Lactobacillus plantarum:Biopreservative prevent cheese contamination,inhibit *Bacillus cereus* growth

Yusra* ,M Baqer and Likaa*,H.Mahdii.
*Department of biology ,College of science,Mustansiriyah university

Abstract(4)concentrations from CFSs of*Lactobacillus plantarum* and standard concentration to Calcium propionate were taken to study their inhibite activity against pathogenic bacteria *Bacillus cereus* .MIC,MBC assay was studied to choose the inhibited concentration used in colony counter method for *B. cereus* in cheeseand compared with calcium propionate concentration .Results in our study refered to high activity for all these concentrations more than calcium propionate with clearly significant deferences ,and when used MIC the effect was too much higher than calcium propionate in decreesing the survival cells of *Bcereus* in cheese**..**

Introduction:

Many food productsare perishable with nature and pathogen microorgauism so, require protection during preparation, storage and distribution to give them desired shelf_ life. Because food products are now often sold in areas of the world for distant from their production sites. The need for extended safe shelf_life for these products has also expanded(16) .The development of food preservation processes has been driven by the need to extent the shelf_life of foods(4).Food preservation is a continuos fight against microorganisms spoiling the food or making it un_safe , food preservation such as heating,refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning , but these techniques frequently have associated adverse changes in organoleptic characteristies and loos of nutrients.(1)

The most common classical preservation agents are the weak organic acids , for example propionic acid and its salt like calcium_propionate , these molecules inhibit the outgrowth of both bacterial and fungal cells and also reported to inhibit the germination and out growth of bacterial spores[2].In manufacture of food it is crucial that preservation are taken to ensure the safety and stability of the product during the its whole shelf_life , In particular , modern consumer trends and food legislation have made the successful attainment of this objective much more of challenge to the food industry(1).Firstly, Consumer require more high quality perservative_free, safe but mildy processed, foods with extended shelf_life , by control of outgrowth of pathogenic spore _ forming bacteria , such as *Bacillus cereus*,addressing this consumer need cells for innovate approaches to ensure preservation of products. Secondly, legislation has restricted the use and permitted levels of some currently accepted preservatives in different foods (3) .

This has created problems for the industry because the susceptibility of some microorganisms to most currently used preservatives is falling (16).An increasing number of consumers prefer minimally processed foods , prepared without chemical preservatvies.Many of these ready_to_eat and novel food types represent new food systems with respect to health risks and spoilage association(4) . Food additivies like preservative can be linked to hyperactivity , asthlma. Cancer_avoid these in your every day died, Allergic and other reactions to food preservatives occur hours and even days later therefore many people cannot notice these connections (5) .Against this back ground, and relying on improved understanding and

knowledge of the complexity of microbial interactions recent approaches are increasingly directed towards possibilities offered by biological preservation(1).

These days Lactic Acid Bacteria (LAB) are used as an (integral) part of hurdle technology ,using them in combination with other preservative technique can effectively control spoilage bacteria and other pathogens and can inhibitingthe activities of awide spectrum of organisms,including inherently resistant,Gram-negative and positive bacteria(13,14,15,16).

Throughout the development of (LAB)as food preservative such as dairy and its produced, *L.plantarum* was reported to apply as an additive in food preservation in the nowly time,because its ability to exhibit antagonistic activity against spoilage and pathogenic organisms in food (10). In the other side *Bacillus cereus* has been recognized as a cause of food_borne disease and food poisoning with increasing frequency in recent years[6].This forming spore bacteria and its pathogenicity is isolated from cheese and milk that reported in many resources(24,7,8,9).

The preservative is very risk (cannot notice) and *Bacilluscereus* infection increasing frequency in cheese (7,8,9). Bio preservative is the alone solution to this problem , so we suggest this study to certify that the biopreservative don't have any risk and at the same time it is usfule in prevent growth of pathogenic bacteria in addition to that give the food desired shelf_life.

