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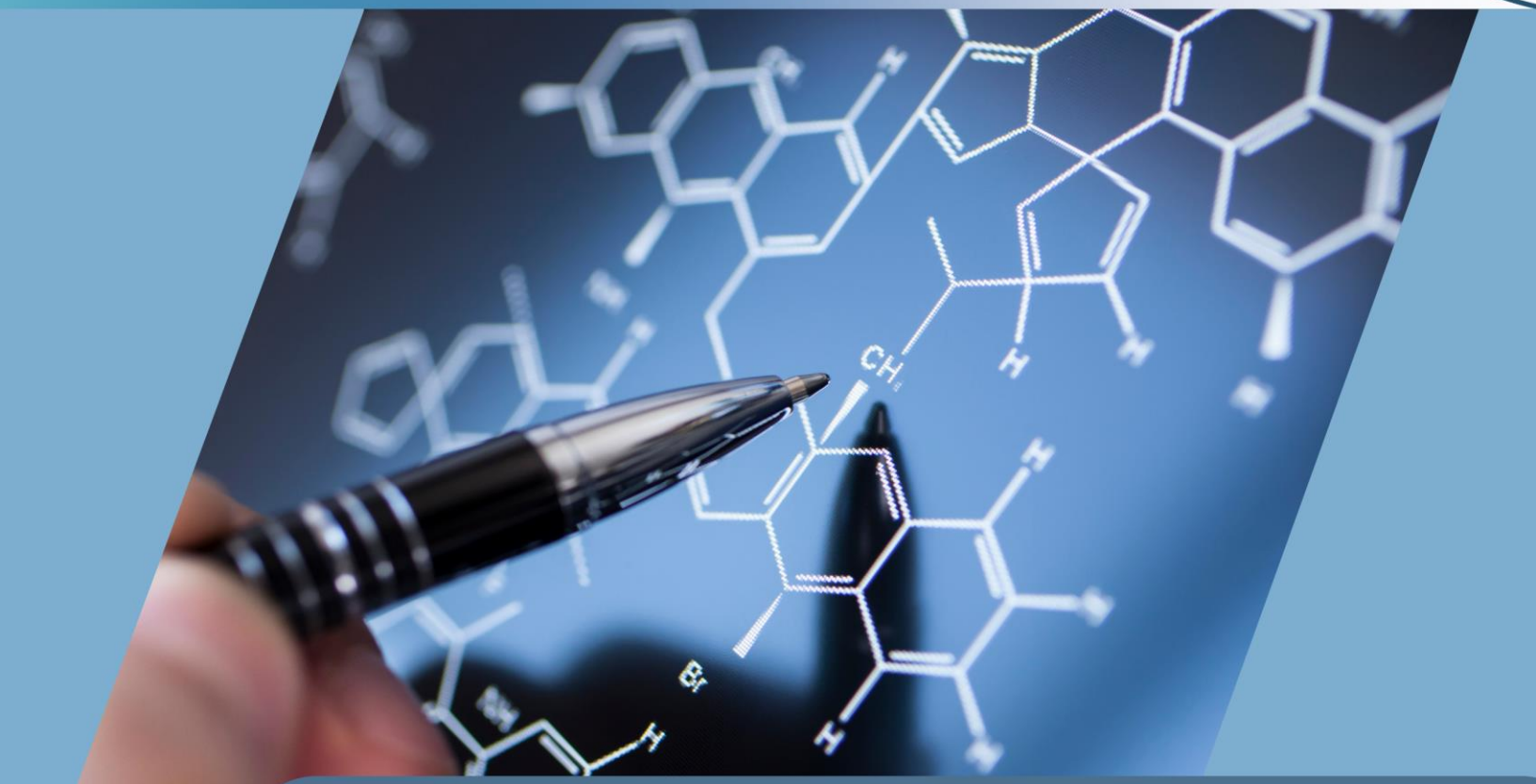

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Isolation and Identification of Pectobacterium Bacteria in Al Bayda, Aljabal Alakhdar, Causing Soft Rot on Potato Plants

Hosnia A. A. Bofarwa

Botany Department, Science Faculty, Omar Al-Mukhtar University, Al-Bayda –Libya.

