Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Isolation and Identification of Lactic Acid Bacteria from Different Sources and Testing their Ability to Produce Cellulases Enzyme

[1]Sabreen Abdullah Mansour, [2]Wael Ali Swadi, [3]Zena Kadhim AL-Younis

[1,2,3] Department of Food Science, College of Agriculture, University of Basrah, Iraq.

ARTICLE INFO

Article History:

Received: 2024/10/30
Accepted:2024/12/1

Keywords:

enzyme-producing bacteria,

cellulolytic enzymes,

cellulolytic microorganisms,

cellulolytic activity.

DOI: 10.22034/FSCT.22.160.77.

*Corresponding Author E-Mail:
agripg.sabreen.mansoor@uobasrah.edu.iq

ABSTRACT

The current study aims to enhance work in the field of isolating microorganisms that produce enzymes with practical uses, sustainability and food industries, by isolating and identifying lactic acid bacteria strains from different sources, some of which contain cellulose, which microorganisms depend on, primarily for their nutrition and reproduction, and which is present in the environment in which they are found, and the possibility of producing the enzyme Cellulase, which is considered one of the important enzymes in the analysis of polysaccharides (cellulose) and the production of monosaccharides and simple sugars. This study included the isolation and identification of Lactobacillus bacteria from different sources, purification, screening, identification and determination of their efficiency in producing cellulase enzymes. The results showed that fifteen isolates were obtained from various sources including soil, fruits, vegetables, pickles, dairy products and live fish entrails. Agar MRS medium with 0.5% (w/v) CaCl2 granules was used to isolate lactic acid bacteria, which were identified by phenotypic, biochemical and Gram staining tests. A preliminary screening was performed by estimating the enzyme activity on the solid medium by measuring the diameter of the transparent halo formed around the colonies in the medium. The results were enhanced by measuring the activity of the enzyme produced on the liquid medium by measuring the light absorption using a spectrophotometer. The results obtained showed that the best isolates in enzyme production are the isolates that produced the highest enzyme efficiency on the solid medium, which is the one isolated from the guts of live fish (fish 3), which formed a transparent halo with a diameter of 3 cm on the solid medium and the highest efficiency in the liquid medium, which obtained the highest absorption in the spectrophotometer, highest enzymatic activity, reaching ٤,٥٤٦ and Specific activity ١٠,٣٣١. After selecting the best isolate from among the isolates, the optimum conditions for enzyme production were studied in different situations, including [temperature, pH, vibrating incubator speed, inoculum quantity, different carbon and nitrogen sources and the period required for fermentation], and the following results were obtained: The optimum temperature for production is 35 °C with an enzymatic activity reached (3.425) and Specific activity (4.502), the pH value was 6 with the enzymatic activity reached (3.437) and Specific activity (4.399), the enzymatic activity was (3.419), and specific activity was (4.804), a fermentation time of 72 hours with an efficiency of (3.065) and Specific activity (4.305),the best carbon source cellulose with an efficiency of (5.44), and the best Nitrogen source wheat bran with an efficiency of (3.634) and a inoculum content of 5% with an efficiency of (3.399).

[DOI: 10.22034/FSCT.22.160.77]

1-Introduction

Lactic acid bacteria (LAB) are important microorganisms that produce lactic acid primarily as a by-product during metabolic activities and play multifaceted roles in the agricultural, food and clinical sectors. Lactic acid bacteria are used in many fermentation processes, as the use of these bacteria is considered one of the most traditional and used sciences in food processing and preservation. Due to the importance of lactic acid bacteria in many food applications and because they have therapeutic properties to enhance human health, research is ongoing to obtain strains with properties that enhance the quality of food products. [1]. These bacteria are associated with humans through fermented foods, dairy products and other sciences and applications [2]. They are heterotrophic, Gram-positive, single rods or short chains [*3*], non-spore-forming, anaerobic but aerobic, and produce lactic acid as one of the main fermentation products by using carbohydrates during fermentation. It produces organic materials that contribute to flavor, taste and smell, which give the products in which it is used unique sensory properties [4]. Lactobacillus plantarum is one of the most widely used genera in the food industry, whether as microbial starters or as probiotic microorganisms. Several strains of L. plantarum have been shown to produce different antimicrobial compounds such as organic acids, hydrogen peroxide, as well as bacteriocins and antimicrobial peptides, each of which has a variable spectrum of action [*5*]. These receptors are thought to be a defense mechanism deployed by bacteria to prevent microorganisms from colonizing their natural environment by inhibiting their proliferation and survival. Other important biological activities such as biofilm formation and the production of biosurfactants play a vital role in suppressing the attachment of pathogens [6,7]. Lactic acid bacteria produce organic acids and other metabolites that enhance the development of flavor in food, prevent spoilage, and are therefore very useful in many applications, especially in the food and dairy industries. The dairy sector in particular greatly benefits from lactic acid bacteria, and therefore the potential of lactic acid bacteria as starters should be investigated as product quality and sensory appeal are greatly influenced by the role of starters in dairy [8]. Lactic acid bacteria have been classified into different genera and species based on their acid-production properties through the fermentation of sugars and their growth at specific temperatures [9]. In addition, lactic acid bacteria can be classified as homozygous or heterozygous organisms based on their ability to ferment carbohydrates [10]. Homogeneous lactic acid bacteria such as Lactococcus and Streptococcus produce two molecules of lactate from one molecule of glucose while heterogeneous lactic acid bacteria such as Leuconostoc and some lactobacilli generate lactate, ethanol and carbon dioxide from one molecule of glucose [11]. There are more than 260 species of lactic acid bacteria that exhibit a wide range of physical, ecological and genetic traits. The genus Lactobacillus was recently reclassified by scientists into 25 genera. This reclassification was necessary because of the diversity of the original genus, which made it extremely difficult to classify, name and differentiate between different lactobacilli. The new genera are Lactobacillus, Paralactobacillus and 23 new genera. The twenty-three new genera include [23] [12] (*Amylolactobacillus*, *Acetilactobacillus*, *Agrilactobacillus*, *Apilactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Dellaglioa*, *Fructilactobacillus*, *Furfurilactobacillus*, *Holzapfelia*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Lapidilactobacillus*, *Latilactobacillus*, Lentilactobacillus, *Levilactobacillus*, *Ligilactobacillus*, *Limosilactobacillus*, *Liquorilactobacillus*, *Loigolactobacilus*, *Paucilactobacillus*, *Schleiferilactobacillus*, and *Secundilactobacillus*) [13].

