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Medical importance of *Lactobacillus fermentum* lysate as a bioactive agent against some pathogenic *Candida* and *Aspergillus* strains

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In search of a safe and new biosource for treating fungal infections, the bacterial lysate of probiotic *Lactobacillus fermentum* was tested for its antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. The antifungal activity of lysate was analyzed by dry weight, disc diffusion and micro broth dilution methods. The growth of *A. niger* and *A. flavus* was inhibited by 31 µg lysate protein/disc but *C. albicans* was firstly inhibited at 62 µg lysate protein/disc. MIC for inhibition of *A. niger* was recorded as 125 µg lysate/ml while, *A. flavus and C. albicans* were inhibited at MIC of 62 µg lysate/ml by micro broth dilution assay. De Man Rogose and Sharpe medium supplemented with wheat bran, corn steep liquer and yeast extract showed the highest yield, 10 g dry biomass/L of *L.fermentum* at pH 6.8. The elution profile of the purified lysate showed five fractions, the first gave more than 60% of the original sample. Its MIC was 25 µg lysate/disc. Its molecular weight was 20 to 30 KDa. Toxicity tests revealed that, up to 150 µg lysate/ml showed no significant haemolysis.

Key words: Haemolysis, *Lactobacillus fermentum*, lysate, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

INTRODUCTION

The number of emerging or re-emerging bacterial, fungal and viral pathogens continues to increase. Additionally, multi drug resistance is also on the increase, in both nosocomial and community-acquired pathogens. The mortality rate due to invasive aspergillosis has risen steadily with a 357% increase which caused significant morbidity and mortality during 1980-1997 (Morgan et al., 2005; Samson et al., 2004). Thus, the need for new antimicrobial agents is perhaps greater now than it has ever been. In recent years, antibacterial and antifungal peptides have gained a lot of interest, due to their potential use as a new generation of therapeutic agents (Zasloff, 2002). Antifungal proteins such as ribosome-inactivating proteins (Ng and Parkash, 2002) and glucanases (York et al., 2004) have been reported from a variety of sources including bacteria, mammals, insects and plants for treating fungal infections. A novel anti-fungal pyrrole derivative was recorded from *Datura metel* leaves (Dabur et al., 2004, 2005). Screening of three novel antimicrobial peptides was assayed for their activity against crop fungal pathogens (Lan et al., 2011).

A protein isolated from *Penicillium* CL showed activity against 21 fungal isolates (Galgoczy et al., 2005). Pro-duction by *Bacillus pumilus*, of an antifungal compound that is active against Mucoraceae and *Aspergillus* species was reported by Bottone and Peluso (2003). A cytosolic

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protein (PPEBL21) was purified from *Escherichia coli* BL21 that demonstrated potent antifungal activity against pathogenic strains of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* (Yadav et al., 2005a). Bacterial lysates are powerful inducers of a specific immune response against bacterial infections, but their mechanism of action is not fully understood. Apparently, they lower bacterial carriage levels in the respiratory tract, decrease hospitalizations owing to exacerbations, and reduce the need for antibiotics (Cazzola et al., 2012).

In view of the importance of probiotics in human health, the present study was undertaken to investigate the effect of cytosolic proteins from *L. fermentum* as a bioactive agent against some human pathogenic fungi.

MATERIALS AND METHODS

Microorganisms

L. fermentum used in the present study was isolated from commercially dairy products in the market and was identified at the center of El Azhar University, Cairo, Egypt according to Bergey's manual of determinative bacteriology, 1986 and 1994. De Man Rogose and Sharpe medium (MRS), is specific for the isolation, growth and enrichment of lactic acid bacteria (Samson et al., 2004). Fungal strains used as indicator organisms for the assessment of the antifungal activity were isolated from garden soil and they are: *A. niger, A. flavus. C. albicans* kindly obtained from Immunology and Microbiology Department, Faculty of Medicine, Alexandria University.

Preparation of the bacterial lysate (BL)

Three days exponential phase cells were separated by centrifugation at 12000 xg for 30 min. The pellets were suspended in 10-15 ml sonication buffer (50 mmol Tris/HCL, 50 mmol EDTA) adjusted to a pH 8. Cells suspension was then sonicated for 1 min/ml of suspension at 50% power/cooling periods using a soincator (Bandelin Sonopuls HD2070 Maxpower). The sonicated samples were centrifuged at 12000 xg for 20 min and the supernatants were used as crude lysate which were then freeze-dried (Yadav et al., 2005b). Protein concentrations of the bacterial lysates were determined by Lowry et al. (1951) before and after lyophilization.

Antimycotic activity determination

Disc diffusion method

Sterilized filter discs were impregnated with different concentrations (31 – 500 µg/ml) of the bacterial lysate and were placed on the surface of agar plates already inoculated with 100 µl of fungal spore suspension. These plates were kept overnight in a refrigerator to allow lysate diffusion, and were then incubated at 37°C for 24 and 48 h. At the end of incubation period, the radial growth and zones of inhibitions were measured in mm. For negative control, boiling inactivation of bacterial lysate of *L. fermentum* was used. The previous procedures were repeated at least three times, mean and standard deviation were calculated.

Micro broth dilution

The test was performed in sterile non-coated 96-well culture plates. Autoclaved glucose peptone broth (90 µl) was added to each well of culture plates. Various concentrations of bacterial lysates (31.9 - 1000.0 µg/ml) were prepared in the wells and inoculated with 10 µl of spore suspension containing approximately 1 x 10^6 spores. The plates were incubated at 37°C and examined macroscopically after 48 h for the growth of the tested strains. MIC was determined.

