

The Study of Lactobacillus gasseri Filtrate Effect on Probionbacterium acnes Isolated From Acnes Patients

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Abstract: The present has been included using of lactobacillus gasseri filtrate against probionbacterium acne in order to study the inhibitory effect of the filtrate on the bacteria after using the antibiotics cotrimoxazole, doxycycline, erythromycin, clindamycin, azithromycin and tetracycline, and differentiate using the same antibiotics without adding Lb. gasseri filtrate. 20 samples isolated from patients suffered from acne and cultured on blood agar, 15 of them gave the species probionbacterium and the rest 5 were Staphylococcus aureus and Staphylococcus epidermidis. Antibiotic sensitivity test has been done by using the above mentioned antibiotics, and then E. coli filtrate used and the sensitivity test repeated. The results showed that incubation time 48 hours with double concentrated Lb. gasseri there for we can recommend to use the metabolic product of the tested bacteria against another pathogenic bacteria.

Introduction

Acne is a long-term skin disease that occurs when hair follicles are clogged with dead skin cells and oil from the skin (1), it most often diffuses in the face, neck and the chest.

It is a common disorder in the world wide prevalence about 70-87% (2). Skin lesions consist of white and black comedones, erythematous papules and pustules, and in severe untreated cases, scarring appears (3).

The disease usually appears in adolescences, the period in which sexual hormones increase, simultaneously and usually disappears in the second half of third decade of age (4,5). Patients with acne always suffer from stress, anxiety and psychiatric problems (4).

The causative agents of acne are mainly *Staphylococcus epidermidis*, *Malassezia furfur* and *Probionbacterium acnes* (6). And the last one is the essential causative agent of acne infection (7). *P. acnes* is Gram-positive anaerobic rods, it has a special culture requirements. Right now, cultivation of *P. acnes* is unnecessary for microbiologic testing

of skin inflammation injuries is to a great extent pointless in light of the fact that it doesn't influence administration, and fruitful anti-infection treatment may not come about because of a diminishment of bacterial numbers (8). There are many antibiotics have been used to treat this disorder cotrimoxazole, doxycycline, erythromycin, clindamycin, azithromycin and tetracycline, they were give significant results in acne treatment. But with the risk of using antibiotics on the skin, their use remains undesirable and alternative treatment is needed.

Recently many microorganisms have been used to treat many diseases as so as to keeping the health of the body which termed by probiotics. There are many types of bacteria have such property like *Lb. gasseri* and lactic acid bacteria (9,10). Actually this bacteria consider one of the most gut flora.

Probiotics characterized by maintaining a healthy body. Through bio-effectiveness and outcomes-offs, as well as through the competition with some pathogenic species, it also activate the immune system and decrease

the side effect of antibiotics(11) , they have a role in diarrhea treatment and Constipation cases . they also participate in lowering blood cholesterol levels, they help to treat liver and urinary tract infections.(12,13) .

There were many studies tried to determinate the role of Lactic acid bacteria and how to make it useful in pathogenic agents inhibition . *Lactobacillus gasseri* was the most used species to study its effect in many bacterial. The aim of the present work is to study the ability of *Lb gasseri* to inhibit probionbacterium acnes which is responsible

Bacteria	CT	DO	ER	CM	AZ	TE
<i>P. acnes</i> 1	R	S	R	R	S	R
<i>P. acnes</i> 2	S	S	S	R	S	R
<i>P. acnes</i> 3	R	S	S	S	S	R
<i>P. acnes</i> 4	S	S	S	R	S	S
<i>P. acnes</i> 5	S	S	R	R	S	R

for acne infection .

Material and Methods

Sample collection

Acne samples were collected from 20 persons suffer from acne their age was 14 -17 d inoculated on blood agar for 48 hours at 37°C and *Probionbacterium acnes* identified by gram positive Indole ,catalase and esculine tests (14).

Lactobacillus gasseri collection

Lb.gasseri was isolated from infant feces. and the filtrate of the bacteria was maid according to Lewus *et al* 1991(15) method.

The study *Lb gasseri* filtrate effect

In order to find out the inhibition activity of the above mentioned bacteria the well-diffusion method(16)was carried out with both un concentrated and twice concentrated *Lb gasseri* filtrate. Then inhibition compared to a filtrate both areas concentrations to see any concentration gives a value of greater inhibition zone(17).

Antibiotics sensitivity test

A disk diffusion test was carried out according to(18).the antibiotics used in test were cotrmixazol, doxycilen, erythromycin

,clindamycin, azithromycin and tetracycline. and then the well- diffusion repeated with the same un concentrated and concentrated twice filtrate of *Lb gasseri* filtrate.