The term “cellulases” refers to a group of enzymes that catalyze the hydrolysis of cellulose into polysaccharides and is an important enzyme in industrial biotechnology today. All living systems regulate their biological activity through enzymes. An enzyme is a protein molecule that acts as a biological catalyst that increases the speed and rate of a reaction, and most cellular reactions occur much faster than they would in the absence of an enzyme. [14]. Cellulolytic enzymes produced by microorganisms have

[DOI: 10.22034/FSCT.22.160.77]

many biotechnological and industrial applications. Due to the use of cellulose in many industries such as textiles, detergents, food, animal feed, biofuel, paper and pulp, pharmaceuticals, and waste management, they are required in large quantities. The first step in developing a process for producing cellulases is the isolation and characterization of bacterial strains capable of producing them. This is a very important step due to the demand for enzymes with many applications in biotechnology. [15] and [16]. The cellulases group consists of three types of enzymes secreted outside the cell: β-glucosidase, 1,4-β-endoglucanase, and 1,4-β- exoglucanase. [17] The most important organisms that produce cellulases are bacteria and fungi, and these microorganisms are usually found in the soil. The potential bacteria to produce cellulases are Cellulomonas, Pseudomonas, Thermoactinomycetes, and Bacillus spp. [18]

2.Materials and methods:

2.1 Sample Collection: 15 samples were collected from different sources to isolate lactic acid bacteria. These samples included fruits [bananas, watermelons, grapes, tomatoes], vegetables [cucumbers, watercress], yogurt, 3 pickle samples, and 4 fish samples. They were brought to the laboratory in sterile, tightly sealed plastic bags, except for fish samples that were brought to the laboratory live, dissected, and the digestive system was extracted. Microorganisms were isolated from the viscera of live fish.

2.2 Isolation of Lactic Acid Bacteria: Twenty-five grams of each sample were added to 250 ml of 0.85% saline solution [NSS] and decimal dilutions of the samples were made by taking one ml of the sample to nine ml of physiological solution and dilution continued to obtain concentration 7-10. After that, 0.1 ml of each dilution was spread on (MRS Agar) with CaCl2 granules at a rate of 0.5% [w/v] and in two replicates for each dilution. The plates were incubated at 37 °C for 48-72 hours under anaerobic conditions. The formation of a transparent halo was observed on MRS agar with CaCl2. The isolates that formed halos were grown individually on MRS agar [the process was repeated three times] to obtain pure isolates. [19, 20]

2.3 Bacterial Diagnosis

- *Microscopic examinations:*

1- Staining bacteria with Gram stain: Colonies growing on a solid MRS medium were selected and cells were stained with Gram stain to identify cell morphology and clustering [21].
2- Motility assay: A drop of 24-hour-old bacteria from the tubes was placed on a motility slide. [21].

- *Biochemical tests:*

1- Catalase test: This test was performed by mixing a bacterial colony aged [24] hours with a drop of water, then adding a drop of 3% hydrogen peroxide prepared on a clean glass slide. Note the formation of bubbles on the slide as evidence of a positive test. [22].
2- Oxidase test: A bacterial colony of 18-24 hours of age was transferred using a sterile wooden stick to a filter paper and a drop of oxidase indicator was added to it. The colony turned dark purple, indicating a positive test [22].
3- SCA: Isolates were cultured at 24 h on the prepared SCA medium and then incubated at 37°C for 7 days. A Change in the color of the medium from green to blue is a positive result [23].
4- Indole test: The tubes containing indole medium (Tryptone Broth were prepared by dissolving 2% g of tryptone and 0.5% NaCl, then the pH was adjusted to 7, then distributed into test tubes at a rate of 10 ml for each tube, then sterilized) were inoculated with a bacterial culture aged [24] hours. It was incubated at a temperature of 37°C for [48-24] hours. After completing the incubation period, a few drops of Kovacs reagent were added [the reagent was obtained from the Al-Basheer Scientific Office in Baghdad Governorate. [24].
5- Growth at temperatures above 45°C: All isolates were grown on MRS agar medium in two replicates at a temperature above 45°C and incubated inverted for 48-24 hours to observe their ability to grow. [21].
6- Growth at temperatures below 5°C: All isolates were grown on MRS agar medium in two replicates at a temperature below 5°C

[DOI: 10.22034/FSCT.22.160.77]

and incubated inverted for 48-24 hours to observe their ability to grow [21].

7- Gas production test from glucose: MRS liquid culture tubes and Durham tubes were inoculated inverted with a drop of 24-hour-old bacterial cultures and incubated at 37°C for 24-48 hours to observe their ability to produce gas from glucose [21].
8- Sugar fermentation test: The medium was prepared using the components of liquid MRS without adding glucose and meat extract, and added to its chlorophenol red converter at a concentration of 0.004%. Then, sugars were added (each separately) at a concentration of 2% to the aforementioned medium, which included (fructose, mannose, glucose, galactose, sucrose, maltose, menthol, radiculose, salicin, xylose, melibiose, raffinose, arabinose, and arabinose) after adjusting the pH (6.2-6.5]. The medium was sterilized, while the sugars (mannose-maltose-xylose) were filtered before adding them to the previously sterilized medium. [21].

2.4 Production of Cellulases Enzyme on Solid Medium: The special solid medium was used for the production of Cellulases, which were prepared from the following components g/l (CaCl2, 0.3 and FeSO4,7H2O, 5 and MnSo4. H2O, 1.6 and CMC, 8 and Tween80, 2 and Peptone, 0.8 and KH2PO4, 2 and Urea, 0.3 and MgSO4,7H2O, 0.5 and ZNSo4, 1.4 and $(NH4)_2So4$, 1.4 and Agar, 20 and distilled water, 1 litre). The solid medium was inoculated with active bacteria aged 24 hours and the plates were incubated at 37°C for 48 hours [25].

2.5 Detection of the Enzyme Product: The production of the enzyme Cellulases is indicated by using the iodine-hydrochloric acid (HCl)-I reagent. This reagent is prepared by taking 100 ml of 0.1 N HCl (+ 500 ml of) + ½ l KI 2% (this reagent is added to the dish containing the pure bacterial colony and left for 5 minutes, then the solution is poured out and the dish is left for 10 minutes. Then a light-colored halo is observed around the colony, indicating the conversion of cellulose into simple sugars] [26]

2.6 Production of Cellulase Enzyme in Liquid Medium

The liquid nutrient medium for the production of Cellulases enzyme was used and prepared from the following materials (FeSO4.7H2O, 0.0004%, CACl2.2H2O, 0.0001%, MgSO4.7H2O, 0.02%, Tryptone, 0.20%, KH2PO4, 0.4%, Na2HPO4, 0.04%, and CMC, 1.0%). The pH was adjusted to 7 and sterilized at 121°C for a quarter of an hour]. 50 ml of the medium was placed in a 250 ml flask for each isolate. The medium was inoculated with 2.5 ml (5%) of the bacterial isolate and incubated in a shaking incubator at 37°C for 48 hours at a speed of 150 rpm. After incubation, the medium was transferred into test tubes and the medium was centrifuged to remove unwanted materials at a speed of 6000 rpm for 10 minutes. The filtrate was collected after centrifugation, which is considered the source of the raw enzyme and is used to determine the enzyme activity. [27]

2.7 Optimal Conditions for the Production of Cellulase Enzyme from the Obtained Isolate

The optimum conditions for the production of the cellulase enzyme from the local isolate were studied by studying the production and enzymatic activity in the cell-free medium and at different temperatures.

