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Protease and lipase activity of *Staphylococcus aureus* obtained from meat, chicken and meatball samples

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ABSTRACT

A total of 270 samples of raw meat (minced calf meat, chicken carcasses) and meatballs (ready-to-eat meat) were analyzed for the presence of *Staphylococcus aureus*. From these samples, 148 *S. aureus* isolates were obtained, which were investigated for proteolytic and lipolytic activity under psychrotrophic conditions (+4 ^{o}C and +20 ^{o}C) associated with meat spoilage. Both proteolytic and lipolytic bacteria can change the quality of raw meat and decrease the shelf-life, resulting in spoilage. *S. aureus* isolates were not showed proteolytic and lipolytic activities at +4 ^{o}C. It was found that 96.2 % , 89.1 % and 75.0 % of the *S. aureus* isolates obtained from meat, meatball and chicken samples showed proteolytic activity at +20 ^{o}C. Few of the isolates obtained from chicken samples (22.8 %) showed lipolytic activity under psychrotrophic conditions (+20 ^{o}C).

Keywords: Protease, Lipase, *Staphylococcus aureus*

1. INTRODUCTION

Staphylococcus aureus is considered the third most important cause of disease in the world amongst the reported food-borne illnesses [1]. *S. aureus* is present on the skin and mucosae of humans and animals, and in the environment [2]. Some strains, so-called “endemic strains”, are present in some processing plants, such as poultry processing lines [3]. As a consequence food products may orginally become contaminated during or after processing. *S. aureus* has been isolated from several foods: meat and meat products, chicken, milk and dairy products, fermented food items, vegetables, fish products, etc. [4].

It is well known that the organism produces various extracellular active substances, such as coagulase, hemolysins, nuclease, acid phosphatase, lipase, proteases, fibrinolysin, enterotoxins, and toxic shock syndrome toxin. These active substances are thought to contribute to the pathogenicity of the organism [5]. *S. aureus* causes a variety of infectious diseases such as furuncles, abscesses, pneumonia, osteomyelitis, and several forms of carditis and meningitidis. Many researchers reported that strains of *S. aureus*, which had strong proteolytic activity, isolated from chickens suffering from endematous and necrotic dermatitis. In addition, they conjectured that there might be a relationship between the protease and the dermatitis in the chickens, because dermatolysis was observed in young chickens and mice when they were inoculated subcutaneously with more than 10^7 cells of protease positive strains [6].

The importance of staphylococcal lipases, like other microbial lipases, results from their significance in bacterial lipid metabolism and their involvement in pathogenic processes [7]. Most of the known staphylococcal lipases are produced by pathogenic members of the genus, i.e., *S. aureus* and *S. epidermidis*. While it is possible that lipases might support the persistance of these strains in the fatty secretions in mammalian skin and thus have an indirect influence on their pathogenic potential, a direct

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involvement of lipases in pathogenesis remains to be demonstrated [8]. *S. aureus* produces lipase in infected patients [9]. Furthermore, lipase interferes with the phagocytosis of the infectious lipase-producing *S. aureus* cells by host granulocytes, thus indicating a direct involvement of lipase in pathogenesis [10].
In previous studies, *S. aureus* was isolated in 3 of 22 (13.6 %) hamburger patties [11], in 17 of 144 (11.8 %) meatball samples [12]. Normanno et al. [13] showed that *S. aureus* was found in 141 out of 293 meat products (48.1 %). Such high levels of *S. aureus* raise concerns and helped determine the type of samples that were analyzed during this study.
However, protease and lipase activity of *S. aureus* isolated from meat/chicken and meatball samples has not been investigated in detail. The prupose of this study was to investigate the incidence of *S. aureus* strains isolated from meat/chicken and meatball samples and proteolytic and lipolytic properties on agar media.

2.MATERIALS AND METHODS

2.1. The collection of samples

Ninety samples of raw calf meat (mince), 90 samples of chicken carcass and 90 samples of meatballs (ready-to-eat-meat; ingredients: minced meat, chopped onions, parsley, garlic and spices. Ingredients are mixed in a bowl and formed by hand and fried in vegetable oil.) were purchased from various supermarkets in Ankara. Individual meat samples (about 100 g) were collected in sterile polyethylene packs, placed on ice, immediately transported to the laboratory and processed within 2h after collection.

2.2. Isolation and identification of *S. aureus*

The samples (25 g) were weighed into sterile stomacher bags diluted with 225 ml sterile buffered peptone water (BPW; Oxoid CM 509) and homogenized in a stomacher (Lab. Lemco 400) for about 2 min. The samples were diluted with BPW, and 0.1 ml portions of dilution levels were streaked on Baird-Parker (BP) agar (Oxoid CM275) supplemented with egg yolk-tellurite emulsion (Oxoid SR54), and incubated at 37 ^{o}C for 24 h. For confirmation a maximum of 5 selected colonies (grey-black, surrounded by a dull halo) per plate were isolated and cultured on slants of Brain-Heart Infusion (BHI, Oxoid CM225). The identification was carried out using the following tests: gram staining, production of coagulase, catalase, DNAse and oxidation and fermentation of mannitol [14]. For comparison, reference strain *S.aureus* ATCC 25923 was included in all test controls.

