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ORGANIC ACIDS PRODUCTION AND ANTAGONISTIC EFFECT OF SOME STRAINS OF PROBIOTICS

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ABSTRACT

Organic acids production and antimicrobial activities of *Lactobacillus acidophilus* KF724889, *Lactobacillus casei* KF724890 and *Streptococcus thermophilus* KF724886, KF724887 and KF724888 strains, which isolated from dairy products as probiotics were screened. Eleven organic acids were detected in the different LAB filtrates, acetic, ascorbic, citric, formic, oxalic, malic, maleic, lactic, propionic, butyric and succinic acids. lactic and acetic acids were the major acids produced by the five strains. *Lactobacillus* in general and *L. acidophilus* in especial were the most active in acids production. Both *L. acidophilus* and *L. casei* produced the highest quantity of lactic acid, being 3257.4 and 2447.75 mg/100 ml, respectively, while *Str. thermophilus* strains KF724886, KF724887 and KF724888 produced 1613.36, 1964.52 and 2031.131 mg/100ml, respectively. Formic acid did not produce by *Str. thermophilus* KF724886. Supernatants obtained from the tested bacteria exhibited varying degrees of inhibitory effect against indicators pathogenic bacteria and yeast. All the tested bacteria have antagonistic effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Protus vulgaris*. Among the isolates, *L. acidophilus* was the most effective strain for inhibiting pathogens growth with strong inhibitory effect against *P. aeruginosa*, *L. monocytogenes* and *Candida albicans*. Only *L. acidophilus* and *Str. thermophilus* KF724888 caused inhibitory effect against *Bacillus cereus*, *L. monocytogenes* and *C. albicans*. *L. casei*, *Str. thermophilus* KF724886 and *Str. thermophilus* KF724887 failed in inhibiting growth of *E. coli*, *B. cereus*, *L. monocytogenes* and *C. albicans*. *L. acidophilus* inhibited growth of *E. coli*, while *Str. thermophilus* KF724888 failed. The results showed that all cell free supernatants (CFSs) of LAB cultures have ability to inhibit all the tested food-contaminating fungi. Based on dry weight measurements of fungal biomass, CFSs of *L. acidophilus* showed high antifungal activity against the tested fungi. CFSs of *Str. thermophilus* KF724887 strain showed strong inhibition percentages against growth of *A. niger*, *T. harzianum*, *P. chrysogenum* and *A. pullulans* (56.63, 54.84, 52.92 and 38.88%, respectively). The study revealed that lactic acid bacteria isolated from Egyptian fermented milk, are capable of producing organic acids and antimicrobial substances which have antagonistic effect on pathogenic organisms, thus, may be promising sources of preservative that may in future be applied to food.

Keywords: Lactic acid bacteria (LAB), Antifungal and antibacterial activity, Pathogens, Probiotic, Organic acids

INTRODUCTION

Lactic acid bacteria (LAB) are the most important group of microorganisms used in food fermentations; they contribute to the fast and texture of fermented products and inhibit food spoilage and pathogenic

bacteria by producing organic acids and antimicrobial substances (Phillip *et al*., 2012). Amongst these substances, the production of lactic acid and acetic acid is obviously the most important. However, certain strains of LAB are further known to produce bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity (De Vuyst *et al*., 2004). Besides the production of bacteriocins, some LAB are able to synthesize other antimicrobial peptides that may also contribute to food preservation and safety (De Vuyst and Leroy, 2007). Mechanisms of probiotic action described to date include adhesion to the intestinal-lumen interface; competition with pathogens for receptor binding, nutrients and colonization; enhancement of mucosal barrier function; promotion of innate and adaptive immune responses; elaboration of bacteriocins; and modulation of cell kinetics, with further mechanisms of action likely to be identified (Howarth, 2010; Lebeer *et al*.,2010; Bassyouni *et al*., 2012).