Materials and Methods :

1.Collection and Identification of isolates :

A.13 isolate of*Lactobacillus plantarum* was isolated from natural sources (dairy product) and indentified according to Hammes and Vogel(1995). Among 13 isolates of *L.plantarum* previously only isolate.*L.plantarum* 5 was able to inhibit the growth of all choosed pathogenic bacterial isolates . In this study, and this isolate had the best activity against the strongest isolate of pathogenic bacteria .

The putative metabolites produced by *L.plantarum* isolate was prepared according to Galphi *et al* (2007). Lypholized and powder dissolved in phosphate_buffer saline (*2m*M Na_2Hpo_4 . 0.5 *m*M KH_2 po_4. 1.3 *m*M Kcl. 135 *m*M Nacl , pH. 7.0). to prepare different concentration.

B.Tested bacteria:

3 isolate from *Bacilluscereus* were isolated from localy cheese, after identification it was submitted to test growth to choose the isolate [3] which had the higher resistant to *L.plantarum*(5).

2. Preparation of cell free supernatants (CFS_S) of *L.plantarum*. (5):

Strains to be tested for an timicrobial activity were incubated in De Man Rogosa Sharpe (MRS) broth for 24 hrs at$37c^0$. Bacterial cells were removed by centrifuging the culture at 8000 xg for 5min at $4c^0$. The pH values of cell free supernatant (CFS_s) were adjusted to (6.5) by the addition of 0.1N(Normality) NaoH . Sterilized , filtered through 0.22 *m*M filter and stored at $4c^0$ (13).

3. Chemical preservation :

Calcium propionate was chosen in this study against pathogenic bacteria isolates : *Bacillus cereus* and prepared by taking 3mg/1 ml which make as CFSs of *L.plantarum* (14) .

4.Antimicrobial activity assay of (CFSs) and Calcium propionate:

Agar well diffusion method was used to detect antimicrobial activities of calcium propionate and CFSs of *L.plantarum* against *Bacillus cereus* isolates. The concentration of (CFSs) were taken at (1000,750,500,250)*M*g/ml to prepare 4 conc. (0.6,0.3,0.15,0.07)ml in this assay (Batdory *etal*. , 2006).

5.The Minimum inhibitory concentration(MIC) and Minimum bacteriocidal concentration (MBC) assay:

(MIC) and MBC assay were performed as described by (Batdory *etal*. ,2006).

6.Use the CFSs in the cheese preserve:

(15)gm from Localy Cheese was weighted. 3ml distilled water was added and put in outo clave(121c^0/15min) after coold it, separated it (3) parts every one (5)gm added to (90ml) normal saline, mixed it in grander and Leavad 15min.

First part: 10^8 *Bacillus cereus* was added (as control) [22].

Second part: 10^8 *Bacillus cereus* and 0.5gm Calcium propionate (26).

Third part : 18^8*Bacillus cereus* and 0.07*m*l CFSsof L. plantarum (25).

1*m*l was taken from each part for using colony counter method to *Bacillus cereus* survival cells , put in incubuter 37c^0 for 24hr , This assay was performed as described in Rukure(1999) .

Statistical Analysis: 7.

Data from experiments were analyzed using students-t-test and p-value of $\leq$ o.o5, $\leq$ 0.01 were considered statistically significant.

Result

It was interesting to investigate the effect of CFSs of *L.plantarum* on *B.cereus* isotates, the inhibition zones were measurments,it can be noted in table (1) that is appear alarge difference in the diameter inhibition zone measurments occurred due to difference effect between CFSs of *L.plantarum* and calcium propionate on *B.cereus* .

CFSs of *L.plantarum* inhibited *B.cereus* growth at(0.6)ml concentration and the inhibition zone reached to(29)mm,compartive with calcium propionate which inhibited *B.*cereus at(7)mm just and this is the standard concentration that allowed to added to food (cheese).graph(1).

