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The Importance of the Enzyme AMIC which is Responsible for Bacterial Division to Develop Antibiotics as a New goal used against *Salmonella* Bacteria

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Abstract

Gram-negative bacteria *Salmonella typhi* are the main cause of typhoid fever not only in developing countries but even in major countries such as the United States of America. Therefore, the World Health Organization (WHO) and health institution have called for infection with this intestinal bacterium to be a warning alaram that may lead to many deaths. The cell wall of bacteria is characterized by distinctive structures, the most important of which is the peptide and glycan layer, due to its importance in the resistance of bacteria to both the immune system and the antibiotics used to eliminate Salmonella bacteria after the Amic enzyme, which is one of hydrolase enzymes, the peptide layer and glycan are essential enzymes that bacteria cannot do without, due to its importance in the division of bacteria and the cellular reconstruction of the peptide and glycan layer. Therefore, the study aimed to investigate the enzymatic activity of the enzyme Amic in the biosynthesis and cleavage of peptidoglycan in Salmonella bacteria, moreover, to test some antibiotics used to treat typhoid and similar diseases on the enzymatic activity of the enzyme Amic by inhibiting or activating that enzyme as anew target for manufacturing or developing novel antibiotics used against Salmonella bacteria.

Keywords

Salmonella, Peptidoglycan, enzymatic activity, AmiC, hydrloses enzyme

Introduction

One of the most important problems facing health organizations and institution in the world is typhoid fever, which is systemic infection disease that affects humans only, caused by salmonella enterica bacterium. Contaminated food and water and people carrying the disease are the main sources of infection (1). Health organization centers and research centers in various parts of the world have recorded many reports and statistics that show the number of people infected with typhoid fever. The World Health Organization recorded in 1984 16milion cases of infection, including 600,000 fatal cases (2). However, with the improvement of water and sanitation systems that some parts of the world have been supplied with, global deaths from typhoid fever have decreased. The latest estimates indicate that there were 21 million cases of typhoid fever and 210,000 deaths resulting from disease around the world in the year 2000 (3). The reason for the high rate of infection with this bacterium is due to its possession of many mechanisms and means that it enables them to cause infection, which are called virulence factors. Among the virulence factors possessed by Salmonella are the protein-degrading enzymes Protease and Lipase, in addition to its resistance to antibiotics. This bacterium invades the blood, tissues, intestinal cells, and macrophages, and multiplies inside them, leading fever lymphatic inflammation and diarrhea (5).

Materials and methods

Salmonella culture media and how to prepare them:

1. Blood agar

Blood agar consists of digested protein source (soybeans), sodium chioride, and 5% sheep blood which includes: Pancreatic digest of casein, Papaic digest of soy meal, NaCl and Distilled water. This medium was adjusted by pH 7.33 and heated and sterilized with Autoclave.

Blood agar prepared procedures:

Blood agar was prepared according to the manufacturer's instructions. Sterilization in the factory using an Autoclave at a temperature of 121 Celsius for 15 minutes. Transferred the blood agar to a water bath at 50 C. The medium was added. When the medium is cooled, blood is added to it at room temperature.

Pour 15 ml into each dish.

(5) set the date of preparation of the medium.

(6) store the dishes at temperatures of 2 to 8 degrees Celsius; and it is preferable to place them in plastic it loses moisture and can be stored without spoiling for 4 weeks.

2. MacConky Agar

MacConkey Agar medium is a culture medium used to grow Gram-negative bacteria by fermentation Lactose. MacConkey Agar contains bile saccharides that inhibit Gram-positive bacteria.

Installation:

Peptone -17 g

Proteose Pepton -3 g

Lactose -10 g

Bile salts -1.5 g

Sodium chloride -5 g

Agar -13.5 g

Water – Add to make 1 liter, adjust pH to 7

There are many variations in the preparation of MacConky agar depending on what medium is required.

The ligand differentiates between Gram-negative bacteria that can ferment lactose(+Lac) and those that cannot ferment lactose(_Lac).

