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Enhanced Production of Fibrinolytic Enzyme from *Pseudomonas aeruginosa* by Optimization Media Components

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Abstract

Fibrinolytic enzyme is factor that lysis fibrin clots. This fibrinolytic factor has prospective use to treat cardiovascular diseases, such as stroke and heart attack. Cardiovascular diseases attracted worldwide attention for their elevation morbidity and mortality. Expensive cost and fatally undesired side effects associated with the available fibrinolytic agents to treat these diseases stimulated the researchers to investigate potentially better agents for curative applications. In the current investigation, fibrinolytic enzyme production from *Pseudomonas aeruginosa* isolated from injuries of wounds and burns patients. Parameters for the promoted production of the enzyme under minimal production media were optimized. It comprised carbon source (glucose), Nitrogen source (Yeast extract), Fibrinogen concentration (0.5 %), inoculum size (1 %), temperature (37°C), and PH (7). Enhanced fibrinolytic enzyme activity (136.2 IU/ml) was obtained after optimization Medium Components compared with that obtained with the minimal medium (60.2 IU/ml) which is 2.2 times higher than the same under non optimized production conditions. Media optimization researches for enhancement of fibrinolytic enzyme production from *Pseudomonas aeruginosa* in Iraq has not been performed so far. This may be the first study to optimization media for the production of fibrinolytic enzyme from *Pseudomonas aeruginosa*. The importance of this study lies in the enhancing the production of the fibrinolytic enzyme with high activity using these bacteria.

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1.Introduction

Thrombotic disorders are the main cause of death world. This is shown in the form of myocardial infarction, embolism, stroke etc. The various cardiovascular disorders (CVDs) caused formation of intravascular thrombus, which leads to death. In 2011 statistics of American Heart Association, presented about 31.3% death was caused by thrombosis, and on the report of the WHO 17 million persons were succumbing to thrombotic disorders every year [1]. Thrombotic disorders were recovered over the previous year's using anticoagulants or antiplatelet agents like warfarin, heparin or by surgeries [2]. Fibrinolytic enzyme production by microorganisms differs

quantitatively and qualitatively depending on the species and strains of microorganisms used, also on their cultural conditions and own nutritional. The total cost of enzyme production is one of the major challenges concerning the cost-effective industrial application of the fibrinolytic Enzymes [3]. Many studied are available on the production of fibrinolytic enzymes from microorganisms, animals and plants, as well as purified from the venom of Elapidae, Viperidae and Crotalidae snake, from earthworms such as *Lumbricus rubellus* and *Eisenia fetida*, *Catharsius molossus* and from plants such as *Spirodela polyrhiza*[4,5]. *P. aeruginosa* is common in nature and causes many diseases for humans and animals including Wound and Burn infection, Endocarditis, Urinary Tract Infection, bacteremia that is why it is considered an important bacterial species [6]. Rarely causes the disease in healthy people and it is an opportunistic hospital bacterium, it is a real risk to patients who are in hospital, especially with patients who suffer from low in the immunity, such as individuals with Acquired immunodeficiency syndrome (AIDS). In addition to those with Burn and Wound infection [7]. The aim of this paper explains studies conducted on the isolation and characterization of the fibrinolytic enzyme producing bacteria and to improve bioprocess for the enhanced production of the fibrinolytic enzyme. *P.aeruginosa*, isolated from patients with wounds and burns infections in (Medical City) that located in the city of Baghdad, was found to be a potential producer of the fibrinolytic enzyme.

2. Materials and Methods

2.1 Patients, specimens, collection

The 43 isolates of *P. aeruginosa* were collected from wounds and burns infections, for the period from September 2020 until December 2020, samples were collected from educational laboratories in (Medical City). The specimens were diagnosed using Vitek 2 system for definitive diagnosis [8].

2.2 Determination of optimal bacterial isolate for the fibrinolytic enzyme production

The optimal bacterial isolate for the fibrinolytic enzyme production was determined by estimated the raw enzyme efficacy was according to Chang method [9], where the specific efficacy of isolates was measured.

2.3. Extraction of enzyme

Extraction of enzyme was done by using centrifugation for production broth that contains the bacterial cells of *P. aeruginosa* at 1000 rpm for 10 min at 4°C.Then the supernatant that contain the crude enzyme was taken [10].