The results of the study revealed that the inhibition zones of CFSs of *L.plantarum* was increased with increasing the concentration (0.07,0.15,0.3,0.6)ml at (15,19,24,29)mm respectively, table (1) is showed the significant differences between the CFSs of *L.plantarum* and calcium propionate effect with each other and with control on *B. cereus* .

Table(1): Inhibition zones of CFSs of *L.plantarum* and Calcium propionate on pathogenic isolate (*B.cereus)*

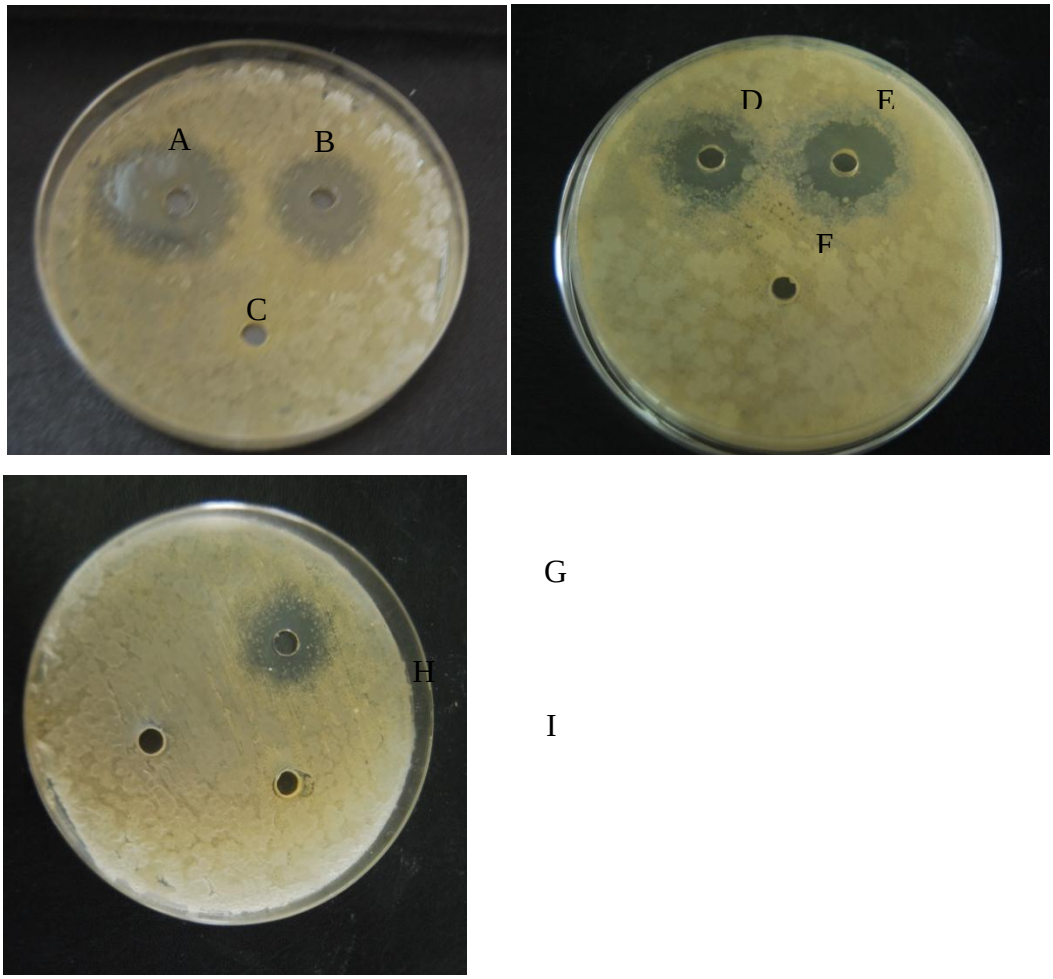
CFSs conc. / Calcium propionate	(0.6)	(o.3)	(0.15)	(0.07)
*(7)	**(29)	**(24)	**(19)	**(15)

Control=(-)

( ): cell free supernatant concentration of *L.plantarum* in ml.

