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Study on Biocontrol Activity of *Lactobacillus plantarum* against Fungal Pathogens

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The present study was carried out to study the inhibitory effect of *Lactobacillus plantarum* isolated from different vegetables and leaf surfaces on fungal pathogens. Inhibitory activity of *L. plantarum* against *Aspergillus* flavus and *Aspergills niger* was studied by growing the fungal cultures singly and in association with *L. plantarum* and the fresh weight and dry weight of the mycelium and percent inhibition was recorded. *L. plantarum* isolate from Coriander showed maximum inhibition in case of *A. flavus* and the isolate from Cauliflower showed maximum inhibition in case of *A. niger*. The inhibitory activity varied among the *L. plantarum*. These results suggest that the inhibitory activity to fungal pathogens varied among the *L. plantarum* isolated from different vegetables and leaf surfaces.

Key words: Inhibitory activity, *Aspergillus flavus, Aspergillus niger.*

Mycotoxinogenic moulds such as *Aspergillus, Fusarium* and *Penicillium* play an undeniable role in the deterioration of the marketable quality and hygiene of foodstuffs by synthesizing highly toxic metabolites known as mycotoxins. Several of these toxins have been identified but, quite a few could be responsible for significant problems in foodstuffs. Concerning the importance and diversity of their toxic effects carcinogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic, the occurrence of mycotoxinogenic moulds in foods is potentially dangerous for public health and also constitutes a major economic problem. Physical and chemical methods have been developed to control the occurrence of these microorganisms and their toxins but, no efficient strategy has yet been proposed to reduce the presence of mycotoxins. Moreover, some moulds have acquired the ability to resist chemical treatments and some preservatives. The reduction of such moulds in food production is thus of primary importance and there is great interest in developing efficient and safe strategies for this purpose. Biopreservation, the control of one organism by another, has received much attention in the last ten years[1] (Magnusson *et al*., 2003).

Among natural biological antagonists, Lactic acid bacteria (LAB) have several potential applications. These microorganisms are widely used for the production of fermented foods and are also part of intestinal microflora. Research reports indicate that LAB has beneficial health effects in humans. These bacteria have a long history of use in foods. They produce some antagonistic compounds able to control pathogenic bacteria and other undesirable spoilage microflora. Using LAB to control mould growth could be an interesting alternative to physical and

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chemical methods because these bacteria have been reported to have strong antimicrobial properties. A limited number of reports have shown that a good selection of LAB could allow the control of mould growth and improve the shelf life of many fermented products and, therefore, reduce health risks due to exposure to mycotoxins[2] (Gourama & Bullerman, 1995).

Antifungal compounds from LAB involve metabolites containing organic acids, proteinaceous compounds and low molecular mass compounds (>1000 Da). Until now, the reported antifungal compounds with low molecular mass are as follows: reuterin from *Lactobacillus reuteri*[3] (Chung *et al*., 1989); 3-hydroxylated fatty acids from *L. plantarum* MiLAB 14 4 (Sjogren *et al*., 2003) and 4- hydroxy-phenyllactic acid from *L. plantarum* 21B 5 (Lavermicocca *et al*., 2000) and several cyclic dipeptides produced by strains of *L. plantarum*, *Pediococcus pentosaceus, L. sakei* and *L.coryniformis*6 (Magnusson *et al*., 2003; 7 Paavola *et al*., 1999; 8 Strom *et al*., 2002).

MATERIALS AND METHODS

In this study inhibitory activity of *L. plantarum* isolated from different vegetables and leaf surfaces against *Aspergillus flavus* and *A. niger* was determined by the method described by 9 Bueno *et al*., (2006). The *L. plantarum* isolates were isolated from different sources viz, Cabbage (CAB), Cauliflower (CAF), Cucumber (CUM), Radish (RAD), Carrot (CAR), Okra (OKR), Turmeric leaves (TUR), Jack leaves (JAK), Coriander leaves (COR) and Banana leaves (BAN) and identified using morphological, physiological characters and using species specific primers. Fungal cultures *A. flavus* and *A. niger* were obtained from Department of Plant pathology UAS, GKVK, Bangalore. These cultures are preserved for further studies.

