

activity of Lactobacillus acidophilus against Klebsiella pneumonia

isolated from hospital-acquired infections by using optical density assay

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Determining the effect of various physicochemical factors on inhibition activity of Lactobacillus acidophilus against Klebsiella pneumonia isolated from hospital-acquired infections by using optical density assay

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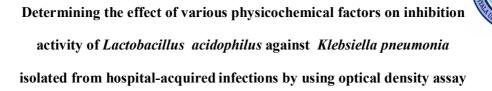
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Abstract

This study aims to determine the effect of using Lactobacillus acidophilus in various physicochemical factors to inhibit the growth of Klebsiella pneumoniae isolated from patients of hospital-acquired infections by using optical density technique. The result revealed that different factors like pH, incubation temperature degree, incubation periods and concentrations, seemed to affect on inhibition activity of L. acidophilus. Maximum activity was noted at pH 3, temperature 45°C, incubation for 48 hr and three fold concentrations which clarified by values O.D reached to 0.111, 0.775, 0.660 and 0.040 respectively whereas the lowest effect appeared after the use of pH 9, temperature 15°C, incubation for 24 hr and one fold concentrations where the inhibition values reached to 1.899, 1.180, 1.161 and 1.411respectively The results ensured that L. acidophilus sufficiency inhibited growth of K. pneumoniae with promising encourage to use the L.acidophilus as biotherapeutic agents against K. pneumoniae infections.

Key words: Klebsiella pneumonia, Lactobacillus acidophilus



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Jameel hadi jiyad

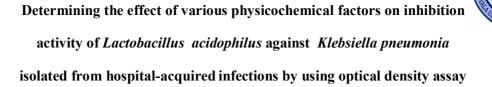
تحديد تأثير مختلف العوامل الفيزيوكيميائية على الفعالية التثبيطية لـ Lactobacillus معزولة من مرضى الإصابات المكتسبة Klebsiella pneumonia معزولة من مرضى الإصابات المكتسبة من المستشفيات بواسطة استخدام تقنية قياس الكثافة الضوئية

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الخلاصة

هدفت الدراسة إلى تقدير فعالية استخدام Lactobacillus acidophilus العوامل الغيزيوكيميائية لتثبيط نمو هدفت الدراسة إلى تقدير فعالية استخدام تقنية قياس المكتسبة من المستشفيات بوساطة استخدام تقنية قياس الكثافة الضوئية وأوضحت النتائج بأن مختلف العوامل مثل درجة حرارة الحضن، فترة الحضن، التركيز و الاس الهيدروجيني، تؤثر على القدرة التثبيطية لـ L. acidophilus اذ لوحظت أعلى قدرة تثبيطية عند الأس الهيدروجيني و،درجة حرارة 45 مئوية، فترة حضن لمدة 48 ساعة والتركيز لثلاث مرات وهذا ما يتضح من خلال القيم التثبيطية التي تصل 1.411 مئوية، مضن لمدة 24 ساعة والتركيز مرة واحدة اذ انخفضت القيم التثبيطية لتصل إلى 1.411 وهذا مئوية ،حضن لمدة 24 ساعة والتركيز مرة واحدة اذ انخفضت القيم التثبيطية لتصل إلى 1.411 وبمختلف الظروف كمثبط ضد نمو L. acidophilus مما يشجع على استخدام هذه العصيات كعلاجات حيوية ضد هذه البكتريا الظروف كمثبط ضد نمو 48 pneumoniae ما يشجع على استخدام هذه العصيات كعلاجات حيوية ضد هذه البكتريا الضارة