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A B S T R A C T

This study sought to identify and characterize the bacteria causing soft rot disease in potatoes in a few Al-Bayda markets. It also sought to determine the pathogenicity of the bacteria and assess their susceptibility to various antibiotics and biochemical tests. From tubers afflicted with soft rot disease and from all samples taken from a few farms and shops in the Al-Bayda marketplaces, a bacterial isolate was obtained. The ability of the bacterial isolate to cause the illness was demonstrated by the pathogenicity test conducted on potato slices. The bacteria were identified as Pectobacterium based on the outcomes of phenotypic and biochemical tests. A test revealed some antibiotic sensitivity. The study's findings demonstrated that, with the exception of the antibiotic tetracyclin, all tested antibiotics are harmful to Pectobacterium, albeit to varied degrees. However, the antibiotic was Erythromycine, at varied degrees. One of the best antibiotics since it prevents erythromycine from being made An region with an average diameter of 3 cm is where bacteria proliferate. Amoxicilline, an antibiotic, was brought with them. It came in second place in terms of effectiveness because the antibiotic Tetracyclin had no harmful effects on the bacteria and the average diameter of the area surrounding the disc containing it free of bacterial growth was 1.5 cm.

1 Introduction

The potato crop Solanum tuberosum, L. is the third most consumed food crop globally and the fourth most strategic crop after wheat, corn, and rice (AL-Razaq et al., 2018; Shayaa and Hussein, 2019). . It is classified as a member of the Solanaceae family. Potato tubers are not only indirectly used in the processing industry, but are also directly consumed as nutrition for humans and animals. after dehydration or freezing (Boras et al., 2006). In light of the challenges the world faces due to rapid population growth and food supply challenges, the Food and Agriculture Organization of the United Nations strongly advocates potato as a key crop for food security. Furthermore, the potato plant is considered one of the most important vegetable crops in the world, as it represents an energy-rich food source and a source of nutrients. It is rich in nutrients such as carbohydrates, sugars, proteins, amino acids, organic acids, minerals and fiber. It is an excellent source of several vitamins, the most important of which are C and B (Alaee, 2018).

Potatoes contain a variety of chemicals that support activity. Antioxidants Hellmann et al. (2021), Suareza et al. (2020), Akrimi et al. (2020), the potato crop ranks fourth, after wheat, corn and rice, and is also very important and forms a large part of the Libyan diet. In 2010, the potato cultivation area in Libya exceeded 15,000 hectares. This year the country's potato production is about 290,000 tons, with a yield of about 19.3 tons per hectare. The areas east and south of Tripoli are important potato production areas due to their favorable soil composition and temperature (Souad

Corresponding author: **E-mail:** Hosnia.bofarwa@omu.edu.ly

Al-Bandaqo 2014). Many bacterial, fungal, viral and nematode diseases can affect potatoes. One of the most serious diseases is bacterial soft rot, caused by the pathogenic bacterium Fruitbacter carotobacter. Infection starts in the field and spreads rapidly during tuber transportation and storage (Youdkes et al., 2020), resulting in heavy agricultural losses (Guttman et al., 2021). Since soft rot is prevalent in both tropical and temperate climates, it is one of the most significant bacterial diseases that harm a wide variety of plants, including members of the Solanaceae family, which includes potatoes and many other crops. It is a disease that harms plants both in the field and in storage, leading to major losses (Motyka et al., 2021).

