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RESEARCH ARTICLE

IN VITRO TESTING OF ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA AND OTHER ORGANISMS AGAINST ONION SPOILAGE ORGANISMS

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ABSTRACT

Maximum post harvest loss in onions is attributed to microbial spoilage. So, LAB and antagonistic organisms were screened for their antimicrobial activities against isolated spoilage organisms. *Aspergillus sp., Penicillium sp., Botrytis sp., Erwinia sp., Bacillus sp.,* and *Staphylococcus sp.,* were isolated from Bangalore rose and Bellary red onion varieties. The antimicrobial activity of Lactic acid bacteria and antagonistic organisms were tested by agar cup method. Out of six Lactic acid bacterial strains NR-1 was found to be effective. Maximum areas of zone of inhibition were recorded in *Erwinia sp*.,(147.8sq.mm) fallowed by *Staphylococcus sp*.(94.2sq.mm) and also inhibit *Aspergillus* sp., (40sq.mm). Among *Pseudomonas* cultures, *Pseudomonas putida* was found to be more inhibitory than *P.fluorescens*. *Pseudomonas putida* inhibited *Staphylococcus sp*.,(188.5sq.mm) *Aspergillus* sp.,(56.5sq. mm). Among yeast cultures, *Saccharomyces cervisiae* inhibits *Staphylococcus sp*.,(308 sq.mm). *Erwinia sp.* (188.5 sq.mm) *Aspergillus* sp., (94.2sq. mm). None of lactic acid bacteria and other antagonistic organisms are not much active against fungal isolates. *Sacharomyces boulardi* could not inhibit any of the bacterial and fungal cultures. The present investigation showed that LAB and other organisms showed potent antimicrobial activity to reduce the post harvest bacterial spoilages of vegetables. These results suggest that this potent isolates could be used as a natural bio preservatives in perishable vegetables.

INTRODUCTION

Onion *(Allium cepa* L.) is one of the most important commercial vegetable crops grown in India. The distinctive characteristics of onion are due to the presence of alliaceous odour which accounts for its use as food, salad, spice, condiment and in medicine. Onion has a paramount effect in preventing heart diseases and other ailments. The pungency in onion is due to volatile oil known as allyl-propyl-disulphide. Nutritionally, fresh onion contains about 86.8 per cent moisture, 11.6 per cent carbohydrates, 1.2 per cent proteins, 0.2-0.5 per cent calcium, 0.05 per cent phosphorus and traces of iron, thiamine, riboflavin and ascorbic acid (Anon., 2003). Many factors operate in successful cultivation, production, storage and marketing of quality onion of which post harvest diseases play an important role. Onion suffers from many post harvest diseases like black mold, blue mold, neck rot, brown rot, soft rot and smudge among which, black mold , blue mold and rots are the predominant ones which restricts the availability of onion to domestic and international trade. In order to regulate the supply and to enable the farmer to get a remunerative price for the produce, long term storage of onion is a pre requisite. Losses of onion during storage are considerable mainly due to sprouting and contamination by several microorganisms. Nearly 40% of the production is lost during post harvest handling and sprouting, of which microbial spoilage alone contributes approximately 15-20% of the total loss (Pantastico and Bantista, 1976; Bhagchandani *et al.,* 1980). The purpose of this study was to examine *in vitro* antimicrobial activity of LAB and antagonistic organisms against postharvest pathogens of onions. Lactic acid bacteria, as mentioned earlier, have a major potential to be used in biopreservation methods because they are safe to consume and they naturally occurring microbiota of many foods and vegetables, and they have been known as a positive influence in the gastrointestinal tract of humans and other mammals as probiotics they are afforded GRAS status (Generally Recognized as Safe) in the United States which suggests the non toxic nature of metabolites produced by these organisms(Stiles, 1996). The preservative action of lactic acid bacteria is due to several antimicrobial metabolites, including organic acids (lactic acid, acetic acid), bacteriocins, hydrogen peroxide and others (Magnusson *et al.,* 2001).

MATERIALS AND METHODS

In vitro tests were conducted with biopreservative agents against spoilage organisms associated with onions. This work

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was carried out in the department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore.

Isolation of spoilage organisms

The spoiled and healthy samples of Bellary red and Bangalore rose onions were collected from local markets in polythene bags. They were subjected to microbiological analysis to isolate spoilage organisms by standard dilution plate count method using Nutrient Agar to isolate bacteria, Martin's Rose Bengal Agar (MRBA) for isolation of fungi.

