

Lipolytic activity of *Staphylococcus aureus* from human wounds, animals, foods, and food-contact surfaces in Brazil

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Abstract

Introduction: *S. aureus* is of great importance to public health due to its pathogenicity. This study aimed to evaluate lipase production by *S. aureus* isolates from different sources.

Methodology: Lipolytic activity was determined using Tween-Calcium agar (48 hours; 35°C).

Results: Eighty-six percent of the isolates from human wounds were positive for lipase production. The frequencies of isolates positive for lipase production were 33.3% from cow udders, 15.4% from the nasal cavities of cattle, 82.9% from ricotta cheeses, and 100% and 91.7% from meat- and vegetable-contact surfaces, respectively.

Conclusion: The production of lipase varied among the isolates according to their source.

Key words: *Staphylococcus*; phenotypic characterization; lipolytic activity; virulence.

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Introduction

Staphylococcus aureus is widespread in nature and is the most frequent bacteria found in skin infections, although it is able to colonize nearly all human tissues [1]. In the nosocomial environment, skin infections caused by *S. aureus* are commonly found during the post-surgical phase; in the general community, these infections are frequently observed as abscesses, impetigo, folliculitis, boils, and necrotizing fasciitis [2].

S. aureus has been recognized worldwide as the etiological agent of most cases of foodborne disease, thereby making it of great interest in the field of the microbiological safety of foods [3,4]. The pathogenesis of infections caused by *S. aureus* depends on the production of surface proteins involved in bacterial adhesion to host tissues and of extracellular proteins such as catalase, thermonuclease, coagulase, and lipase [5]. *S. aureus* is known as a potential lipase-producing, opportunistic

pathogenic bacterium that is able to interfere with the immune host defense by eliminating granulocyte chemotaxis and reducing phagocytosis [5-7].

In addition to its role in the pathogenicity of *S. aureus*, the influence of lipase on food quality as a result of its ability to hydrolyze lipid components in foods is a concern. Microorganisms with lipolytic activity can increase the rate of food deterioration through their action on lipids and as a result of the accumulation of intermediate- and end-products that change the flavor of foods [4,8].

This study compared lipase production by Brazilian strains of *S. aureus* isolated from animals, human wounds, foods, and food-contact surfaces.

Methodology

The *S. aureus* isolates were obtained from infected human wounds in a college public hospital in the city of João Pessoa, Paraíba, Brazil (50 isolates); from cattle (udder: 30 isolates; nasal cavities: 13 isolates)

from a small farm in the city of Patos, Paraíba, Brazil; from bovine ricotta cheeses of different brands that were marketed in local supermarkets in the city of João Pessoa, Paraíba, Brazil (41 isolates); and from meat-contact surfaces (24 isolates) and vegetable-contact surfaces (24 isolates) in food service facilities of a public hospital in the city of João Pessoa, Paraíba, Brazil.

S. aureus was isolated as described elsewhere [9,10]. The samples (swabs of samples from wounds, animals, and food-contact surfaces; 25 g of cheeses) were first selectively enriched in brain-heart infusion broth (225 mL) supplemented with NaCl (75 g/1000 mL) for 24 hours. Then, tenfold serial dilutions of the cultures were prepared (10^{-1} – 10^{-4}) in sterile peptone water (10 g/1000 mL), and 100- μ L aliquots were plated on salt mannitol agar (for samples from wounds and animals) or Baird-Parker agar (for samples from food-contact surfaces and cheeses), both supplemented with potassium tellurite (10 g/1000 mL) and egg yolk emulsion (50 mL/1000 mL), at 35°C. Typical colonies were selected and placed on nutrient agar slants. The isolated strains were then identified using biochemical tests (Gram stain, catalase activity, coagulase reaction, thermonuclease production, and mannitol and glucose fermentation). The strains that were confirmed as *S. aureus* were refrigerated (7°C) in nutrient agar slant tubes.

The lipolytic activity of the isolates was determined according to Sierra (1957) [11]. The isolates were first cultivated in brain-heart infusion broth for 24 hours at 35°C. Next, an aliquot (100 μ L) of the culture was grown on sterile plates containing Tween-Calcium agar (10 g/1000 mL peptone, 5 g/1000 mL sodium chloride, 0.1 g/1000 mL calcium chloride, 15 g/1000 mL agar, and 10 mL/1000 mL Tween 80) for 48 hours at 35°C. Afterwards, lipase-producing strains were identified by the presence of a halo of calcium soap crystals around their colonies that resulted from the action of lipase on Tween 80 (oleic acid ester). The experiments were carried out in

triplicate on three different occasions and showed consistent results.