1. The temperatures (45, 40, 35, 30, 25) °C were used, and the enzyme production was then estimated by Enzymatic activity through measuring optical absorption at 550 nm.
2. The effect of the PH was studied, the values (10, 9, 8, 6, 5, 4) were used and the enzyme production was then estimated by Enzymatic activity through measuring optical absorption at 550 nm,
3. The effect of the speed of the incubator Shaker was studied, and the following speeds (180, 160, 140, 120, 100) rpm were used, after which the enzyme production was estimated.
4. The effect of the fermentation time to produce enzymes using the local isolate was also tested at (144, 120, 96, 72, 24) minutes and the enzyme production was estimated.
5. Studying the effect of different carbon sources on enzyme production from the local isolate was done, as the following carbon sources were used (glucose, molasses, sunflower oil, cellulose, glycerol, mantol),

[DOI: 10.22034/FSCT.22.160.77]

6. and the enzyme production was then estimated.
7. The effect of the nitrogen source was also studied using the components of the previous medium with a change in the nitrogen source used in production. The following nitrogen sources were used (wheat bran, urea, glutamic acid, whey, ammonium nitrate), and the enzyme production was then estimated.
8. The effect of the inoculum ratio on the enzyme production from the local isolate was studied, as the following inoculum ratios were used (7, 6, 4, 3, 2, 1).

2.8 Statistical analysis

All measurements were done in triplicate and data presented are mean values ± standard deviation. A one-way analysis of variance (ANOVA) was applied to assess the diffusion diameters of cellulase activity, however, a two-way analysis was performed to analyze the screening of cellulase producing lactic acid bacteria, enzymatic activity and Specific activity and Optimal Conditions for the Production of Cellulase Enzyme from the Obtained Isolate. Mean comparisons were carried out using the L.S. D test at a level of significance $pp \leq 0.05$. SPSS program ver. 25 was applied to analyze data

3.Results and Discussion

The results of the isolation of Lactobacillus bacteria resulted in obtaining 15 isolates belonging to this genus. The isolates obtained varied according to the sources of isolation used in this study, as the sources varied to include (milk, cucumber, banana, watermelon, soil, grapes, fish four isolates, pickles three isolates, watercress and tomatoes). Figure [1] shows the results of the morphological examinations of the bacteria.

The results showed that the colonies of Lactobacillus bacteria growing on the solid MRS medium were circular in shape and small in size, some were convex and others were flat, smooth, soft and shiny. As for their color, some were white while others were creamy in color. Microscopic examination showed that they were cells of different shapes, as some were rod-shaped while others were spherical rod-shaped, some were single and some were double, while some were in the form of long or short chains, according to what was stated in [28]. It is a Gram-positive, non-spore-forming, non-motile bacterium, and the results were consistent with what was mentioned by [29].

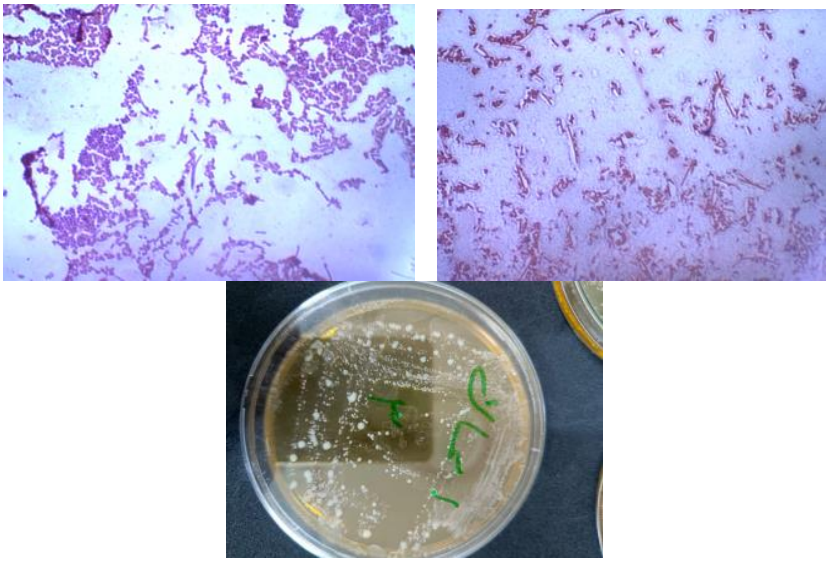

Figure [1] Phenotypic tests of lactic acid bacteria

3.1 Biochemical characteristics

When conducting biochemical tests on Lactobacillus isolates, it was found that all isolates were positive for Gram stain, did not produce catalase except for isolate [Fish 2] and oxidase, did not consume citrate, did not produce indole, could ferment glucose, and grew at 45°C but did not grow at 5°C. Table [1]

[DOI: 10.22034/FSCT.22.160.77]

Table [1] Biochemical tests of bacterial isolates

Indole	Growth at 5	Growth ٤٥	Gram stain	Gas production	consume citrate	Catalase	Oxidase	
-	-	+	+	+	-	-	-	**Yogurt**
-	-	+	+	+	-	-	-	**cucumber**
-	-	+	+	+	-	-	-	**banana**
-	-	+	+	-	-	-	-	**Watermelon**
-	-	+	+	+	-	-	-	**soil**
-	-	+	+	-	-	-	-	**Grapes**
-	-	+	+	+	-	-	-	**Fish 1**
-	-	+	+	+	-	+	-	**Fish 2**
-	-	+	+	+	-	-	-	**Fish 3**
-	-	+	+	+	-	-	-	**Fish 4**
-	-	+	+	-	-	-	-	**Pickle 1**
-	-	+	+	+	-	-	-	**Pickle 2**
-	-	+	+	+	-	-	-	**Pickle 3**
-	-	+	+	+	-	-	-	**Watercress**
-	-	+	+	-	-	-	-	**tomatoes**

3.2 Sugar Fermentation

The ability of Lactobacillus bacteria to ferment some types of sugars was tested, which included [galactose, mannitol, sucrose, melibiose, sorbiose, lactose, raffinose, glucose, fructose, sorbitol, and xylose]. The results in Table [2] showed that Lactobacillus isolates had different abilities to ferment sugars, as there were [4] isolates that could ferment galactose sugar, [4] isolates that could ferment mannitol sugar, [6] isolates that could ferment sucrose sugar, two isolates that could ferment melibiose sugar, none of the isolates could ferment sorbose sugar, three isolates could ferment lactose sugar, and there were [5] isolates that could ferment raffinose sugar, [5] isolates that could ferment glucose sugar, [4] isolates that could ferment fructose, and [6] isolates that could On fermentation of sorbitol and all isolates have the ability to ferment xylose.

