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EXTRACELLULAR LIPASE PROFILE OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM WOUND.

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ABSTRACT
Staphylococcus aureus has always been reported for the clinical importance in infection while the industrial potential can easily be overlooked; therefore this research was designed to study the extracellular lipase activity of *Staphylococcus aureus* isolated from surgical wound culture. Forty-one isolates of *Staphylococcus* sp. were collected, inoculated and incubated; the isolates were confirmed using cultural, morphological and biochemical characteristics. The lipolytic activity was determined qualitatively using Tributyrin agar. The lipase produced was further quantified. Twenty-six (63.4%) of the isolates fermented mannitol. The isolates of *Staphylococcus aureus* were coagulase and catalase positive. All the confirmed twenty-six isolates were also positive for lipase production by hydrolysing the media. The specific activities of lipase produced by the *Staphylococcus aureus* strain ranged from 0.008µm/min/mg to 0.050µm/min/mg. The *Staphylococcus aureus* strains reached their peak of lipase production at different time intervals. Twenty-seven percent of the isolate reached a peak of lipase production between 20-25hrs of incubation while twenty-three percent (23%) each reached peak between 10-15hrs and 15-20hrs, the next twelve percent (12%) reach their peak between 35-40hrs and approximately four percent (4%) attained their peak between 25-30hrs and 30-35hrs. The differences in the growth rate and attainment of peak of lipase production may be an indication that the *Staphylococcus aureus* varied widely in their physiological and biochemical properties.

KEYWORDS: Enzyme, Lipase, Mannitol, *Staphylococcus aureus*

INTRODUCTION
Staphylococcus aureus is a leading cause of bacteremia as well as skin and soft tissue infection in animals and humans. The severity of infection caused by this pathogen is due to its ability to rapidly acquire and lose resistance and virulence determinants (Noto *et al.*, 2008). It is a frequent colonizer of the nasal epithelium of animal and humans. In addition to that, studies have shown that nasal colonization predisposes an individual to be at greater risk of infection with *S. aureus.* Furthermore, 20 to 30% of healthy individuals are frequently or persistently colonized by *S. aureus*, while about 70% are intermittently colonized (Kennedy and DeLeo, 2009; Aqel *et al.*, 2015;Jenkins *et al.*, 2015).

Staphylococcus aureus is an aerobic or facultative anaerobic which colonises the skin, nasal passage and axillae of humans. It occurs in grape like clusters when viewed through the microscope and has large round golden yellow colonies often with beta haemolysis when grown on blood agar (Murray *et al.*, 2003; Ryan and Ray, 2004).

Staphylococcus aureus is always considered to be a potential pathogenic bacteria. It is responsible for many of the human diseases. The clinical importance and syndromes caused by this bacteria can be grouped as: cutaneous infections which includes folliculitis, impetigo, wound infections, toxin-mediated infections that includes toxic shock syndrome, food poisoning, scalded skin syndrome which is seen in children under the age of four, Other ailments associated with this organism includes pneumonia, bacteremia, endocarditis, osteomyelitis and septic arthritis (Moura *et al.*, 2012).

Members of the genus *Staphylococci* are known to harbor a large repertoire of virulence gene coding for pyogenic superantigens, exfoliative, leukocidine, hemolysin, toxic shock syndrome toxin -1, Lipase, biofilm formation proteins, coagulase, fibrinolysin, protease and lipase (Peacock *et al.*, 2002). These virulence factors are associated with attachment, persistence, evasion/destruction of host defenses, tissue

invasion/penetration and toxin-mediated disease symptoms (Spanu *et al*., 2012).

This bacteria forms large yellow colonies and it is often haemolytic in blood agar. Nearly all strains of *Staphylococcus aureus* produce the enzyme Coagulase; being coagulase positive is an important feature of this bacterium (Ryan and Ray, 2004). A number of cell surface proteins and secretory toxins produced by S. *aureus* have been shown to promote virulence by facilitating evasion of the host immune response. In addition to that, *S. aureus* is regarded as one of the most prevalent cause of food poisoning worldwide and known to produce more than 30 different extracellular metabolites (Aydin *et al.*, 2011).

It has both bound and free coagulase while the other staphyloccocci are coagulase negative. The surface of this bacterium is coated with Protein A and Protein A is not found on the surface of coagulase negative staphylococci. It ferments mannitol and most other staphylococci are mannitol negative. *Staphylococcus aureus* can grow at a temperature range of 15 to 45^{o}C and at NaCl concentrations as high as 15 percent. Salt is useful as a selective constituent in isolation media.