Dry weight method

Flasks containing 30 ml of glucose peptone medium were inoculated with 100 µl of spore suspension of the provided fungal strain. Bacterial lysate under test was added after filter sterilization at different concentrations. One set of flasks was incubated for 24 h and the other for 48 h, under shacked conditions (150 rpm) at 32 ± 2°C. Hyphal growth of the tested fungus was collected by filtration, washed with sterile physiological solution, allowed to dry at 60°C for 24 h. The fungal dry mass was determined gravimetrically. By measuring the percentage of reduction in biomass of the tested fungal strains using the gravimetrical method (Rybka and Kailasaparty, 1996), these assays were repeated at least 3 times.

Optimization of a bioactive bacterial lysate production from *L. fermentum*

Selection of the most suitable carbon source

Carbon source which was found in MRS medium (glucose) was substituted with molasses, wheat bran, whey, sucrose, manitol and lactose each at a time. Each carbon substrate was used as 2% (wt/vol) and all other medium components left unchanged.

Selection of the most suitable nitrogen source

Yeast extract, beef extract and peptone of the MRS medium was substituted with yeast extract only, yeast extract with CSL (corn steep liquor), CSL only, NH_4Cl with CSL, and peptone each at a time. Each nitrogen source was used as 1% (wt/vol) and all other medium components left unchanged.

Fractionation of L. fermentum lysate

The lysate prepared from the *L. fermentum* was fractified by two methods. (1) Ultra filtration using Vivaspin® dividing it into 2 parts according to its molecular weight (KDa). (2) Fractionation by ion-exchange chromatography, DEAE-Cellulose (Patterson et al., 1980). A 15.0 mg of lysate protein was dissolved in Tris/HCl, pH 7.4, and loaded onto a DEAE-cellulose column pre-equilibrated with the same buffer. The non-adsorbed proteins were eluted with Tris/HCl, pH 7.4. The elution of adsorbed proteins was carried out using a 200 ml linear gradient from 0 to 1 mol of NaCl in Tris/HCl, pH 7.4. The flow rate was adjusted to 1.0 ml min^{-1} and 2.0 ml fractions were collected. The OD_{280} (optical density) of the fractions was measured. The OD_{280} values of various fractions were plotted against the human fractions numbers. The peaks were pooled and analyzed for antifungal activity. The proteins recovered in peaks showing antimycotic activity were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli and Favre, (1973).

Table 1. Antifungal bioactivity of lysate from *L. fermentum* against *A. niger*, *A. flavus* and *C. albicans* investigated by measuring the diameter of inhibition zone (disc diffusion assay).

L. fermentum (µg protein lysate/disc)	Diameter of inhibition zone (mm)		
	A. niger	*A. flavus*	*C. albicans*
31	1.8 ± 0.2	1.5 ± 0.1	0.0 ± 0.0
62	2.0 ± 0.2	2.0 ± 0.1	1.5 ± 0.1
125	3.2 ± 0.3	3.3 ± 0.4	2.5 ± 0.2
250	3.6 ± 0.4	3.7 ± 0.2	2.9 ± 0.1
350	3.9 ± 0.2	3.8 ± 0.2	3.1 ± 0.1
500	4.0 ± 0.3	4.2 ± 0.3	3.8 ± 0.2

Table 2. Antifungal bioactivity of lysate from *L. fermentum* against *A. niger*, *A. flavus* and *C. albicans* investigated by Microbroth-Dilution Assay.

L. fermentum (µg protein lysate/ml)	Microbroth-dilution assay		
	A. niger	*A. flavus*	*C. albicans*
1000.00	+	+	+
500.00	+	+	+
250.00	+	+	+
125.00	+	+	+
62.25	−	+	+
31.25	−	−	−

+, Complete inhibition; −, growth was observed.

Haemolytic assay

Erythrocytes were collected from apparently healthy individuals under physician supervision according to the basic method of Latoud et al. (1986). Erythrocytes were then separated by centrifugation. The pellets remained were washed three times with PBS and centrifugation at 1500 xg for 10 min. Different concentrations of bacterial lysates were prepared and 2% erythrocyte suspension was added to each concentration. The mixtures were incubated at 37°C for 1 h. After incubation period, erythrocytes were pelleted at 5000 xg for 10 min. The supernatant was collected and the absorbency at 450 nm was determined using a spectrophotometer. In negative control sets, only buffer was used for background lysis, whereas in positive controls, lysis buffer was used for completely lysing the erythrocytes.

RESULTS

Antifungal activity of the bacterial lysate

The antifungal activity of the prepared lysate from *L. fermentum* was examined by determination of growth inhibition firstly by measuring the diameter of inhibition zone by disc diffusion assay method. Table 1 shows that *L. fermentum* lysate exhibited a mild to moderate activity against *A. niger, A. flavus* and *C. albicans*. 31 µg protein lysate/disc inhibited the growth of *A. niger, A. flavus* but did not show any effect on *C. albicans* using the Disc-Diffusion Assay. One fold increase (62 µg protein lysate/ disc) of lysate concentration, leads to an increase of inhibition reaching 10 and 25% when using *A. niger* and *A. flavus* as test strains. However, an inhibition zone of 1.5 mm was first observed using *C. albicans* at the same concentration of lysate.