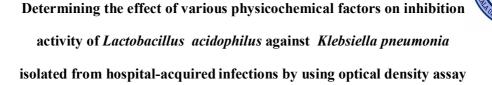


Bushra J . Mohamed

Jameel hadi jiyad

Introduction

The genus Klebsiella is generally classified into seven species and subspecies, some of them are regarded as "environmental" isolates were also isolated from human clinical specimens and animals (1,2). Pathogenic Klebsiella strains belonging to K. pneumoniae and K. oxytoca are known to cause hospital-acquired infections such as septicemia, pneumonia, and urinary tract and soft tissue infections (3). As a cause of nosocomial infection due to gram-negative bacteria, Klebsiella ranks next to Escherichia coli, accounting for 8% of endemic hospital infections and 3% of epidemic outbreaks (4). Meanwhile, the appearance of multiresistant strains among clinical Klebsiella isolates, especially those producing extended-spectrum betalactamases, which show resistance to extended-spectrum cephalosporins, has been increasing over the past several years., thus complicating the therapy of this infection (5,6) Therefore, Klebsiella nosocomial-infection surveillance is necessary to use the alternative treatment by using biotherapeutic agents in the prevention and control of Klebsiella infection (7)Several studies have assessed the potential of Lactobacilli particularly Lactobacillus acidophilus in prevention or treatment of certain infections by producing antimicrobial substances. addition, Lactobacill or probiotic therapy is considered as "natural" and without side effects in contrast with conventional pharmaceutical treatments. The purpose of this study is to determine the effectiveness of using lactobacillus acidophilus to inhibit the growth of K. pneumoniae isolated from patients of hospital-acquired infections by using optical density technique



Bushra J . Mohamed Jan

Jameel hadi jiyad

Methods Materials and

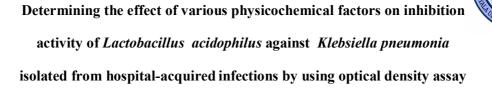
Isolation and identification of L. acidophilus

A portion of 0.1 ml of yogurt samples were drawn aseptically to a previously sterilized test tubes containing 9.9 ml Manns-Regoz and Sharpe (MRS) broth, then incubated at 37 °C for 24 hr. under anaerobic conditions. After incubation, serial dilutions were made, and 0.1 ml from last dilution was streaked on the surface of MRS agar in Petri dishes, and then incubated for 24 hr. at 37 °C under anaerobic conditions (anaerobic jar). After that, part of the growth was transferred to MRS broth in a test tube and incubated under anaerobic condition.

The suspected *L. acidophilus* isolates were identified by microscopic examination, biochemical tests and carbohydrate fermentation test according to (8,9).

Isolation and identification of K.pneumonia.

K.pneumonia isolated from hospitalized patients whose blood culture yielded *Klebsiella* and identified by using standard microbiology techniques. The isolates were culture in Brain Heart Infusion (BHI) broth(Difco, USA) and routine susceptibility testing of some antibacterial agents(Trimethoprim- Sulphamethoxazole 30μg, Azithromcin 15μg, Cefotaxim 30μg, Chloramphenicol 30μg, Ciprofloxacin 5μg Lincomycin 2μg, Gentamycin 10μg, Tetracycline 30μg, Ampicillin 10μg, and Amikacin 30μg from Oxoid - England) was performed by disk diffusion method on Mullor-Hinton agar(Difco- USA) described in The Bergey's Manual of Determinative Bacteriology(10).



Bushra J . Mohamed

Jameel hadi jiyad

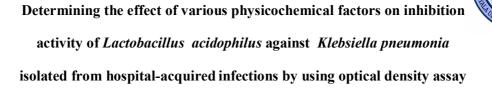
Performed L.acidophilus filtrate

L. acidophilus was inoculated in MRS – broth and incubated at 37^{0} C for 24 hrs in anaerobic condition. After incubation period, Cell – free supernatants were collected by centrifugation at 3000 rpm for 40 min. These supernatant was filtrated through Millipore filter unite (0.22 μ m) as described in (11).

Detection of antagonistic a activity of L. acidophilus against the K. pneumonia

Optical density(O.D), The measure of the amount of light absorbed by a suspension of bacterial cells with the use of spectrophotometer As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria is present. The amount of light scatter can be measured in a spectrophotometer.

In present research we were inoculated L.acidophilus filtrate with K.pneumonia culture as a ratio (1:1) then incubated for overnight at 37°C. Typically, when working with a particular type of cell, you would determine the optical density at a particular wavelength that correlates with the different phases of bacterial growth. Generally we will want to use cells that are in their mid-log phase of growth, thus we estamated the antagonistic activity in the approach of optical density assay(O.D. $_{600}$) nm.