(*):significant differences with control massured (mm).

(**):very significant differences with control and calcium propionate massured (mm).



A,B,D,E :Concen.(29,24,19,15) ml of *L.plantarum* respectively
C,F,H,I:Control G:Calcium propionate concen.

Fig. Comparsion the antibacilli activity of *L.plantarum* and Calcium propionate

In MIC ,MBC assay (0.07)ml was the minimum inhibition and bactericidal concentration,and a comparsion to number of *B. cereus* survival cells in cheese With each of CFS of *L.plantarum* and calcium propionate, table (2) was made to identify that ,two definite association were noted that is : decreasing the Number of*B.cereus* survival cells in cheese and significant differences between CFS of *L.plantarum* and calcium propionate effect on these numbers in the same growth conditions.

The results appear the using of CFS of *L.plantarum* that resulted 10^3*4.85 survival cell of *B. cereus* , versus calcium propionate using was showed 10^4*1.70 survival cell of *B.cereus*.,when we notice the control we found the second *association* ,there is a significant differences between CFS and calcium propionate with each other towards the numbers of cells and with control on these cells.

Table(2):Effect of CFS of *L.plantarum* and calcium propionate on number of survival *B.cereus cells* in three parts of cheese.

Part 1	Part 2	Part 3
B.cereus +Control	*B.cereus*+Calcium propionate	*B.cereus* +CFS of *L.planta*rum
10^5*3.02 5.3+0.9	10^4*1.70 4.1+0.6 A	10^3*4.85 3.2+0.11 AB

A:significant differences with control
AB:significant differences with control and calcium propionate

Discussion

The study revealed the larg inhibition zone of CFSs of *Lctobacillus plantarum* against *B.cereus*,because of special interest of them ,and causative by the antagonistic properties which make them particulary useful as biopreservative (17).

When these bacteria concept for nutrients, their metabolites often include end compounds have antimicrobials properties such as lactic acid ,acetic acid ,hydrogen peroxide and peptide bacteriocins, inaddtion the bacteria produce nisin which have very important antimicrobial and a particulary effective preservative (15,16).

Many of studies refers to direct antimicrobial effect of organic acids (Lactic, Acetic and Propionic)which they are being end products of *L .plantarum* (18,21).So ,the inhibition zones and the decreased number of survival cells of *B.cereus* may be causative by antagonistic action of acids and it is believed to be/or interferance with the maintenance of cell membrane potential ,then inhibition of active transport and mechanisms enable the beneficial bacteria to kill the others because they have a broad mode of action and inhibit both gram –negative and gram positive bacteria as well as yeast and molds ,this result agreed exactly with our study(18,27).

Another study (19,27) refered to the *L.plantarum* ability to inhibition and reduction *B.cereus* growth through produce hydrogen peroxide (H_2O_2) through the oxidation of reduced Nicotinamide Adenine dinucleotide(NADH),H_2O_2 can have a strong oxidizing effect on membrane lipids and cellular proteins.

Rose *et al.*,2002 confirmed that *B.cereus* was inhibited by acids and H_2O_2 in addition to others because *L. plantarum* can produce a range of end product have antimicrobial activity such as Bacteriocins,these proteinaceous inhibitors target the cell membrane and depolarize it ,and also inhibit synthesis of the cell wall,and *Bcereus* is one of the common targets in context to this mechanisms .While Pitt *et al ,2000* refered to that besides the production of inhibitory compounds ,this bacteria (*L.plantarum)* have the ability to compete with pathogens for nutrients during growth process, the combined influence of competing *L.plantarum* and the resulting decrease in pH produce an unfavourable environment for many pathogens such as *B.cereus* .