Determination of the minimum inhibitory concentration of lysate (MIC) as well as examining the morphological characteristics of the tested lysate-treated fungal strains was carried out using the Microbroth-Dilution Assay. Table 2 showed that for inhibiting the growth of *A. niger,* 4 folds of *L. fermentum* lysate concentration were required and the MIC was 125 µg lysate/ml. On the other hand, *A. flavus and C. albicans* were inhibited at MIC of 62.3 µg lysate/ml. The morphological appearance of the tested strains was observed microscopically from the microdilution wells as shown in Figure 1. *A. niger* showed normal appearance of heads however, the lysate treated spores showed atrophied heads and spores rarely germinated. *A. flavus* on the other hand, was grown normally in untreated spores and no growth was observed in case of lysate treated spores. *C. albicans*, unicellular fungal cells grown individually in lysate untreated cells, nevertheless, lysate treated cells showed frequent formation of pseudomycelia in which daughter cells did not separate from the mother cells. Fungal spores which were treated with Exoderil drug showed collapsed spores mass in the case of *A. niger* and *A. flavus* but *C. albicans* showed no pseudomycelium formation, but cells appeared empty or with abnormal vacuolation.

The percentage of reduction in biomass of the tested fungal strains was determined by gravimetrical method in which spores of *A. niger, A. flavus* and *C. albicans* were allowed to grow in lysate free-glucose peptone medium as a control test and in glucose peptone medium supplemented with 40 to 200 µg lysate/ml. A linear trend of inhibition was observed in all strains, however, the slope was higher in the case of *C. albicans* indicating strong inhibition effect, followed by *A. flavus* and then *A. niger* which showed the lowest slope but an inhibition percenttage in biomass of 65 as compared to spores grown in glucose peptone lysate free medium as shown in Figure 2.

Effect of carbon source on the growth and antifungal activity of *L. fermentum*

As shown in Table 3, MRS medium supplemented with wheat bran showed the highest productivity reaching 10 g dry biomass/L. The antifungal activity of lysate derived from *L. fermentum* cells grown on molasses showed the highest antifungal activity, which resulted in 2.1, 2.3 and 2.8 mm inhibition zones by using 300 µg lysate/ml when it was tested against *A. niger, A. flavus* and *C. albicans*, res-

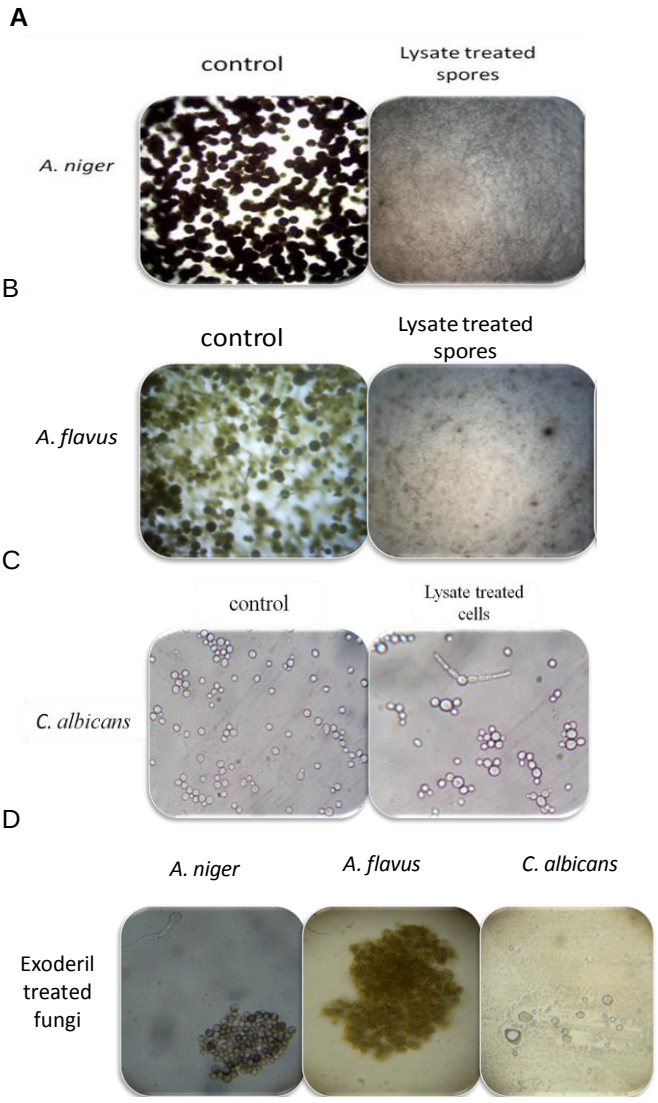

Figure 1. Micrographs of microwells showing growth of: A, *A. niger* spores without the addition of lysate (control) and lysate-treated spores with 125 µg ml^{-1} lysate. B and C, *A. flavus* and *C. albicans* spores without the addition of lysate (control) and lysate-treated spores with 62 µg ml^{-1} lysate. D, growth of *A. niger, A. flavus* and *C. albicans* in medium containing 20 µg ml^{-1}Exoderil. The microwells plates were incubated at 28°C for 48 h, examined microscopically and micrographs were taken.

pectively. The percentage of reduction in biomass of the tested fungal strains was determined. Spores were allowed to grow in lysate free-glucose peptone medium as a control test and in glucose peptone medium supplemented with 200 and 300 µg lysate/ml. Figure 3a, b and c showed that MRS medium supplemented with molasses provided a high and significant inhibition against *A. niger* and *A. flavus*, while MRS medium supplemented with wheat bran showed slight inhibition against *C. albicans.*

Effect of nitrogen source on the growth and antifungal activity of *L. fermentum*

Results illustrated in Table 4 indicated that lysate prepared from MRS supplemented with yeast extract and CSL showed the highest antifungal activity against all tested fungal strains at all tested lysate concentrations. Furthermore, by increasing the lysate concentration to 3-folds the inhibition increased by about 15 - 20%. About 6 g dry biomass/L of *L. fermentum* were produced using CSL and yeast extract (1% each). The reduction percent in biomass of the tested fungal strains was determined, reduction in biomass against all the tested fungal strains at all tested lysate concentrations (Figure 4a, b and c) with *A. flavus* as the most affected strain (51.6%). Moreover, *L. fermentum* lysate prepared from MRS containing NH_4Cl and CSL resulted in a high and significant inhibition against *A. niger, A. flavus* and *C. albicans* using the disc diffusion method as shown in Figure 5.

