



وزارة التعليم العالي والبحث العلمي

جامعة القادسية / كلية العلوم

قسم علوم الحياة

The inhibitory effect of *Lactobacillus spp*
isolated from different sources on
Proionbacterium acnes which caused acnes

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بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

((وِیَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّیْ

وَمَا أُوتِیْتُمْ مِنَ الْعِلْمِ إِلَّا قَلِیْلًا))

صَدَقَ اللّٰهُ الْعَلِیُّ الْعَظِیْمُ

الإهداء

إلى ...

من أسس العلم وبرع فيه .. معلمنا الأول .. إلى أماننا الصادق عليه السلام .

إلى ...

من جعل الله الجنة تحت أقدامها

ومقرت مرضاه برضاها .. أمي

إلى ...

بمجر المحنان والمحنة إلى من غرس بذور العلم وسقاها .. حباً .. وحناناً ومعرفة

إلى ...

معلمي الأول والأخير ... أبي

إلى ...

مرياح الشمال العالية التي تشد شراع سفيني لتبحر بي إلى الإمام

إلى ...

أخواني وأخواتي

إلى ... صانع الأجيال وباني المجتمع

إلى من كاد أن يكون رسولا ...

إلى ... أساتذتي

شكر والتقدير

بدأنا بالكثير وقاسينا أكثر من هم وعانينا الكثير من الصعوبات وه نحن اليوم والحمد لله نطوي
سهر الليالي وتعب الأيام وخلاصة الأيام ومشوارنا بين دفتي هذا العمل المتواضع .

أتقدم بالشكر الجزيل إلى الأستاذة **ضحى مهدي** على ما قدمته من جهود مضية في سبيل إكمال
هذا المشروع .

والى جميع الأساتذة والتدريسيين الذين ساهموا ولو بنصيحة من أجل الامتقاء بهذا المشروع الى المستوى
الأعلى وأخيراً أشكر زملائي الذين كانوا عوناً في سبيل تحقيق هذا الانجاز .

الفصل الأول

Introduction

Abstract

This present study was done to detect the antigenic activity for lactobacillus spp isolated from un pasteurized milk and stool sample against probionbacter acnes . 30 milk samples and 20 infant stool samples were cultured on blood agar and transmitted to MRS agar ,and the mixed probion bacterium Acnes

The results were inhibition zone between 11-28mm .

Introduction

Lactobacillus is a species that converts lactose and a number of other sugars into lactic acid. This is called Lactic acid bacteria. Lactobacillus is spread in many places including normal flora, including the mouth, intestines and vagina, and have a major effect on maintaining the natural bacterial balance of these parts, as well as in the digestive tract of many animals, as well as in fermented fruits and vegetables (Longh and Wadstrom 2009). Lactobacillus is a class of Lactobacillaceae (Lactobacillaceae), which is part of the Division of Firmicutes. The genus Lactobacillus has 120 species classified by the ratio of cytosine / guanine (32-53%). The most important species are *L. acidophilus*, *L. casei*, *L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *bulgaricus*, *L. fermentum*, *L. gasseri*, *L. helveticus*, *L. johnsonii*, *L. plantarum*, *L. rhamnosus*, *L. sakei* (Holt et al., 1994).

Lactobacillus was characterized by its medicinal importance as it was found to contribute to the promotion of human health. A number of researches were carried out in this field and studied the effect of its different types of 120 species on many infections caused by bacteria and parasites as well as their effect on cancer tumors and their motivation for immunity. Gastrointestinal disorders and gastric ulcers (Francavilla et al. 2008)). Because of the importance of these biologically active bacteria and their enzymes, bacteriocines and

polysaccharides, many researchers have studied the effect of *Lactobacillus* on a large number of pathogenic bacterial species such as *Escherichia coli*, *Vibrio cholera*, *Pseudomonas*, *Streptococcus* and *Staphylococcus*. These researchers, Liu and his group who studied Effect of Multiple Sclerosis Produced from *Lactobacillus* on *E. coli* Epidemics in Newly Depressed Pigs As weaning phase is a critical stage due to rapid changes in the intestine and development of immune response. An effective antiviral against *E. coli* by promoting the growth of microbial bacteria and prevention of diarrhea (Liu et al., 2008). Turner and his group also found that *L. reuteri* bacteria such as bacteriosin, lactic acid and hydrogen peroxide inhibited the growth of *Staphylococcus aureus* (Turner et al., 2006).

The case of fever-infected *L. casei* can be used against *Pseudomonas aeruginosa* infections, which cause acute infections and reduced immunity in humans. Miake and his group (1985) found that mice treated with fever-resistant bacteria for five days had the ability to survive and resist pathogenic bacteria Compared with non-treated *Lactobacillus* mice. The immune system secrete cytokines against foreign bodies, including bacteria, but what Veckman and his group found (2004) that monocyte-derived CD8 can distinguish pathogens and pathogens, as CD8 stimulate the product of lymphocytes against

L. rhamnosus Lead to a slight rise in the expression of auxiliary molecules to the cell surfaces and a weak response to cytokine and chemokine while stimulation .(against pathogenic bacteria

The aim of this study is to study the effect of lactobacilli isolated from different sources against acnes bacteria.

الفصل الثاني

Materials and methods

Materials and method

(2-1) materials

(2-1-1)tools

1-tubes, 2-petridishes, 3-loop, 4- cotton, gloves, 6-Aseptic material ,
7-needle , benzene lamp .