The genus Pectobacterium is a member of the family Enterobacteriaceae. It is one of the most important families in charge of soft rot in economically important crops like corn, tomatoes, and potatoes, according to Oulghazi et al. (2021). Moreover, the main culprit behind black leg illness is a genus of Pectobacterium called Dickeya (Werra et al., 2020). Pectobacterium bacteria l infections also cause soft rot in a variety of crops (Fan et al., 2020, Koh et al., 2012). Plant tissue components can be destroyed by it due to the action of enzymes that break down plant cell walls (Lee et al., 2013, Li et al., 2018, Giovannoni et al., 2020). According to Paul et al. (2020), the activity of these enzymes released by these bacteria that cause the breakdown of cell walls is what causes soft rot illness. Plant cell wall-degrading enzymes (PCWDEs), like pectinases, are the wall-degrading enzymes released by these bacteria. In addition to cellulases, hemicellulases, and proteinases. In order to cause disease symptoms, these bacteria require specific environmental conditions, such as humidity and the availability of nutrients that are gotten through wounds or naturally occurring plant holes. Once within the plant, they embed themselves in the interstitial spaces or vascular tissues, where they produce an enzyme. as per Colmer et al. (2009) and Paul et al. (2020), who disintegrate the cell wall of plants.

Given the significance of soft rot on potatoes and its extensive spread in the city of Al-Bayda as a result of the paucity of research on this illness, the study's objective is to identify and isolate the bacteria that cause the condition.

2 Materials and Methods

2.1 Gather samples, isolate the pathogen, and purify it

Pathogen Isolation and purification

In the fall of 2023, samples of potato tubers exhibiting soft rot symptoms were gathered from Al-Bayda local markets. With a few minor adjustments, the methodology of Doololbeldieva et al. (2016) was used to isolate the pathogen. Selected potato tubers exhibiting signs of soft rot disease were taken from the Al-Bayda local markets and cleaned under running tap water to eliminate dust. After that, it was surface sterilized for three minutes using sodium hypochlorite NaCl (chlorine 5%, concentration 2%). Next, after removing the potato's outer peel with 10 ml of sterilized normal saline, washed three times with sterile distilled water to remove the sterile substance, and mashed using a ceramic mortar. (0.85% NaCl). Using a sterile carrier loop, a portion of the bacterial suspension was collected, and the planing procedure was used to inoculate the NA medium. After that, the inoculation-treated dishes were kept in the incubator for a whole day at 28 °C. Using a sterile loop, a piece of the developing bacteria was transferred to Nutrient Agar medium, where it was striped to create single colonies and cultured for 24 hours under the same circumstances. Fig (1).

2.2 Examining the isolate of bacteria that is causing the soft rot on potatoes for pathogenicity

2.2.1 Examining potato slices for pathogenicity

To test for pathogenicity, the tubers that were as healthy, regular, and constant in size as feasible were chosen. After repeatedly washing them with water to get rid of dust, they were surface sterilized for three minutes with NaCl (5% chlorine) (2% concentration), and then they were repeatedly cleaned with sterile distilled water. They cut the tubers. The potatoes were sanitized and then sliced into uniform pieces that were about 10 mm thick. Using a sterile cork drill with a 5 mm diameter, a hole was drilled in the center of each slice, which was then put in sterile Petri plates (with sterile filter paper placed underneath). At 24 hours old, all of the bacteria under investigation were put into the holes. microliter/hole (610 colony-forming units/ml) including six slides per isolate, each kept in its own plate. After inoculating the slides, the dishes were kept in an

incubator set at 28 °C for six days to monitor the infection's progress Fig (2).

2.3 Phenotypic and biochemical testing

2.3.1 3.1- Bacterial isolate's microscopic and phenotypic features:

After the bacteria were cultured on NA media for 24 hours, smears were made on slides. After clean glass was stained with Gram staining, it was viewed using an optical microscope with an objective lens set to 100 x magnification. This allowed researchers to record the morphology, aggregations, and interactions between the bacterial cells and the dye .Table 1. in addition to the type, color, and form of the bacterial colonies' development on NA medium. Properties embraced the diagnostic standards that Schaad (1988) and Holt et al. (1994) mentioned.