An extra experiment was designed to find out the effect of boiled inactive lysate on the growth of test fungal strains. In that case increase in fungal growth indicated that the bacterial lysate consists of peptides with antifungal activity which were probably denaturated by boiling and yielding a solution rich in amino acids and small peptides that stimulate the fungal growth more than control by about 10 - 15% (data not shown).

Fractionation of *L. fermentum* lysate

L. fermentum crude lysate was subjected first to ultra filtration using Vivaspin® through 30 KDa cut off membrane which resulted in two fractions according to their molecular weights. The antifungal activities of the both fractions were tested by disc diffusion assay. As shown in Table 5, the inhibition zones determined for all concentrations of both fractions ranging in their molecular weights from less to more than 30 KDa indicated the poly dispersal nature of the antifungal compounds that ranging in their molecular weights and found on both sides of membrane. A further purification has been done to the crude lysate using DEAE-cellulose column. Results illustrated in Figure 6 for elution profile of the lysate indicated that the lysate contain five peaks (FI to FV). Investigating the yield of the total recovery of about 80% of the applied protein, the first elution fraction (FI was represented in fractions from 3 to 6) revealed the highest fraction in its protein concentration reaching about more than 60% of the original loaded lysate, while from fraction II to V constitute the remaining amount of original protein in crude lysate. A major protein peak was obtained by the first elution which was covered by fractions from 2 to 10. The antifungal activities of the five fractions were examined by disc diffusion assay method. It was observed that the antifungal activity resided mainly in FI against *A. niger, A. flavus* and *C. albicans*. All other fraction peaks

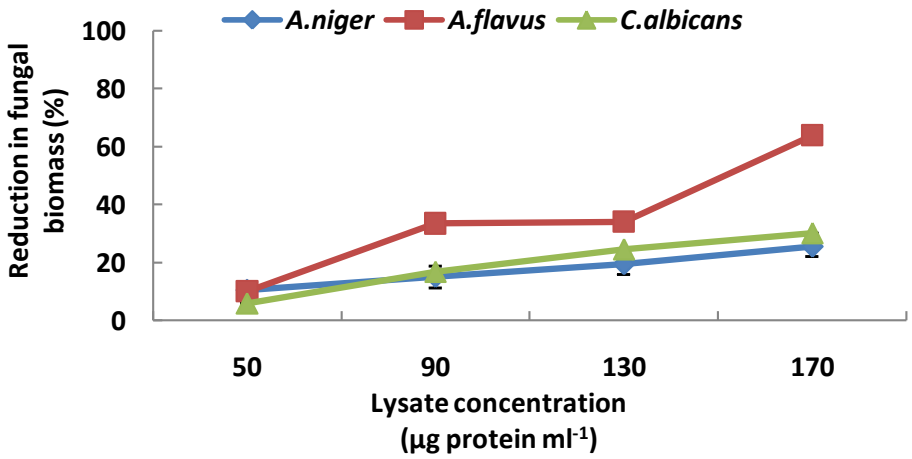

Figure 2. The percentage of reduction in fungal biomass of *A. niger, A. flavus* and *C. albicans,* by *L. fermentum* lysate.

Table 3. Antifungal bioactivity of *L. fermentum* lysate derived by variable carbon sources in MRS medium investigated by measuring the diameter of inhibition zone (disc diffusion assay).

Carbon source*	*L. fermentum* lysate (µg lysate/ml)	Diameter of inhibition zone (mm)		
		A. niger	***A. flavus***	***C. albicans***
Molasses	100	1.1 ± 0.1	1.7 ± 0.2	2.1 ± 0.1
	200	1.5 ± 0.1	1.8 ± 0.2	2.5 ± 0.1
	300	2.1 ± 0.1	2.3 ± 0.2	2.8 ± 0.1
Whey	100	Reduction of the sporulation around the disc	Reduction of the sporulation around the disc	0.0 ± 0.0
	200			
	300			
Wheat bran	100	Reduction of the sporulation around the disc	Reduction of the sporulation around the disc	1.1 ± 0.1
	200			1.4 ± 0.1
	300			1.8 ± 0.1
Lactose	100	1.03 ± 0.1	0.9 ± 0.1	
	200	1.3 ± 0.1	1.1 ± 0.1	0.0 ± 0.0
	300	1.9 ± 0.2	1.5 ± 0.1	

*Also, sucrose and manitol were tested but showed only weak inhibition to growth.

showed low antifungal activity as the reduction of sporulation and growth of the tested fungal strains but no inhibition zone is noticed in the case of *A. niger* and *A. flavus*. On the other hand, fraction no III and IV showed inhibition zone against *C. albicans*. The MIC of FI was determined by disc diffusion assay and it was found to be 25 µg lysate/disc (data not shown).

