

Probiotic Efficacy of *Lactobacillus reuteri* Culture Filtrates against Certain Groups of Proliferating Bacteria Isolated from Acne Abscess

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Abstract: Acne is a common disease in Saudi nationals particularly among adolescents and young adults. The disease has substantial effects on quality of life which making treatment essential. Probiotics nowadays is becoming a method of first choice to combat the disease. The present study was carried out to evaluate and to identify the causative agents of acne disease in Shaqra region. Subsequently, the effects of culture filtrate of the bacterium *Lactobacillus reuteri* on the *in vitro* growth of bacterial isolates obtained from acne i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Propionibacterium granulosum* were also carried out along with the subsequent inhibitory impacts on acne symptomatology. It was observed that the culture filtrates of *L. reuteri* were significantly inhibitory to the growth of the four bacterial species. Increase in zone of inhibition was directly correlated with concentration of *L. reuteri*. *L. reuteri* at both diluted and concentrated doses was inhibitory at 24, 48 and 72 h of incubation periods. The most inhibitory incubation period was 48 h using well-diffusion agar technique with concentrated filtrate. The toxicity of the culture filtrate was largely influenced by the dilution and incubation period. Antibiotics sensitivity tests against different bacterial isolates was tested using Triclosan, Benzoyl peroxidase, Erythromycin, Retinoid, Tetracycline and Clindamycin. All of the bacterial isolates were vulnerable (susceptible, sensitive) to Triclosan and Benzoyl peroxidase, Retinoid, Tetracycline and Clindamycin, but all of the isolates were resistant to Erythromycin antibiotic. It could be concluded that the culture filtrate of *L. reuteri* could be used as probiotics and a source of biological control agents to cure acne disease in the area.

Keywords: Acne, culture filtrates, kingdom of Saudi Arabia, *Lactobacillus reuteri*, probiotics

INTRODUCTION

Acne is one of the most common skin diseases all over the world. It was estimated that more than 80% of all people being affected at some periods in their life (Karim and Rameid, 2011). The disease may cause psychosocial problems such as social embarrassment and isolation (Leyden, 1997). The affected area in humans are face, chest and back are most commonly affected. These areas containing large oil glands (Leyden, 1997). The symptoms of the disease are characterized by two phases. Non-inflammatory acne phase is characterized by open and closed comedone formation. Open comedones, also known as blackheads, are papules within a dilated follicle. They are composed of shed keratin and may have a black color due to melanin deposition and lipid oxidation. Closed comedones, or whiteheads are usually small skin or cream-colored papules with no apparent follicular opening and no associated inflammation. Inflammatory lesions phase occur when comedones expand and evolve into papules, pustules, nodules and cysts.

The disease is affected by several factors such as bacterial infections, stress, food, hormonal imbalances

and cosmetic application (Burkhart *et al.*, 1999). The disease is caused by different types of bacteria i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Propionibacterium granulosum*. All of these pathogenic bacteria are proliferate rapidly during puberty and are often involved in the disease symptoms development (Kang *et al.*, 2012; Holland *et al.*, 1979). Previous studies showed that the causal agents are obligate anaerobic organisms and has been developed the disease through inflammatory acne by their ability to activate complements and metabolize sebaceous triglycerides into fatty acids that attract neutrophils chemo tactically (Webster *et al.*, 1980).

Therapeutic agents for acne so far are antibiotics (Katsambas and Dessinioti, 2008). The main goals of pharmacotherapy for acne are to prevent new lesions and reduce the number of severity of existing lesions; prevent scarring and hyperpigmentation and limit the duration of the disease (Strauss *et al.*, 2007). This is specially for mild to moderate papulopustular acne without scarring together with topical retinoid or with other topical antimicrobials such as antibiotics or azelaic acid (Ofori, 2011). The most commonly used

topical antibiotics are Erythromycin and Clindamycin (Kang *et al.*, 2012). First-line antibiotics are usually Tetracyclines. Lymecycline, Doxycycline and minocycline are more effective than tetracycline. Erythromycin is a second-line drug, although a bacterial resistance is a problem. Trimethoprim and azithromycin can be used as third-line agents (Kang *et al.*, 2012).