2.3.2 Capacity to grow in certain media with differential cultures.

2.3.2.1 Dextrose-Yeast Extract CaCO3 Medium

To make the YDC medium, mix 1000 milliliters of sterile distilled water with 10 grams of yeast extract, 20 grams of dextrose, 20 grams of calcium carbonate, and 15 grams of agar. In one liter of hot distilled water, dissolve the aforementioned elements, and thoroughly mix the mixture with a Vortex mixer. Adjust the pH to 7.2 and incubate for 20 minutes at 121 degrees Celsius and 15 pounds per square inch of pressure. Transfer the medium into sterile plates and introduce the studied bacterial isolates into them. The plates were incubated for seventy-two hours at a temperature of 28 °C. Krieg and Dobereiner (1984), Schaad et al. (2001), and Wilson et al. (1967) all documented the type of growth that occurred on this medium.

2.3.2.2 Kings Medium (KB)

Add 20.0 g of peptone, 2.5 g of 4HPO2K, 6.0 g of O2.7H4MgSO, 15.0 g of agar, and 15 ml of glycerol to 1000 ml of distilled water to prepare the medium. After dissolving the medium's ingredients in hot distilled water and adjusting the pH to 7.2, the autoclave was sterilized for 20 minutes at 121 degrees Celsius and 15 pounds per square inch of pressure. The planned approach was used to inoculate the medium with the bacterial isolate under investigation after it had been poured into dishes. For seventy-two hours, bacterial cultures were cultured at 28 °C (Schaad et al., 2001). Table (2).

2.3.3 Biochemical assays for the investigated bacterial isolate:

2.3.3.1 Catalase test:

The bacterial growth on NA media was given a few drops of a 3% aqueous solution of hydrogen peroxide (H2O2) at the 24-hour mark, as the development of gas bubbles after a few seconds indicated a positive test (2000, MacFaddin, Winn et al., 2006).

2.3.3.2 Oxidase test:

A portion of the bacterial growth was transferred to the area of the filter paper saturated with the reagent and gently rubbed with a stick after several drops of the reagent Tetramethyl-P-phenylendiamine dihydrochlorie (made by adding 1 g of the listed substance to 100 ml of sterile distilled water) were placed on the paper. Wooden, the test is positive if the violet color appears within ten seconds (MacFaddin, 2000).

2.3.3.3 Growth test at 35°C :-

After preparing and sterilizing NA medium in an incubator for 20 minutes at 121 degrees Celsius and 15 pounds per hour of pressure, the medium was inoculated using 24-hour-old bacterial colonies and incubated for 48 hours at 35 degrees Celsius for observation. Expansion or Absence Scchad 1980.

2.3.3.4 Growth test in a medium containing 5% NaCL

After preparing 100 milliliters of NA medium and adding five percent sodium chloride to it, autoclave it for twenty minutes at 121 degrees Celsius and 15 pounds per hour of pressure. Following that, it was injected with 24-hour-old B. teria colonies and let to incubate for 24 hours at 28±2°C in order to observe it. Development or not (Schaad et al. (2001).

2.4 Pectobacterium sensitivity to specific antibiotics:

The working methodology:

1- Readyed a test tube with three milliliters of distilled sterile water in it.

2- To adequately mix the bacteria in the container containing bacteria in water, equal amounts of bacterial growth were poured into a tube using a sterile transfer needle (loop). The needle was then placed on top of the container and shaken several times.

3- The Hilton Muller's surface is contaminated with microorganisms, and Cotton Swab publishes the information by covering the entire surface of the center—not just the bust.

4- After the inoculated dishes were in the incubator for two hours at 28 °C, they were taken out and placed near the flame using sterile tongs. I then added three disc containers to each dish and repeated the process with different antibiotics spaced equally apart. Set aside three discs for each antibiotic and place the dishes in the incubator at the same temperature.

5- Following two days of good bacterial growth on the medium, all the dishes were taken out and the diameter of the empty circle around each disc—where the bacteria could not grow was measured.

6- The disk will be measured (by millimeter) using thementioned and comparing ruler to ascertain the degree of resistance if a transparent loop forms around it. Table (4) and Fig (3). Tetracyclin, Amoxicillin, and Erythromycin are the medications that.