Lipase is the most abundant enzyme produced by this bacterium. Lipases support the colonisation and growth of the bacteria by the cleavage of the triacylglycerols derived from the sebum of the skin (Longshaw, 2000). The clinical studies have proven that *Staphylococcus aureus* that were isolated from the deep wound infections produced higher amounts of lipase than those isolated from the more superficial ones (Rollof*et al.*, 1987) suggesting that lipase activity might be important for nutrition or dissemination of the bacteria. Enzymes from bacteria are used for industrial purpose. The Isolation and characterization of these enzymes are crucial steps in biotechnology. The extracellular enzymes derived from the bacteria serve for many purposes in the industry**.** To date, enzymologists have turned their attention to bacteria as a source of the enzymes as the bacterial origins of enzymes are cheaper and less time consuming than the animal sources. The advent of enzymology represents an important breakthrough in the biotechnology industry (Kirk *et al.*, 2002). In recent times, lipases (triacylglycerol acylhydrolase, E.C. 3.1.1.3) have emerged as key enzymes in swiftly growing biotechnology, owing to their multi- faceted properties, which find usage in a wide array of industrial applications, such as food technology, detergent, chemical industry and biomedical sciences (Pandey *et al.*, 1999).

Lipases (acylglycerol hydrolases, EC 3.1.1.3) catalyse both the hydrolysis and Synthesis of esters formed from glycerol and long-chain fatty acids. The microbial lipases are important in the biotechnological industry as they are stable in organic solvents, they do not require cofactors and they have a broad substrate specificity. Therefore, this research was aimed at determining the extracellular lipase profile of *Staphylococcus aureus* isolated from wound of patients attending Federal Medical Centre, Ido-Ekiti.

MATERIALS AND METHODS

Source of Organisms

The bacterial isolates used in this study were *Staphylococcus aureus* strains isolated from wounds of patients attending Federal Medical Centre, Ido-Ekiti. The identity of the isolates was confirmed by mannitol salt agar, blood agar, as well as the cultural, morphological and biochemical characteristics.

Screening for Lipase Activity

Tributyrine agar was used to screen the lipolytic activity of the various samples. The already prepared agar was poured into plate. It was allowed to gel and the organism was introduced by streaking gently in a single line on the agar plate, incubated for 24hours at 37ºC. The presence of the lipase activity of the colonies was detected as opaque halos occurred around the colonies positive for the lipase activity (Haba *et al.*, 2000).

Preparation of crude extract for enzyme activity

Inoculum was prepared by inoculating each bacterial culture (OD600~1.5) into a basal medium containing olive oil as carbon source in a well labeled flask. The flasks were kept in shaking incubator at 30℃ with 150 revolution per minute (RPM) for 18 h. One millilitre of inoculum was pipetted and incubated. Samples were drawn from each of the flasks at intervals of 6 h for a period of 48 h and later centrifuged at 5000 x g for 30 minutes at 4℃. Cell free supernatant corresponding to growth phase was used as the crude enzyme for assay and further analysis.

Lipase Assay

The crude enzyme preparation was the culture broth after separation of cells and particles. The enzyme was normally stored at 4℃ until used. Lipolytic activity was determined by colorimetric method based on the activity in cleavage of p-nitrophenylpalmitate (p-NPP) at pH 8.0 (Lotrakul and Dharmsthiti, 1997). The reaction mixture contained 180 µL of solution A (0:062 g of p-NPP in 10 mL of 2-propanol, sonicated for 2 min before use), 1620 µL of solution B (0.4% triton X-100 and 0.1 % gum Arabic in 50 mMTris-HCl, pH 8.0) and 200 µL of properly diluted enzyme sample. The product was detected at 410 nm wavelength after incubation for 15 min at 37℃. Under this condition, the molar extinction coefficient (410 nm) of p-nitrophenol (p-NP) released from p-NPP was 15000 M−1. One unit of lipase activity was defined as 1 µmol of p-nitrophenol (p-NP) released per minute by 1 mL of enzyme.

Protein Determination

Protein concentration was determined using the method developed by Lowry *et al*. (1951). Reagent A: 2% $NaCO_3$ in 0.1 N NaOH; Reagent B: 0.5% $CuSO_4.5H_2O$

in 1% Na or K tartarate; Reagent C: 100 ml of Reagent A + 2 ml of reagent B and Reagent E: 1:2 dilution of John's reagent water. Graded concentrations of Bovine Serum Albumin (BSA) in tubes were prepared. Then 0.3 ml of each concentration was measured into test tubes. 3 ml of reagent C was added, mixed and left for 10 min. Then 0.3ml of reagent E was added, mixed and left for 30 min. The optical density was read at 600nm. The graph of OD versus concentration of BSA was obtained as standard curve of BSA. The same was done for unknown substance and the protein concentrations from the standard curve were read off and obtained by multiplying with dilution factor. All readings were obtained in triplicates.

Determination of Specific Activity

The specific activity of each of the strains of *Staphylococcus aureus* was determined by dividing the lipase produced by the amount of protein produced.

RESULTS

All the Forty-one (41) isolates had visible growths on Nutrient agar. These organisms are gram positive, cocci shaped and catalase positive. Only 63.4% (Twenty-six) of the forty one isolates were able to ferment mannitol when grown on Mannitol Salt Agar, the rest fifteen isolates (36.5%) did not ferment mannitol. The morphology of those that fermented mannitol presents round and smooth colonies with golden yellow pigmentation. Sixteen (39.0%) of the isolates produced α-haemolysis, Twenty-four (58.5%) produced β-haemolysis while only one (2.4%) did not grow on blood agar. Thirty-six (87.8%) of the isolates are coagulase positive. These include all the twenty six (63.4%) isolates tested to ferment mannitol (Table 1).

