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# Capability of *Lactobacillus acidophilus* supernatant to inhibit production of lipase from methicillin-resistant *Staphylococcus aureus*

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**ABSTRACT**

The ability of acid supernatants of *Lactobacillus acidophilus* isolated from a vinegar were tested against methicillin resistant *Staphylococcus aureus* (MRSA) with lipolytic activity isolated from acne pimple. Results revealed a significant effect of cell free supernatant on MRSA isolates in comparison with control. Furthermore, subinhibitory concentrations of acid supernatants found to be very effective in inhibiting the production of lipase from biofilm and planktonic cells of MRSA isolates. What's more, acid supernatants of *L. acidophilus* showed inhibiting effect on the crude lipase in egg yolk agar plates.

## Introduction

The staphylococci are gram-positive spherical cells, usually arranged in grape-like irregular clusters. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. Staphylococci rapidly develop resistance to many antimicrobial agents and show difficult therapeutic problems (1).

Methicillin-resistant *S. aureus* (MRSA) and other multidrug resistant bacteria have emerged as serious pathogens over the past decade and, despite major research efforts aimed to find an effective drugs; increasing resistance has compromised therapy (2).

*Staphylococcus aureus* have a greater likelihood of developing biofilms this mode of colonization facilitates infections that are often difficult to treat and produce high morbidity and mortality. Antimicrobial agents and host response are often unable to clear these biofilms, and alternative approaches to controlling *S. aureus* infections are needed (3).

Clinical isolates of *Staphylococcus aureus* that were isolated from deep infections produced higher amounts of lipase than those derived from superficial locations (4). This indicates that lipase activity may be important for nutrition and/or dissemination of bacteria. Beyond that, lipases may play a direct role in virulence. It has been postulated that these particular enzymes may exert an influence on pathogenesis of *Staphylococcal* skin infection (5).

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In vitro studies have shown that purified lipase influenced functions of different human immune cells, such as the chemotaxis of neutrophils and granulocytes (6). The strongest hint that secreted lipases are involved in pathogenesis is the detection of anti-lipase IgG antibodies in patients suffering from *S. aureus* infections fortifying the virulent potential of extracellular lipases (7).

Previous work at out laboratory (dept. of Biology, College of Science, University of Baghdad) found that *Lactobacillus acidophilus* supernatants succeeded in eliminating wound infection caused by *S. aureus* (8). Accordingly, present work aimed to investigate the role of *L. acidophilus* crude supernatant in the inhibition of lipase production from planktonic cells as well as biofilm of methicillin-resistant *S. aureus* (MRSA). To our knowledge this is the first time that *Lactobacillus* supernatant has been used to inhibit lipase production.

## Materials and methods

### Microorganisms

Nine clinical isolates of MRSA were isolated from acne pimples by inoculating the pimple materials on Mannitol salt agar plates (HiMedia, India, pH7.2) and incubating at 37 °C for 24 hr. and identified according to Holt *et al.* (9). While *L. acidophilus* was isolated from a vinegar sample by spreading 1 ml of vinegar onto MRS agar (HiMedia, India, pH5.5) plates which were subsequently incubated anaerobically at 37 °C for 48 hr. Identification of *L. acidophilus* was performed by phenotypic criteria. It was initially tested for colony morphology, Gram reaction, catalase

activity, motilily test, and gas production from glucose. It was further characterized by its carbohydrate fermentation (10,11).

*Staphylococcus aureus* ATCC 6538P standard strain was used as a positive control.

**Inhibitory effect of L. acidophilus supernatant on S. aureus**

Overnight *L. acidophilus* cultures contained 1.5 × $10^8$ colony forming unit/ml at 37°C for 24 hr. These cultures were centrifuged at 6000 rpm/min for 10 min at 4°C. The resulting supernatants were filtered through a 0.2-µm membrane filter to remove the remaining bacteria and debris. All supernatants were cultured on MRSA in order to confirm the absence of lactobacilli cells (12). Aliquots of supernatants were neutralized with 8N NaOH to pH7 were prepared as well (13). Thereafter, double fold serial dilutions were made from these supernatants and stored at 4°C until usage.

Well diffusion method described by Ikeagwu *et al*. (14) was followed to detect *L. acidophilus* supernatant inhibition activity by spreading *S. aureus* suspension (1.5 × $10^8$ cfu/ml) over Mueller Hinton agar (HiMedia, India, pH7.2) plates using sterile cotton swab. Then 5 mm in diameter wells were cut out from the surface of previously cultured Mueller Hinton agar plate. Wells, in triplicates, were filled with 50 µl of *L. acidophilus* acid supernatant, neutralized supernatant and MRS broth only. After an aerobic incubation at 37ºC for 24 hr., the diameter of inhibition zones caused by *L. acidophilus* supernatants, were measured.The MIC values were defined as the lowest concentration inhibiting completely the bacterial growth (15).