Lactic acid bacteria such as *Lactobacillus spp.* has an effects against different pathogenic bacteria. The bacteria produce a wide range of antimicrobials and have been studied extensively with regard to their possible use in the field of probiotics. Probiotics are useful alternatives for the treatment and prevention of infectious diseases (Shornikova *et al.*, 1997). *Lactobacillus reuteri* is a probiotic bacteria used widely in viral diarrhea diseases (Saavedra, 2000). It is an obligatory fermentative resident in the gastrointestinal tract of the humans and usually produce an antimicrobial compound called reuterin that have inhibitory effects against many Gram negative and Gram positive bacteria (Talarico *et al.*, 1988).

A few studies have been done on the interaction between *L. reuteri* and infectious pathogenic bacteria (Kang *et al.*, 2012). In Saudi Arabia, no any documented records has been found concerned with such type of research. Therefore, the objective of the present work is to assess the effect of using *L. reuteri* culture filtrates on certain groups of proliferating bacteria isolated from acne abscess in Shaqra province.

MATERIALS AND METHODS

The study was carried out at the department of clinical laboratory sciences, Shaqra University, KSA, during October to December, 2013.

Strains of *Lactobacillus reuteri* and culture filtrates:

Different isolates of *L. reuteri* as a probiotic agent was isolated and provided from the feces of infants from Shaqra general hospital. Around 10 isolates of the bacteria was carefully collected. The bacteria was identified and authenticated in the laboratory of microbiology using Gram stain and biochemical tests. The isolates were cultured on De Man, Rogosa, Sharpe broth medium (Difco, USA). Then incubated at 37°C for 24 h under aerobic conditions using the technique

adopted by Kang *et al.* (2012). Furthermore, the bacterial cultures of *L. reuteri* were used subsequently in all of the experiments. The Culture filtrate of the bacterium was obtained by filtering pure cultures through filter paper (Whatman No1) and through sterilized filter (0.22 µm pore size; Millipore, USA), under aseptic conditions. Then clarified by centrifugation at 6000 rpm for 10 min and were taken as standard inoculums (S). Another dilutions i.e., S/2 of each culture filtrate was prepared by adding required amount of deionized distilled water.

Acne strains of bacteria: Four types of bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Propionibacterium granulosum* were used. Strains of the bacteria were isolated from clinical cases of acne symptoms. All of the bacteria were cultured on nutrient broth (Oxoid) at 37°C for 24 h under aerobic or anaerobic conditions.

In vitro assessment of culture filtrate of *Lactobacillus reuteri* on different acne strains of bacteria:

To determine the effects of *L. reuteri* culture filtrates on the growth of pathogenic bacteria namely *S. aureus*, *S. epidermidis*, *P. acnes* and *P. granulosum* well-diffusion agar disk method was used (Groove and Randall, 1955). In accordance with this method, plates containing nutrient agar media were swabbed entirely with the four types of acne abscess bacteria. Then five (Disks) in each plate was impregnated with 0.1 mL/disk of *L. reuteri* culture filtrate. The plates were then incubated at 37°C for 24, 48 and 72 h. The zone of inhibition around each disk was measured in mm and the results are presented as means SD. In another set of experiments, Triclosan, Benzoyl peroxidase, Erythromycin, Retinoid, Tetracycline and Clindamycin antibiotics at 25 µg/disk were used as a reference standard drugs for comparison (Table 1).

Statistical analysis: Data obtained from repeated experiments were pooled and the means were used in analysis for significant differences using appropriate statistical procedure (SAS Institute Inc., Cary, NC, USA).

