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Isolation and Molecular Detection of *Bacillus subtilis* Isolated from Bowel Syndrome Patients with Demonstrating the Bacteria's Ability to Produce Amylase

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ABSTRACT

Objective: *Gram-positive, rod-shaped bacteria and spore-producing species that can endure in highly severe environments are described by the morphology of Bacillus subtilis. Antibacterial medications play a major role in prevention and treatments for bacterial illnesses at both animals and humans, which feeds the global problem of antibiotic overuse. Eventually, this causes pathogens to become more resistant to different antibacterial medications, and super bacteria may even emerge. At the moment, neither the bacteria that are not resistant nor that are susceptible to antimicrobial medications, and the rise in pathogenic strains that are resistant to these medications is a major barrier to the disease's prevention and treatment, making it more challenging to administer.* ***Method:*** *Study include 50 samples were collected for the current investigation. 10(20%) isolates were found to be bacterial after the required testing were completed, and medication sensitivity tests were then carried out. After testing for 8 antibiotics and the result were high resistant against Rifampicin and Ticarcillin and percentage was (80%), (70%) respectively. The bacteria were then subjected to morphological examinations in order to identify the production of the amylase enzyme.* ***Results:*** *A current study show that bacteria was resistant into number of them. According to the study's noteworthy findings, the enzyme was secreted by 4(40%) isolates.* ***Novelty:*** *The bacteria were then subjected to morphological examinations in order to identify the production of the amylase enzyme.*

INTRODUCTION

The lipopolysaccharide layer of the bacterial cell membrane is linked to the harmful action of G-ve bacteria. G-ve bacteria are hence more resistant to antibiotics. Serious illnesses affecting human and public health may result from this resistance. A few genus and species for Gram-negative bacteria which contain the majority of significant human pathogenics pathogens were listed. Some of these genera are bacilli, such as *Helicobacter pylori, Neisseria meningitidis, Rickettsia rickettsii, Salmonella typhi,* and *Shigella sonnei,* while others are cocci, such as *Bordetella pertussis, Brucella melitensis,* and *Brucella canis* [1], [2]. *Bacillus* species are spore-forming, Gram-positive bacteria that are distributed throughout the ecosystem in a variety of locations. In tobacco, lettuce, cucumber, and wheat, *Bacillus* species like *Bacillus, subtilis*, *Bacillus*. amyloliquefaciens, also *B. vitezensis* have existed demonstrated into have a negative influence on a range of diseases and to have a substantial impact on plant development. One of the main characteristics of *Bacillus* species is their ability to produce wide-spectrum antibiotics, including fengycin, and surfactin. Due to its antifungal broad spectrum effect, less toxicity, and allergenicity, the iturin family has garnered more interest recently [3].

Additionally, *Bacillus subtilis* can develop defenses against a range of stressors, such as heat, oxidative, alkaline, osmotic, and acidic environments, more quickly, according to [4]. in contrast to supplies for Gram-negative class bacteria, recombinant goods produce by *Bacillus. subtilis* cells that not be Introduce lipopolysaccharide into the environment. endotoxins, and the bacteria has no known unfavorable interactions with either humans or animals [5]. *Bacillus subtilis* probiotic strains are well known for being reliable, safe, and non-toxic to humans and animals. This bacterium produces the bacteriocin protein or polypeptide as it grows and reproduces. This content has strong antibacterial activity, a broad spectrum of antibacterial action, and exceptional thermal stability [6].

Stability and activity are not significantly impacted by pH levels. As a result, it has a lot of promise for usage as a substitute for antibiotics. This rod-shaped, Gram-positive germ is called *Bacillus subtilis*, where cultivated to ordinary nutritional medium, a circular colony for these bacteria itis jagged edges and a rough, opaque, fuzzy white or somewhat yellow appearance [7]. The fermentation cycle of Saccharomyces cerevisiae is 180 hours or longer, while that of *Bacillus. subtilis* is shorter and typically lasts 48 hours. This species also has access to excellent expression methods with exceptional genetic stability and does not exhibit a high preference for codons. *Bacillus. subtilis* has a single cell membrane, in contrast to *Escherichia coli,* which decreases process costs, streamlines downstream processing, and encourages protein secretion. Finally, it is widely acknowledged that this species is safe [8].