**Detection of lipase production**

**1- Planktonic cells of *S. aureus***

Egg yolk agar plates were streaked with thick line of *S. aureus* isolate in triplicates. The plates were incubated at 37 °C for 24 hr. and the zone of clearance, which corresponded to the lipase activity of the isolate, was noticed.

**2- Biofilm of *S. aureus***

The air-liquid interface (ALI) assay described by Caiazza and O'Toole (16) was followed to produce biofilm. Glass test tubes were placed at an angle of 45 ◦ relative to horizontal, and an overnight brain heart infusion broth culture of *S. aureus* was slowly applied to test tubes. All tubes incubated at 37 °C for 48 h. after the incubation period, biofilms grew radially and concentrically on the surface of the liquid media were harvested after 24 h of growth by lifting the biofilm intact from the broth surface using sterile forceps and placed on egg yolk agar for the detection of lipase production as mentioned earlier.

**3- Crude *S. aureus* lipase**

Overnight growth of all isolates in BHI at 37 °C was centrifuged at 6000 rpm/min for 10 min at 4°C, filtered through 0.22 µm membrane filters and submitted for sterility checking by inoculated 0.1 ml of resultant supernatant onto nutrient agar plates and cultivated at 37 °C for 24 hr. Lipase activity in cell free supernatant was detected by egg yolk agar method as mentioned earlier.

**Inhibition of lipase production**

Subinhibitory concentration (1:16 dilution) of *L. acidophilus* acid supernatants was added to the egg yolk agar plates, thereafter, the same procedure mentioned previously was followed to investigate the lipase production either by cell free supernatant, biofilm or planktonic cells of *S. aureus*.

**Statistical analysis**

Results were assessed by analysis of variance (ANOVA) and Duncan test, using SPSS version 7.5. Differences were considered significant at $P \leq 0.05$.

## Results and discussion

**Inhibitory effect of *Lactobacillus acidophilus* supernatant on *S. aureus***

Data presented in table 1 revealed that acid supernatants of *L. acidophilus* had a significant effect on all MRSA isolates in comparison to control. Therefore it was used for lipase inhibition assays.

Table 1: Inhibitory effect of *Lactobacillus acidophilus* acid supernatant on methicillin resistant *Staphylococcus aureus.*

| *S.aureus isolate* | Diameter of inhibition zone (mm)* Dilution of acid supernatant | | | | |
|---|---|---|---|---|---|
| | 1/1 | 1/2 | 1/4 | 1/8 | 1/16 |
| *S.aureus* 1 | 20.6 | 14.3 | 11.6 | 6.6 | 0 |
| *S.aureus* 2 | 10.3 | 7 | 4.3 | 0 | 0 |
| *S.aureus* 3 | 5.3 | 3.6 | 0 | 0 | 0 |
| *S.aureus* 4 | 7.6 | 5.3 | 2 | 0 | 0 |
| *S.aureus* 5 | 5 | 2.3 | 0 | 0 | 0 |
| *S.aureus* 6 | 11.6 | 9.6 | 5 | 0 | 0 |
| *S.aureus* 7 | 8.6 | 6.6 | 2.3 | 0 | 0 |
| *S.aureus* 8 | 12 | 8 | 3.6 | 0 | 0 |
| *S.aureus* 9 | 7.3 | 3 | 0 | 0 | 0 |
| LSD | 1.650 | 1.264 | 1.11 | 0.33 | 0 |

Each datum is a mean of triplicate. LSD= least significant difference.

Generally, the lactic acid bacteria (LAB) are the most implicated of the probiotic organisms, particularly those of the genera *Lactobacillus* and *Bifidobacterium*, which protects their territory by secreting acids, thereby creating an environment which is inhospitable to disease-causing bacteria. Lactobacilli change the oxidation-reduction potential through its production of metabolites by making the environment less encouraging for organisms requiring oxygen. This action contributes to the overall inhibiting effect of these probiotic bacteria (17).

Voravuthikunchaia (18) stated that the antimicrobial effect of the lactobacilli might be fundamentally attributed to cause both growth inhibition and cell death. A number of metabolic by-products such as $H_2O_2$ and bacteriocin are believed to contribute to their antibacterial activity. Both substances produced from this bacteria appeared to have significant activities against classical antibiotic-resistant bacteria, MRSA. However, not all lactobacilli are effective against pathogenic bacteria.