Table [2] Fermentation of sugars by the isolates under study

Sugar Fermentation											
Xylose	Sorbitol	Fructose	Glucose	Raffinose	Lactose	Sorbiose	Melibiose	Sucrose	Manthol	Galactos	
+	+	+	+	-	+	-	-	+	+	+	Yogurt
+	+-	+	+	+	+	-	-	+	+	+	cucumber
+	-	-	-	-	+	-	-	-	-	-	banana
+	-	-	-	-	-	-	-	-	-	-	Watermelon
+	+	-	-	-	-	-	-	-	-	-	soil
+	-	-	-	-	-	-	-	-	+	-	Grapes
+	-	-	-	+	-	-	-	-	-	-	Fish 1
+	-	-	-	+	-	-	-	-	-	-	Fish 2
+	+	+	+	+	-	-	-	+	-	+	Fish 3
+	-	-	+	-	-	-	∓	-	-	-	Fish 4
+	-	-	-	-	-	-	+	-	+	-	Pickle 1
+	-	+	+	-	-	-	-	+	-	-	Pickle 2
+	-	-	-	+	-	-	-	-	-	+	Pickle 3

[DOI: 10.22034/FSCT.22.160.77]

+	+	-	-	-	-	-	-	+	-	-	Watercress
+	+	-	-	-	-	-	-	+	-	-	tomatoes

3.3 Screening of Isolates for the Production of Cellulases

A preliminary screening of the isolates obtained through the study was conducted Table [3] by measuring the diameter of the transparent halo formed around the colonies growing on the solid medium containing CMC as a carbon source. The results showed that the isolates isolated from live fish aggregations were superior to the other isolates from different sources. If the isolate Fish 3 obtained the highest halo diameter, which reached 3 cm, then the isolate Fish 1 had a halo diameter of [2.6] and Fish 4, which was equal to it, the isolate Pickle 2, which had a halo diameter of 1.4 cm. These results were close to the results of the isolates isolated from pickles, which ranged between 1.4 and 1.2 cm. It was followed by watercress, which was equal to pickle 1 with a result of 1.3 cm, then tomatoes, then watermelon, soil, grapes, cucumbers, yogurt, and finally the isolates isolated from bananas, which had a diameter of 0.4 cm.

Table [3] Diameter of the transparent halo around the colonies on the solid medium for the production of Cellulases

isolation	Yogurt	cucumber	banana	Watermelon	soil	Grapes	Fish 1	Fish 2	Fish 3	Fish 4	Pickle 1	Pickle 2	Pickle 3	Watercress	tomatoes
zone diameter	٠,٥	٠,٦	٠,٤	٠,٩	٠,٨	٠,٧	٢,٦	0	٣	١,٤	١,٣	١,٤	١,٢	١,٣	١,١

The isolates that excelled in production were then selected and a secondary screening was conducted for them by estimating the activity of the enzyme produced in the liquid medium containing CMC as a carbon source. After preparing and sterilizing the medium and inoculating it with the active isolates aged 24-48 hours, and through three replicates for each isolate, the results in Table [4] were obtained, which showed the superiority of Isolate (Fish 3), which obtained the highest absorption in the spectrophotometer, which represents the highest enzymatic activity, reaching ٤,٥٤٦ and Specific activity ١٠,٣٣١. It was followed by Fish Isolate 4 and Pickle 2, which were ٢,٦٩٣ and ٢,٦٣٩, respectively and a Specific activity ٤,٩٠٥, ٤,٨٥١, respectively. The results showed a decrease in the enzymatic activity and Specific activity of the enzyme produced by the other isolates, which included Fish 1, Pickle 1 and Pickle 3, which were equal to ٢,١٦٩, ١,٧٧٥ and ٢,٠٤٩, with Specific activity ٣,٨٠٩, ٣,٧٦٠ and ٣,٦١٢. as in Figure [2]. Fish Isolate 3 was chosen as the best isolate for enzyme production.

Table 4 Secondary screening of some lactic acid bacteria isolates producing the enzyme Cellulases

Isolate	Specific activity	Enzymatic activity
Fish 1	٣,٨٠٩ ± 0.002	٢,١٦٩ ± 0.003
Fish 3	١٠,٣٣١ ± 0.003	٤,٥٤٦ ± 0.004
Fish 4	٤,٩٠٥ ± 0.002	٢,٦٩٣ ± 0.002
Pickle 1	٣,٧٦٠ ± 0.002	١,٧٧٥ ± 0.003
Pickle 2	٤,٨٥١ ± 0.004	٢,٦٣٩ ± 0.002
Pickle 3	٣,٦١٢ ± 0.004	٢,٠٤٩ ± 0.003

[DOI: 10.22034/FSCT.22.160.77]

3.4 Study of the optimum conditions for the production of the enzyme Cellulases

The study of the optimum conditions for the production of the enzyme from the selected isolate [Fish 3] included all of the temperatures, acidity function, incubator speed, storage period, carbon and nitrogen sources, and the percentage of vaccine used.

4.Temperature

The results in Figure [2] showed that the effectiveness increased with the increase in the fermentation temperature and that the best temperature for enzyme production from local fish isolates [3] was 35 degrees Celsius, as the enzymatic activity reached (3.425) and Specific activity (4.502) compared to temperatures (25, 30, 37, 40, 45) in which the enzymatic activity was (2.١٦٩, 2.497, 2.214, 1.645, 0.٥20) and Specific activity (3.309, 3.504, 3.66, 3.051, 1.015) respectively. The reason for the high effectiveness at temperatures of 35 degrees and temperatures close to it is due to the high ability of the bacterial isolate under study to produce the enzyme, as the temperature is ideal for the growth of these bacteria. The results of the statistical analysis showed significant differences in both enzyme activity and specific activity during the temperature experiment. The results agreed with the results obtained by [30], who confirmed that the best temperature for production is 35 degrees Celsius and agreed with what was reached by [31] and the results agreed with what was stated by [32], who stated that the best temperature was 35°C, while it differed from what [33] reached, who stated that the best temperature is 30°C. The reason for the best temperature for production may be due to the source of the isolation and the conditions in which the isolation lives, as its source is fish that live in waters that are often moderate to low temperatures.