Table 1: Antibiotic sensitivity tests against different strains of pathogenic bacteria*

Antibiotics (25 ug/disk)	Sensitive	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium granulosum</i>	<i>Propionibacterium acne</i>
Triclosan	20	25	22	21	24
Benzoyl peroxide	18	21	23	16	18
Erythromycin	24	26	25	22	20
Retinoid	16	22	20	21	18
Tetracycline	15	24	24	19	20
Clindamycin	15	20	22	20	18
Control	-	-	-	-	-

Values are means of 3 replicates. Data represents inhibition zone (mm)* No - = inhibition zone

RESULTS AND DISCUSSION

As a general rule, culture filtrates of bacteria are considered active against other bacteria when the zone of inhibition is greater than 6 mm (Boyd and Beveridge, 1979). Results presented in Table 2 to 4 indicated that the bacterium *L. reuteri* culture filtrates in the two different concentrations significantly ($p = 0.05$) suppressed the growth of all types of pathogenic bacteria isolated from acne abscess namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Propionibacterium granulosum*. The inhibition zone was highest in the standard concentrated inoculum. It was found to be 22 mm in *Staphylococcus aureus* and 18 mm in *Staphylococcus epidermidis* and 17 mm in *Propionibacterium granulosum* and 14 mm in *Propionibacterium acnes* after 48 h of incubation. The inhibition zone was reduced in diluted concentration

(S/2). It was 17, 17, 15 and 11 mm in *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium granulosum* and *Propionibacterium acnes*, respectively in the same incubation period. The least inhibition zone was noticed in *Propionibacterium acnes* (11 mm) in the same 48 h incubation period. Reduction in the zone of inhibition in all types of acne proliferating bacteria was directly correlated to the concentration of the *L. reuteri* filtrate. The tested culture filtrates of the bacterium *L. reuteri* were differed in their influence on pathogenic bacteria isolated from acne abscess depending on the dilution used (Table 2 to 4; Fig. 1 to 4). However, the reduction in inhibition zone is decreased when the culture dilution of *L. reuteri* was increased with no significant differences between incubation periods extended more than 72 h in each treatment (Table 2 to 4 and Fig. 4).

The culture filtrates of the two concentrations were found to be inhibitory to all bacterial stains,

Table 2: Influence of different dilution of *L. reuteri* culture filtrates on the growth of certain pathogenic bacteria isolated from acne abscess after 24 h incubation period*

Culture filtrate (<i>L. reuteri</i>) Concentration (0.1 mL/disk)	Pathogenic isolates of bacteria			
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium granulosum</i>	<i>Propionibacterium acnes</i>
Concentrated (S)	20±0.76	16±0.76	15±0.77	18±0.27
Diluted (S/2)	13±1.62	10±1.98	12±1.94	15±0.75
Control (no culture filtrate added)	-	-	-	-

*Values are means±SD of 3 replicates. Data represents inhibition zone (mm)

Table 3: Influence of different dilutions of *L. reuteri* culture filtrates on the growth of certain pathogenic bacteria isolated from acne abscess after 48 h incubation period*

Culture filtrate (<i>L. reuteri</i>) Concentration (0.1 mL/disk)	Pathogenic isolates of bacteria			
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium granulosum</i>	<i>Propionibacterium acnes</i>
Concentrated (S)	22±0.89	18±1.96	17±0.41	14±0.74
Diluted (S/2)	17±0.41	17±0.46	15±0.75	11±0.75
Control	-	-	-	-

*Values are means±SD of 3 replicates. Data represent inhibition zone (mm)

Table 4: Influence of different dilutions of *L. reuteri* culture filtrates on the growth of certain pathogenic bacteria isolated from acne abscess after 72 h incubation period*

Culture filtrate (<i>L. reuteri</i>) Concentration (0.1 mL/disk)	Pathogenic isolates of bacteria			
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium granulosum</i>	<i>Propionibacterium acnes</i>
Concentrated (S)	13±1.62	11±1.99	15±0.75	13±1.62
Diluted (S/2)	15±0.75	14±0.73	14±0.74	10±1.98
Control	-	-	-	-

*Values are means±SD of 3 replicates. Data represent inhibition zone (mm)

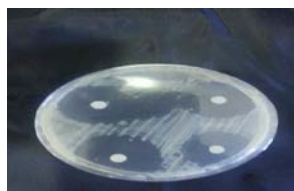


Fig. 1: Inhibitory effects of *L. reuteri* culture filtrate on *Staphylococcus aureus*