(2-1-2) Devices

1-Autoclave , 2-hood ,3-incubator ,4-sensitive balance

Culture media

1-nutrient agar :to prepare one liter of nutrient agar about (28gm) was weighed by sensitive balance and dissolve sterilizing distilled water and then autoclave for 15 min.

2-Blood agar: about(39.5gm) of the medium was dissolved in distilled water and then autoclaved for 15 min

3-preparing of MRS media: it's the selective media for

lactobacillus

1% peptone ,1% beef extract ,0.4% 1% beef extract ,0.4% yeast extract , 2% glucose , 0.5% sodium acetate trihydrate , 0.1% poly sorbate80 , 0.2% dipotassium hydrogen phosphate, 0.2%

triammonium citrate , 0.02% magnesium sulfate heptahydrate ,
0.005% manganese sulfate tetrahydrate, 1% agar, pH adjusted to 6.2
at 25°C.

Stains and Reagents

Gram stain , Catalase R

Sample collection

50 different samples have been collected from two sources 20 fetal
feces and the other 30 were obtained from non pasteurized fresh
milk .

The samples were transported to the lab as soon as possible .

Stool samples were mixed with normal saline and then
shaken well, then cultured on blood and nutrient agar by pour
plate method without any dilution.

and the while ,milk samples were diluted with distilled water until

10^{-3} The three dilutions were cultured on both blood and nutrient agar

All the dishes were incubated at 37°C
temp for 24 hours .

After 24 hours of incubation the dishes which gave a clear growth
were stained with gram stain :

a) crystal violet; 30 seconds , b) rinse for 5 seconds,

c)cover with gram iodine for 1min ,d)rinse with water for 5 secons
e)decolonize for 15-30 seconds ,f)rinse with water for 5 seconed,
g)counterstain safanin for about 60 seconed,h)rinse for 5 seconed.

Cultivation on MRS agar

Only the dishes which give gram positive bacilli colonies were cultured on MRS medium by power plate method and incubated at 37°C For 24 hours

After incubation period the colonies were transferred to test tubes contained MRS agar by using deep agar method.

Catalase test :this test was done by mixing the growing bacteria with (H₂O₂) on clean slide and waiting the bubbles according to (holet et ,at 1994)

Preparing of lactobacillus filtrate

The pure colonies were concentrated with normal saline

Testing of the activity of the bacteria against acnes bacterium

Pronbionbacteriumacene isolate cultured on blood agar were obtained from lab and then transmited to a petridishes and cultured for 24 hours.To test the activity of lactobacillus bacteria against acne bacterium the well diffusion method was used with concentrated lactobacillus filtrate (Gupta et., al 1998)

الفصل الثالث

Results and discussion

Results and discussion

This previous study was aimed to find out the activity of *lactobacillus* bacteria against acne disorder as try to find a natural material to treat this problem.

About 20 of 30 milk samples were give growth colonies on blood agar

and only 5 of them were gram positive bacilli and catalase positive

as regarding to stool samples only 4 of the 20 samples were gram positive

bacilli and catalase positive and the rest 16 were gave growth with gram

negative bacilli and cocci so they neglected

table1)

Samples	Milk samples	Stool samples
Total samples	30	20
Growth on blood agar	20	20
Bacilli ,gram + and catalase +	5	4

to studying the activity of *lactobacillus* bacteria against acne bacterium

the isolated bacteria was grown on blood agar to gather with probionbacterium

for 48 hours in order to ensure of the inhibition zone .

by well diffusion method maximum and the result was about 28 at maximum rate and 11 minimum as seen in table 2 below

Milk samples	Stool samples
NO.1 :12	15
NO.2:10	14
NO.3:13	17
NO.4:20	13
NO.5:28	20

rate so this result can ensure the activity of *lactobacillus* against acne as so as the results showed that the activity of lactobacillus isolated from infant feces is more effective against acnes bacteria than these isolated from unpasteurized milk which is many studies takes lactobacillus as antibiotic for many disorders because the last bacteria contain many compounds like lactic acid and acetic acid the activity of these acids because they can enter the plasma membrane of acne bacterium and blocked as soon as the nutrient to reach to the cell and this agrees with (Isolauri et al., 2003). Lactobacillus contains bacteriocin which can conjugate with receptors on the cell membrane of acne bacterium. This matter results in uncontrolled amino acids and this agrees with (Delgado et al., 2001).

conclusions.

In a brief we can use lactobacillus bacteria for treatment of many disorders because it can be considered a safe and cheap way instead of using drugs and chemicals and avoiding their side effects .

Recommendations

The study of lactobacillus effects on many other disorders

Trying to isolate lactobacillus from many food products

Adding lactobacillus as a probiotic to the food.

الفصل الرابع

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الخلاصة

اجريت الدراسة الحالية لغرض التحري عن قابلية بكتريا *Lactobacillus* spp في تثبيط بكتريا المسببة لحب الشباب .

جمعت حوالي ٥٠ عينة (٣٠ حليب غير مبستر + ٢٠ براز اطفال حديثي الولادة) وبعد الزرع على وسط أكار الدم ووسط MRS الخاص ببكتريا *Lactobacillus* ومن ثم مزجت مع بكتريا *probionbacterium acnes* المسببة لحب الشباب أظهرت مناطق تثبيط واضحة تصل إلى 28mm مما يدل على أهمية هذه البكتريا في مقاومة الأنواع الممرضة .



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دراسة التأثير التثبيطي لبكتريا *Lactobacillus spp*

المعزولة من عينات مختلفة على بكتريا *Probionbactrium*

Acnes المسببة لحب الشباب

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