Lactic acid bacteria isolated from dairy products have received increased attention as a potential food preservative due to their antagonistic activity against many food-borne pathogens such as *Listeria monocytogenes* (Jamuna and Jeevaratnam, 2004; Al Askari *et al*.,2012) and other pathogens, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Bacillus subtilis*, *Bacillus cereus*, or *Escherichia coli* (Aslam and Qazi, 2010; Al Askari *et al*., 2012; Ali *et al*. 2013). In addition, LAB have been found to show antifungal activity. In this respect, *Lactobacillus casei* and *Lactobacillus acidophilus* possess good antifungal properties and are able to protect immunocompromised people from opportunistic infections and adhesion by *Candida albicans* or other *Candida* species as described by many researchers in their works (Anokhina *et al*., 2007). A strong antifungal activity of cell-free supernatants from *L. casei* subsp. *rhamnosus* and *L. acidophilus* metabolites against growth of *Trichoderma viride*, *Aspergillus niger, Penicillium chrysogenum, Aureobasidium pullulans* was reported by (Yang and Clausen, 2005). Lactobacilli isolates including *L. cruvatus*, *L. lactis* subsp*. lactis*, *L. casei*, *L. pentosus*, and *L. sakei* were reported to have a wide range of antifungal activity against *Aspergillus fumigatus, A. flavus, Fusarium moniliforme*, *Penicillium commune*, and *Rhizopus oryzae* (Kim, 2005).

The aim of this study was to screen organic acids production and the antibacterial and antifungal activity of local selected lactic acid bacteria, *Lactobacillus acidophilus* KF724889, *Lactobacillus casei* KF724890 and *Streptococcus thermophilus* strains KF724886, KF724887 and KF724888, isolated from Egyptian fermented dairy milk products, against several of indicators food contaminating and spoilage microorganisms.

Materials and Methods

Lactic acid bacterial strains:

The five lactic acid bacteria (LAB) used are *Lactobacillus acidophilus* KF724889, *Lactobacillus casei* KF724890 and *Streptococcus thermophilus* strains (KF724886, KF724887 and KF724888). All bacteria were isolated from Egyptian fermented dairy milk and identified (Data not shown).

Food contaminating microorganisms:

Seven pathogenic bacteria and 5 pathogenic fungi were used for testing antimicrobial activity of LAB isolates. Strains included:

I. *Staphylococcus aureus* ATCC® 33591, *Pseudomonas aeruginosa* ATCC® 19429, *Escherichia coli* ATCC® 13706 [obtained from the American Type Culture Collections (ATCC) USA.],
II. *Listeria monocytogenes* [obtained from Animal Health Research Institute (AHRI) Agriculture Research Center, Giza, Egypt],
III. *Proteus vulgaris*, *Shigella* sp., *Bacillus cereus* and *Candida albicans* [obtained from city of Scientific Research and Technology Applications, Arid lands Cultivation Research Institute (ALCRI)].
IV. *Trichoderma harzianum*, *Penicillium chrysogenum, Aspergillus niger* and *Aurobasidium pullulans* were kindly supplied by the Plant Pathology Research Institute (PPRI) Agricultural Research Center, Giza, Egypt.

Culture media:

The following media were used for growing the used strains: *L. monocytogenes* and *B. cereus* strains were grown onto Trypticase™ Soy Agar Yeast Extract (TSA-YE) (Atlas, 1995).

- *E. coli*, *Stap. aureus* and *P. aeruginosa* were grown on Enriched Nutrient Agar (ENA) (Atlas, 1995).
- *P. vulgaris* and *Shigella* sp. were grown on Deoxycholate Agar. *Candida albicans* was grown onto Sabouraud dextrose agar (SDA) (Atlas, 1995),
- The fungal strains *T. harzianum*, *P. chrysogenum, A. niger* and *A. pullulans* were grown on Potato dextrose (PD) broth (Oxoid®, Inggris) sebanyak.
- The strains *L. acidophilus*, *L. casei*, *S. thermophilus* strains KF724886, KF724887 and KF724888 were grown on Man Rogosa Sharpe broth (MRS) (MERCK, 1996-1997) or onto MRS plates [(MRS supplemented with 1.5% (w/v) agar)]. Afterwards, strains were grown in the condition described for the virulence assays.

HPLC analysis of organic acids produced by the lactic acid bacterial strains:

Organic acids produced by lactic acid bacterial strains were determined by a HPLC according to the method by Zbigniew *et al*., 1991. Organic acid standard from Sigma Co. including acetic acid, ascorbic acid, butyric acid, citric acid, formic acid, succinic acid., lactic acid, malic acid, maleic acid, oxalic acid and propionic acid were used.