3 Results

3.1 1. Gather samples and separate disease-causing bacteria

From every sample taken from the local markets in the city of Al-Bayda, where the growth first emerged, a bacterial isolate linked to the soft rot illness on potatoes was identified. Bacteria were cultured on NA culture media for 12 hours, and individual colonies were identified after 24 hours of incubation at 28°C. The colonies had a smooth, convex surface, a cream to white color, and regular, flawless edges.

Figure (1) (a and b) demonstrating the gathering of samples exhibiting disease symptoms; (c) demonstrating the emergence of cream-colored to brilliant white bacterial growths following isolation, 24 hours of culture in Na medium, and 28-degree Celsius incubatio

3.2 Evaluating the pathogenic potential of different microorganisms linked to soft rot illness

3.2.1 A test for pathogenicity using potato slices

The results of the pathogenicity test on potato slices showed that the studied bacterial isolate has the ability to cause rot disease on potato slices and the appearance of distinctive symptoms, the most important of which is tissue decomposition and the emission of unpleasant odors due to bacterial activity.

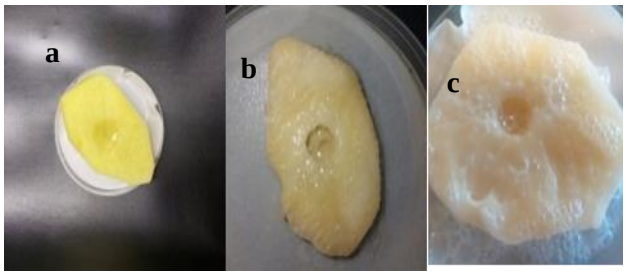


Figure (2) Effects *Pectobacterium* of potato slices, a. Control , b and c Symptoms of tissue decomposition and the emission of unpleasant odors appear a result of bacterial activity

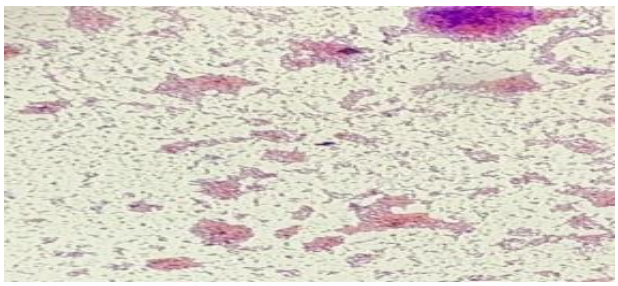
3.1- Evaluations both biochemical and phenotypic

3.1.1- Microscopic and morphological features of the bacterial isolates being examined:

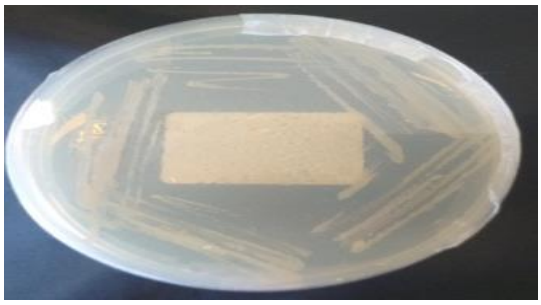
Table (1) shows the results obtained from studying the phenotypic and microscopic characteristics of bacteria isolated from potato tubers and stems infected with potato soft rot disease:

Table (1) Microscopic and phenotypic characteristics of the bacteria under study

Adjective	Notes
Reaction with Gram stain	Negative
Cell shape	Short rod
Cell assembly	Most of them are solitary and some are clustered
Color and shape of colonies on NA medium	Creamy to shiny white, round, with regular, perfect, convex edges



Reaction with Gram stain:- Negative



Color and shape:- Creamy to shiny white, round, with regular, perfect, convex edges

3.1.2 - Growth ability in some differential culture media

Table (2) shows the nature of growth of the bacterial isolates under study and the characteristics of the colonies growing on some selective and differential media.