Results

A total of 182 isolates of *S. aureus* from different sources were tested for lipase production. From the isolates obtained from human wounds, 43/50 (86%) produced lipase. In the isolates from animals, the highest frequency (53.3%; 16/30) of lipolytic activity was found in isolates from udders, while few isolates from nostrils showed lipolytic activity (15.4%; 2/13). Of the isolates obtained from cheeses, 82.9% (34/41) produced lipase. All isolates from meat-contact surfaces and 91.7% (22/24) of the isolates from vegetable-contact surfaces produced lipase. Of the total number of isolates that were tested, 77.5% (141/182) were positive for lipase production (Table 1). Repeating the experiments showed consistent results.

Discussion

The higher frequency of positive lipase production (86%) among the *S. aureus* isolates from human wounds (nosocomial environment) may be related to the need in these isolates of the lipolytic ability for colonization and establishment of the infectious process in human tissue. Early studies reported on the influence of lipases on abscess formation in rats and on the greater severity of infections caused by lipase-producing *S. aureus* strains [12]. In the same study, higher counts of *S. aureus* (10^4 - 10^7 CFU/g) in the kidneys, livers, and spleens of rats were found in infections from positive lipase strains, which indicates the importance of this enzyme for the formation of abscesses and the invasion of organs.

Some researchers have reported that most *S. aureus* isolates obtained from cattle are not able to produce lipase, as opposed to isolates from humans, which show the ability to produce lipase [13,14]. A study carried out in Brazil found that most *S. aureus* cattle isolates were negative for lipase production and

Table 1. Lipase activity of isolates of *S. aureus* from different sources in Brazil

| Source | Number of isolates | Isolates with lipolytic positive activity | |
|-----------------------------|--------------------|---|-------------|
| | | N° | % |
| Human wounds (hospital) | 50 | 43 | 86 |
| Animals (udder) | 30 | 16 | 53.3 |
| Animals (nostrils) | 13 | 2 | 15.4 |
| Cheese (ricotta) | 41 | 34 | 82.9 |
| Meat-contact surfaces | 24 | 24 | 100 |
| Vegetables-contact surfaces | 24 | 22 | 91.7 |
| Total | 182 | 141 | 77.5 |

that most of the lipase producers were obtained from udders, which comes into contact with the handler during milking. This contact could increase the possibility of human contamination of the udder with lipase-producing *S. aureus* [15]. Of the isolates that were obtained from cow udders in this study, 53% were positive for lipase production. Our findings confirm other studies that demonstrated the lack of lipase production among isolates of *S. aureus* from cattle.

Most *S. aureus* isolated from foods (ricotta cheese) and from meat- and vegetable-contact surfaces (food processing environments) were positive for lipase production. A low frequency of lipase production among isolates of *S. aureus* from chicken samples (22.8%) [8] and an absence of lipase production among isolates of *Staphylococcus* spp. from fermented sausages [16] were observed in earlier studies, while lipase production in all isolates of *S. aureus* obtained from cow's milk was found in another study [17].

The idea that *S. aureus* isolates obtained from ricotta cheeses in this study are of human origin is provocative due to the high frequency of lipolytic activity observed among these isolates and the already reported ability to produce lipase in most of the *S. aureus* isolates obtained from humans [13,14]. Contamination of these samples (cheeses) may be related to improper handling, suggesting that meat- and vegetable-contact surfaces are contaminated in a similar manner. Nevertheless, the high frequency of lipolytic activity among the isolates from foods and food-contact surfaces reveals the potential of these isolates to spoil foods, particularly through their action on lipids, which would result in decreased food quality and shelf life.

Although only a limited number of isolates of *S. aureus* from animals and food-contact surfaces were analyzed, the findings of this study show a clear difference in the frequency of lipase-producing isolates from different sources, which suggests that this feature could be related to the environment in which the isolate is harbored. The higher frequency of lipase-producing isolates in human wounds, foods, and food-contact surfaces demonstrates the need to control *S. aureus* in health care and food-processing environments because lipase production enhances pathogenicity and food-spoiling ability of this bacterium.

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