2.4 Determination of Protein Concentration

Determined Protein concentration was done according to the method of Bradford (1976) [11]. A 50µl of 1 M NaOH was mixed with 20µl of crude enzyme and shacked for 2-3 minutes, then 1 ml of Bradford solution was added with shaking. The absorbance was measured at 595 nm by spectrophotometer.

2.5 Determine the optimum carbon source production

The optimal carbon source for the fibrinolytic enzyme production was determined by testing five different carbon sources (1.0%), which are) Sucrose - maltose - glucose-glycerol-starch)that added to the culture media. Then the medium was inoculated with a volume of 100 µl of *P. aeruginosa* and incubated for 24 hours at 37 ° C and pH 7, after which the activity of the fibrinolytic enzyme produced from the bacteria was determined through extracting the enzyme to find the optimum carbon source for fibrinolytic enzyme production

2.6 Determine the optimum nitrogen source production

The optimal nitrogen source for the fibrinolytic enzyme production was determined by testing five different nitrogen sources (1.0%), which are (peptone – casein – meat extract – yeast extract – ammonium sulfate) that added to the culture media. Then the medium was inoculated with a volume of 100 µl of *P. aeruginosa* and incubated for 24 hours at 37 ° C, and pH 7 with fixation of the optimal carbon source, after which the activity of the fibrinolytic enzyme produced from the bacteria was determined through extracting the enzyme to find the optimal nitrogen source for the production of the fibrinolytic enzyme [13].

2.7 Determination of the optimum fibrinogen concentration production

The optimal fibrinogen concentration for the fibrinolytic enzyme was determined by testing five different concentrations of fibrinogen (0.2, 0.4, 0.5, 0.6, and 0.8) % were added to the culture media, then the media was inoculated with a volume of 100 µl of *P. aeruginosa* and incubated for 24 hours at 37 ° C and pH 7 with the fixation of the optimal carbon and nitrogen sources. The activity of the fibrinolytic enzyme produced from the bacteria was determined after extracting the enzyme to find the optimum fibrinogen concentration [14].

2.8 Determine the optimum inoculum size production

The inoculum size for optimal production of the fibrinolytic enzyme was determined by inoculated with different sizes (0.5,1,1.5,2,2.5%) of *P. aeruginosa*, and incubated for 24 hours at 37 ° C and pH 7. The optimal conditions of carbon, nitrogen and fibrinogen concentration had fixation, then the activity of the fibrinolytic enzyme produced from the bacteria was determined after extracting the enzyme to find the optimum inoculum size for production [15].

2.9 Determine the optimum temperature enzyme production

The optimum temperature for the fibrinolytic enzyme production was determined by culture medium containing 1000 µl of *P. aeruginosa* at different temperatures (20 ,25 ,30 ,37, and 40) ° C in incubator for 24 hours. Then, the activity of the fibrinolytic enzyme produced from the bacteria was determined after extracting the enzyme, to find the optimum temperature for production [16].

2.10 Determine the optimum pH for enzyme production

The optimal pH for the fibrinolytic enzyme production was determined by changing the pH of the culture media (5, 6, 7, 8, and 9). The media inoculated with 1000 µl of *P. aeruginosa* for 24 hours at 37 ° C, and then the activity of the fibrinolytic enzyme was determined after extracting the enzyme to find the optimum pH for production [16].

3. Results and Discussion

3.1 Identification of bacteria

The first Identification of bacteria done by used Vitek 2 system. The specimens were culturing on nutrient agar and incubates for 24 hours at 37 ° C. So, the result of study agreed with [17] (Table 1).

Table 1: Characteristics of *P. aeruginosa.*

Characteristics of *P.aeruginosa*	Result
Morphology	Rod ,compose round colonies with fluorescent greenish color by Pyocyanin stain, fruity order, 2-3mm in diameter, smooth with irregular surface
Gram stain	Pink

3.2 Determine the optimum carbon source for production

The rate of carbon source metabolism can effect on the rapid multiplication of cells and subsequent production of a necessary substance for metabolism [18]. The following carbon sources (Sucrose - maltose -glucose-glycerol-starch) were adding to enzyme production media and incubated for 24 hours at of 37 ° C and pH 7, to find out the best carbon source for producing the enzyme. Results showed that the best carbon source is glucose and the highest specific efficacy was 108 U/mg. When using glucose, while the lowest specific efficacy was 14 U/mg when using glycerol (Figure 1). The current study does not agree with [19] as the best carbon source was sucrose, but the least effective carbon source is glycerol. The difference in results depends on the conditions and location of the bacterial isolation.