Immunoprecipiation assay by Native Amic Native enzyme isolation. When the bacterial growth reaches the exponential phase, the bacterial are harvested by centrifugation, where the culture medium containing the growing bacteria is transferred to centrifuge tubes (Bekman Coulter tubes 250 ml) at a speed of 6000 r.p.m for 30 minutes. Then, the supernatant represented by the culture medium (LB broth) is disposed of and the bacterial cells are kept at a temperature of 4 C (refrigerator). Bacterial lysate is prepared by adding the lysis buffer to the bacterial cells of Staphylococcus aureus, then impurities and unwanted materials are disposed of by transferring the Bacterial lysate to Eppendrof tubes and centrifuging at a speed of 12000 r.p.m for an hour. Then, the clear (2ml Eppendrof tubes) is transferred to new tubes Cleared bacterial lysate. The clear bacterial lysate containing wall-dissolving enzymes is added. The cell lysate including Amic is added to the column containing anti-Amic Sepharose column. The column is then washed with TBS washing buffer to remove the other enzymes and contaminations not associated with the Amic enzyme sulfur. Add elution Ependrof tubes from the column and collect it in native Amic tubes to remove the enzyme buffer and store it at 200℃ (freezer) until the necessary tests are performed.

SDS-PAGE electrophoresis

To confirm the isolation of the native Amic enzyme, electrophoresis of the isolated enzyme is performed 10x of sample buffer, where SDS-PAGE electrophoresis is added using 50µ of Amic, then the samples are loaded into the wells of the gels. The sample is then electrophoresed using a running buffer at a voltage of 200 volts for an hour. The gel is then transferred to Coomassie Brillant Blue to reveal the bands specific to the enzyme or protein.

Zymography and FITC-peptidoglycan hydrolysis assay

The enzymatic activity of AmiC enzyme was measured using Zymography and FITC Peptidoglycan hydrolysis assay. In the Zymography method, 80.2% cell wall was mixed with an Acrylamide buffer and left for 30 minutes to solidify in SDS-PAGE gel. Then, 1x sample buffer was added to Amic enzyme and loaded onto the gel containing the bacterial cell wall and was run at voltage 200 For one hour, after the end of the transfer, the gel was placed in the enzyme activation buffer (Renauation) with the Shaker replaced and left for two hour on the buffer (50 Mm Tris-HCl, ph.(6.5-7)four times every half hour. Then the gel was incubated at 37°C and left for 24 hours in the incubator. The buffer was replaced, and the gel was placed in methylene blue to view the bands of enzymatic activity of the Amic enzyme. For the FITC-Peptidoglycan method, 50µg of Amic 3 was mixed with 35 µg of FITC-Peptidoglycan in Eppendrof tubes and incubated at 37°C in a shaker incubator for 24 h. The lysozyme and enzyme were then filtrated or purified using 0.2 um syring Filters. The filtered samples were transferred to 96 well micro plates and the amount of FITC released because of the enzyme activity in both methods was measured in nm in a Varioskan plate reader 540 on an Amic.

Results and discussion

Immunoprecitation assay showed the successful isolation of the native Amic enzyme from Anti-Amic using a bacterial lysate Staphylococcus aureus sepharose column Figure (1). The results showed the presence of Amic enzyme bands in the SDS-PAGE gel alone at the expected molecular weight of 32 DA, indicating the purity of the enzyme. The result also showed Zymography of the presence of the enzymatic activity of Amic by analyzing the bacterial cell wall, represented by bands present in the gel stained with methylene blue