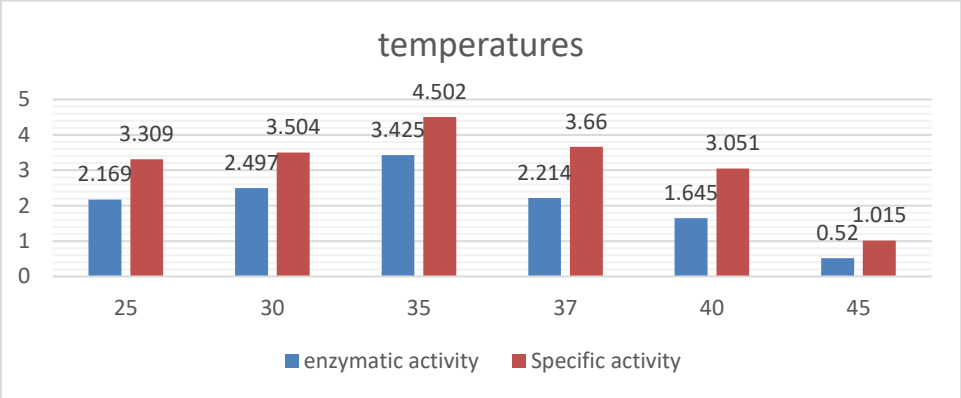

Figure [2] The effect of temperature on the production of the enzyme Cellulases.

L.S.D.= 0.831 for the interference between enzymatic activity and temperature.
L.S.D.= 1.021 for the interference between specific activity and temperature.

1- The PH

The results of the study confirmed that the best production of the cellulase enzyme and the highest enzymatic activity of the enzyme produced from the local isolate [fish 3] were at (PH 6) where the enzymatic activity reached its highest levels and reached (3.437) with Specific activity (4.399) and while the results for the rest of the acid functions were 0.278 at (PH 10) , 1.010 at (PH 9) , 1.989 at (PH 8) and 2.125 at PH 7 while the activity decreased from its highest value at the acid function (PH 6) to 1.295 at PH 4 and 2 at PH 5 and Specific activity (1.201, 2.02, 2.505, 3.101, 2.333, 3.534) respectively as shown in Figure [3] The results of the pH study showed highly significant differences in both enzyme activity and specific activity at the ($p \leq 0.05$). and the results agreed with what was reached by [30,34] who confirmed that the best enzyme production and activity were at (PH 6) and did not agree with the results of [35] and [31]] who found that the best degree of production was (PH 6) and

[DOI: 10.22034/FSCT.22.160.77]

(PH 8) and [36] found that the optimal pH was [7].

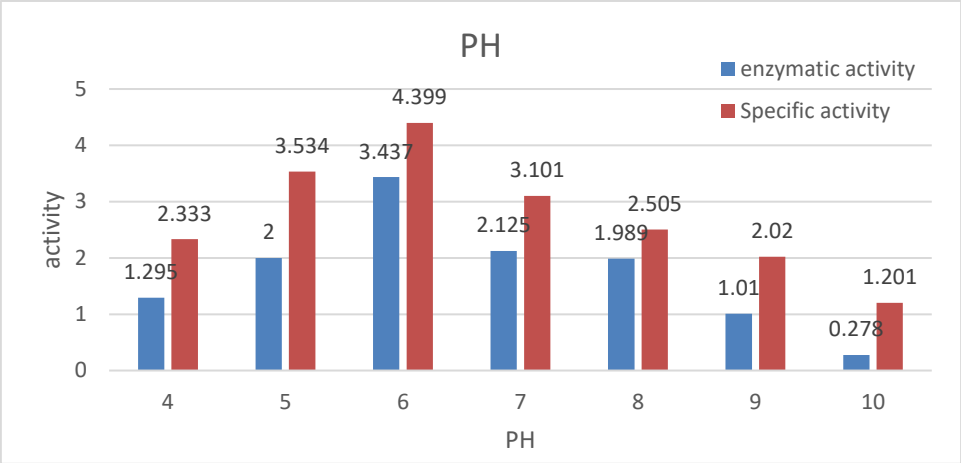

Figure [3] Effect of pH on Cellulase production

L.S.D.= 1.121 for the interference between enzymatic activity and Effect of pH.
L.S.D.= 0.8.02 for the interference between specific activity and Effect of pH.

2- Incubator Speed

The results of the study confirmed that the enzyme activity began to increase with an increase in the speed of the vibrating incubator, as it reached the highest activity (3.419) and with Specific activity (4.804) at a speed of 150 rpm, and the activity decreased when the speed increased or decreased from this level. The enzymatic activity ranged between 2.879 at a speed of 160 rpm and 2.459 at a speed of 100 rpm, Also, the Specific activity reached its highest levels at a speed of 150 rpm, reaching 4.804. After that, Specific activity decrease with an increase or decrease in the speed of the incubator, reaching its lowest level of 3.694 at a speed of 100 rpm. as shown in Figure [4]. The results of the research were consistent with the results obtained by researchers (37, 38, 33, 31) who proved that the best speed of the vibrating incubator for the production of the cellulase enzyme was 150 rpm. The results differed from (35, 30, and 39), who showed that the best production speed was 120 cycles/minute.

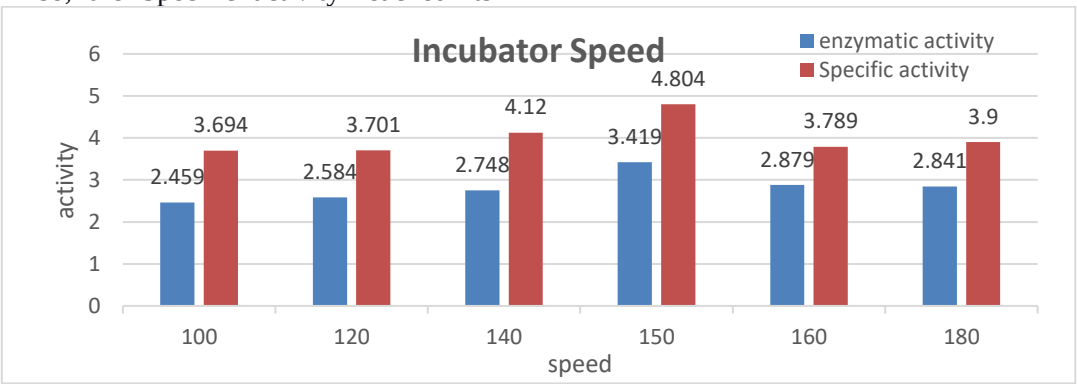

Figure [4] Effect of the vibrating incubator speed on Cellulase production

L.S.D.= 0.560 for the interference between enzymatic activity and Effect of the vibrating incubator speed.
L.S.D.= 0.587 for the interference between specific activity and Effect of the vibrating incubator speed.

3- Fermentation Time

Figure [5] shows the results of estimating the enzymatic activity of the cellulase enzyme produced by the local isolate Fish 3. It was found that the activity began to increase from the beginning of fermentation and after 24 hours from the start of the experiment, the

[DOI: 10.22034/FSCT.22.160.77]

enzymatic activity reached 2.672 and began to increase after 48 hours and reached 2.722.