SDS-PAGE of crude and partially fractified lysate obtained from *L. fermentum*

As shown in Figure 7, the first lane showed the freeze-dried lysate composed mainly of a protein band at an average molecular weight of 20 to 30 KDa. The same was observed with crude lysate as shown in SDS-PAGE graph. The DEAE-cellulose-unbound proteins illustrated in FI lane demonstrated many bands of proteins in the molecular mass range of 20 to 45 KDa. Also, fractions from FII to FV showed mainly 2 protein bands in the range of 20 to 30 KDa, approximately. Peak FI showed the highest activity in relation to the other peaks, due to the high differences in protein concentration basically proteins at 20-30 KDa which where eluted continuously along the 5 fractions.

Haemolytic assay of the *L. fermentum* lysate

Results of toxicity experiments revealed that the total

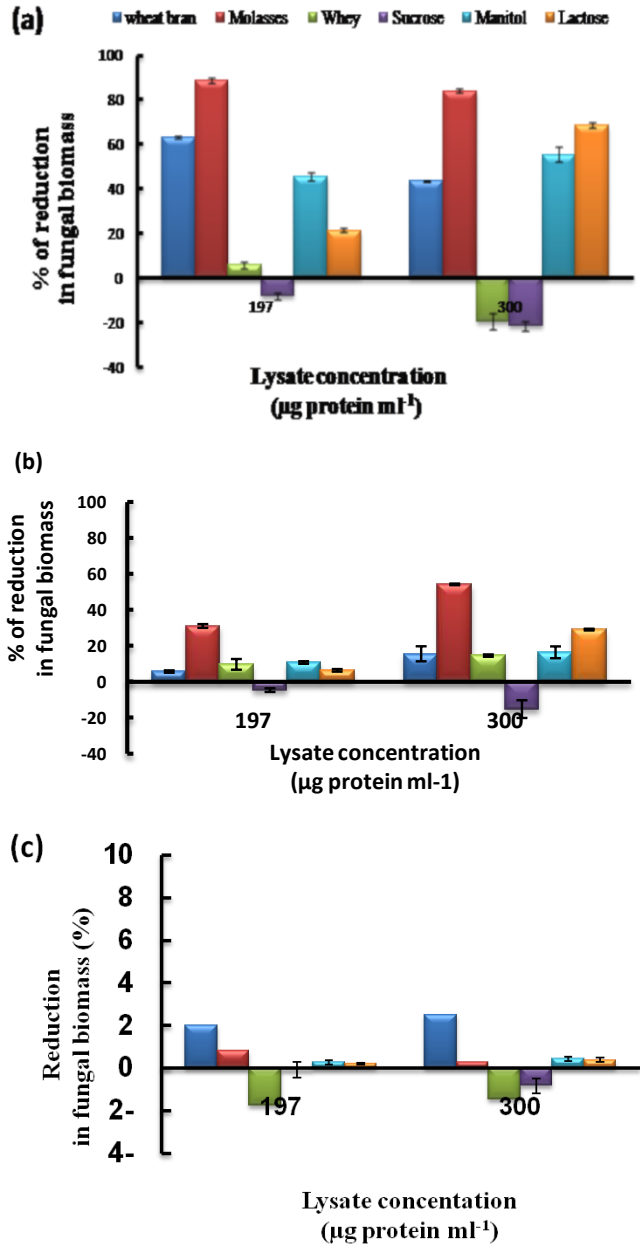

Figure 3. The effect of different carbon sources on the antifungal activity of the resulted bacterial lysate derived from *L. fermentum* against (a) *A. niger*, (b) *A.flavus* and (c) *C. albicans* by measuring the percent of reduction in fungal biomass. ■: Wheat bran; ■: molasses; ■: whey; ■: sucrose; ■: manitol; ■: lactose.

protein lysate of *L. fermentum* investigated in the present study was non toxic to human erythrocytes up to 150 µg lysate/ml. The higher doses exerted insignificant toxicity and only marginal haemolysis (30%) was detected at 300 µg lysate/ml as illustrated in Figure 8. Exoderil, a chemotherapeutic antifungal drug caused lysis of all erythrocytes at a range of 20 to 30 µg exodril/ml. By testing the cytotoxicity of the fractified part that resulted from ultra filtration and fraction FI that was obtained via ion exchange chromatography, it was found that all the concentrations tested showed no significant haemolysis and hence no toxicity to human erythrocytes as compared to

Table 4. Antifungal bioactivity of *L. fermentum* lysate derived by variable nitrogen sources in MRS medium investigated by disc diffusion assay.

Nitrogen source (1%) of each*	*L. fermentum* lysate (µg lysate/ml)	Mean diameter of inhibition zone (mm)		
		A. niger	*A. flavus*	*C. albicans*
NH_4Cl + CSL**	100	1.9 ± 0.1	1.9 ± 0.2	1.4 ± 0.2
	200	2.4 ± 0.2	2.4 ± 0.1	2.1 ± 0.1
	300	2.8 ± 0.2	2.9 ± 0.1	2.5 ± 0.1
Yeast + CSL	100	1.3 ± 0.3	2.1 ± 0.2	2.5 ± 0.1
	200	2.4 ± 0.1	2.7 ± 0.3	2.8 ± 0.2
	300	2.8 ± 0.2	3.3 ± 0.2	3.1 ± 0.1
CSL	100	Reduction of the sporulation around the disc	1.6 ± 0.1	1.5 ± 0.1
	200		1.9 ± 0.2	1.8 ± 0.2
	300		2.0 ± 0.2	1.9 ± 0.1
Yeast	100	Reduction of the sporulation around the disc	1.0 ± 0.1	
	200		1.3 ± 0.2	0.0 ± 0.0
	300		1.4 ± 0.2	

*Also, peptone was tried but showed no result. **Corn steep liquor (CSL).

the chemotherapeutic drug and the lysis buffer.