Characterization of isolated cultures

Spoilage organisms isolated from onion samples were characterized based on their colony characteristics, microscopic observations and physiological characteristics. Predominant fungal isolate slides were prepared for microscopic observation with intact fruiting bodies. The specimens were identified up to generic level based on the type of mycelium and spore as described by Benek (1957).

Preparation of Lactic acid bacterial suspension

Lactic acid bacterial suspension was prepared using Mann, Rogosa and Sharpe's (MRS) broth and the cultures used were NR-1, Ch-5, Lc-4, EL-1, MRS-2 and LAB-2.The cultures were incubated at 37^0C for four days. Cultures were obtained from Department of agricultural Microbiology, GKVK, Bangalore.

***In vitro* testing of antimicrobial activity of lactic acid bacteria**

Inhibitory activities of LAB against spoilage bacteria and fungi were studied using the Agar Cup method or well diffusion assay technique (Collins and Lyne, 1970).

Yeast suspension preparation

Yeast suspensions were prepared using Davis Yeast Salt broth and the cultures used were *Saccharomyces cerevisiae* and *Saccharomyces boulardii*. The cultures were incubated at 37°C for four days. The cultures were obtained from Department of agricultural Microbiology, GKVK, Bangalore.

Bacterial suspension preparation

Bacterial suspensions were prepared using Nutrient broth and the cultures used were *Pseudomonas putida*, and *Pseudomonas fluorescens*. The cultures were incubated at 37°C for four days. The cultures were obtained from Department of agricultural Microbiology, GKVK, Bangalore.

***In vitro* testing of antimicrobial activity of antagonistic microorganisms**

Inhibitory activities of antagonistic microorganisms against spoilage bacteria and fungi were studied using the agar cup method and the zones of inhibition were recorded.

RESULTS AND DISCUSSION

Isolation of spoilage organisms

Representative bacterial and fungal isolates were obtained from both the varieties of onion *viz*. Bangalore Rose and Bellary Red varieties. Based on microscopic observations some physiological and biochemical tests, the isolated bacteria were found to be *Bacillus sp*., *Erwinia sp*., and *Staphylococcus sp*., The fungal isolates were characterized based on their morphological structures viz., fruiting body and mycelial characteristics. The dominant ones belonged to the genera *Aspergillus sp., Penicillium sp., and Botrytis sp.*The Surface microflora of onions is total bacterial counts varied from 10.1 × 10^3 CFU/g to 83.4× 10^3 CFU/g. Highest population was recorded in Bellary red onions (83.4 × 10^3 CFU/g). Lowest bacterial counts were in Bangalore rose (10.1 × 10^3 CFU/g). Fungal population ranged from 4.9 × 10^2 CFU/g to 16.09 × 10^2 CFU/g. Maximum fungal population was recorded in Bellary red onions (16.09 × 10^2 CFU/g) and minimum in Bangalore Rose (4.90 × 10^2 CFU/g). (Table 1) *Penicillium* generally causes a soft watery rot on the surface of, which are borne broom like conidiophores covered by masses of blue green conidia. These symptoms were caused by *Penicillium cyclopium* in stored onions (Wijeratnam and Lowings, 1978). Bhattacharya and Mukherjee (1986) found that *Bacillus* and *Pseudomonas* were responsible for causing several post harvest diseases of vegetables at room temperature.

Table 1. Total surface micro flora of spoiled and healthy bulbs of Bellary red and Bangalore rose onions

S.No.	Source	Bacteria CFU x 10^3/g		Fungi CFU x 10^2 /g	
		Bangalore Rose	Bellary Red	Bangalore Rose	Bellary Red
1.	Bulbs (Spoiled)	65.0	71.40	14.06	16.09
2.	Bulbs (Healthy)	20.1	45.80	5.30	7.30
3.	Leaves	10.1	13.90	4.90	6.43
4.	Soil	55.25	83.40	9.70	12.49

* Values represent mean of four replications

***In vitro* testing of antimicrobial activity of lactic acid bacterial isolates**

The antimicrobial properties of LAB against spoilage organisms were tried in *in vitro* (Table 2).