Efficacy of LAB for inhibition pathogenic bacteria:

Antibacterial activities of LAB strains against some food-borne pathogens and spoilage bacteria were determined using agar diffusion technique described by Herreros *et al*. (2005).

Extraction of cell-free supernatants (CFS):

Cultures of LAB were propagated in 10% sterilized Skim milk. The active cultures were used to inoculate individual 100 ml Erlenmeyer flasks containing 50ml MRS broth at level of 2% (v/v). The inoculated media were incubated at 37°C for 48 hrs according to Khedkar *et al.* (1990) and were centrifuged at 6000 rpm for 15 min. The clear supernatant was divided into two portions, one was sterilized by passing through sterile 0.45µm syringe

filter for obtaining cell free filtrate (CFF), and the second was heated at 121°C for 20min and designated as heated cell-free filtrate (HCFF). The antimicrobial activity of the two filtrates of each culture was studied.

Amount of 20 ml of appropriate Agar medium of each pathogen at 45°C were vigorously mixed with 500µl of an overnight culture of the indicator bacterial and yeast strains and poured into a 9cm diam. Petri dish. Wells with a 7mm diameter were made in the agar layer and 100 µl of cell-free supernatants were placed in each well. The Petri-dishes were kept in the refrigerator for 2h for diffusion then incubated at 37°C for 24 hrs before examination for zones of inhibition.

Efficacy of LAB for inhibition of pathogenic fungi:

One ml aliquots of supernatant described before was placed in 100 ml flasks containing 20ml potato dextrose broth and incubated in triplicate with actively growing culture of each test fungus. Stationary cultures were incubated at 27°C for 7 days. At the end of incubation period, the mycelium was filtered and washed several times with distilled water on preweighed Whatman 1 filter paper (Whatman International Maidstone England) then dried in an oven at 80°C till constant weight. The antifungal activities of the cell-free tested bacteria were expressed as percentage inhibition of mycelial growth in comparison with the untreated medium, according to the formula: MGI%= (A-B)/A×100

Where, MGI (%) is the percent of mycelial growth inhibition, A: dry weight of the pathogen when growing without cell-free extract and B: dry weight of the pathogen for each cell-free filtrate of LAB.

RESULTS AND DISCUSSION

HPLC analysis of organic acids

Data in Table (1) showed that eleven organic acid (acetic, ascorbic, citric, formic, oxalic, malic, maleic, lactic, propionic, butyric and succinic acids) were detected in the different filtrates. Results indicated that, lactic and acetic acids were the major acids produced by the five strains. Results are in agreement with those reported by (Liptáková *et al*., 2007 and Zalán *et al*., 2010), who found that lactic and acetic acid are regarded as the main organic acids produced by lactobacilli. *Lactobacillus* in general and *L. acidophilus* in especial were the most active in acids production. *L. acidophilus* and *L. casei* produced the highest quantity of lactic acid, being 3257.4 and 2447.75 mg/100ml, respectively, while *Str. thermophilus* strains KF724886, KF724887 and KF724888 produced 1613.36, 1964.52 and 2031.131 mg/100ml, respectively. Lactic acid exerts strong antagonism activity against many microorganisms, including food spoilage organisms and pathogens (Amenu, 2013). At low pH, the lactic acid is in undissociated form, and it is toxic to many bacteria, fungi and yeast. Acetic acid came in the second order, the quantities of acetic acid produced by *L. acidophilus*, *L. casei* and *Str. thermophilus* strains KF724886, KF724887 and KF724888 were 2469, 1510.66, 960.85, 1044.85 and 823.12 mg/100 ml, respectively. Acetic and propionic acids produced by LAB may interact with cell membranes, and

caused intracellular acidification and protein denaturation (Urga, 1992). They are more antimicrobialy effective than lactic acid due to their higher pKa values (Lactic acid 3.08, acetic acid 4.75 and propionic acid 4.87) and higher percent of undissociated acids than lactic acid at a given pH (Earnshaw, 1992). Maleic and ascorbic acids were detected in lower quantities compared with the other organic acids. Hladíková *et al*. (2012) reported similar results; they produced lactic acid, acetic and succinic acids from some lactic acid strains. Lactic acid concentration was 2.877-15.2829 g/L while acetic and succinic acids concentration were 0.696-0.954 g/L and 0.187-0.421 g/L, respectively. The other organic acids recorded moderate values. On the other hand, Formic acid was not detected in the filtrate of *Str. thermophilus* KF724886.