DISCUSSION

There is presently much active research focusing on the development of target-specific probiotics containing well-characterized bacteria that are selected for their health-enhancing characteristics. In this research, the lysate of *L. fermentum* demonstrated a significant activity against *A. niger, A. flavus* and *C. albicans.* It was found that the MIC of *L. fermentum* lysate was 125 µg/ml against *A. niger* and 62 µg/ml against *A. flavus* and *C. albicans.*

The results also indicated that *A. flavus* and *C. albicans* were more susceptible than *A. niger* to the lethal effect of lysate. While higher concentrations were required to inhibit the growth of *A. niger*. This may be why other investtigators suggested that the surface molecules in different species of pathogens may determine the susceptibility for treatment with bacterial lysate (Yadav et al., 2005b). Similar results were obtained by Magnusson et al. (2003) who screened 1200 isolates of lactic acid bacteria for anti-*Aspergillus* activity and observed a strong inhibitory activity against *A. fumigatus* but several isolates showed reduced antifungal activity after storage and handling.

The results obtained from lysate of *L. fermentum* was similar to that of Yadav et al. (2005a) who indicated that when lysates are prepared from BL21, DH5α, HB101 and XL Blue strains of *Escherichia coli* when examined by using microbroth-dilution assay, these strains exhibited a mild to moderate activity (MICs in µg/ml: DH5α, 62.50; HB101, 125.00; XL Blue, 250.00). They revealed that *E. coli* reported a very important medicinal value which was also supported by Matricardi et al. (2003). The supernatant of *Streptomyces thermonitrificans* showed better anti-*Aspergillus* activity (MIC, 62.5 µg/ml) than lysate (MIC, 125 µg/ml) Yadav et al. (2005a, 2007). *C. albicans* remains the most susceptible of the yeasts studied for fluconazole and itraconazole when compared with non *C. albicans* (Panizo et al., 2009).

No studies have been carried out on the optimization of lysate production from bacteria, however, there was a research by Leal et al. (2002) showing that bacteriocins production changes dramatically upon altering of environmental conditions and optimum production may require a specific combination of environmental parameters. The best result of biomass yield was obtained in MRS medium supplemented with 2% wheat bran (10 g/l) of *L. fermentum*. Wheat barn is a good source of proteins, starch and some vitamins, which confers excellent substrate for growth and reduce the possibility of biosynthesis of secondary metabolites in the relatively short period of incubation. Also, the best nitrogen source that resulted in a large biomass yield (5.8 g/l) of *L. fermentum* was obtained from MRS medium supplemented with 1% yeast extract and 1% CSL furthermore. The significance of this funding lies in the low cost of molasses, yeast extract and CSL. As compared to glucose, peptone and beef extract, the aim was achieved in this research. CSL is of great importance in the production of antibiotics where it is considered as a source of free amino acids, many vitamins and minerals that are incorporated in protein synthesized ribosomally and non ribosomally (Zabriskie et al., 1982).

The fractifaction of *L. fermentum* lysate yielded 5 fractions of protein peaks. The protein profile of the crude lysate gave large number of bands when run on SDS–PAGE and the fractified peak with the highest antifungal activity showed many protein bands in the molecular