RESEARCH METHOD

Gathering of samples and Molecular detection

50 samples were collected from patients in Al Diwaniyah's municipal hospitals who had a variety of symptoms, including fever, diarrhea, and stomach ache. The samples were then prepared for culture, and several diagnostic tests were performed. The unidentified bacteria were isolated and cultivated by streaking them on nutritional agar, blood agar, and culture media. They were then vigorously shaken for 18 to 24 hours at 37°C.

Smearing, staining, and morphology of bacteria: The unidentified bacteria were stained using the Gram technique and examined optically. And After being collected, the samples were carefully transferred in sterile containers to the microbiological laboratory. bacterial cultures were grown for 12 hours in 30°C after a nutrient broth was prepared. The bacterial DNA were then extracted by way of a bacterial cultures by use reagent kit (InterLabServis, Russia). Extraction of DNA were carried out inin accordance with the manufacturer's instructions. Samples of DNA were kept at -20°C. The PCR technique was used to amplify the 16S rRNA gene. The gene 16S rRNA was chosen. in order to molecularly identify bacterial colonies. Bacterial biochemical testing: The unidentified bacteria underwent the methyl red (MR), indole synthesis, carbohydrate fermentation, and VP tests, respectively. Unknown bacterium preparing the fermentation broth [9].

Amylase activity is ascertained via measure a quantity for reduce sugar generated by way of starch use the dinitrosalicylic acids method.

Amylase activity measurement

Starchagar was used to test the *Bacillus subtilis* isolates for the production of amylase. After being Starch agarmedium was inundated with iodine solution after being infected with the organism. The aforementioned iodine solution is applied to the arch after it has grown for 72 hours, plates. Around amylase/producing colonies, cleared zones are visible against a blue backdrop.

Antimicrobial susceptibility testing

The several zones for inhibitions was measure into closest millimeter also categorized such sensitive, moderately resistant and sensitive, based on interpretation tables provided via discmanufacturer [10].

RESULTS AND DISCUSSION

the outcomes of the biochemical assays used to diagnose bacterial isolates, since the majority of the isolates produced a positive. These isolates produced a negative urease secretion test and a positive nitrate reduction test, but they produced a negative result for the catalase enzyme test and a positive result for the citrate test.

Figure 1. Show the *Bacillus subtilis* colony.

After 24 to 48 hours, increase of *Bacillus subtilis*. The production for enzyme decreased after this time. One explanation for the observed peaking and throughing of extracellular enzyme production is an action for component one cause synthesized for other, and different inhibitions via substrate hydrolysis products, and a decreases at growth after 48h for *Bacillus. subtilis* growth. these more like resulte via lysis of cellular, as evidence via a previously reporte generate for extracellular amylase by *B. subtilis*, that is consistents with finding from another researchers [11], [12] . Also was use TSA agar medium, the *Bacillus subtilis* strain that was identifie to MacConkey medium as well

Chapman media didnt grown, instead, also grew more effective, producing medium-sized colonies with smooth, moist, sticky ridges. Colonies on LB and nutritional agar were flat, almost round, and dry. The rabbit blood agar was entirely fluid, smooth, and elevated, colonies that are hemolytic. formation of Surface biofilms, fluids and transport culture, and bottom following shocks in LB broth medium all show aerobic bacterial growth, which is in line with *Bacillus subtilis* traits. Additionally, while the broth was being prepared.

The isolates listed above were then subjected to drug sensitivity testing, as indicated in the table below as at Table 1. The following percentages for results show high antibiotic resistance. The result was high resistant of Rifampicin (80%), Imipenem and Ticacillin was both (70%) while the intermediate include the Chloramphenicol was (60%)and Cefepime was also the same percentage , while the less resistant or sensitive was Amikacin (0%).

Table1. Antibiotic sensitivity test of *Bacillus subtilis.*

Antibiotic types	Resistance	Intermediate	Sensitive
Cotrimoxazole	5(50%)	3(%30)	2 (%20)
Chloramphenical	0(0%)	6(%60)	4(%40)
Amikacin	6(60%)	4(%40)	0(%0)
Ticarcillin	7 (%70)	2 (%20)	1(%10)
Meropenem	2 (%20)	5(50%)	3(%30)
Rifampicin	8(%80)	0(%0)	2 (%20)
Cefepime	2 (%20)	6(%60)	2 (%20)
Imipenem	7 (%70)	1(%10)	2 (%20)

Public health was seriously threatene via the incidenced for antibiotics resistance at bacterial strains, that necessitates immediate research into a new antibiotics or antibacterial compounds [13]. Over a past less decades, the large number for studies on production for a new antibiotics from differential strains for plants and microbes that have reporte [14]. Bacteria produced and utilized antibiotics at their organic habitats into defend againsts invasion for another bacterial species. this antibiotics no only provided a form for defenses but also function such as vital signaling molecules that facilitate communication between the bacterial population's cells [15].