The inhibitory effect was measured in terms of area of zone inhibition around the wells. Lactic acid bacterial isolate EL-1 could not inhibit any of the cultures tested. Inhibitory effect of lactic acid bacterial cultures against spoilage bacteria revealed that *Erwinia sp*. was inhibited to the maximum extent of 147.8 sq mm by NR-1 followed by MRS-2 (138.2 sq mm). In case of fungal isolates there was either little or no inhibition. *Aspergillus* sp., was inhibited by NR-1 with the inhibition zone of 40.0 sq mm. fallowed by MRS-2 (25.1 sq mm). None of the lactic acid bacterial isolates produced inhibition zone against *Penicillium* sp. and *Botrytis* sp. *In vitro,* lactic acid bacterial isolate NR-1 was found to be effective against *Erwinia* sp. *Staphylococcus* sp. *Bacillus* sp. and *Aspergillus* sp. due to the production antimicrobial compounds. Lactic acid bacteria

Table 2. in vitro testing of antimicrobial activity of Lactic acid bacterial cultures against onion spoilage organisms (Area of zone of inhibition in sq.mm)

Sl.No.	Test Organism	Lactic Acid Bacteria					
1.	Spoilage Bacteria	NR-1	Ch-5	LC-4	MRS-2	EL-1	LAB-2
	Erwinia Sp.	147.8	56.5	94.2	138.2	0.0	0.0
	Bacillus Sp.	147.8	56.5	94.2	0.0	0.0	25.1
	Staphylococcus Sp.	94.2	25.1	56.5	0.0	0.0	0.0
2.	Spoilage Fungi						
	Aspergilus Sp.	40.0	0.0	25.1	0.0	0.0	0.0
	Pencillium Sp.	0.0	0.0	0.0	0.0	0.0	0.0
	Botrytis Sp.	0.0	0.0	0.0	0.0	0.0	0.0

* Values represent mean of four replications

Table 3. *In vitro* testing of antimicrobial activity of bacterial and yeast cultures against onion spoilage organisms (Area of zone of inhibition in sq.mm)

S.No.	Test Organisms	*Pseudomonas fluorescens*	*Pseudomonas putida*	*Saccharomyces cerevisiae*	*Saccharomyces boulardii*
1.	Spoilage Bacteria				
	Erwinia Sp.	138.2	11.7	188.5	0.0
	Staphylococcus Sp.	138.2	188.5	308.0	0.0
	Bacillus Sp.	0.0	56.5	25.1	0.0
2.	Spoilage Fungi				
	Aspergilus Sp.	0.0	56.5	94.2	0.0
	Botrytis Sp.	0.0	25.1	11.7	0.0
	Pencillium Sp.	0.0	11.7	25.1	0.0

* Values represent mean of four replication

might be suppressing other pathogens by modifying the environment either by acid production or by the production of antimicrobial antibiotics or compounds. Gouramma *et al.,* (1995) observed reduction in growth and inhibition in aflatoxin production of *Aspergillus flavus* sub sp. *parasiticus* by a mixture of *Lactobacillus* sp. Lactic acid bacteria were also found to inhibit various food borne pathogens like *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* (Dahiya and Speck, 1968; Haynes and Harman, 1973), *Bacillus* sp. was inhibited by *Pediococcus* strains (Zhenni *et al.*, 1997) and *Lactococcus* (Spelhang and Harlander, 1984)