Results and Discussion

About 20 acne isolates were obtained ,they have been collected from many secondary schools in Diwaniya city , all 20 samples were gram positive .these isolates were 15 of them *Probionbacterium acens*, 4 were *Staphylococcus aureus* , and the

Rest 1 was *S .epidermids*. from these 15 *P. acnes* samples we had obtain 5 different isolates .

Antibiotics used in this work were Co-trmixazol(CT), doxycilen(DO), erythromycin (ER),clindamycin(CM), azithromycin (AZ)and tetracycline(TE). the results of antibiotics sensitivity test are shown in table 1 below

Table 1: antibiotics sensitivity test

R:resistance

S:sensitive

An inhibitory activity of *Lb. gasseri* un concentrated and twice concentrated filtrate was assayed by milliliter for three incubation times , the mentioned bacteria was inoculate on MRS agar for 24 ,48 and 72hours .the tables (2,3,4)below explain the diameter of inhibition zone for these 3 periods.

Table 2: the inhibition zone after 24 hour incubation time

Pathogenic isolate	Inhibition zone against <i>P .acnes</i> after using unconcentrated <i>Lb. gasseri</i> filtrate	Inhibition zone against <i>P .acnes</i> after using twice concentrated <i>Lb. gasseri</i> filtrate
<i>P. acnes</i> 1	13	15
<i>P. acnes</i> 2	12	14
<i>P. acnes</i> 3	-	-
<i>P. acnes</i> 4	10	11
<i>P. acnes</i> 5	-	-

-:non inhibition zone

Table 3: the inhibition zone after 48 hour incubation time

Pathogenic isolate	Inhibition zone against <i>P. acnes</i> after using unconcentrated <i>Lb. gasseri</i> filtrate	Inhibition zone against <i>P. acnes</i> after using twice concentrated <i>Lb. gasseri</i> filtrate
<i>P. acnes</i> 1	15	18
<i>P. acnes</i> 2	16	26
<i>P. acnes</i> 3	-	-
<i>P. acnes</i> 4	13	28
<i>P. acnes</i> 5	-	-

Table 4: the inhibition zone after 72 hour incubation time

Pathogenic isolate	Inhibition zone against <i>P. acnes</i> after using unconcentrated <i>Lb. gasseri</i> filtrate	Inhibition zone against <i>P. acnes</i> after using twice concentrated <i>Lb. gasseri</i> filtrate
<i>P. acnes</i> 1	12	14
<i>P. acnes</i> 2	13	15
<i>P. acnes</i> 3	-	-
<i>P. acnes</i> 4	9	11
<i>P. acnes</i> 5	-	-

As obvious from tables the highest inhibitory zone was for *Lb. gasseri* twice concentrated and the for 48 hours incubation time . thus it can be consider the optimum conditions to inhibit *probionbacterium acens* ,Conversely the using of un concentrated bacterial filtrate for 24and 72 hours less or non-effect of 48 hours.

In order to understand the nature of probiotics and its role in bacterial inhibition there were many studies to explain that , one of them which presented by (19) who said that the probiotics have the ability to inhibit therewith of pathogenic bacteria *in vivo* and *in vitro* by producing antimicrobial compounds and

Antibacterial growth like cytokines and butyric acid .also (20) mentioned that a special strains of *Lactobacillus* have an anti-infective properties as so as capable to inhibit the growth of *S. aureus* which companied wounds

infections cases .the inhibitory effect of *Lb. gasseri* against acne bacteria may belong to contain the filtrate an inhibitory compounds as lactic and acetic acids which consider the main acids produces by *Lb. gasseri*, those acids can penetrate the bacterial cellular membranes and prevent arrival of nutrients to acne bacteria. As well as *Lb. gasseri* contain anther inhibitory material known as bacteriocin which has the ability to conjugate with a special receptors present on plasma membrane of acne bacteria this lead to uncontrolled fellow of amino acids and positive charged ions this result in cell membrane explosion thus susceptible bacterial cell death . In deed the many studies find that the treatment of *Lb. gasseri* filtrate with trypsin and pepsin (protein digestive enzymes) lead to losing inhibition activity so it may have a protein nature which is responsible for acne bacteria killing .as so as *Lactobacillus* bacteria can change the pH of the medium which cause bacteriocin production and conversion to active form this result in many organic acids production can lowering pH levels and disabled metabolic activates like oxidation and transport .

In a brief we have used one of the most important probiotics filtrate to study its effect on a common infection Acne and the results of inhibition were can not be underestimated therefore we can recommend to use probiotics in many bacterial infection treatment .

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