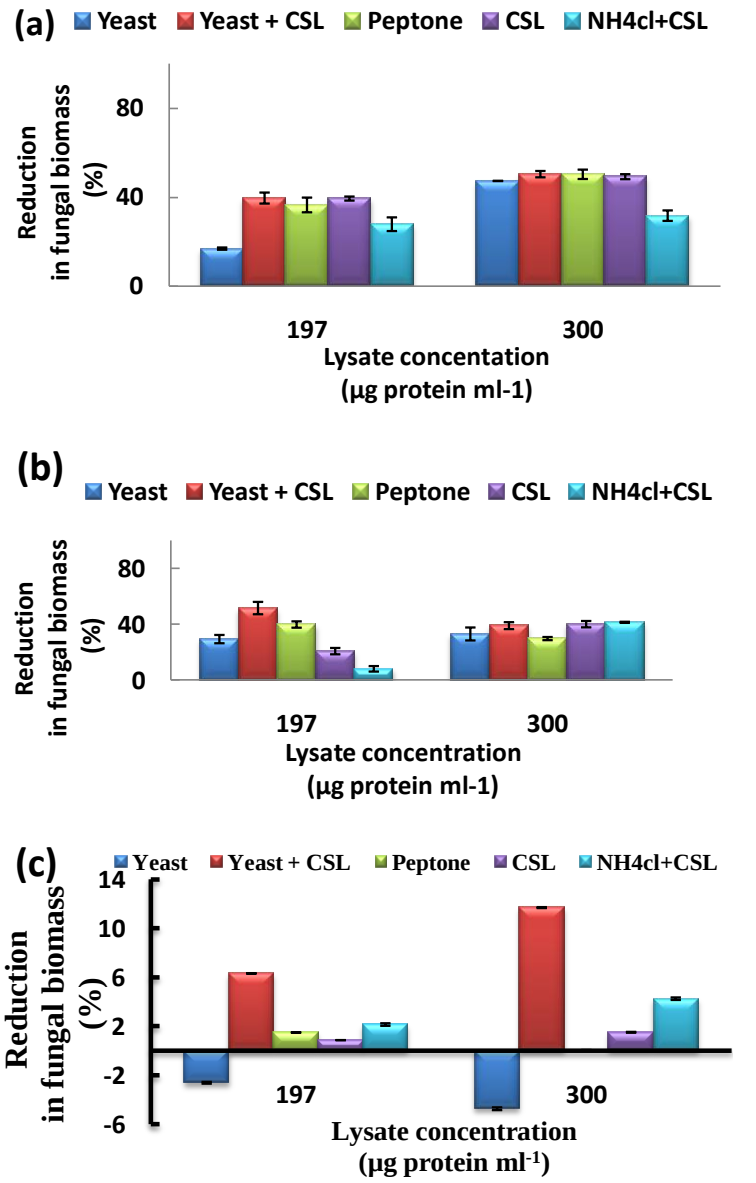

Figure 4. Antifungal activity of the lysate derived from *Lact. fermentum* grown on different nitrogen sources tested against (a) *A. niger,* (b) *A. flavus,* (c) *C. albicans* by measuring the percent of reduction in fungal biomass.

mass range of 20 – 45 KDa. This result was in accordance with that observed by Yadav et al. (2005a and 2007) showing that the fractifaction of *E. coli* BL21 lysate showed 5 fractions of protein. The protein profile of crude *E. coli* BL21 lysate and the active fraction with antifungal activity showed that crude lysate gave various bands when run on SDS-PAGE, while the active fraction with antifungal activity demonstrate two major bands in the molecular mass range of 29 to 43 KDa. The MIC of the active fraction was found to be 3.90 µg/ml against *A. fumigatus* by microbroth dilution assay while by disc diffusion method its MIC was 1.25 µg/disc.

In the present study, crude lysate was non toxic up to a test concentration of 200 µg/ml on the other hand, the fractified fraction with antifungal activity FI showed no toxicity with all tested concentrations up to 2000 µg/ml against erythrocytes, as compared to this results with that obtained by Yadav et al. (2005a and 2007) who showed that *E. coli* BL21 total lysate and completely purified fraction with antifungal activity PPEBL21 were non toxic

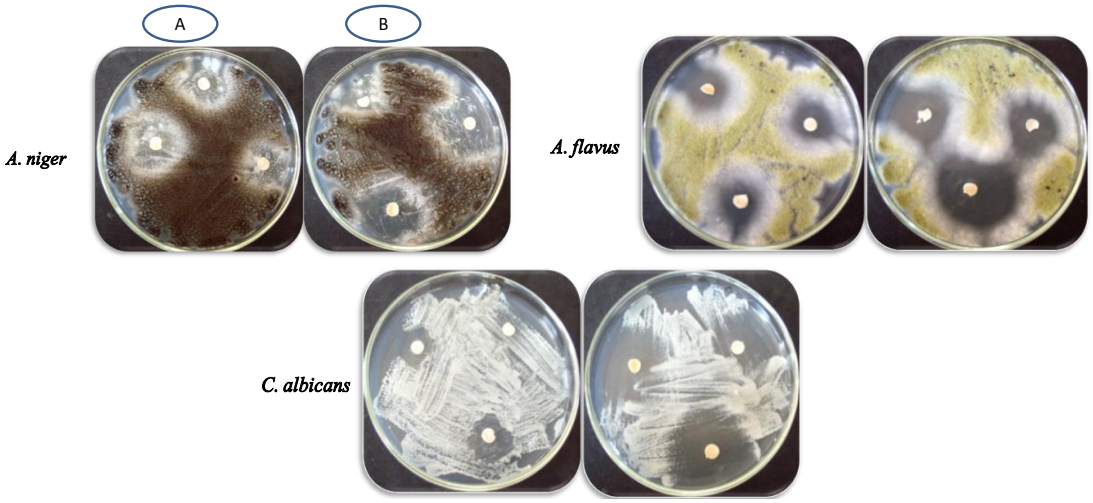

Figure 5. Inhibition of fungal growth by disc diffusion assay method using 100, 200 and 300 µg ml of *L. fermentum* lysate prepared from A) MRS-NH_4Cl with CSL, B) MRS-yeast extract with CSL.

Table 5. Antifungal bioactivity of the fractified part of lysate against *A. niger, A. flavus* and *C. albicans* studied by measuring the diameter of inhibition zone (disc diffusion assay).

***L. fermentum* lysate (µg disc^{-1})**		**Mean diameter of inhibition zone (mm)***		
		A. niger	***A. flavus***	***C. albicans***
M.wt. over 30 KDa	62.25	1.8 ± 0.6	4.5 ± 0.7	3.3 ± 0.3
	125.00	1.9 ± 0.2	4.1 ± 0.1	3.5 ± 0.6
	250.00	1.9 ± 0.2	4.3 ± 0.4	3.8 ± 0.0
	350.00	2.0 ± 0.1	4.7 ± 0.2	5.0 ± 0.0
M.wt. lower than 30 KDa	62.25	1.3 ± 0.2	2.8 ± 0.4	2.3 ± 0.3
	125.00	1.5 ± 0.3	3.7 ± 0.3	3.0 ± 0.2
	250.00	1.8 ± 0.3	4.1 ± 0.1	3.8 ± 0.3
	350.00	1.9 ± 0.1	4.3 ± 0.1	4.2 ± 0.3

*Mean diameter of inhibition zone using fraction of bacterial lysate obtained by ultra filtration using Vivaspin® 30 KDa cut off membrane.