The efficiency of produced organic acids originates from their effect on the bacterial cytoplasmic membrane, where they affect the membrane potential and therefore inhibit the active transport through the membrane (Caplice and Fitzgerald, 1999).

Table1. HPLC analysis of organic acids (mg/100 ml) produced by LAB strains.

Organic acid	*L. acidophilus*	*L. casei*	*Str. thermophilus strains*		
			KF724886	KF724887	KF724888
Oxalic	50.17	37.86	4.06	3.86	3.8
Citric	253.23	151.99	90.27	102.58	84.74
Lactic	3257.4	2447.75	1613.36	1964.52	2031.131
Ascorbic	2.75	4.79	5.05	1.97	3.88
Formic	33.48	28.98	-	25.48	4.11
Succinic	77.34	40.45	33.43	28.3	43.75
Malic	51.04	37.91	27.31	27.38	5.08
Propionic	35.85	91.78	101.36	49.54	24.61
Butyric	113.09	159.16	108.02	20.15	111.21
Acetic	2469	1510.66	960.85	1044.85	823.12
Maleic	0.1	0.09	0.07	0.05	0.04

Efficacy of LAB for inhibition pathogenic bacteria:

Data presented in Table (2) showed the inhibitory activities caused by LAB strains against pathogens. Among the isolates, *L. acidophilus* was the most effective strain for inhibiting all pathogenic organisms; it exerted strong inhibitory activities against *P. aeruginosa, L. monocytogenes* and *C. albicans.* Only *L. acidophilus* and *Str. thermophilus* KF724888 were able to inhibit growth of *Candida albicans*, *L. monocytogenes* and *B. cereus.* The supernatants of *L. acidophilus* and *Str. thermophilus* KF724888 did not lost their anti-listerial activity by autoclaving. In contrast, Yang *et al*. (2012) found that *Str. thermophilus* and *L. casei* supernatants totally lost their anti-listerial activity subsequent to exposure to 121°C for 15 min. Coconnier *et al*. (1997) reported that *L. acidophilus* was able to kill intracellular *Salmonella typhimurium* in human intestinal Caco-2 cell culture model. Aslim *et al*. (2005) found that all lactobacilli isolated from Turkish dairy products have

antimicrobial activity against *Staphylococcus aureus*, *E. coli* and *Yersinia entrocolitica*.

Concerning*, L. casei*, *Str. thermophilus* KF724886 and KF724887, they were not able to inhibit growth of *E. coli*, *B. cereus*, *L. monocytogenes* and *C. albicans.* In addition, *Str. thermophilus* KF724888 failed to inhibit *E. coli* growth. Results clearly indicated that all the LAB strains have antibacterial effect against *Staph. aureus, P. aeruginosa*, *Proteus vulgaris* and *Shigella* sp. The data demonstrated that the heat sterilized cell-free extracts nearly have the same inhibitory effect of sterilized cell free extract that sterilized by filtration. Data are in agreement with results of Moghadamd *et al*. (2006) who reported that bacteriocins of *L. acidophilus* and *L. bulgaricus* were resistant to heating at 56, 80 and 100ºC for 10, 30 and 60 min; as well as were stable between pH 3 and 10. Similarly, Aslam and Qazi (2010) reported that cell free culture supernatants of *L. acidophilus* showed highest inhibitory activity against *E. coli* isolates and a strain of *Staphylococcus* sp. Also all LAB isolated from raw fruits and vegetables inhibited *E. coli* isolated from human sources. The same results were obtained by Sharpe (2009) who found that when *L. lactis* and *Ent*. *faecium* were inoculated onto fresh-cut salad, the growth of *Pseudomonas* sp., yeasts and total coliforms were remarkably reduced. Similarly, Yang *et al*. (2012) found that addition of LAB onto fresh-cut onions significantly inhibited the growth of *Pseudomonas* sp. during storage at 5°C.

