKV yeast isolates in different sugar (sucrose) concentration was studied using the sucrose percentage from 0-50. The results (Table 4) indicated that all the four yeast isolates had full growth within 24 hours on the medium containing 20 per cent sucrose concentration whereas they produced scanty growth on 25-35 per cent concentration and no growth on 40-50 per cent sugar concentration. In 48 hours time the scanty growth of these isolates on 25-35 per cent sugar concentration turned into full growth whereas no growth on 40- 50 per cent turned into scanty growth and at 72 hours all the four yeast isolates got full growth on 50 per cent sugar concentration.

These results indicated that all the four yeast isolates could grow in the sucrose concentration upto 50 per cent. As the sugar concentration increases beyond 20 per cent, the period required for full growth also increases. On the basis of sugar utilization pattern all the 4 yeast isolates seems to be same.

Table 4. Growth pattern of MPKV yeast probiotic in different sugar concentration

Incubation period for growth	Probiotic yeast isolate	Growth on sugar concentration (% sucrose)										
		0	5	10	15	20	25	30	35	40	45	50
1 Day	I	+ +	+ +	+ +	+ +	+ +	+	+	+	-	-	-
	II	+ +	+ +	+ +	+ +	+ +	+	+	+	-	-	-
	III	+ +	+ +	+ +	+ +	+ +	+	+	+	-	-	-
	IV	+ +	+ +	+ +	+ +	+ +	+	+	+	-	-	-
2 Days	I	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+
	II	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+
	III	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+
	IV	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	+	+
3 Days	I	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	II	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	III	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	IV	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

+ + = Full growth, + = Scanty growth, - = No growth, I,II,III,IV = Yeast

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