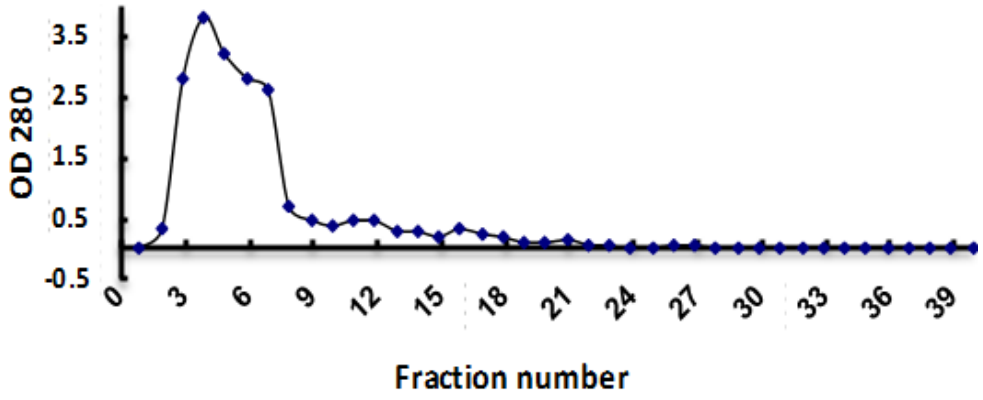

Figure 6. Elution profile of *L. fermentum* lysate on DEAE-cellulose represented five peaks from FI to FV: FI (3-5), FII (6-8), FIII (9-12), FIV (15-17), FV (20-22). OD, optical density.

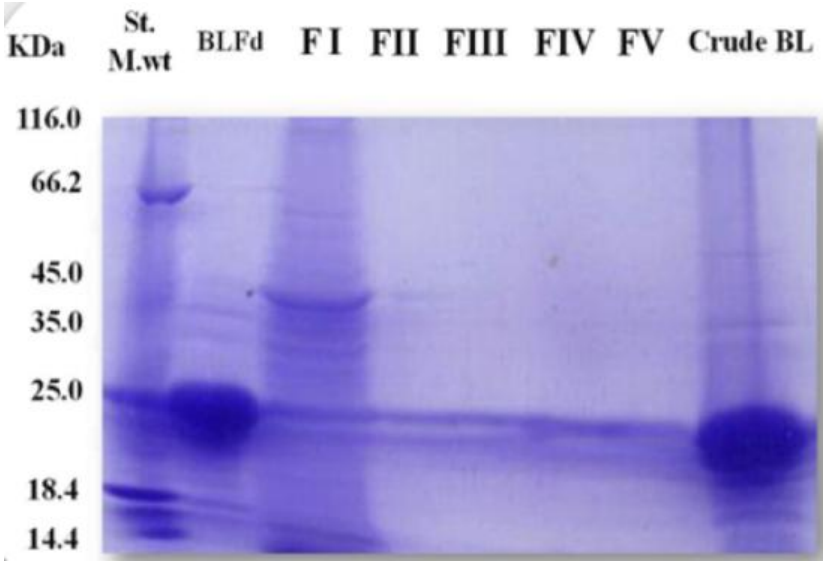

Figure 7. SDS-PAGE showing the protein profile of a purified (FI, FII, FIII, FIV, FV), freeze dried bacterial lysate (BLFd) and crude (BL) lysate obtained from *L. fermentum* previously cultivated under optimum conditions for lysate production.

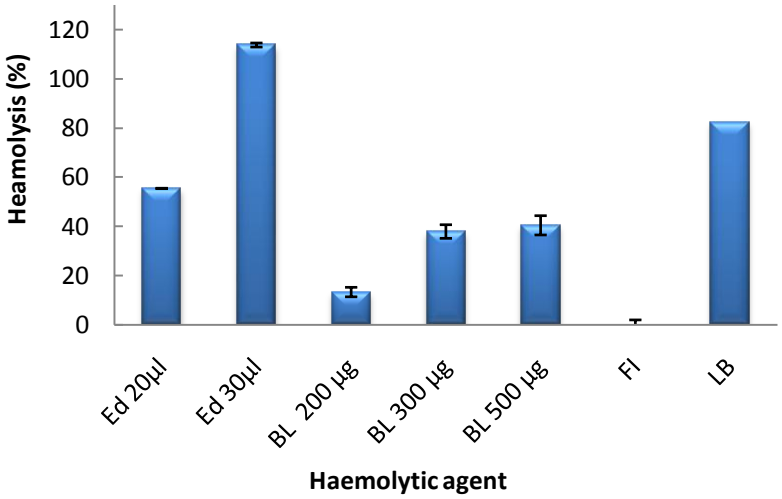

Figure 8. Cytotoxicity of lysate obtained from *L. fermentum* previously cultivated under optimum conditions investigated by lysis of healthy human erythrocytes using the haemolytic assay. Ed, Exoderil drug; BL, total bacterial lysate; FI; peak 1, LB, lysis buffer.

up to a test concentration of 1250 µg/ml to human erythrocytes. These results gave evidence that the partially purified *L. fermentum* lysate is totally safe for producing potent antifungal compound.

Conclusion

There are few studies in the emerging field of bacterial lysates which provides evidence that the lysate of the *L. fermentum* which was naturally occurring probiotic strains in human dairy food had a potential and promising antifungal activity. As compared to chemotherapeutic antifungal drugs, it had extremely very low toxicity to human cells, therefore, it could be an important source of biologically active and less toxic compounds useful for developing new better antifungal preparations.

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