Bushra J .Mohamed

Jameel hadi jiyad

Determining Bacterial Concentration

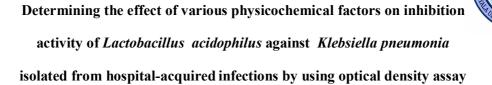
To evaluate O.D, the following procedure was done according to (12).

1- with sterile pipette tips Place a 500 µl sample of the blank (BHI broth) in a cuvette using sterile technique to avoid contamination of the sterile media stock and also to protect the cultures from contamination. Place the cuvette in spectrophotometer.

- 2-After the spectrophotometer has read the blank, remove the cuvette, replace it with a cuvette containing 500µl of the *K. pneumonia* culture (control) and let the spectrophotometer read it.
- 3. Pipet 500 µl of each sample into a labeled cuvette and allow the spectrophotometer read them.
- 4-Record the O.D's. of each read.

The effect of different *L. acidophilus* incubation temperature on antagonistic a activity

Tubes containing 10 ml MRS broth were inoculated with 1% of L. acidophilus culture then incubated at 15 °C, 37 °C and 45 °C for 24 hr. After incubation, L. acidophilus filtrate was prepared and inoculated with K. pneumonia then incubated at 37 °C for 24 hr. then the antagonistic activity was estimated for each temperature. according to (13)



Bushra J . Mohamed

Jameel hadi jiyad

The effect of different *L. acidophilus* concentrations on antagonistic a activity

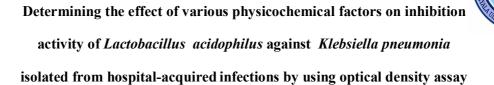
The filtrates of *L. acidophilus* which has equal volume 200 ml were concentrated by freeze – dryer to one fold (100 ml), two fold (50 ml), three fold (25 ml). Each concentrations was inoculated with *K. pneumonia* then incubated at 37 °C for 24 hr. then antagonistic activity was estimated as described in (14).

The effect of different incubation periods of *L. acidophilus* on antagonistic a activity

Tubes containing 10 ml MRS broth were inoculated with 1% of *L. acidophilus* culture then incubated at 37 °C for different incubation periods (24, 48 and 72) hr. After incubation, *Lactobacillus acidophilus* filtrate was prepare and inoculated with *K.pneumonia* then incubated at 37 °C for 24 hr. and the antagonistic activity was estimated for each period according to (15).

The effect of different pH value of *L. acidophilus* culture on antagonistic a activity.

Tubes containing 10 ml MRS broth were inoculated with 1% of *Lactobacillus acidophilus* culture, the pH of *Lactobacillus* culture was adjusted to 3, 6 and 9 respectively with 6 M HCl or 6M NaOH and autoclaved, then incubated at 37 °C for 24 hr. After incubation, *L. acidophilus* filtrate was prepare and inoculated with *K.pneumonia* then incubated at 37 °C for 24 hr. The antagonistic activity was estimated for each pH value of *L. acidophilus* culture as described in (16)



Bushra J . Mohamed

Jameel hadi jiyad

Results and discussion

The result showed that *K. pneumonia* in current investigate was associated with frequent adverse effects and resistance to antibiotics like Amikacin, Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamycin, Tetracycline, Azithromcin and was sensitive to Lincomycin, Cefotaxim and Trimethoprim-sulphamethoxazol, therefore alternative methods to control this bacteria are needed (17).

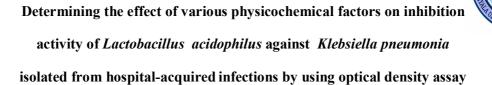
The effect of different Lactobacillus incubation temperature on antagonistic a activity

Our finding revealed that L.acidophilus was effective against K. pneumonia in different incubation temperature and had the best effect at 45 °C which reached to 0.775, while the less effect was observe when incubate L.acidophilus at 15 °C with inhibition values reached to 1.180, whereas the minimum values indicate less growth of K. pneumonia as shown in table (1).