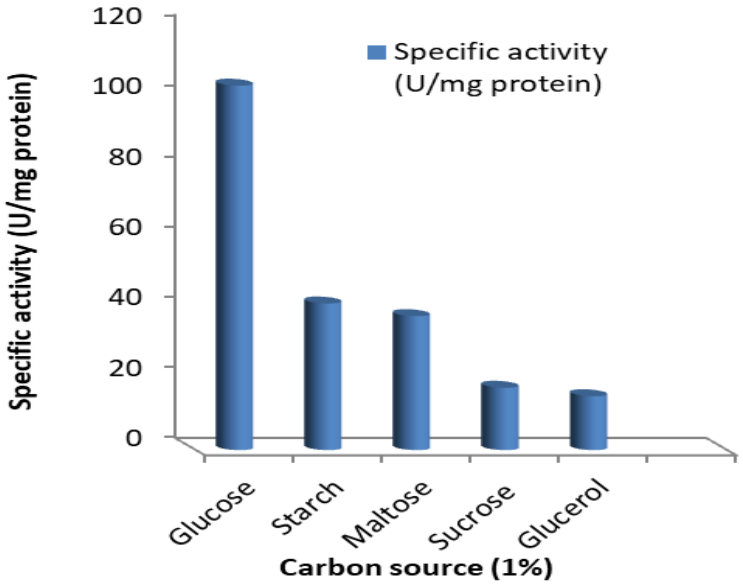

Figure 1: The optimum carbon source for production.

3.3. Determine the optimum nitrogen source for production

The following nitrogen sources were tested (peptone - casein –meat extract -yeast extract - ammonium sulphate) to find out the best nitrogen source for enzyme production, through adding nitrogen sources to the enzyme production media and incubated for 24 hours at 37 ° C and pH 7 with fixation of the optimum carbon source. The results show that the best nitrogen source is Yeast extract and the highest specific efficacy reached 116.3 U/mg when using glucose, while the lowest specific efficacy was 47.4 U/mg when using Peptone (Figure 2). The result agreed with result on the fibrinolytic enzyme production by Proteus [20].

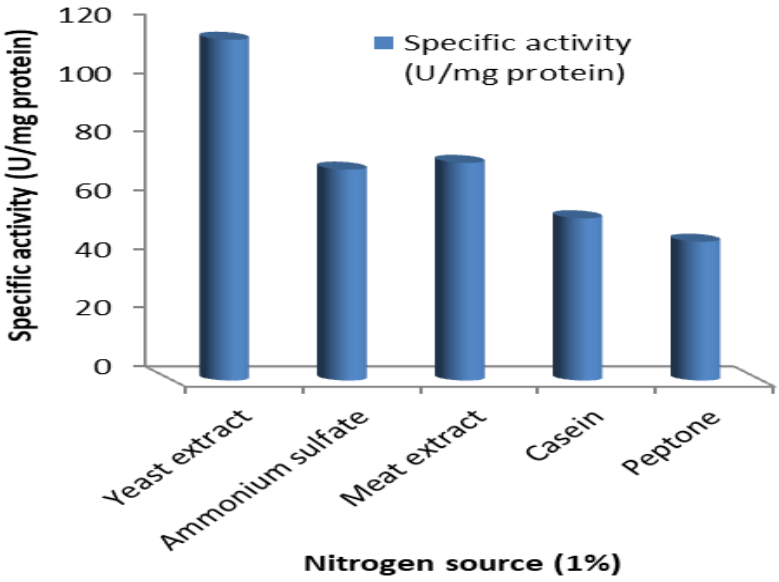

Figure 2: The optimum nitrogen source for production.

3.4. Determination of the optimum fibrinogen concentration for production

Five concentrations of fibrinogen (0.2, 0.4, 0.5, 0.6, and 0.8) % were tested to find the best concentration of fibrinogen for the production of the enzyme. The results showed that the best concentration of fibrinogen is 0.5% and the highest specific efficacy reached to 127.2 U/mg at concentration 0.5%, while the lowest specific efficacy was 18.5 U/mg when using 0.8% (fig 3).