The inhibitory activity of LAB is mainly due to accumulation of primary metabolites such as lactic acid and acetic acid, ethanol and carbon dioxide. LAB is also capable to produce antimicrobial compounds such as bacteriocins and other compounds with small molecular mass. The differences observed in inhibitory activity among LAB strains may be due to the biochemical properties of the strains used and chemical conditions of growth (Tannock, 2004). This may be also due to production of bacteriocins, which are peptides with bactericidal activity usually against strains of closely related species (Abriouel *et al*., 2012). Bacteriocins may enhance survival of LAB in complex ecological systems that focused on prevention of growth of harmful bacteria in the fermentation and preservation of dairy products. It is more interesting with respect to probiotics that individual strains may inhibit growth of or adhesion of pathogenic microorganisms by secreted products, and not merely an effect of acidic pH (Atta, 2009). Bacteriocins produced by yogurt Lactobacilli including *L. acidophilus* were reported to have inhibitory effect on growth of *E. coli* O157: H7 and it was deduced that the dilutions lower than minimum inhibitory dilution of each bacteriocin would inhibit the production of verotoxins (Moghadamd *et al*., 2006). An important property of probiotic strains is their antagonistic activity against pathogenic bacteria due to organic acids secretions. In this respect, propionic acid bacteria can produce antimicrobial substances capable of inhibiting the growth of pathogenic and spoilage microorganisms. Propionic acid, acetic acid, and diacetyl in addition to the antimicrobial peptides are included among these compounds (Havenaar, 1992). Abriouel *et al*. (2012), reported similar results on antagonistic activity of LAB. Yang *et al*. (2012) hypothesized that organic acids act on the cytoplasmic membrane by neutralizing its electrochemical

potential and increasing its permeability, thus leading to bacteriostasis and eventual death of susceptible bacteria.

The obtained results indicated that, only *L. acidophilus* and *Str. thermophilus* KF724888 caused inhibitory effect against the *B. cereus*, *Listeria monocytogenes* and *Candida albicans,* this indicate that the two LAB are capable of synthesizing inhibitive substances on *Candida albicans* and these substances according to (Jimenez-Diaz *et al*., 1993) may be mainly proteins. *C. albicans* resistance to antagonist activities exerted by some LAB strains has been described previously by Grimoud *et al*. (2010) and explained by yeast resistant to acidic conditions, oxidative stress or bacteriocins, which are among the main mechanisms involved in probiotic antibacterial activities. From results, a broader antibacterial activity could be obtained by combining the tested LAB tested strains that were the most effective against the differant pathogens.

Table 2. Antagonistic effect of cell free extracts of LAB against some pathogenic bacteria and *Candidia albicans* (size of inhibition zones mm).

Tested organism	LAB isolates									
	L. acidophilus		*L. casei*		*St. thermophilus* strains					
					KF724886		KF724887		KF724888	
	CFF[a]	HCFF[b]	CFF	HCFF	CFF	HCFF	CFF	HCFF	CFF	HCFF
Staphylococcus aureus	13.7	13	11.2	11	11	11	13	13	12.2	12
Pseudomonas aeruginosa	17	17.1	15	14.5	14.5	14	16.5	16	15.2	15
Proteus vulgaris	14.8	14.5	12.1	11.9	12.3	13	12.3	12	12	12
Escherichia coli	11.3	11.2	0	0	0	0	0	0	0	0
Shigella sp.	15	15.1	12	12	11.8	11.5	12.2	12	12.5	12.5
Bacillus cereus	12	12	0	0	0	0	0	0	11	11
Listeria monocytogenes	19	18.5	0	0	0	0	0	0	16.3	16.5
Candida albicans	17.5	17.5	0	0	0	0	0	0	19.5	20

[a]**CFF= Cell-free sterilized LAB filtrate**
[b]**HCFF= Heated cell-free sterilized LAB filtrate**

Efficacy of LAB for inhibition pathogenic fungi:

Data in Table (3) showed the antagonistic effect of cell-free supernatant of *L. acidophilus* and *L. casei* on the four tested fungi. Results indicated that cell-free supernatants of *L. acidophilus* showed high antifungal activity against the tested fungi. The percentages of reduction of supernatants were 25.33, 36.69, 36.45 and 23.87 against *Aspergillus niger*, *Trichoderma harzianum, Penicillum chrysogenum* and *Aureobasidium pullulans*, respectively. It could be noticed that *P. chrysogenum* was very sensitive to the supernatants of *L. acidophilus*; these results are similar to those reported by De Muynck *et al*. (2004).