The enzymatic activity reached its highest levels after 72 hours from the start of the experiment and reached the highest value of the activity (3.065) and a Specific activity of (4.305). Then the activity began to decrease with time until it became (2.551, 2.377, 2.246, and 1.939 after 96, 120, 144 and 150) hours, respectively.

The reason for this increase in enzyme production and enzyme activity is due to the availability of suitable conditions and nutrients for the bacterial isolate, which reached its highest levels after 72 hours, after which the concentration of nutrients began to gradually decrease, which was accompanied by a decrease in the concentration of the produced enzyme as well as the inhibitory effect of fermentation products, which include the enzyme and other metabolic products.

The results were consistent with the findings of [40], [35], and [36], who found that the optimum time for enzyme production from lactic acid bacteria was 72 hours. The results differed from [14], [41], and [42], who found that the optimum period for fermentation was [24], [48], and [96], respectively.

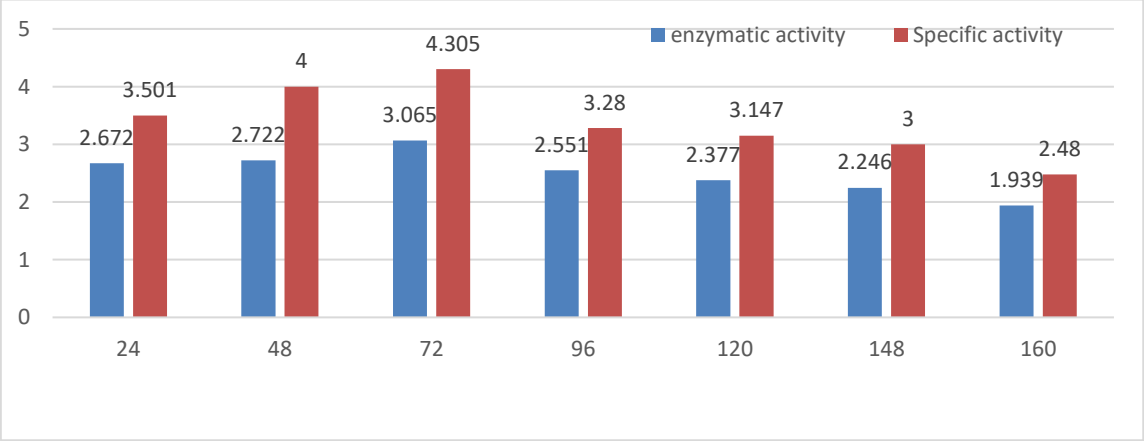

Figure [5] Effect of fermentation time on **Cellulase** production.

L.S.D.= 0.340 for the interference between enzymatic activity and Effect of fermentation time**.**
L.S.D.= 0.301for the interference between specific activity and Effect of fermentation time**.**

4- Carbon Source

The results of the study showed as in Figure [6] that the best carbon source for the production of the Cellulases enzyme by the local isolation of fish 3 was cellulose, where the enzyme activity reached its highest values when used and was (5.44) and a Specific activity of (6.8), followed by CMC which reached an activity of (5.229), then glucose, which reached an activity of 3.169 when used, then molasses, then mannitol, sunflower oil, and finally glycerol (2.896, 1.868, 1.786, 1.617) respectively. This work is similar to the work of [43], [30] and [33].

[DOI: 10.22034/FSCT.22.160.77]

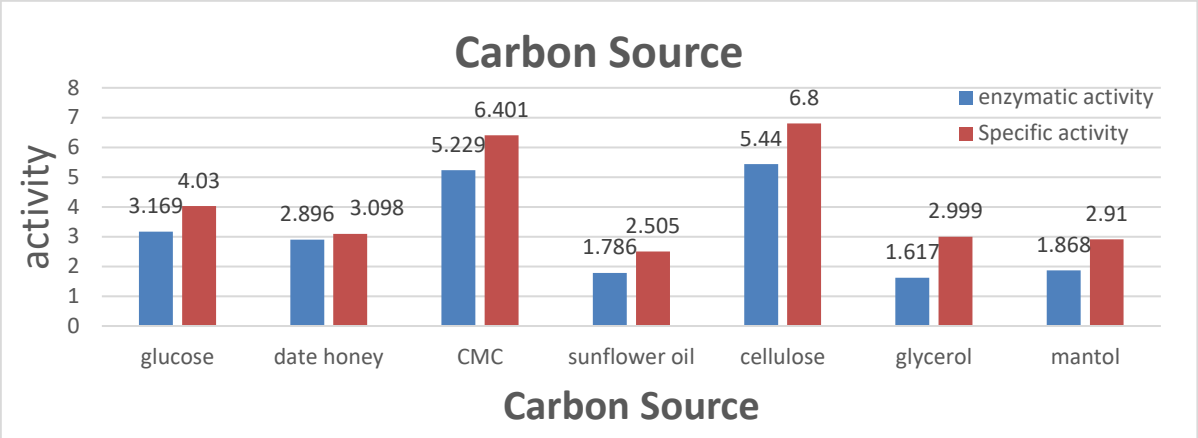

Figure [6] The effect of the carbon source on the production of the **Cellulase.**

L.S.D.= 0.380 for the interference between enzymatic activity and Effect of the carbon source**.**
L.S.D.= 0.410 for the interference between specific activity and Effect of the carbon source**.**

5- Nitrogen Source

Figure (7) shows the results of the effect of the carbon source used in the production of the Cellulases enzyme by the isolate under study, which is Fish 3. Wheat bran outperformed all the nitrogen sources used, as the effectiveness reached its highest value (3.634) and a Specific activity of (4.402), followed by tryptone as the second-best nitrogen source with effectiveness of 3.185, while the results were close when using urea and whey, whose effectiveness reached 2.786 and 2.535, respectively, then came glutamic acid with an effectiveness of 2.076, and finally came ammonium nitrate with effectiveness of 1.863, as in Figure [7]. This work is similar to the work of

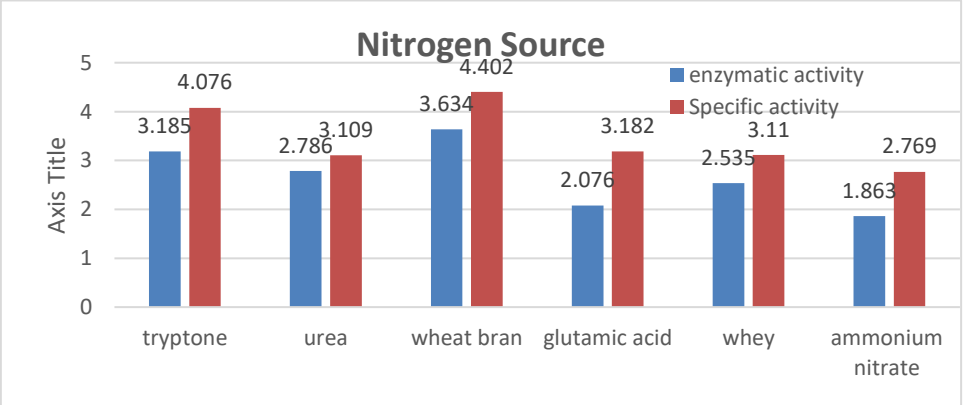

Figure [7] Effect of the nitrogen source on **Cellulase** production.