The agricultural medium	Notes
Nature of growth on YDC medium	Colonies are yellow to cream, convex, shiny, circular with a perfect edge
Nature of growth on Kings Medium (KB)	White to cream colonies with a round, convex, opaque entire edge

3.1.3- Biochemical tests for the bacterial isolates under study:

Table (3) shows the overall results of the biochemical tests obtained for the bacterial isolates under study that are associated with soft rot disease on potatoes

Biochemical tests	**Results**
Catalase	**+**
Oxidase	**-**
growth at temperature 35 °C	**+**
Growth in a medium containing 5% sodium chloride +	**+**

(+) Positive test result

(-) Negative test result

4- Sensitivity of *Pectobacterium* to some antibiotics :

The study's findings demonstrated that, with the exception of the antibiotic tetracyclin, all tested antibiotics are harmful to *Pectobacterium*, albeit to varied degrees. However, erythromycine was the antibiotic to varied degrees. One of the best antibiotics since it prevents erythromycine from being made An region with an average diameter of three centimeters is where bacteria proliferate. Table Four: Amoxicilline, an antibiotic, was brought with them. It came in second place in terms of effectiveness because the antibiotic Tetracyclin had no harmful effects on the bacteria and the average diameter of the area surrounding the disc containing it free of bacterial growth was 1.5 cm. Figure (3)

Table (4): Effect of various antibiotics against *Pectobacterium* isolate

Antibiotics	**Diameter of growth-free zone/cm**			
	Duplicates			**the average**
Erythromycine	**3**	**2**	**2**	**3**
Amoxicilline	**1.5**	**1.0**	**0.6**	**1.5**
Tetracyclin	**0**	**0**	**0**	**0**

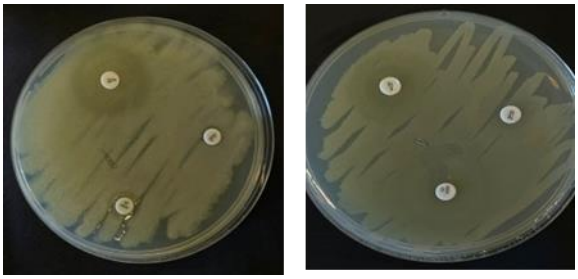


Figure (3) Effect of various antibiotics against Pectobacterium isolate

4 Discussion

According to Dees et al. (2017), the Pectobacterium genus is a member of the Enterobacteriaceae family. According to Oulghazi et al. (2021) it is one of the most significant families responsible for soft rot in economically significant crops including corn, tomatoes, and potatoes. Furthermore, black leg illness is primarily caused by the genera Dickeya and Pectobacterium (Werra et al., 2020). Pectobacterium bacterial infections induce soft rot in a variety of crops and generate large-scale economic losses globally (Fan et al., 2020; Koh et al., 2012). It is capable of destroying plant tissue components through the activity of plant cell wall-degrading enzymes (Lee et al., 2013; Li et al., 2018; Giovannoni et al., 2020). Paul et al. (2020) indicated that soft rot disease occurs as a direct result of the activity of these enzymes, which Secreted by these

bacteria, which leads to the decomposition of cell walls, these wall-degrading enzymes secreted by these bacteria are called (PCWDEs), such as Pectinases , Cellulases, Hemicellulases and Proteinases. These bacteria need certain environmental factors, such humidity, and the availability of nutrients that are obtained through wounds or natural plant holes in order to create illness symptoms. They enter the plant and become embedded in the vascular tissues or interstitial spaces, where they manufacture an enzyme. which (Colmer et al., 2009 and Paul et al., 2020) break down the plant cell wall. The pathogenicity test on potato slices revealed its results. It demonstrated that the investigated bacterial isolate can induce potato slice rot illness and manifest many symptoms, the most significant of which are tissue degradation and malodorous emissions due to bacterial activity. These outcomes agree with the research conducted by Zhou (2019) and Goszczynska et al. (2000).