The acidogenic capacity of lactobacilli significantly depressed culture pH as is characteristic of this genus. We suggest that this, rather uniform trait, may be one of the contributing factors to the consistently observed *S. aureus* growth inhibition phenomenon. It has been suggested that the role of organic acid as contributing to such inhibition and, certainly, lactobacilli are known to play a role in helping to maintain an acidic-type vaginal environment (19).

**Inhibition of lipase production**

Subinhibitory concentrations of acid supernatants of *L. acidophilus* were found to be very effective in inhibiting lipase activity. Since no zone of clearance was noticed on the egg yolk agar plates neither in planktonic (figure 1) nor in biofilm of MRSA (figure 2). Figure 2, on the other hand, illustrated the effect of acid supernatants of *L. acidophilus* on biofilm as the diameter of biofilm has reduced.

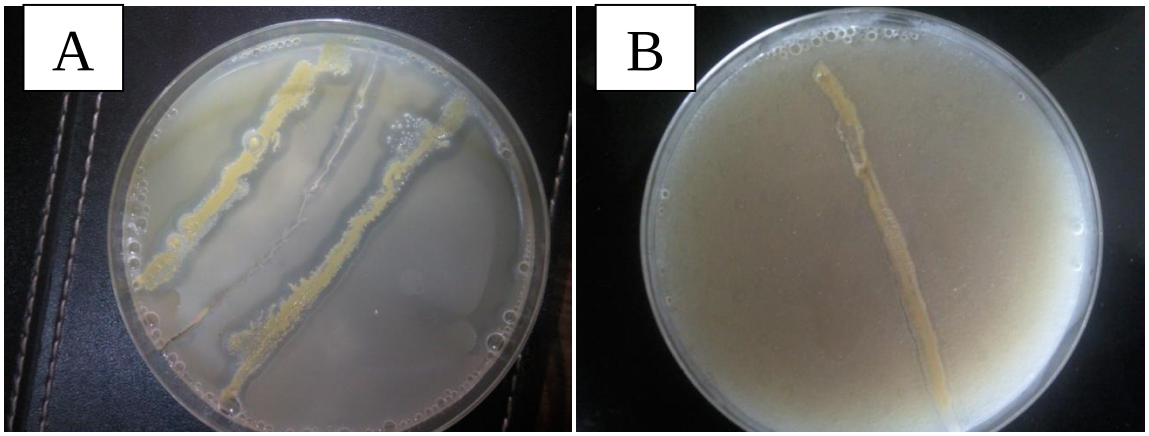


Figure 1: Inhibitory effect of *Lactobacillus acidophilus* acid supernatant on planktonic cells of methicillin resistant *Staphylococcus aureus*. A) Before adding the supernatant. B) After the addition.

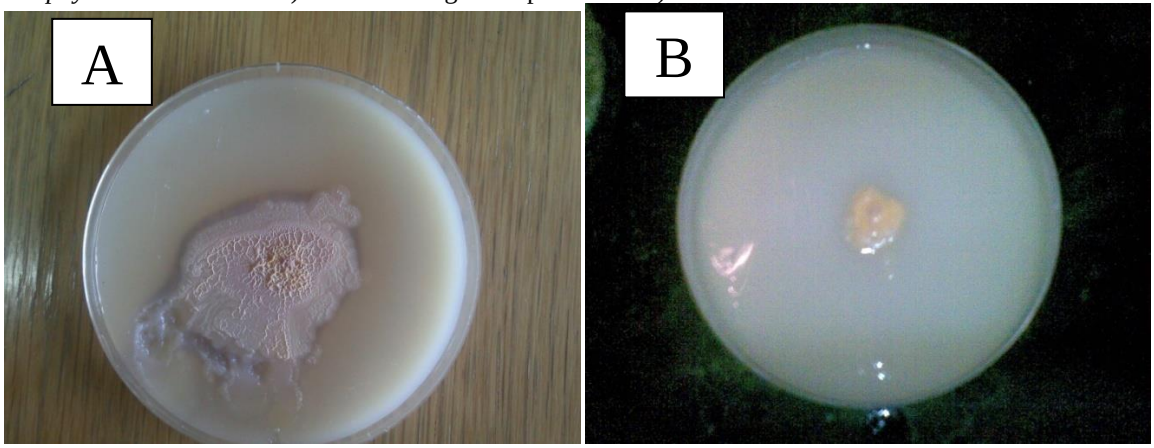


Figure 2: Inhibitory effect of Lactobacillus acidophilus acid supernatant on Biofilms of methicillin resistant Staphylococcus aureus. A) Before adding the supernatant. B) After the addition.

Cell-density-dependent gene regulation by quorum sensing systems has a crucial function in bacterial physiology and pathogenesis. Queck *et al.* (20) demonstrated that the *S. aureus agr* quorum-sensing regulon is controlling lipase production. Therefore, *L. acidophilus* supernatant may have a crucial role in intercepting the quorum sensing signaling molecules involved in lipase production.