Table 1: Quantitative result of *S. aureus* on growth media and reactions to some tests. Parameter Number of *S aureus*

Isolates	41 (100%)
Mannitols fermenter	26 (63.4%)
β-haemolysis	16 (39.0%)
α-haemolysis	24 (58.5%)
Coagulase positive	36 (87.8%)

The specific activity of the isolates ranged between 0.008µm/min/mg to 0.050µm/min/mg as shown in Table 2.Twenty-seven percent of the isolate reached a peak of lipase production between 20-25hrs of incubation while twenty-three percent (23%) each reach peak between 10-15hrs and 15-20hrs, the next twelve percent (12%) reach their peak between 35-40hrs and approximately four percent (4%) each reach their peak between 25-30hrs and 30-35hrs (Table 3).

Table 2: Specific activities of the lipase producing organisms.

Isolates	Lipase(ml/min/ml)	Protein(mg/ml)	Specific activity(µml/min/mg)
1	0.813	19.862	0.041
3	0.627	16.000	0.039
4	0.458	19.862	0.023
5	0.658	13.241	0.050
7	0.658	27.862	0.024
9	0.462	15.724	0.029
10	0.627	27.310	0.023
12	0.342	13.793	0.025
15	0.587	20.690	0.028
17	0.556	20.690	0.027
18	0.347	15.724	0.022
19	0.404	13.241	0.031
20	0.231	6.345	0.034
22	0.467	24.552	0.019
23	0.569	26.207	0.022
26	0.413	30.621	0.014
27	0.396	19.862	0.020
28	0.791	17.379	0.046
29	0.311	18.207	0.017
30	0.689	17.379	0.040
31	0.671	26.483	0.025
32	0.404	16.828	0.024
34	0.147	17.655	0.008
37	0.333	8.552	0.039
38	0.493	23.448	0.021
40	0.427	16.276	0.026
41	0.447	16.026	0.022

Note: Specific Activity = Lipase/ protein

Table 3: Rate of attaining peak of lipase production.

Incubation Period (Hrs)	Percentage Production (%)
5-10	2 (7.69%)
10-15	6 (23.08%)
15-20	6 (23.08%)
20-25	7 (26.92%)
25-30	1 (3.85%)
30-35	1(3.85%)
35-40	3 (11.54%)

DISCUSSION

It is estimated that 20% of the human population are long-term carriers of *Staphylococcus aureus* (Kluytmans *et al.*, 1997) which can be found as part of the normal skin flora and in anterior nares of the nasal passages (Cole *et al.*, 2001). *Staphylococcus aureus* is the most common species of staphylococcus to cause Staph Infection; it is a successful pathogen its bacterial immuno-evasive strategies (Cole *et al.*, 2001). *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteraemia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections.

The clinical importance of *Staphylococcus* sp. is often documented as they cause diseases among many different organisms. Although not fully described the enzymes proteinase and lipase are studied for their role in the pathogenicity and illness process (Arvidson, 2000). All the *Staphylococcus aureus* isolates used in this study produced appreciable amount of the enzyme lipase ranging from 0.008µm/min/mg - 0.050µm/min/mg, this result is also similar to what Xie *et al*., (2012) reported for both *Staphylococcus aureus* and *S. epidermids* where considerable amount of lipase was produced using different substrates. However, they attain a peak of lipase production at different phases of growth (Table 3). Approximately, Twenty-seven percent (27%) of the organisms reached a peak of lipase production between 20-25hrs of incubation period while about 7.69% reached a peak of enzyme production after 5-10hrs. The differences in the growth rate and attainment of peak of enzyme production may be an indication that the *Staphylococcus aureus* varied widely in their physiological and biochemical properties. This same trend was reported by Odunfa and Oyewole (1986) for *Bacillus subtilis* used in fermentation of African locust beans. Pogaku *et al*., (2010) earlier reported that there was maximum lipase production at 48 hour of bacterial growth which is different from what is observed in this research. The difference may have resulted from the fact the cell of *Staphylococcus aureus* used in this research are already in their exponential phase as at the time the colonies were inoculated.

There are many extracellular enzymes used for the industrial purposes; it was seen that *Staphylococcus* sp. produce lipases and proteases more than another enzyme group. From the industrial view, pathogenic bacteria are not usually used as there would be health risks but if the risks are overcome, the enzymes could be purified and used in the industry. The search of the extracellular enzyme profiles of the different staphylococci would in the future give clues about the site of infection that *Staphylococcus* species have. In this study, the different strains of *Staphylococcus aureus* produce the enzyme lipase reaching the peak of their production at different and/or various interval of incubation period depicting that differences in the growth rate and attainment of peak of lipase production may be an indication that the *Staphylococcus aureus* varied widely in their physiological and biochemical properties.

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