Treatment for bioassay

For each treatment *Aspergillus flavus* and *A. niger* cultures were grown in Potato Dextrose Broth (PDB) for 5-6 days at room temperature as a single culture and in association with *L. plantarum*. Inhibitory activity of *L. plantarum* was recorded by taking the fresh weight and dry weight of the mycelia. Inhibitory activity was tested for all the ten isolates and the difference in fresh and dry weight of the mycelia was recorded. For each isolate control was maintained without inoculation and inoculation with only *L. plantarum*. Per cent inhibition was of cultures was calculated using the formula:

$$Percent\ Inhibition \frac{Control - Treated}{Control} \times 100$$

RESULTS AND DISCUSSION

The fungal cultures singly and in association with *L. plantarum* was grown and The fresh weight and dry weight of the fungal cultures singly and in association with *L. plantarum* was recorded, and in all the cases the mixed cultures had a lower fungal growth compared to fungal cultures only. The difference in fresh weight and dry weight and percent inhibition is presented in (Table 1 and 2). The values are predicted graphically using bar graph. Per cent inhibition was calculated and found that the *L. plantarum* isolate from Coriander showed maximum inhibition in case of Aspergillus flavus and the isolate from Cauliflower showed maximum inhibition in case of *Aspergillus niger*. Similar result was reported by [10] Lavermicocca *et al*. (2000) in which *L. plantarum* isolated from sourdough was shown to possess an efficient antifungal activity against *Aspergilus niger*, *A. flavus* and *Fusarium graminearum*. Similarly[11] Laitila *et al*. (2002) reported that *L.*

Table 1. Fresh weight and dry weight of *A. flavus* cultures under inoculated and uninoculated with *Lactobacillus plantarum* cultures

S. No.	Isolate	*Aspergillus flavus* (Wt in g)		
		Fresh weight	Percent inhibition	Dry weight
1	CAB	4.0	31.04	2.4
2	CAF	3.9	32.75	2.2
3	CUM	4.0	31.04	2.4
4	RAD	3.2	44.83	1.3
5	CAR	3.5	39.67	1.9
6	OKR	3.1	46.55	2.0
7	TUR	3.8	34.50	2.1
8	JAK	3.3	43.10	1.6
9	COR	2.6	55.17	0.8
10	BAN	3.4	41.38	1.4
11	Control	5.8	-	3.2
	S. Em ±	0.10	-	-
	CD@1%	0.34	-	-

Table 2. Fresh weight and dry weight of *A. niger* cultures under inoculated and uninoculated with *Lactobacillus plantarum* cultures.

S. No.	Isolate	*Aspergillus flavus* (Wt in g)		
		Fresh weight	Per cent inhibition	Dry weight
1	CAB	3.1	44.64	1.1
2	CAF	2.9	48.21	0.7
3	CUM	4.3	23.21	2.0
4	RAD	3.5	37.50	1.6
5	CAR	3.7	33.93	1.5
6	OKR	4.4	21.43	2.1
7	TUR	4.0	28.57	1.8
8	JAK	3.9	30.36	1.7
9	COR	3.3	41.07	1.3
10	BAN	3.2	42.86	1.2
11	Control	5.6	-	2.9
	S. Em ±	0.08	-	-
	CD@1%	0.28	-	-

plantarum strains VTTE-78076 and VTTE-79098 were effective against different plant pathogenic, toxigenic and gushing active *Fusarium* fungi.12 Bueno *et al*. (2006) also reported the effect of two species of *Lactobacilli, Lactobacillus casei* CRL 431 and *Lactobacillus rhamnosus* CRL 1224, on growth of different *Aspergillus flavus* strains. Thus from the present study, the growth of *Aspergillus flavus* and *Aspergillus niger* in the presence of *Lactobacillus plantarum* was reduced.

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