Bacillus subtilis. isolates were identified at the molecular level by using the universal primer to amplify the 16s rDNA gene. All of the bacterial isolates' DNA samples were used in place of one negative control (one that lacked a DNA template) for performing Polymerase Chain Reaction (PCR). The PCR-amplification of the DNA was used for electrophoresis in a 1. 25% agarose gel. And the result showed as at Figure 2. The results showed that 10 isolates belonged to *Bacillus subtilis*, In many nations, bacterial antibiotic resistance is a serious health issue. In accordance with earlier research, an assessment of the drinking water's bacteriological quality verified the existence of *Bacillus subtilis*

bacteria that are susceptible to multiple antibiotic classes. In a different investigation, *Bacillus sp*. Isolate The results were consistent with what had been concluded with study of Khaled, 2017.

The Amylase enzyme production rate of the bacteria was also measured, and the percentage was 4 isolates that secreted the enzyme.

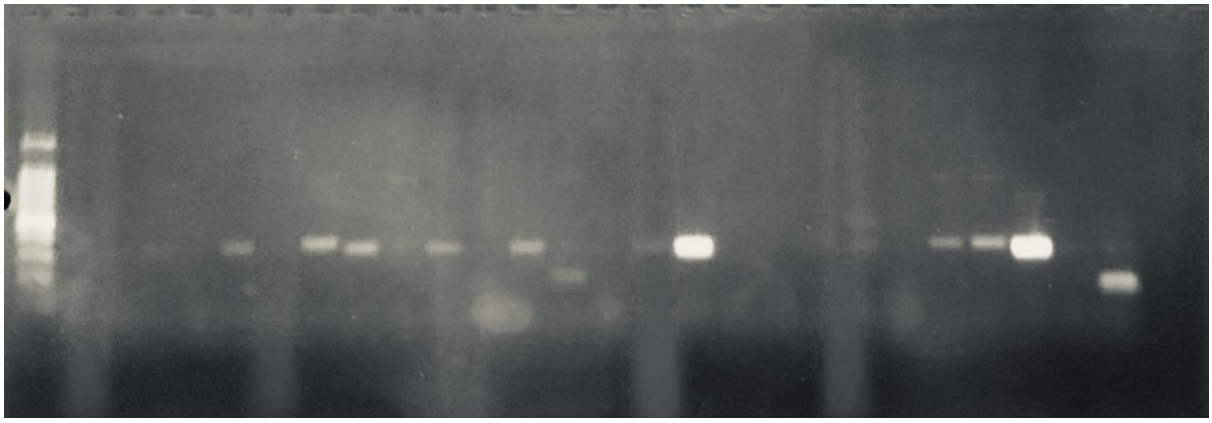

Figure 2. Show Molecular detection of *Bacillus subtilis* isolates.

Isolated bacterial strains were molecularly described using universal bacterial primers and conserved 16S rRNA gene sequences. To determine the size of the amplified fragments, the final result was subjected to 1% gel electrophoresis after the targeted gene sequence was amplified using the conventional PCR technique. After sending the amplified samples and pertinent sequencing fragments for sequencing, the obtained nucleotide sequences were phylogenetically analyzed According to a report that supports our research in Uzbekistan [16].

CONCLUSION

Fundamental Finding : The study found that this bacterium can be either beneficial or harmful depending on the context, particularly when antibiotic misuse creates favorable conditions, and it also demonstrated the bacterium's capacity to produce nicotinic amylase with relevance for industrial starch degradation. **Implication :** Elevated antibiotic resistance associated with this bacterium may reduce treatment effectiveness and highlights its potential significance for both clinical management and industrial processes. **Limitation :** The study provides limited molecular-level evidence to fully explain the bacterium's behavior and resistance mechanisms. **Future Research :** Further comprehensive investigations, including molecular studies, are recommended to better understand the bacterium and its applications.

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