Consumers require more high quality preservative_free ,safe but mildy processed food with extended shelf_life(1),This may mean that to be have foods preserved at in controlled conditions ,Lactic Acid Bacteria is the alone and best solution to do that ,and according to that ,*Lplantarum* is the efficiency species of LAB because of each antimicrobial compound produced by *L.plantarum* during growth process provides an additional hurdle for *B.cereus* (pathogen and spoilage food bacteria) to overcome before they can survive and /or proliferate in the cheese (or in any food another)from the time of manufacture to the time of consumption ,Since *L.plantarum*

may produce a number of inhibitory substances,its' antimicrobial potential is defined by the collective action of its' metabolic product on undesirable bacteria.

Conclusion:

We can conclude from our study that CFSs of *L.plantarum* have high efficiency in both of well diffusion and plate count assay against *B. cereus* more than calcium propionate,and this antimicrobial activity able to prevent pathogen *B.cereus* in cheese.*L.plantarum* must be applied as an additive to food preservation in the future,.it is from generally regarded as safe (GRAS) and have arisen agreat deal of attention as anovel approach to control pathogens in food stuffs ,must been widely studied .

References

1.Rosali,I.(2007).Food preservation-A Biopreservative Approach .Food Global Science Books .p112.

2.McCann,D.;Barrett,A.;Crumpler,D.(2007).Food additives and hyperactive behavior in 3 – year-old and 8/9 year old children in the community .The Lancet .370 :9598-1560.

3.http://www.food.gov.uk/safereating/chemsafe/additiviesbranch/preservatives.2011.

4.http://www.understanding food additives.org/pages/ch2p5-2.htm.

5.http://www.traditionaloven.com/articles..additivies-to-avoid.

6.htpp://www.nejm.org/doi/full/10-1656/NEJM 19701192980306.

7.http:text book of bacteriology.net/B.cereus-2html.2011 Kenneth

8.Gitahi,J.:Ombui,J.;Nduati,D.and Gicheru,M.(2009).Genetic characterization of food borne *Bacillus cereus* strains from milk ,cheese and rise by multiplex PCR assay .International Journal of Integrative Biology.5(2):82-86.

9.Ombui,J.;Gitahi,J. and Gicheru,M.(2008).Direct detection of *Bacillus cereus* enterotoxin genes in food by multiplex polymerase chain reaction .International Journal of Integrative Biology .,2(3):172-182.

10.Puttalingamma,V. and Begum,K.(2012).Influence of temperature,carbon and nitrogen sources on Antimic robial Activity[AMA]of Lactic acid bacteria against *Bacillus cereus* .Int.J.of .Food Science .,4(1):71-79.

11.Ghalfi ,H.;Benkerroum, N.;Doguiet,D.and Bensaia ,M.(2007).Effectiveness of cell adsorbed bacteriocin produced by *Lactobacillus curvatus* CWB1-B28 and selected essential oils to control *Listeria monocytogenes* in pork meat during cold storage .,Soci.Appl.Microbial .,268-273.

12.Hammes,W.P. and Vogel ,R.F.(1995).The Genus Lactobacillus .In :The Genera of Lactic Acid Bacteria .Edited by Wood ,B.J. and Holzapfel, W.H.

13.Vuyst,L.D. and Leroy ,F.(2007).Bacteriocin from Lactic acid bacteria:Production ,Purification and food Applications.J.Molec.Microbial.Biotecnol.,13:94-199.

14.Lavermiccoca,P.;Valerio,F.;Evidente,A. and Gobbetti,M.(2000).Purification and characterization of Novel Antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21 B. Microbiol.,66(9):4084-4090.

15.Batdory,B.;Dalgalarrondo,M.;Choieset,Y.Pedroche,J. and Prevost,H.(2006).Purification and Characterization of tow bacteriocins produced by lactic acid bacteria isolated from Mongolian airag.J .Appl.Microbial.,101:837-848.

16.Mecitoglo,C.;Ahmet ,Y.;Arslanoglu,A.;Zahra,E.and Ali,C.(2005).Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging .,Food .Reser.Intern.39:12-21.