Table 1 The effect of different L.acidophilus incubation temperature on antagonistic a activity

incubation	O.D values of growth of treated
temperature of	K. pneumonia
L.acidophilus	CUSITY COLLEGE
	1.525 K. pneumonia only(control)
15 °C	1.180
37 °C	0.856
45 °C	0.775

^{*}The less value indicate good inhibition activity



Bushra J . Mohamed Jameel hadi jiyad

Thus indicate the temperature degree 45 °C that optimum incubation temperature for produce the inhibition agents like bactriocine, and this result accordance with (18)as which showed that the optimum degree to produce bactriocine was 45 °C.

The effect of different L. acidophilus concentrations on antagonistic a

The optimum inhibitory effect shown during use three fold of L. acidophilus filtrate where the inhibition values reached to 0.040 and the lowest effect appear with one fold L. acidophilus filtrate when the inhibition values reached to 1.411, as shown in table (2).

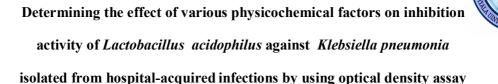
Table 2. The effect of different L.acidophilus concentrations on antagonistic a activity

L.acidophilus	
filtrate concentrations	O.D values of growth of treated <i>K. pneumonia</i>
1	1.525 K. pneumonia only(control)
One fold	1.411
two fold	0.760
three fold	0.040

^{*}The less value indicate good inhibition activity

Our result indicated that L.acidophilus filtrates concentrated to three fold have the highest inhibitory effect than the other folds because a variety of compounds with antimicrobial activity was concentrated therefore minimize the growth of K.pneumonia, an observation which come in accordance with other workers (19,20 and 21).

Vol: 8 No: 3, July 2012 129 ISSN: 2222-8373



Bushra J . Mohamed

Jameel hadi jiyad

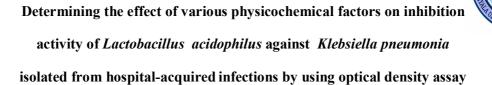
The effect of different incubation periods of *L.acidophilus* on antagonistic a activity

The target of this experiment was to test the ability of *L. acidophilus* for inhibition the growth of *K. pneumonia* with different incubation periods our result showed that incubation for 48 hr. hade high effect reached to 0.660 as shown in table (3). Similar observation were recorded by other investigators like (22, 23 and 24) who found that inhibitory effect of LAB increased after 48hr of incubation, that indicated to many antimicrobial substance are produced in this period than other periods thus may be due to that the inhibitory materials (acidophilin) are secreted outside the cells after increasing the incubation time causing decrease in the inhibitory effect so the amount of antibacterial substance in the period after 24 hr. not enough to reduce the growth of *K. pneumonia* and after 72 hr. the antimicrobial substance loss some of their inhibition activity.

Table 3 The effect of different incubation periods of *L. acidophilus* on antagonistic a activity

incubation periods of Lacidophilus	O.D values of growth of treated <i>K. pneumonia</i>
	1.525 K. pneumonia only(control)
24 hr.	1.161
48 hr.	0.660
72 hr.	0.960

^{*}The less value indicate good inhibition activity



Bushra J . Mohamed

Jameel hadi jiyad

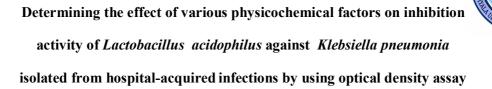
The effect of different pH value L. acidophilus culture on antagonistic a activity

Regarding pH the maximum inhibitory activity was observed at pH 3 which reached to 0.111 nm and minimum was observed at pH 9 which reached to 1.899, it was clear that the acidic pH was a better inhibitory effect than alkaloid and that confirmed by Aasen (25) who mention that acidic pH stimulated inhibitory effect against Gram positive (*Staph. aureus*, *Baccillus subtalis*) and Gram negative bacteria (*E.coli,klebsiella spp.,Proteus spp.*). Tagg *et al.* (26) reported that *L. acidophilus* bacteriocin are resistant to acidic pH more than basic pH. Similar results were obtained by other investigator such as (27) when found that best inhibitory effect was gained during used acidic pH and *L. acidophilus* bacteriocins were active at as low as pH3.