Results presented in Table (4) showed the antagonistic effect of the three *Str. thermophilus* strains against the tested fungi. Results showed that the strains supernatants were capable of inhibiting fungi growth. Based on dry weight measurments of fungal biomass, *Str. thermophilus* KF274887 inhibited *A. niger*, *T. harzianum, P. chrysogenum* and *A. pullulans* percent in 56.63, 54.84, 52.92 and 38.88 growth, respectively. On the other hand, *Str.*

thermophilus KF274888 inhibited 22.29, 52.48, 30.05 and 26.19 percent in growth of *A. niger*, *T. harzianum, P. chrysogenum* and *A. pullulans*, respectively. In addition, results indicated that *Str. thermophilus* KF274886 recorded 20.86, 14.15, 15.25 and 17.37 percent reduction in growth of *A. niger*, *T. harzianum, P. chrysogenum* and *A. pullulans*, respectively.

These results are similar to those reported by De Muynck *et al*. (2004), they reported that *L. acidophilus* exudate showed to be excellent producers of antifungal metabolites. Furthermore, these metabolites were heat stable, as they remained active after a pasteurization process. Yang and Clausen (2005) found that cell-free supernatants from *L. casei* subsp. *rhamnosus* and *L. acidophilus* inhibited 95-100% growth of three mould fungi and one strain fungus associated with wood-based building materials. Results of heated cell-free supernatants showed that the autoclaving reduced antagonistic effect of the supernatants.

The obtained results showed clearly that lactic acid bacteria produce substances, which have ability to inhibit or prevent the growth of food-contaminating fungi. Many reports have suggested that antifungal activity is a combination of organic acids such as lactic, acetic, and phenyllactic acid (Yang and Clausen 2005; Hladíková *et al*., 2012) or bacteriocins (Mortvedt *et al*., 1991) and low molecular weight antimicrobial agents and peptides (Strom *et al*., 2002). El Sanhoty (2008) suggested that the antifungal effect of LAB could not simply the result of low pH, but is most probably due to the formation and secretion of pH dependent antifungal metabolites.

Finally, results showed that the isolated LAB strains are able to produce organic acids and to inhibit the growth of some pathogenic bacterial and fungal strains. The thermal stability of the supernatants of bacteriocinogenic LAB isolates may constitute an advantage for potential use as bioreservatives in combination with thermal processing in order to preserve food products.

Table 3:Effect of supernatants of *L. acidophilus* and *L. casei* (1 ml/20ml) on growth of the tested fungi

Tested fungi	*L. acidophilus*					*L. casei*			
	Control	CFF[a]		HCFF[b]		CFF		HCFF	
		Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction
Aspergillus niger	0.1879	0.1403	25.33	0.1487	20.83	0.1632	13.15	0.1657	11.78
Trichoderma harzianum	0.2622	0.166	36.69	0.1906	27.33	0.2405	8.28	0.2445	6.75
Penicillium chrysogenum	0.1128	0.0717	36.45	0.0758	32.85	0.1034	8.33	0.1054	6.59
Aureobasidium pullulans	0.1163	0.0885	23.87	0.0951	18.27	0.0897	22.87	0.0927	20.29

CFF[a] = Supernatantes sterilized by filtration
HCFF[b]= Supernatants sterilized by autoclaving

Table 4. Effect of supernatants of *Str. thermophilus* strains (1 ml/20ml) on growth of the tested fungi

Tested fungi	Control	*Str. thermophilus* strains											
		KF724886				KF724887				KF724888			
		CFF[a]		HCFF[b]		CFF		HCFF		CFF		HCFF	
		Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction	Dry weight (g)	% reduction
A. niger	0.19	0.15	20.86	0.15	18.5	0.08	56.63	0.1	47.04	0.15	22.29	0.15	18.51
T. harzianum	0.26	0.23	14.15	0.23	10.95	0.12	54.84	0.13	50.8	0.13	52.48	0.13	48.93
P. chrysogenum	0.11	0.1	15.25	0.1	11.32	0.05	52.92	0.06	45.66	0.08	30.05	0.08	28.1
Aureobasidium pullulans	0.12	0.1	17.37	0.1	14.02	0.07	38.88	0.07	35.74	0.09	26.19	0.09	22.54

CFF[a] = Supernatantes sterilized by filtration
HCFF[b]= Supernatants sterilized by autoclaving

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انتاج الأحماض العضوية والتأثير التضادي لبعض سلالات البكتريا العلاجية

