

Isolation of *Bacillus* spp. from some sources and study of its proteolytic activity

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Abstract

In view of the importance sources that employ in our study that deals with human poisoning , this study was aimed to isolate *Bacillus* spp. from some sources that deals with human food poisoning and to identify this microorganisms morphologically and biochemically . Twelve samples including (air , water , egg , milk , cheese and meat) in duplicate were taken in this study , after isolation of the *Bacillus* spp. from these samples an investigation was taken up to find out the activity of proteolytic enzymes by this bacteria under controlled condition as one of its important virulence factors . The results showed that all the sources were contaminated with *Bacillus* spp. And the average numbers of colonies were found to be more in air samples followed by meat , milk , egg , water and the lowest in cheese samples. Also all the colonies of *Bacillus* which isolated were positive to proteolysis activity by forming clearing zones around the growth of the bacteria , because of its ability to degradation proteins to amino acids .

Introduction

Bacillus species are gram positive, rod-shaped, aerobic and endospore forming ⁽¹⁾. Many species of this genus exhibit a wide range of physiologic abilities that allow them to live in many natural environment⁽²⁾. It play an increasingly important role in food and beverage industries ⁽³⁾. Contamination with *Bacillus* spp. deals with food poisoning and causing illness ⁽⁴⁾. Most of these bacteria have the ability to disintegrate proteins as named proteolytic activity ⁽⁵⁾.

Proteases are degradative enzymes which catalyze the specific and selective modifications of proteins ⁽⁶⁾. Which were characterized for some gram positive species often playing a role as important virulence factors through interactions with the host defense ⁽⁷⁾. The vast diversity of proteases in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications ⁽⁸⁾. *Bacillus* spp. represent an excellent source of enzymes owing to their broad biochemical diversity and susceptibility to genetic manipulation. Protease represent on the largest groups of industrial enzymes and account 60 % of the total worldwide sale of enzymes ⁽⁹⁾. Generally proteases produced by *Bacillus* spp. are most importance due to the stability of their enzymes under different environmental conditions ⁽¹⁰⁾. The objectives of this research deals with a study of many environmental samples from different sources that used to isolate *Bacillus* spp. to detect the proteolytic activity and to study some of biochemical and morphological features of this important bacteria ⁽¹¹⁾.

Materials and methods

Samples

Duplicate samples were collected from different sources involved (water , air , milk , cheese , meat and eggs) (12 samples) to isolate *Bacillus* spp.

- Air samples were collected by exposure two plates containing nutrient agar to the atmospheric air for 1/4 hrs.
- Water and milk samples were diluted in to concentrations by adding 1 ml in 9 ml sterile distilled water to achieve 10^{-1} .

- Egg, cheese and meat samples:10 gm of each were dissolved in 90 ml of sterile distilled water then serial dilutions in sterile distilled water up to 10^{-5} were prepared. The initial dilution of all samples were heat activated in water bath at 80°C for 15 min. ⁽¹¹⁾

Culture media

Lecithinen (N. agar with 10% egg yolk) locally prepared ⁽¹²⁾ ⁽¹³⁾, Urea agar base , Nutrient gelatin , Sulfide indole motility (SIM) , Nitrate medium , Skim milk agar , Starch agar. (Hi Media / India) .

Methods

One ml of each pasteurized dilution was poured into Petri dishes containing nutrient agar medium. The inoculated Petri dishes were then incubated at 37°C for 24 hrs. ⁽¹⁴⁾. The suspected isolates were identified microscopically for morphological study, gram stain and motility as well as biochemical tests were performed such as catalase, oxidase, lecithinase production, citrate utilization, V. P reaction, indole production, nitrate reduction, urease activity, starch hydrolysis, gelatin hydrolysis and sugar fermentation (glucose , arabinose , lactose , mannitol , galactose , maltose , salcin , xylose , fructose , sucrose) ⁽¹⁵⁾. Also we examined the ability of bacteria to grew in nutrient broth with 5% and 7% NaCl and to grew in 42°C and under anaerobic conditions ⁽³⁾. Protease production test was screened for proteolytic activity on skim milk agar plates . the inoculated plates were incubated for 24 hrs. at 37°C . Proteolysis activity was observed by formation halo zone around the colonies which indicated degradation of proteins into amino acids by the bacteria ⁽¹⁶⁾.

Results and Discussion

According to the results obtained , the isolates were belong to the genus *Bacillus* spp. The average numbers of colonies were found to be more in air samples followed by meat , milk , egg and water (the same range) and the lowest in cheese samples as shown in table (1) which represent the percentage of

Bacillus species calculated from the all of the microorganisms isolated from these samples .