L.S.D.= 0.567 for the interference between enzymatic activity and Effect of the nitrogen source**.**
L.S.D.= 0.398 for the interference between specific activity and Effect of the nitrogen source**.**

6- Inoculum Size

Figure [8] shows a noticeable increase in the enzymatic activity of the enzyme produced with the increase in the percentage of vaccine from the active culture at the age of 24-48 hours. The figure shows that the activity increased from its lowest level of 0.169 when using a vaccine percentage of 1% to the highest level (3.399) at a vaccine percentage of 5% and the Specific activity was (5.002). It then began to decrease at a vaccine percentage of 6% and 7%, and the activity reached 2.786 and 2.628.

[DOI: 10.22034/FSCT.22.160.77]

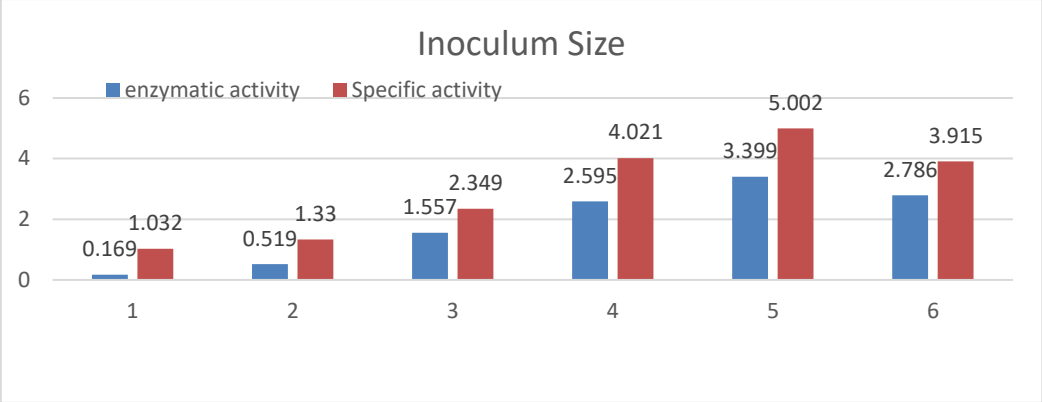

Figure [8] Effect of the vaccine percentage on Cellulase production

L.S.D.= 0.586 for the interference between enzymatic activity and Effect of the vaccine percentage**.**
L.S.D.= 0.102 for the interference between specific activity and Effect of the vaccine percentage**.**

Conclusion: The current study showed that 15 isolates of lactic acid bacteria were acquired and diagnosed by morphological and biochemical tests. the ability of lactic acid bacteria to produce cellulase enzyme was tested as well as the screening by estimating the enzyme activity and Specific activity.

It was found that there are 14 isolates capable of producing the enzymes by measuring the diameter of the clear zone around the colonies as a result of the enzyme activity, as well as estimating the enzyme activity and Specific activity by measuring the light absorption, which proved that the isolate (fishes 3) is the best isolate.

The optimum conditions for enzyme production were studied using the same light absorption method, which included (temperature, acidity function, incubator speed, fermentation time, inoculum size, carbon source and nitrogen source).

5.References

[1] Bintsis, T. [2018], Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. Aims Microbiol., 4, 665–684.
[2] Hill, D., Sugrue, I., Arendt, E., Hill, C., Stanton, C., & Ross, R. P. [2017]. Recent advances in microbial fermentation for dairy and health. F1000Research, 6, 751.
[3] Fidanza, M., Panigrahi, P. and Kollmann, T.R. [2021] Lactiplantibacillus plantarum–Nomad and Ideal Probiotic, Frontiers in Microbiology, V[12] 13.
[4] Quinto, E.J.; Jimenez, P.; Caro, I.; Tejero, J.; Mateo, J.; Girbes, T. [2014] Probiotic Lactic Acid Bacteria: A Review. Food Nutr. Sci. 5, 1765–1775.
[5] Arena, M.P., Silvain, A., Normanno, G., Grieco, F., Drider, D., Spano, G., and Fiocco, D. [2016] Use of Lactobacillus plantarum Strains as a Bio-Control Strategy against Food-Borne Pathogenic Microorganisms, Frontiers in Microbiology, [7] 464.
[6] Abruzzo, A.; Giordani, B.; Parolin, C.; De Gregorio, P.R.; Foschi, C.; Cerchiara, T.; Bigucci, F.; Vitali, B.; Luppi, B. [2021] Lactobacillus crispatus BC1 Biosurfactant Delivered by Hyalurosomes: An Advanced Strategy to Counteract Candida Biofilm. Antibiotics [Basel].10, 33.
[7] Markande, A.R.; Patel, D. and Varjani, S. [2021] A review on biosurfactants: properties, applications and current developments. Bioresour Technol., 330.
[8] Hati, S.; Mandal, S.; Prajapat, J.B.[2013], Novel Starters for Value Added Fermented Dairy Products. Curr. Res. Nutr.Food Sci. J. [1], 83–91.
[9] Parvez, S.; Malik, K.A.; Ah Kang, S.; Kim, H.Y.[2006] Probiotics and their fermented food products are beneficial

[DOI: 10.22034/FSCT.22.160.77]

for health. J. Appl. Microbiol.100, 1171–1185.

[10] Mokoena, M.P. [2017] Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules, 22, 1255.

[11] Salminen, S.; Bouley, C.; Boutron-Ruault, M.C.; Cummings, J.H.; Franck, A.; Gibson, G.R.; Isolauri, E.; Moreau, M.C.; Roberfroid, M.; Rowland, I. [2013] Functional food science and gastrointestinal physiology and function. Br. J. Nutr. 1998, 80 [Suppl. 1], S147–S171.

[12] Zheng, J.; Wittouck, S.; Salvetti, E.; Franz, C.M.; Harris, H.M.; Mattarelli, P.; and Watanabe, K. A [2020] taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol., 70,2782–2858.

[13] Ayivi. R.D., Gyawali, R., Krastanov, A, Aljaloud, S.O., Worku, M., Tahergorabi, R., da Silva, R. C. and. Ibrahim. S.A [2020] Lactic Acid Bacteria: Food Safety and Human Health Applications, Dairy , 1, 202–232.

[14] Islam, M., Kumar Sarkar, P., Mohiuddin, A.K.M., Suzauddula, Md. [2019]. Optimization of fermentation condition for cellulase enzyme production from Bacillus sp ,Malaysian Journal of Halal Research Journal [MJHR] V[2], ISSUE 2.