17.Ananou,S.;Maqueda,M.;Martinez,M.and Valdivia,E.(2007). Biopreservativation,an ecological approach to improve the safety and shelf –life of foods .Communicating current Research and Educational Topics and Trend in Applied Microbiology.ISBN 9788461194230.
18.Molin,G.(2006):*Lactobacillus plantarum* 299 V,3:21.
19.Roos,R.P.;Sullivan,L. and Hill,C.(2002).Potential of bacteriocine-producing lactic acid bacteria for improvements in food safety and quality.,Nation .Food Biotech.,84(5):593-604.
20.Pitt,F.;Christoph,C. and Cadavani,F .(2008).Hydrogen peroxide production by *Lactobacillus* and its role in anti-bacterial activity ,Euro.Microbial.Societ.,283(2):210-215.
21.Lefoka,M.(2009).The survival of microbial pathogens in dairy products.,Faculty of Natural and Agricultural Sciences,Department of Microbial,Biochemical and food Biotechnology.,University of the free state,Bloemfontein.
22.OPH.(2004).Infectious Disease Epidemiology Section .,Office of Public Health ,Louisiana Dep. Of Health and Hospitals. *Bacillus cereus* Toxi-Infections.www.oph.dhh.state.la.us.
23.Ko,J.;Yang,H.;Chang,J.and Seo,J.(2007).*Lactobacillus plantarum* inhibits epithelial barrier dysfunction and interleukin-8 section induced by tumer necrosis factor –α.World J. of Gastroentero.13(13):1962-1965.
24.Foster,J.W.and Foester,H.F.(1966).Endotrophic Calcium,Strontium and Barium Spores of *Bacillus megaterium* and *Bacillus cereus* .J.of Bacteriol.,19(3):1333-1345.
25.Mills,S.;Serrano,L.;Griffin,C.and others.(2011).Inhibitory activity of *Lactobacillus plantarum* LMG P-26358 against *Listeria innocua* when used as an djunct starter in the manufacture of cheese .,Microbial.Cell Factories.10(1):57.
26.Valerio,F.; Bellis,P.;Lonigro,S.and others.(2008).Use of *Lactobacillus plantarum* fermentation products in bread –making to prevent *Bacillus subtilis* ropy spoilage.,Instit.Scien.Food Product(ISPA).Nation.Reser.Counc.(CNR).Via Amendola ,122/0.70126 Bari-Italy.
27.Begum,K.and Puttalingamma,V.(2010).Influence of temperature,carbon and nitrogen sources on antibacterial activity[AMA] of Lactic Acid Bacteria against *B.cereus.*,Int.J of food Science .,Technol. And Nutri.,4(1):71-79.
28.Rukure,G.T.(1999).Survival and growth of *Bacillus cereus* during Gouda cheese manufacturing .Depart.food Science university of Pretoria,South Africa.

Bacillus cereus ماده حافظه حيويه تمنع تلوث الاجبان , تثبط نمو: ***Lactobacillus plantarum***

يسرى* محمد باقر محسن ولقاء* حميد مهدي
*الجامعه المستنصريه,كلية العلوم ,قسم علوم الحياة

الخلاصه
تم اخذ (4)تراكيز من عالق بكتريا *Lactobacillus planta*rum والتركيز القياسي للكالسيوم بروبيونيت لدراسة فعاليتهم التثبيطيه ضد البكتريا المرضيه*Bacillus cereus*,اختيرالتركيز المثبط بتجربتي MIC,MBCليستخدم في طريقة عد خلايا *B.cereus في الجبن و قورنت مع نتائج* الكالسيوم بروبيونيت.اشارت نتائج الدراسه الى الفعاليه التثبيطيه لجميع التراكيز وبصوره اعلى من تركيز الكالسيوم بروبيونيت وبفروق معنويه واضحه جدا