Table (1) : The average percentage of *Bacillus* spp. isolated from some sources under study

| Sources | % of <i>Bacillus</i> spp. |
|---------|---------------------------|
| Air | 70 |
| Water | 50 |
| Milk | 60 |
| Cheese | 30 |
| Meat | 65 |
| Egg | 50 |

In addition different types of bacteria isolates from sources under study but mostly of them was *Bacillus* spp. see Fig. (1) a , b , c , d

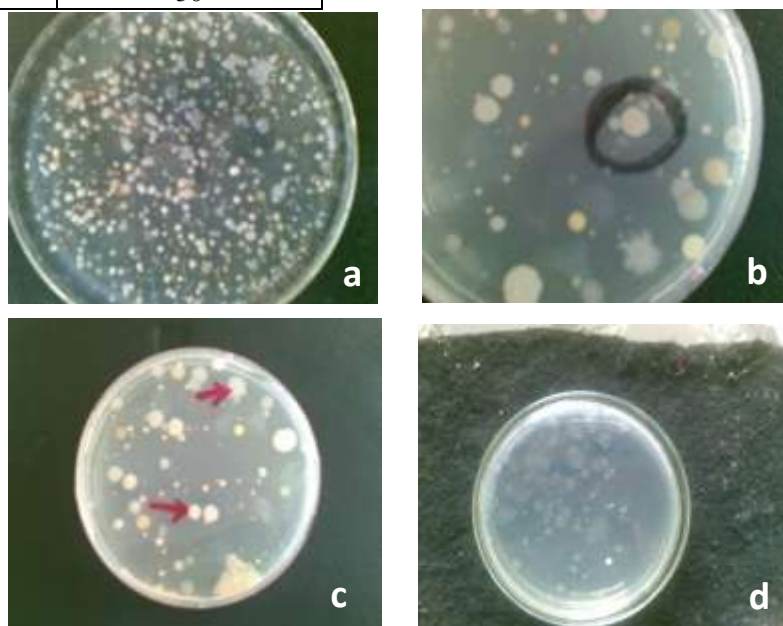


Figure (1) : Showing growth of *Bacillus* spp. with different types of bacteria isolates from some sources under study a. Water b. Air c. Meat d. Milk

Microscopic observation were revealed a straight gram positive , spore-forming bacilli and motility . In

addition table (2) summarize the results of biochemical characteristics .

Table (2) : Biochemical characteristics obtained from *Bacillus* spp. isolated from different samples

| Sources Tests | Air 1 | Air 2 | Water 1 | Water 2 | Milk 1 | Milk 2 | Cheese 1 | Cheese 2 | Meat 1 | Meat 2 | Egg 1 | Egg 2 |
|---------------------|-------|-------|---------|---------|--------|--------|----------|----------|--------|--------|-------|-------|
| Gram staining | + | + | + | + | + | + | + | + | + | + | + | + |
| Spore formation | + | + | + | + | + | + | + | + | + | + | + | + |
| Cell shape | rod | rod | rod | rod | rod | Rod | rod | rod | rod | rod | rod | rod |
| Motility | + | + | + | + | + | + | + | + | + | + | + | + |
| Citrate utilization | + | + | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | - | + | + | - | + | + | + | + | + | + | + |
| Urea hydrolysis | - | + | + | + | - | + | - | - | + | + | + | - |
| Gelatin hydrolysis | + | + | - | + | + | + | + | + | + | + | + | + |
| Catalase | - | + | + | + | + | + | + | + | - | + | + | + |
| Lecithenase | - | - | + | + | + | - | + | - | - | + | - | + |
| Nitrate reduction | + | - | + | + | - | - | - | + | + | + | + | + |
| Voges proskauer | + | + | - | - | + | + | + | + | + | + | + | - |
| Starch hydrolysis | + | + | + | + | + | + | + | + | + | + | + | + |
| Indole | - | - | - | - | - | - | - | - | - | - | - | - |
| Growth in 5% NaCl | - | + | + | + | + | + | + | + | - | + | + | + |
| Growth in 7% NaCl | - | - | + | + | + | + | - | + | + | + | + | - |
| Anaerobic growth | + | + | + | + | + | - | + | + | + | + | + | + |
| Growth at 42° c | + | - | + | - | + | + | + | + | + | + | - | + |
| Protease | + | + | + | + | + | + | + | + | + | + | + | + |

All the strains were found to be positive in citrate utilization, oxidase, urease, gelatin hydrolysis, catalase, lecithinase, motility, nitrate reduction, voges-proskover and starch hydrolysis as well as they were grew well in 5 % and 7 % NaCl at 42°C and an aerobically, it gave negative reaction to indole, see Fig. (2) .

The results shown in table (3) reflect the ability of different isolates to ferment sugars , the positive results indicate that the bacteria was able to ferment these sugars due to have specific enzyme that responsible for sugar fermentation and production of acid and gas and/or gas.



Figure (2) : Showing some results of biochemical tests of *Bacillus* spp.