[15] Mandels M [1985] Applications of cellulases. Biochem Soc Trans 13:414–415

[16] Park J, and Park K [2001] Improvement of the physical properties of reprocessed paper by using biological treatment with modified cellulase. Bioresour Technol 79:91–94

[17] Patel, A.K., Singhania, R.R., Sim, S.J. and Pandey, A. [2019] Thermostable cellulases: Current status and perspectives. Bioresour. Technol., 279, 385–392.

[18] Sadhu, S. and Maiti, T.K.[2013] Cellulase Production by Bacteria: A Review. Br. Microbiol. Res. J. 3, 235–258.

[19] Giyatno DC, Retnaningrum E:[2020] Isolation and characterization of exopolysaccharide producer lactic acid bacteria from kersen fruit [Muntingia calabura L.]. J. Sains Dasar. 2020; 9[2]: 42–49.

[20] Fatin, R, and Pumnat, C. [2018] Isolation and Characterization of Biosurfactant Produced by Lactic Acid Bacteria from Indigenous Thai Fermented Foods, International Journal of Food Engineering Vol. 4, No. 4,

[21] Harley, J.P. and Prescott, L.M. [2002] Laboratory Exercises in microbiology, 5thEd, The McGraw-Hill companies, 449 p.

[22] Hemraj, V.; Diksha, S. and Avneet, G. [2013]. A Review on Commonly Used Biochemical Test for Bacteria. IJLS. 1[1]: 1-7.

[23] Brown, A. E. and Smith, H. R. [2017]. Benson's Microbiological Applications, Laboratory Manual in General Microbiology. 14th ed. McGraw-Hill Higher Education. New York. 438pp.

[24] Forbes, B. A.; Saham, D. F. and Weissfeld, A. S. [2007]. Baily and Scott's Diagnostic Microbiology. 12thed. Mosby, Inc., an anffilliate of Elsevier, Inc. 1031pp.

[25] Bhagat, S.A and Kokitkar, S.S. [2021] Isolation and identification of bacteria with cellulose-degrading potential from soil and optimization of cellulase production, Journal of Applied Biology & Biotechnology Vol. 9[06], pp. 154-161.

[26] Al- habeb

[27] Shaikh, N.M., Patel, A.A., Mehta, S.A. and Patel, N.D., [2013] Isolation and Screening of Cellulolytic Bacteria Inhabiting Different Environment and Optimization of Cellulase Production" Universal Journal of Environmental Research & Technology, 3[1].

[28] Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T.; Williams, S.T. [1994]. Bergey's manual of determinative bacteriology, pp. 559-564. 9th ed. William and Wilkins, MD.

[DOI: 10.22034/FSCT.22.160.77]

[29] Desa, A. [2008]. Strain identification, viability and probiotics properties of *Lactobacillus casei* Ph. D. Thesis. Victoria university, Werribee Campus Victoria, Australia.

[30] Abada EA, Elbaz RM, Sonbol H, Korany SM. [2021]. Optimization of cellulase production from Bacillus albus [MN755587] and its involvement in bioethanol production. Polish J. Environ. Stud. 30: 2459-2466.

[31] Yakubu1, A., Uba, G. and Vyas, A. [2021] Optimization of Culture Conditions for the Production of Alkaline Cellulase Enzyme Produced from Fusarium oxysporum VSTPDK, JEMAT, [9], No 1, 3-9

[32] Rabby, R. I., Ahmed,Z.B., Paul, G. K.,. Chowdhury, N. N., Akter, F., Razu, M. H. Karmaker, P. and Khan, M. [2022] A Combined Study on Optimization, In Silico Modeling, and Genetic Modification of Large Scale Microbial Cellulase Production, Biochemistry Research International, 14 pages

[33] Zhang, S., Wang , Z., Shen, J., Chen, X. and Zhang, J. [2023] Isolation of an Acidophilic Cellulolytic Bacterial Strain and Its Cellulase Production Characteristics, *Agriculture*, *13*, 1290

[34] Gimba, Y. A., Idris, A., Hassan, A. and Hassan, O. N. [2021] Isolation and optimization of the fermentation condition of cellulolytic microbial isolates from cassava waste water, GSC Biological and Pharmaceutical Sciences, 14[01], 011–017.

[35] Li, H., Dou, M. Wang , X., Ping Kou , N., Jiao, J. and Fu, Y. [2021] Optimization of Cellulase Production by a Novel Endophytic Fungus *Penicillium oxalicum* R4 Isolated from *Taxus cuspidate Sustainability*, *13*, 6006.

[36] Bai, S M., kumar, R., D.J. Mukesh kumar, P. Balashanmugam, M.D. Bala kumaran, P.T. Kalaichelvan [2012] Cellulase Production by *Bacillus subtilis* isolated from Cow Dung Archives of Applied Science Research, 4 [1]:269-279.

[37] Chantarasiri, A. [2020] Diversity of cellulolytic bacteria isolated from a freshwater wetland reserve in Thailand and their cellulolytic activity, Applied Ecology and Environmental Research 18[4]:5965-5983.

[38] Hossain, Md., Ahammed, A., Sobuj, I.S., Shifat, S.K. and Somadder P. D. [2021] Cellulase Producing Bacteria Isolation, Screening and Media Optimization from Local Soil Sample *American Journal of Microbiological Research*,[9] *3, 62-74.*

[39] Faizah, M. Ardyati, T. and, Suharjono [2020] Isolation and Identification of Indigenous Cellulolytic Bacteria from Sago Pith Waste at Palopo, South Sulawesi, Indonesia, J.Exp. Life Sci. 10[2].

[40] Vimal, J., Venu, A. and Joseph, J [2016] Isolation and identification of cellulose degrading bacteria and optimization of the cellulase production, International Journal of Research in Biosciences, [5] 3 [58-67].

[41] Shanmugapriya, K., Saravana, P.S., Krishnapriya, Manoharan, M.Mythili1, A, AND Joseph, S. [2012]. isolation, screening and partial purification of cellulase from cellulase producing bacteria, International Journal of Advanced Biotechnology and Research, [3], 1, pp 509-514.

[42] Lugani, Y., Singla, R. and Sooch, B.S. [2015] Optimization of Cellulase Production from Newly Isolated Bacillus sp. Y3, Journal of Bioprocessing & Biotechniques, [5], [11].

[43] Ariffin, H., Abdullah, N., Kalsom, M.S., Shirai, Y. and Hassan, M.A. [2006]. production and characterisation of cellulase by bacillus pumilus eb3, International Journal of Engineering and Technology, Vol [3] 1, pp. 47-53.

[44] Hayek, S.A.; Gyawali, R.; Aljaloud, S.O.; Krastanov, A.; Ibrahim, S.A. Cultivation media for lactic acid bacteria used in dairy products. J. Dairy Res. 2019, 86, 490–502.

[DOI: 10.22034/FSCT.22.160.77]