Table 4 The effect of different pH value of *L.acidophilus* culture on antagonistic a activity

pHof	
L.acidophilus	O.D values of growth of treated
culture	K. pneumonia
	Tr COD
	1.525 K. pneumonia only(control)
pH 3	0.111
p11 3	0.111
pH 6	1.656
pH 9	1.899

^{*}The less value indicate good inhibition activity



Bushra J . Mohamed Jameel hadi jiyad

Our outcomes showed that *L.acidophilus* have the high inhibitory effect on *Klebsiella pneumonia* and this may due to bacteriocin (acidophilus) production from *L. acidophilus* furthermore antimicrobial activities of *L.acidophilus* were very stable under a series of different conditions with promising inhibitory spectrum but this activity strongly dependent on pH, incubation periods, concentrations and incubation temperature as claimed by (28, 29)

From the results we can conclude that *L.acidophilus* could offer alternatives treatment and the studies should be aimed at opening new possibilities of bacteriocins synthesis to increase their production on an industrial scale.

Reference

- 1- Carter, J. S.; Bowden, F. J.; Bastian I; Myers, G. M.; Sriprakash, K. S. and Kemp, D. J. (1999). Phylogenetic evidence for reclassification of *Calymmatobacterium granulomatis* as *Klebsiella granulomatis* comb. nov. Int. J. Syst. Bacteriol. 49:1695-1670.
- 2-Monnet, D. and Freney, J. (1994). Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. J. Clin. Microbiol. 32:1121-1126.
- 3. Jonas, D.; Spitzmuller, B.; Daschner, F. D; Verhoef ,J. and S. Brisse. (2004). Discrimination of *Klebsiella pneumoniae* and *Klebsiella oxytoca* phylogenetic groups and other *Klebsiella* species by use of amplified fragment length polymorphism. Res. Microbiol. 155:17-23.
- 4. Podschun, R. and Ullmann, U. (1998). *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev. J. 11:589-603.
- 5. Cordery, R. J.;Roberts ,A.; Cooper,F.; Bellinghan ,C. H; S. J. G. and Shetty, N.(2011). Evaluation of risk factors for the acquisition of bloodstream infections with extended-

Vol: 8 No: 3, July 2012 132 ISSN: 2222-8373

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Bushra J . Mohamed Jameel hadi jiyad

spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species in the intensive care unit. Hosp. J.Infect. 68:108-115.

6- García San Miguel, L.; Cobo, J.; Valverde, A.; Coque, T. M.; Diz, S.; Grill, F. and Canton, R.(2007). Clinical variables associated with the isolation of *Klebsiella pneumoniae* expressing different extended-spectrum beta-lactamases. Clin. Microbiol. Infect.J. 13:532-538.

7-Nyirjesy ,P.; Weitz, M.V.; Grody, M..H.T. and Lorber, B.(1997). Over-the counter and alternative medicines in the treatment the chronic vaginal symptoms. Obstetrics and Gyncology .J.90:50-53.

8-Kandler, O. and Weiss, N. (1986).Genus *Lactobacillus* In: Bergey's Manual of Systematic Bacteriology.Eds by Sneathy, P.H.A.; Mair, N.S. and Holt, J.G., William and Wilkins Co. Baltimore.MD.Vol.2.1208-1234.

9-Atlas, R.M.; Parks, L.C. and Brown, A.E.(1995). Laboratory manual of experimental microbiology. 1st ed. Mosby-Year Book. Inc. U.S.A.

10- Holt, J. G.; Krieg, N. R.; Sneath, P. H. A. Staley, J. T. and Williams ,S. T. Ed W. R. Hensyl. (1994) .Genus *Klebsiella*,. in Bergey's Manual of Determinative Bacteriology. Ninth Edition. p. 461-465

11-AL-Obidy, N.N.M. (1997) effect of bacteriocin-producing lactic acid bacteria on some pathogenic bactria. M.Sc.thesis, College of Science. Mustansyria University.

12- Dolan, R. M. and Costerton, J. W. (2002). Clinical Microbiology Review. thired Edition. p. 351-355

15, 167

13-Vander Meulen, R.; Markas, L.; Verbruggh, R. and Vuyst, T. (2006). *In vitro* kinetic analysis of oligofructose consumption by bacteroides and *Bifidiobacterium spp*. Appl. Environ. Microbiol.J. 72: 1006-1012.