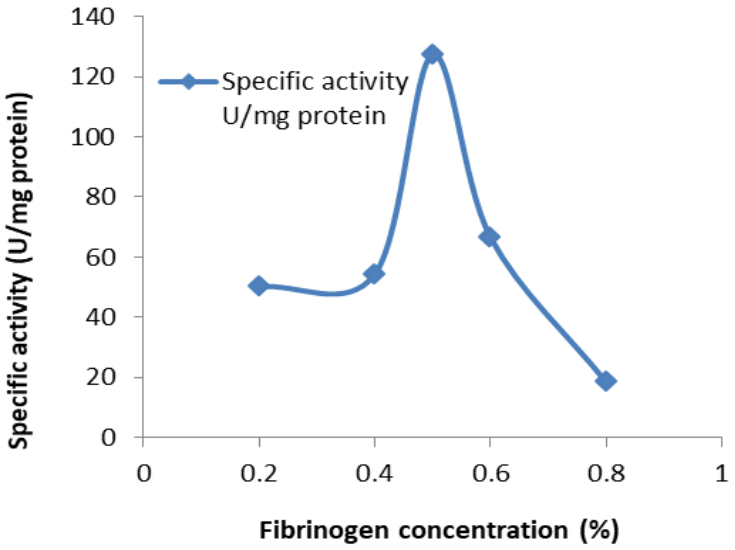

Figure 3: The optimum fibrinogen concentration for production.

3.5. Determine the optimum inoculum size for production

The results showed that the best inoculum size of the bacterial was 1% and the highest specific efficacy reached to 129.5 U/mg at a concentration of 1%, while the lowest specific efficacy was 47.4 U/mg when using 2.5 (Figure 4) .This study agreed with [19] where the best size of the inoculum was 1%, noting that the specific effectiveness of the enzyme decreases when increasing the concentration of the inoculum size.

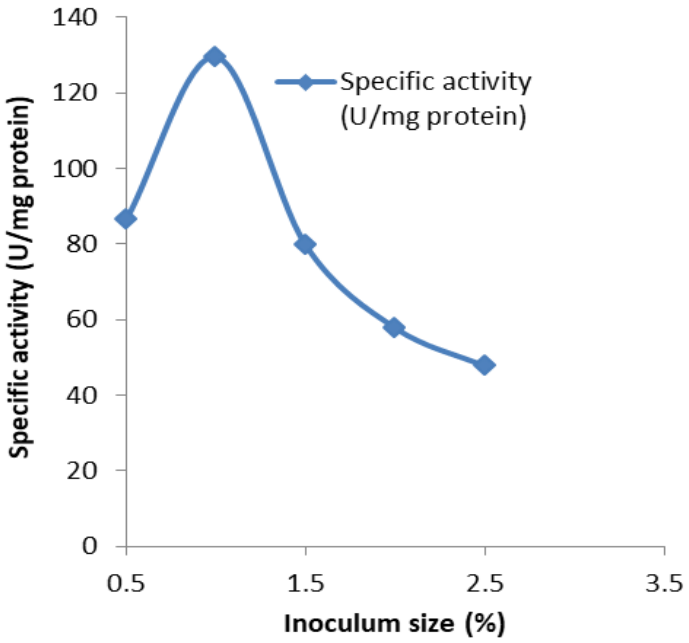

Figure 4: The optimum inoculum size for production.

3.6. Determine the optimum temperature for enzyme production

The bacteria were incubated at five different temperatures to find the best temperature for the production of the enzyme, adding 1000 µl of the bacterial inoculum to the enzyme production medium and incubated for 24 hours at pH number 7 with fixation of the sources of carbon and nitrogen and the optimal concentration of fibrinogen. The results showed that the best temperature was 37 ° C and the highest specific activity reached to 135.1 U/mg at a temperature of 37 °C, while the lowest specific activity reached to 129.2 U/mg at 25 ° C (Figure 5). The strain used in this study showed growth and ability to produce the enzyme, which indicates the wide range of temperature adaptability of the bacteria for enzyme production. The production of the enzyme from bacteria is affected by the temperature [21].