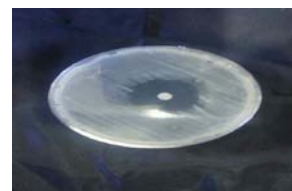
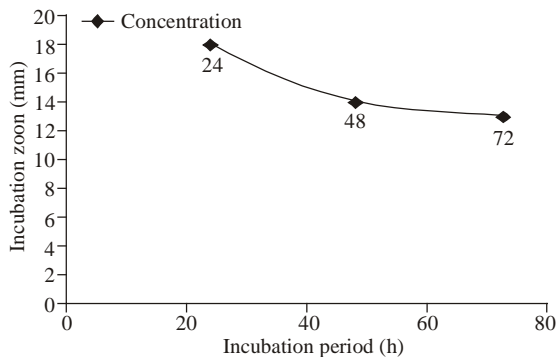


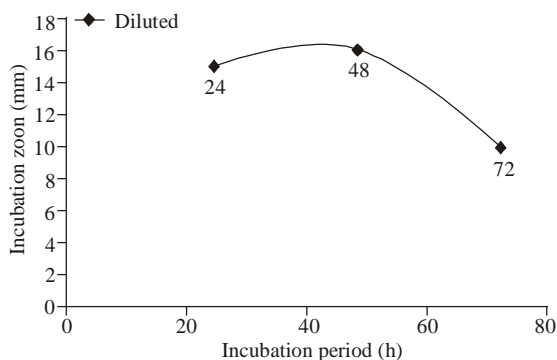
Fig. 2: Inhibitory effects of *L. reuteri* culture filtrate on *Staphylococcus epidermidis*



Fig. 3: Antibacterial effects of some standard antibiotics against *Staphylococcus aureus*



(a)



(b)

Fig. 4: Influence of the concentrated/diluted culture filtrate of *L. reuteri* on the growth of *P. acnes* isolated from acne abscess

whereas the inhibition zone was increased gradually with the increase of culture filtrate concentration. In case of *Staphylococcus aureus* and in the incubation period of 48 h the inhibition zone was 22 and 17 mm in S and S/2 concentration respectively (Table 3 and Fig. 1 to 4). Moreover, the maximum inhibition zone was observed against *Staphylococcus aureus* (22 mm) followed by 18 mm in *Staphylococcus epidermidis*, 17 mm in *Propionibacterium granulosum* and 14 mm for *Propionibacterium acnes*, with the same trend in the trend in the different incubation periods (Fig. 1 and 2 and Table 2 to 4).

Results obtained from the *in vitro* antimicrobial activity of *L. reuteri* showed that the culture filtrate of the bacterium have substantial inhibitory effects against the four tested bacterial strains. Still the standard concentration of the bacterial culture filtrate was superior in suppressing the bacterial growth, followed by diluted one. The antimicrobial activity of the *Lactobacillus reuteri* culture filtrate might be due to or attributed to the production of certain toxic metabolites i.e., reuterin and/or enzymes in the culture medium (Karim and Rameid, 2011). The bacterium produces lactic acid and other organic acids that can exhibit antimicrobial activity (Taniguchi *et al.*, 1998). The study within hands examined the antimicrobial activity of *L. reuteri* strain on the bacteria involved in the development of acne. The study is also considered as first documented report on the inhibitory effects of *L. reuteri* on the proliferation of four pathogenic bacteria isolated from acne abscess in Shaqra city of Saudi Arabia. It was concluded that the strain of *L. reuteri* bacterium was a good producer of organic acids that works as antimicrobial agents towards pathogenic bacteria isolated from acne abscess. The results of the present study suggest that *L. reuteri* bacterium has potential as a probiotic for the skin.

The results are in close agreement with other findings obtained by other workers (Karim and Rameid, 2011; Kang *et al.*, 2012). When the obtained results were compared to antibiotics findings (Table 1 and Fig. 3); it could be concluded that the culture filtrates obtained from *L. reuteri* was more or less effective than some of the standard antibiotics used. But in some other bacteria, on the other hand, was more effective. According to high antimicrobial activity of *L. reuteri* culture filtrate which can justifies its uses in probiotic medicine; further research work should be done using this bacterial culture filtrate involving the edaphic factors such as PH. More studies are also needed to isolate and characterize the active compounds from *L. reuteri* to be tested on the skin *in vivo* to determine the toxicity and the optimum dose to be used as effective management tool for acne.

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