Based on variations in the metabolic activity of various bacteria, biochemical tests are performed to identify different species of bacteria (Schaad et al., 2001). The ability to use specific compounds, produce specific enzymes differently in different species, and have diverse metabolisms all aid in the identification of different species. According to Schaad et al. (2001), Terta et al. (2013), and Li et al. (2020), this kind of testing helps. Phenotypic differences cannot differentiate one type of bacteria from another based solely on appearance, so they cannot be used to make a diagnosis in the case of bacteria. the arrangement and size of bacteria because many different types of bacteria have the same sizes and shapes. The findings demonstrated that the bacteria were round, cream to bright white, gram-negative, short rod-shaped, and found singly or in colonies. These characteristics are in line with the descriptions of P. given in Holt et al. (1994), Schad (2001), Perombilon (2006), Galilei et al. (2009), and Olgazi et al. (2021). Perfect, convex, and regular edges. These outcomes align with Thus, in the end, the primary method for identifying bacteria is based on variations in their metabolic activities (Ztruk et al., 2018). According to Terta et al. (2013) and Agyemang et al. (2021), 2020), each type of bacteria has a unique set of metabolic activities that set it apart from all other types. These biochemical fingerprints are characteristics that are controlled by bacterial enzymes, which can be divided into two categories: those that operate outside of bacterial cells and are primarily in charge of producing cellular energy from simple substances required for cell survival and function, as well as those that work inside bacterial cells Bacterial cells produce enzymes into their surroundings that break down complex molecules with a high molecular weight, such proteins, carbohydrates, and fats, which are too big to fit through a cell membrane. Bacterial bacteria, this is because to their complex makeup, such as lipids, or their huge size, such as proteins and carbohydrates (Kraepiel and Barny 2016). Additionally, the bacteria tested positive for catalase and negative for oxidase. As it was grown on media containing 5% sodium chloride, bacteria also grew on NA medium kept at 35°C. These results were in line with those of Gasik et al. (2014) and Ramadan and Al-Mashhadani (2006). Additionally, bacteria developed on the examined electoral media, with the following outcomes. It agrees with findings from other research on Pectobacterum growth in a few selective media (Holt and Krieg, 1984; Terta et al., 2010; Ravari Baghaee et al., 2011).

The bacterial ribosome is made up of the S50 unit and the 30S unit. A number of antibiotics block the function of ribosomes. Any of these can be impacted by antibodies. Antibodies Aminoglycosides bind to a certain type of ribosome to function. Glycosidic linkages bind complex sugars together to form substances known as aminoglycosides. Tetracyclines, for example, have a different molecular nucleus than streptomycin or deoxystreptidine because the latter inhibits the 30S unit. Daniel Robert (1988).

Three major classes of antibiotics, including chloramphenicol, block the 50S subunit. The broad-spectrum bacterial inhibitor, like erythromycin, inhibits both Gram-positive and Gram-negative bacteria. Manzer J. (1989) The study's findings demonstrated that, with the exception of the antibiotic tetracyclin, all tested antibiotics are harmful to Pectobacterium, albeit to varied degrees. However, in different amounts, the antibiotic was Erythromycin One of the best antibiotics since it prevents erythromycine from being made An region that has an average diameter of 3 cm is where bacteria proliferate. The investigated antibiotics' effects varied. Where the bacteria were exposed to the antibiotic Erythromycine Extremely successful while the medication Amoxicilline was It had a moderate impact. Tetracyclin, an antibiotic, was ineffective in this regard. This discrepancy can result from the tested antibiotics' varied interactions with the contents or parts of the bacterial cell wall. The findings of Khalil et al. (2021) and Maha Raouf Al-Saad (1980) suggest that

certain antibiotics impede the processes of protein synthesis and amino acid metabolism, whereas other antibiotics impede cell division or the action of enzymes that attach peptide side chains to the peptidoglycan portion of the cell wall.

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Conflict of interest: The author declares that there are no conflicts of interest.

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