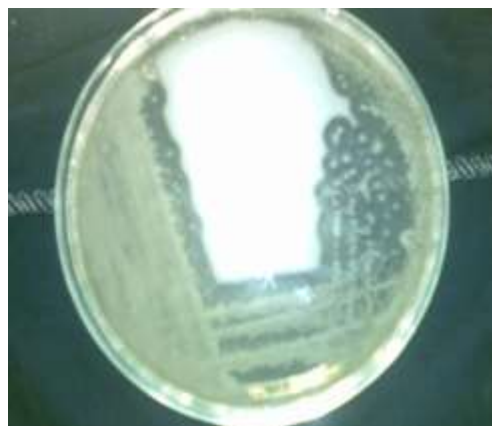
a. Motility b. Lecithinase c. Citrate utilization d. Starch hydrolysis e. Nitrate reduction
f. Voges-proskauer g. Lactose fermentation h. Salicin fermentation

Table (3) : The ability of *Bacillus* spp. to ferment sugars

| Sugar \ Source | Air 1 | Air 2 | Water 1 | Water 2 | Milk 1 | Milk 2 | Cheese 1 | Cheese 2 | Meat 1 | Meat 2 | Egg 1 | Egg 2 |
|----------------|-------|-------|---------|---------|--------|--------|----------|----------|--------|--------|-------|-------|
| Glucose | + | + | + | - | + | + | - | + | + | + | + | + |
| Arabinose | - | - | - | - | - | - | - | - | - | - | - | - |
| Lactose | + | + | + | + | - | + | + | - | + | - | + | - |
| Mannitol | - | - | - | - | - | - | - | - | - | - | - | - |
| Galactose | + | - | - | - | - | + | - | - | - | - | - | + |
| Maltose | + | + | + | - | + | + | + | + | + | + | - | + |
| Salcin | - | - | - | - | - | - | - | - | - | - | - | - |
| Xylose | + | + | - | + | + | - | + | + | - | + | + | + |
| Fructose | + | + | + | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + | + | + | + |

Protease production of *Bacillus* spp. on skim milk agar were measured by observing a clear halo zone

around a colonies of substrate hydrolysis which indicate proteolytic activities Fig. (3) .

**Fig. (3) : Showing a halo zones around the colonies of *Bacillus* spp. isolates from some sources under study**

The size of halo zones showed in table (4) which indicate that air samples were the largest zone around the growth of bacteria (38 mm) while the water samples were of the smallest one (10 mm).

Table (4) : Size of zones around colonies on skim milk agar medium

| Samples | Hydrolysis zones (mm) |
|----------|-----------------------|
| Air 1 | 37 |
| Air 2 | 40 |
| Water 1 | 25 |
| Water 2 | 28 |
| Milk 1 | 30 |
| Milk 2 | 40 |
| Cheese 1 | 30 |
| Cheese 2 | 35 |
| Meat 1 | 40 |
| Meat 2 | 36 |
| Egg 1 | 26 |
| Egg 2 | 30 |

Similar observation was reported by ⁽¹⁵⁾ in Korea who isolated *Bacillus* spp. and identified from rotating biological contactor based on their biochemical properties. Also ⁽⁴⁾ in India identified *Bacillus* from fresh water lake and studied its biochemical characteristics. Finally ⁽⁷⁾ obtained 12 strains of *Bacillus* spp. from traditionally fermented African locust Bean in Nigeria. These strains were evaluated in respect to production of protease on skim milk agar

and the bacteria exhibited the highest proteolytic activity with a diameter of clear zone measuring 35.0 mm.

This results agreed with ⁽¹⁷⁾ in Tamil Nade , they isolated *Bacillus* spp. from pasteurized milk , and studied its proteolytic and lipolytic activities . These organisms survive heat treatment and high temperature used for processing of the products , activates spore germination and out growth , resulting in spoilage of products .

Followed by ⁽³⁾ in Germany who studied food poisoning caused by *Bacillus cereus* and the related activity of protease with causing poisoning in food . while ⁽¹⁸⁾ studied spores of *Bacillus* as associated with protease activity by washing spores with 1 M KCl . A proteolytic activity associated with the dormant spores and can be solubilized by washing the spores with 1M KCl. This proteolytic activity is responsible for the attack of β chains of ribonucleic acid polymerase in extracts of dormant spores of this organisms. Thus indicating that it is loosely band to the spore.

From this study we concluded that our samples were of poor hygienic quality and this bacteria considered to be one of the most important microbial contamination.

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عزل جرثومة *Bacillus* spp. من بعض المصادر ودراسة قابليتها على إفراز أنزيم البروتيز

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الملخص

تم في هذا البحث عزل جرثومة *Bacillus* spp. من بعض المصادر شملت عينات من (هواء ، ماء ، بيض ، حليب ، جبن ، لحم) والتي لها علاقة مباشرة بصحة الإنسان وبواقع مكررين لكل عينة (١٢ نموذج) .

شخصت الجرثومة اعتمادا على الاختبارات الشكلية و الزرعية و الكيموحيوية ، أثبتت النتائج أن جميع العزلات كانت ملوثة بجرثومة *Bacillus* وكانت أعلى نسبة لعدد المستعمرات في عينات الهواء ثم اللحم ، الحليب ، البيض والماء وأخيرا أقل نسبة ظهرت في عينات الجبن ، فضلا عن دراسة فعالية أنزيم البروتيز باستخدام وسط أكار الحليب المنزوع الدهن وقد لوحظ أن جميع عزلات الجرثومة تمكنت من إفراز أنزيم البروتيز بتشكيل مناطق رافقة حول نمو الجرثومة وذلك لقابليتها على تحليل البروتينات إلى أحماض أمينية .