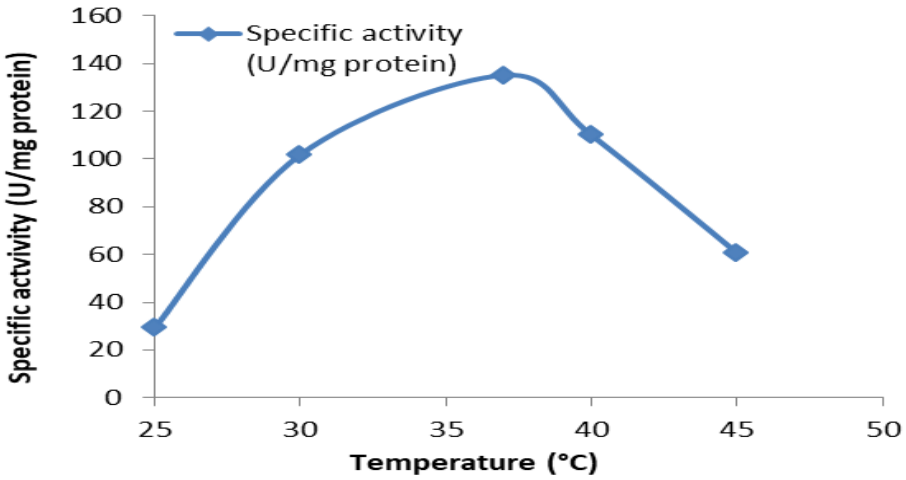

Figure 5: Optimum temperature for enzyme production.

3.7. Determine the optimum pH for enzyme production

The results showed that the best pH was 7and the highest specific activity reached to 136.2 U/mg at pH 7, while the lowest specific activity was 39.5 U/mg at pH 9 (Figure 6). The current study agreed with [22] where the best pH was 7 and with an observation that the specific activity of the enzyme decreased when the pH was increased to 9. The enzyme production from bacteria is affected by pH. The reason is that the pH has an effect on the growth of microorganisms through changes in the production or inhibition of proteins or the change in oxidation and reduction reactions inside the cell, as well as the production and consumption of energy important to the performance of all the vital functions of bacteria [23].

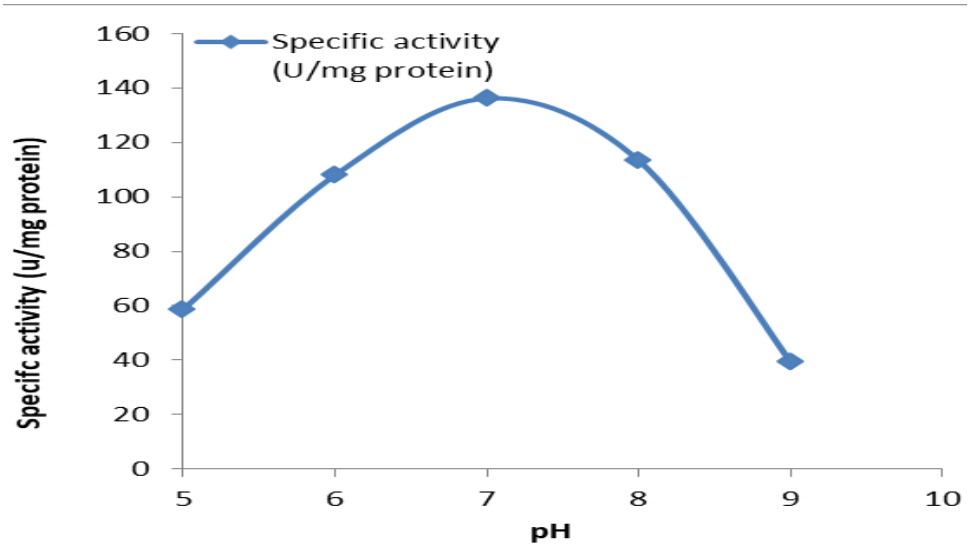

Figure 6: Optimum pH for enzyme production.

Conclusions

As a conclusion, an improved production of the fibrinolytic enzyme can be obtained by dominating the culture conditions and by modifying the composition of media. The use of one factor at a time was useful in selecting the independent variables contributing towards enzyme production and determining maximum/minimum level of these distinct variables for more optimization. This study not only reduces the time but as well the cost of production of the fibrinolytic enzyme.

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Conflict of Interest

There are no conflicts of interest regarding the publication of this manuscript.

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