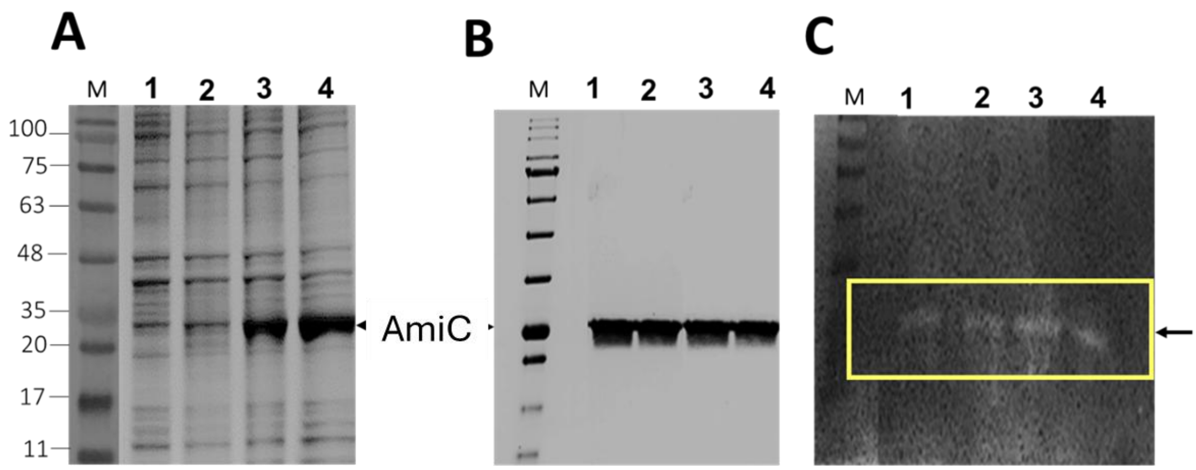
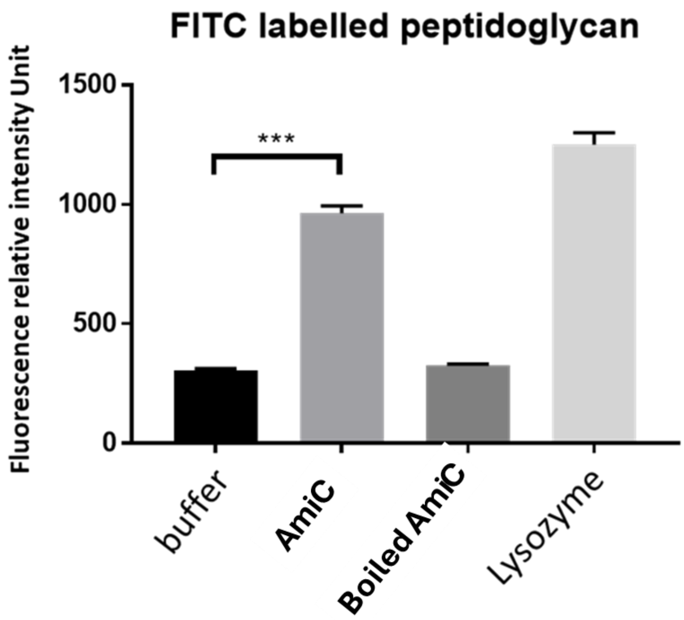


Figure 1(A) Unpurified *Salmenla typhibacterial* lysate (B) MW 32kDa enzyme in molecular weight Immunoprecipitation assay isolated by Amic technique (C) Enzymatic activity of Amic using Zymography method

The enzymatic activity of Amic was also measured by FITC-Peptidoglycan method. The results showed the presence of the enzymatic activity of the enzyme AmiC, which indicates that this enzyme is active and effective towards the cell wall. The results were compared with the positive control sample Lysozyme positive control and the sample Negative control represented by boiled AmiC **Figure (2)**



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Negative control represented by boiled AmiC **Figure (2).** And its pathogenicity [20] Investigating whether this enzyme is effective or not is very important to open the door to investigating the most important inhibitors or antibiotics that can be used against enzyme activity and thus inhibit the growth of bacteria and eliminate them. Especially since most bacterial species have shown resistance to many antibiotics and the emergence of new strains known as Antibiotic resistant bacteria has made treatment with these antibiotics ineffective in eliminating bacteria [21]. Finding alternative methods has become necessary to find new targets for antibiotics against bacteria. One of the most important new goals is to investigate enzymes AmiC (including Peptidoglycan hydrolases enzyme that degrades the bacterial cell wall). Finding the necessary inhibitor or developing an antibiotic such as Vancomycin is very important to eliminate Staphylococcus aureus bacteria that cause blood poisoning during burns. *Salmonella typhi* bacterium showed resistance to the antibiotic Vancomycin used through the mutations that the bacteria make, such as changing the sites of the antibiotic's binding to the cell wall [22]. It is well known that the antibiotic Vancomycin targets the D-Alanine end of the stem peptide that connects the peptide and glycan strands together. Therefore, this antibiotic prevents the binding of the peptide and glycan strands, which makes the bacteria susceptible to osmosis and decomposition, thus eliminating them. Therefore, the bacteria were forced to change the binding site from the 4-3 site to 3-3, thus nullifying the effect of Vancomycin