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****قسم الميكروبيولوجى-معهد بحوث الأراضي والمياه والبيئة-مركز البحوث الزراعية-الجيزة-مصر**

استخدمت في هذه الدراسة خمس عزلات من بكتريا حامض اللاكتيك معزولة من منتجات الألبان كبكتريا علاجية. حيث درست قدرتها على إنتاج الأحماض العضوية والنشاطات المضادة للميكروبات. وجد أحد عشر حامضا عضويا في الرواشح المختلفة للبكتريا المعزولة وتضم أحماض: الخليك، الاسكوربيك، الستريك، الفورميك، الأكساليك، الماليك، المالنيك، اللاكتيك، والبروبيونيك، البيوتريك والسكسينيك. وقد مثل اللاكتيك والخليك الأحماض الرئيسية التي تنتجها السلالات الخمس. وبصفة عامة فقد أظهر جنس *Lactobacillus* خاصة بكتريا *L. acidophilus* نشاطا مرتفعا في إنتاج الأحماض. كما كانت بكتريا *L. acidophilus* و *L. casei* هي الأكثر انتاجا لحامض اللاكتيك حيث وصل الى ٣٢٥٧.٤ و ٢٤٤٧.٧٥ ملليجرام/١٠٠ مل، على التوالي، بينما أنتجت سلالات بكتريا *Str. thermophilus* KF274886، KF274887 وKF274888 الحمض بتركيزات ١٦١٣.٣٦ و ١٩٦٤.٥٢ و٢٠٣١.١٣١ ملليجرام/١٠٠مل، على التوالي. هذا ولم يكن لبكتريا *Str. thermophilus* KF724886 قدرة على انتاج حامض الفورميك. وعند دراسة التأثير التضادي للرواشح الخلوية (سواء المعقمة بالفلتر أو حراريا) للعزلات في مقاومة المسببات المرضية البكتيرية، أظهرت النتائج أن جميع رواشح السلالات الخمس لها قدرات تثبيطيه متنوعة ضد جميع سلالات المسببات المرضية البكتيرية والخمائر المستخدمة محل الدراسة. حيث كان لجميع العزلات تأثير تضادي لبكتريا *Staphylococcus aureus*، *Pseudomonas aeruginosa*، *Protus vulgaris* و*Listeria monocytogenes*. وكانت بكتريا *L. acidophilus* هي الأكثر كفاءة في تثبيط المسببات المرضية *P. aeruginosa*، *L. monocytogenes* و*Candida albicans*. بينما انفردت عزلتا *L. acidophilus* و KF724888 *Str. thermophilus* بإحداث تأثير تثبيطي ضد ميكروبات *B. cereus*، *Listeria monocytogenes* و *C. albicans*. على النقيض، لم يكن لعزلات بكتريا *L. casei*، KF724886 *Str. thermophilus* و KF724887 *Str. thermophilus* أي تأثير تثبيطي على نمو ميكروبات *Escherichia coli*، *Bacillus cereus*، *L. monocytogenes* و *C. albicans*. كما كان لبكتريا *L. acidophilus* تأثير تثبيطي قوى في إيقاف نمو بكتريا *E. coli*، بينما لم يلاحظ لعزلة KF724888 *Str. thermophilus* أي تأثير. على هذا وعند دراسة التأثير التثبيطى على بعض الفطريات الملوثة للمواد الغذائية، أظهر الراشح الخلوي لبكتريا *L. acidophilus* تأثير تثبيطيا مرتفعا لنمو جميع الفطريات محل الدراسة. كما أظهر الراشح الخلوي لبكتريا KF724887 *Str. thermophilus* تأثير تثبيطي قوى ضد فطريات *Aspergillus niger*، *Trichoderma harzianum*، *Penicillium chrysogenum* و*Aureobasidium pullulans* بنسب (٥٦.٦٣، ٥٤.٨٤، ٥٢.٩٢ و٣٨.٨٨٪، على التوالي) مقارنة بالكونترول. علية فان السلالات المعزولة أظهرت قدرة على إنتاج الأحماض العضوية والمواد المثبطة للميكروبات الممرضة وبالتالي، فهي تعتبر أحد مصادر المواد الحافظة الواعدة التي قد يتم تطبيقها في المستقبل في صناعة الغذاء وكذلك استخدامها كبكتريا علاجية.

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