In vitro antimicrobial activity of antagonistic organisms

The antimicrobial activities of antagonistic organisms against spoilage organisms were tried *in vitro* (Table 3). *Saccharomyces boulardii* could not inhibit any of the cultures tested. Among bacteria, *Staphylococcus sp*. was inhibited to the maximum extent of 308 sq.mm by *Saccharomyces cerevisiae* followed by *Pseudomonas putida (188sq.mm)* and *P. fluorescens* (138.2 sq.mm). Lesser inhibition was observed in case of fungi. *Aspergillus sp.* was inhibited by *Saccharomyces cerevisiae* (94.2 sq mm) followed by *Pseudomonas putida* (56.5 sq mm). Only *Aspergillus* sp was inhibited by *P. fluorescens* (25.1sq.mm) Test antagonistic organisms of yeasts were employed against bacteria and fungi. *Saccharomyces cerevisiae* was found to be effective against all bacteria and fungi tested. Maximum area of zone inhibition was (308 sq. mm) recorded against *Erwinia* sp. followed by *Bacillus* sp. *Debaromyces hansenii* exhibited the highest antagonistic activity against *Penicillium* sp. (Chalutz and Wilson, 1990). *Sporobolomyces roseus* inhibited blue mold *Penicillium expansum* from 100 to 0 % and gray mold *Botrytis cinerea* from 78 to 0 %. Bacterial isolate *Pseudomonas putida* recorded maximum inhibition against *Erwinia* sp. followed by *P. fluroscens*. Colyer and Mount (1984) reported that potato tuber treated with *Pseudomonas putida* was antagonistic to *Erwinia* sp. The per cent of rotted tissue, visual ratings reduced by 50 % and this was due to the production of secondary metabolites, which prevent the growth of organisms. Laine *et al.* (1996) studied the antimicrobial activity of *Pseudomonas* sp.against food poisoning bacteria and fungi. In conclusion, the results obtained from this study demonstrated the remarkable antimicrobial activity of lactic acid bacteria and antagonistic organisms. According to present studies, suggest that this potent isolates could be used as a natural bio preservatives in perishable vegetables other food products.

REFERENCES

Anonymous, 2003, *FAO- Bulletin of Statistics*, pp: 38-44.

Benek, E.S., 1957, In Medical Mycology – A Laboratory manual. Burgess publishing Co., pp. 185.

Bhagchandani, A., Netrapal, P.M., Narendra Singh and Choudhuri, B. (1980). White onion for dehydration. Indian Hort. 24: 7-9.

Bhattacharya, I., and Mukherjee, N., 1986, Soft rot of storage tissue due to some uncommon bacteria: Some epidemiological aspects. *Indian Agriculturist.,* 30: 75-82.

Chalutz, E., and Wilson, C.L., 1990,Post harvest bicontrol of green, blue mold and sour rot of citrus fruit by *Debaryomyces hansenii*. *Plant Dis*., 74:134-137.

Collins.C.H., and Lyne,P.M., 1967, Antimicrobial susceptibility and assay Techniques. In: *Microbiological Methods*. Butter worth-Heinemann, Oxford.pp.155-169.

Colyer, P.D., and Mount, M.S., 1984, Bacterization of potatoes with *Pseudomonas putida* and its influence on post harvest soft rot diseases.*Plant.Dis*., 68:703-706.

Dahiya, R.S., and Speck., 1968, Hydrogen peroxide formed by *lactobacilli* and its effect on *Staphylococcus aureus. J. Dairy Sci.*, 51: 1568-1572.

Gouramma, Hassan and Lloyd, 1995, Inhibition of growth and a aflatoxin production of *Aspergillus flavus* by *Lactobacillus* sp. *J. Food Prot*., 58: 1249-1256.

Haynes, W.C. and Harmon, L.G., 1973, Effect of selected lactic acid bacteria on growth of *Staphylococcus aureus* and production of enterotoxin. *Appl. Microbiol*., 25: 436-441.

Laine, M.H., Karwoski, M.T., and Mattila Sandholm., 1996, Antimicrobial activities of *Pseudomonas spp.* against food poisoning bacteria and moulds. *Lett.Appl. Microbiol*., 22:214-218.

Magnusson J., Schnu¨rer J. 2001. *Lactobacillus coryniformis subsp. coryniformis* Strain Produce a Broad-Spectrum Proteinaceous Antifungal Compound. *Applied and Environmental Microbiology*, 67, 1–5.

Pantastico, B. and Bantista, O.K. (1976). Post harvest handling of tropical vegetable crops. Hort Sci. 11:122-124.

Spelhaug, S.R., and Harlander.,1989, Inhibition of food borne bacterial pathogens by bacteriocins from *Lactococcus lactis* and *Pediococcus pentosaceous*. *J.Food.Prot*., 52:856-862.

Stiles, M.E., 1996, Biopreservation by lactic acid bacteria. *Anton Van Leewenhock*, 70: 331345.

Wijeratnam, R.S.W., and Lowings, P.H., 1978,Watery rot of stored onions caused by *Penicillium cyclopium* Westling. *Pl.Pathol*., 27:100.

Zhenni, Y., Soumaline, T., Makinen., A.M., and Huttunen, E., 1997, Antimicrobial activity of 2-pyrrolodine-5 carboxylic acid produced by lactic acid bacteria. *J.Food.Prot*., 60:786-790.

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