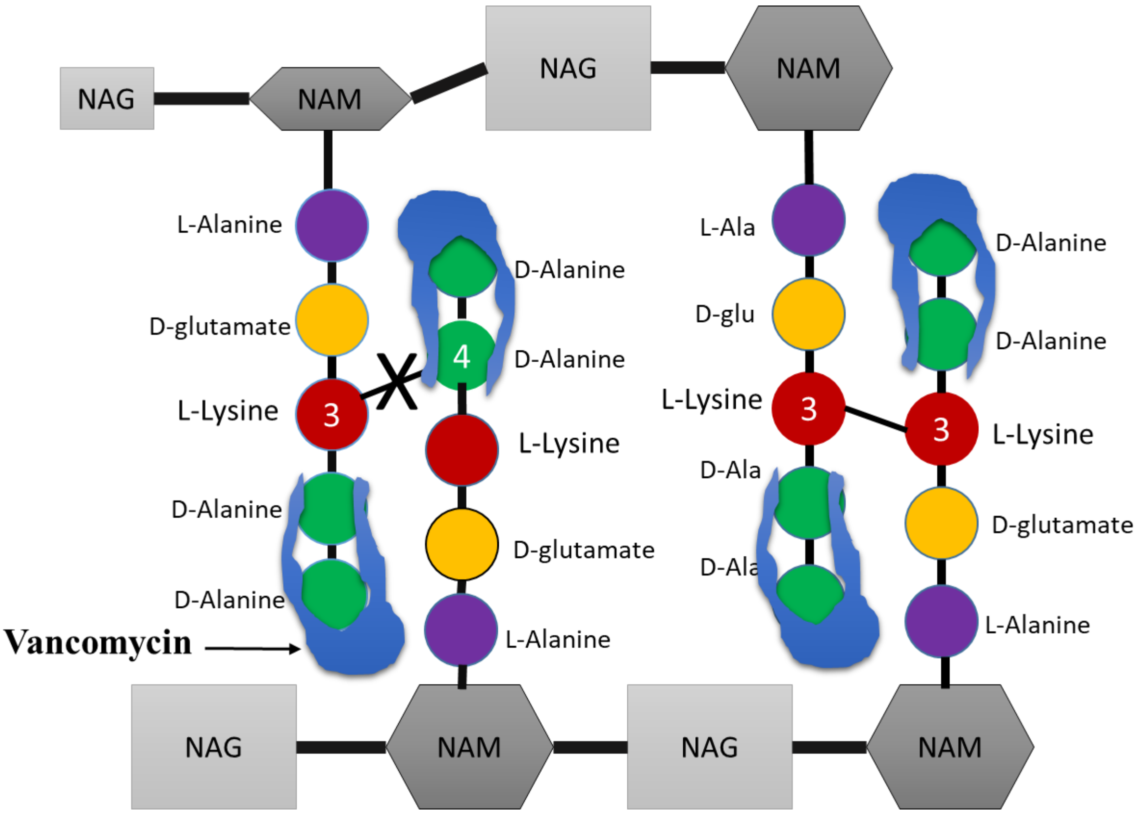

Figure (3) The site of the antibiotic Vancomycin's binding to D-Alanine to prevent the binding of the peptide and glycan strands to each other and thus eliminate the bacteria. The right side shows the bacteria's resistance to the antibiotic Vancomycin by changing the binding sites from 4-3 to 3-3 and then the bacteria survive and remain alive and active.

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