Vol: 8 No: 3, July 2012 133 ISSN: 2222-8373

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Bushra J . Mohamed Jameel hadi jiyad

- 14 -Velraeds, M. M.; van der Mei, H. C.; Reid,G. and Busscher H. J.. (1996). Physiochemical and biochemical characterization of biosurfactauts released by *Lactobacilltus* strains. Coll, Sud. B: Biointerfaces.J. 8:53-61.
- 15-Vignolo, G.M, de Kairuz, M.N, de Ruiz Holgado, A.A. and Oliver, G. (1995). Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by *Lactobacillus casei* CRL705 Appl Bacteriol . J. 78, 5-10.
- 16-Vignolo, G.M.; Suriani, F.; Holgado, A. and Oliver, G.(1993).

 Antibacterial-activity-of-*Lactobacillus*-strains-isolated-from-dryfermented sansnges. App. Bac. J. 75: 344-349.
- 17-Petti, S.; Tarsitani, G.; Simonetti, D and Arca ,A (2012). Antibacterial activity of yoghurt againts viridans streptococci in vitro. Arch. Oral Biol.J. 53: 985-990.
- 18. Rogelj, I.; Bogovic Matijasic, B.; Canzek Majhenic, A and Stojkovic, S
- (2002). The survival and persistence of *Lactobacillus acidophilus* LF221 in different ecosystems. Int J .Food Microbiol .76:83–91.
- 19-Šušković, J.; Kos, B.; Beganović, J.; Leboš Pavunc, A.; Habjanič, K.and Matošić, S. (2010). Antimicrobial activity The most important property of

probiotic and starter lactic acid bacteria. Food Technol. Biotechnol.J. 48(3): 296-307.

- 20-Todorov, S.D. and L.M.T. Dicks, 2004. Comparison of 2 methods for purification of plantaricin ST31, a bacteriocin produced by *Lactobacillus plantarum* ST31 Enzyme and Microbial. Technol.J. 36: 318-326.
- 21-AL- Dulaimy,J.(2005). Using Lactic acid bacteria isolated from human and food sources to inhibit the growth of some bacteria causing diarrhea. Doctorate thesis,College of Science.Al Mustansiriyah University.

Vol: 8 No: 3, July 2012 134 ISSN: 2222-8373

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Jameel hadi jiyad

22-AL- Yas,M.G.(2006). Evaluation of different methods for detecting *Hylicobacter Pylori* isolate from human and the effect of propiotics on the bacteria growth . Doctorate thesis, College of Science. AL-Nahrain University.

Bushra J . Mohamed

23-Aziz ,Z.Z.(2007). Studing the effect of *Lactobacillus aciophilas* on the pathological effect of *Proteus mirabilis* in mice.M.Sc.thesis, College of Science Al-Nahrain University.

24-Kubba,M.A.(2006).Improvement of inhibition effect of probiotic against some bacterial isolate using prebiotic.MSc thesis.AL-Nahrain University

25-Aasen, I.M.; Moretro ,T.; Katla, T.; Axelsson, L. and Storro, I.(2000). Influence of complex nuterient, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG42687. Appl. Microbiol. Biotechnol.J. 53:

159-166.

26-Tagg, J.R and McGiven, A.R. (2002). Assay system for bacteriocins. Appl.

Microbiol.J. 21: 943-944.

27-Flythe, M.D. and Russell, J.B.(2004). The effect of pH and a bacteriocin (bovicinHC5) on *Clostridium sporogenes* MD1, a bacterium that has the ability to degrade amino acids in ensiled plant materials. FEMS Microbiol Ecol.J. 47: 215-222.

28-Karthikeyan ,V and Santhosh, S.W.(2009). Study of Bacteriocin as a Food Preservative and the *L. acidophilus* Strain as Probiotic. Pakistan Journal of Nutrition 8 (4): 335-340.

29-Rossland, E.; Langsrud, T.; Granum, P.E. and Sorhaug, T. (2005) Production of antimicrobial by strains of *Lactobacillus or Lactococcus* co-cultured with Bacillus cereus in milk. Int. J. Food Microbiol. J. 98: 93-100.

Vol: 8 No: 3, July 2012 135 ISSN: 2222-8373