Although the role of bacterial lipase activity in chronic skin diseases has not been well documented, lipase might play an important role in facilitating bacterial colonization in nutrient-limited environments such as the human skin. Human skin is weakly acidic, and indeed *S. aureus* lipase activity showed a preference for acidic conditions of around pH 6 (21). Moreover, *S. epidermidis* lipase SEL-3 shows a similar pH preference to *S. aureus* lipase (22): this is unusual for bacterial lipases, which generally exhibit the highest activity at alkaline pH. Such different pH preferences imply that *S. aureus* lipase might be one of the crucial virulence factors that is persistent on human skin.

Regarding figure 3, acid supernatants of *L. acidophilus* has an inhibitory action on crude lipase, i.e. the lipase was deactivated in the presence of *Lactobacillus* supernatant.

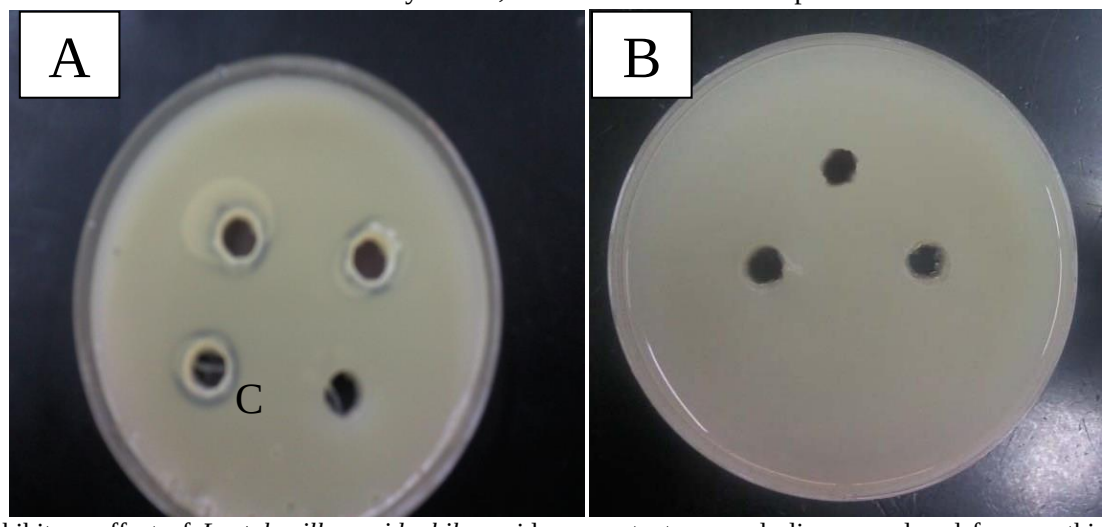


Figure 3: Inhibitory effect of *Lactobacillus acidophilus* acid supernatant on crude lipase produced from methicillin resistant *Staphylococcus aureus*: A-Before adding the supernatant, C=control (sterile MRS broth). B-After the addition of supernatant.

Kuroda *et al* (23) found that a sub-minimum inhibitory concentration of farnesol inhibits lipase activity. A quantitative lipase assay revealed that the inhibitory action of farnesol appears to be the result of the inhibition of lipase activity rather than of its secretion into the culture medium.

As a conclusion, *Lactobacillus* supernatant was able to prevent lipase production from planktonic cells and biofilm of *S. aureus* in addition to deactivation of lipase activity.

Further studies should be carried out to identify the interference of *Lactobacillus* supernatant with quorum sensing mechanism responsible for lipase production. Also much work is needed in order to isolate and purify the active antimicrobial compounds in the supernatant responsible for the inhibition of lipase production in order to decrease the level of the effective dose.

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# مقدرة طافي *Lactobacillus acidophilus* على تثبيط انتاج اللايبيز من العنقوديات الذهبية المقاومة للميثيسيلين.

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**الخلاصة:**

اختبرت قدرة الطافي الحامضي لبكتريا *Lactobacillus acidophilus* المعزولة من الخل ضد العنقوديات الذهبية المقاومة للميثيسيلين (MRSA) المحللة للدهون المعزولة من حب الشباب. اظهرت النتائج تاثيرا معنويا للطافي في عزلات MRSA مقارنة مع السيطرة. كما وجد ان التراكيز تحت المثبطة للطافي الحامضي كانت مؤثرة جدا في تثبيط انتاج اللايبيز من الخلايا الهائمة و الغشاء الحياتي لعزلات MRSA. فضلا عن ذلك امتد التأثير التثبيطي الى اللايبيز الخام في اطباق مح البيض.