2.3. Determination of proteolytic and lipolytic activity of *S. aureus* on agar media

Proteolytic and lipolytic activities were assessed as described by Harrigan and McCance [15]. The enzymes lipase and protease were detected on the following media: Proteolytic count was performed using the spot technique by plating bacterial suspension on Skim Milk Agar (1.5 % agar and 10 % skim milk) and incubated at 20 ^{o}C and +4 ^{o}C for 10 days. The presence of transparent zones around the spots was recorded as positive strains referring to protease production [15]. Lipolytic counts (LP) were determined using Nutrient Agar (NA) containing tributyrin. The medium was prepared 10 g of tributyrin (PM4, Oxoid) and 28 g of NA (CM4, Oxoid). Plates were incubated at 20 ^{o}C and 4 ^{o}C for 72 h determine viable lipolytic counts, and lipolytic activity was determined by measuring clear zone around each colony [15].

2.RESULTS AND DISCUSSION

A total of 148 strains of *S.aureus* were used in this experiment. Of these isolates, 54 were obtained from minced meat, 48 from chicken and 46 from meatball samples.

Staphylococcus aureus is one of the most important foodborne pathogens found in meat and meat products and *S. aureus* intoxication in debilitating illness [1]. Although staphylococci are commonly found on the skin of a wide variety of mammals and birds and on environmental surfaces, humans are thought to be the primary source of strains associated with food matrix staphylococcal intoxication [16].

The high prevalence of *S. aureus* detected in the samples examined (Table 1). The majority isolates obtained from 90 calf minced meat samples were *S. aureus* (60.0 %). Of the 90 chicken carcass samples examined, 53.3 % were *S. aureus.* Of the 90 meatball samples examined, 51.1 % were *S. aureus*. Similar results were also reported by Schlegelova et al. [17], Alvarez-Astorga et al. [18], and Aycicek et al. . [12] in beef, and chicken parts and processed chicken products and meatballs respectively. Jay [2] reported that the presence of *S. aureus* in foods commonly indicates contamination that may be directly introduced into the food by workers who have skin lesions containing *S. aureus*, or sneezing or coughing.

A number of factors contribute to the virulence of *S. aureus*, including deoxyribonuclease (DNase), catalase, lipases, proteases, and hemolysins [19]. The importance of protease and lipase, which causes bacterial virulence, was proven in several studies. However, there was less study in the protease and lipase activities of *S. aureus* strains isolated from meat/chicken and meatball samples. According to our results *S. aureus* isolates are not showed proteolytic and lipolytic activities at +4 ^{o}C. The proteolytic and lipolytic activities at +20 ^{o}C of the *S. aureus* isolates are shown in Table 2. Fifty-four *S. aureus* isolated from meat samples and 52 of them produced protease, none of them produced lipase. Forty-eight *S. aureus* isolated from chicken samples and 36 of them produced protease, 11 of them produced lipase. Forty-six *S. aureus* isolated from meatballs and 41 of them produced protease, none of them produced lipase (Table 2). It is important to note that 96.2 % , 89.1% and 75.0 % of the *S. aureus* obtained from meat, meatball and chicken samples displayed proteolytic activity under psychrotrophic conditions. Takeuchi et

al. [5] used skim milk agar to detect protease of the isolates and reported that protease positive strains of *S. aureus* are isolated frequently from diseased chicken. Kuramasu et al. [6] isolated strains of *S. aureus*, which had strong proteolytic activity, isolated from chickens suffering from edematous and necrotic dermatitis.

Up to date most of the staphylococci lipases have been purified and some biochemical properties investigated. Lipase studies were performed mainly with the coagulase-positive species *S. aureus*, a species pathogenic for humans and animals. Rosenstein and Götz [20] reported that most of the known staphylococcal lipases are produced by pathogenic members of the genus, i.e., *S. aureus* and *S. epidermidis*. According to our results, *S. aureus* isolates in meat, chiken and meatball samples are proteolytic rather than lipolytic. Few of the isolates obtained from chiken samples (22.8 %) displayed lipolytic activity under psychrotrophic conditions.

In conclusion, the large amount of *S. aureus* found in meat and meat products, represents a health hazard to the consumers, and emphasises the need for improved hygiene practise at levels in the food industry. Proteolytic psychrotrophic bacteria are the main microorganisms responsible for spoilage of meat and meat products, due to their ability to produce proteases. This study also indicates that *S. aureus* isolates from meat, chicken and meatball samples displayed proteolytic activity under psychrotrophic conditions.

Table 1. Prevalence of *S. aureus* in the 270 samples of meat/chicken and meatball samples

Source of samples	No. of samples	No. of *S.aureus*	%
Calf minced meat	90	54	60.0
Chicken carcass	90	48	53.3
Meat balls	90	46	51.1
Total	270	148	

Table 2. Proteolytic and lipolytic activities at +20 ^{o}C of the *S. aureus* strains isolated from meat samples

Samples	No. of strains	Proteolytic activity		Lipolytic activity	
		No	%	No	%
Meat	54	52	96.2	-	-
Chicken	48	36	75.0	11	22.9
Meatball	46	41	89.1	-	-
Total	148	129		11	

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