Research Article

Isolation and Identification of a Local Bacterial Isolates from Soil Producing Chitinase and Optimization of its Production

Reem A. Abdullah[1]* and Ziad T. Sedrah[2]

[1]*Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.*

Abstract | Nine pure isolates were recovered from various soils, with only three identified as Bacillus sp based on culture and morphological characterisation. The isolates utilized as producers of chitinase enzyme. The highest activity (5.85 U/ml) of the enzyme was achieved from Bacillus sp. (A). This isolate has been recognized as Bacillus licheniformis using 16S rRNA gene sequencing, with an accuracy of 99.79%. This work enhanced the synthesis of chitinase by Bacillus licheniformis through modifying several process variables, including the period of incubation, pH, and temperature, colloidal chitin, sources of nitrogen, as well as aeration. Findings indicate that optimal chitinase synthesis occurs at pH 8, when cultured in a stable environment at 40°C for 48 hours, utilizing a 1% concentration of colloidal chitin and 1% yeast extract as the nitrogen source.

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***Correspondence** | Reem A. Abdullah, Department of Food Science, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq; **Email:** reem.ali2302m@coagri.uobaghdad.edu.iq
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Introduction

Enzymes constitute proteins that have extraordinary catalytic activity and function as biological catalysts. Furthermore, they catalyse chemical events in cells and are crucial for all kinds of life due to their great specificity (Al_Azawee and Sedrah, 2024). They are used in many industries, including the food industry (Ahmed *et al*., 2018). Chitinolytic enzymes are extensively found in diverse species, such as fungi, bacteria, viral infections, plants, and mammalian (Hamed, 2016; Yang *et al*., 2021). The chitinase (EC 3.2.11.14) enzyme hydrolyzes insoluble chitin into its oligomer and monomers constituents (Henrissat, 1991). Chitinases degrade chitin by hydrolyzing β-1,4-glycosidic linkages, resulting in the formation of chitooligosaccharides (COS), further Chitobiases catalyzing N-acetylglucosamine (NAG) production. Contingent upon their engagement (Le & Yang, 2019) Chitin is a structure polysaccharide that exists in the cell walls of fungi, exoskeletons of arthropods, and soils. This enzyme belonging to the GH18 and GH19 families are essential for breaking down this polysaccharide. Chitinases have a molecular mass ranging from 20 to 120 kDa, according to (Kumar *et al*.,2018; Alwan *et al*., 2025; Talib *et al*., 2025). The production of single-cell proteins, pharmaceutically important chit oligosaccharides and N-acetyl D-glucosamine, separation of yeast and fungal protoplasts, treatment of chitinous waste, control of

harmful fungi, and prevention of malaria transmission are just a few of the many utilizes for chitinases (Vyas & Deshpande, 1991) Numerous bacteria inhabiting soil and aquatic environments exhibit chitinase activities and have been isolated or taken from several strains of bacteria, including Streptomyces, Pseudomonas, Paenibacillus, Serratia, Alteromonas, and Bacillus (Meena *et al.*, 2022). Bacillus species represent a significant source of bioactive natural chemicals (Emmert & Handelsman, 1999) They are not only harmless to humans and other organisms, but they also create a great deal of secondary metabolism through their endospore formation process (Shoda, 2000). Chitinolytic activity have been demonstrated by many Bacillus species, including B. pumilus (Agarwal *et al.*, 2017), B. subtilis (Ashwini *et al.*, 2014), and B. licheniformis strain LHH100 (Laribi-Habchi *et al.*, 2015). An increase in the synthesis of extracellular chitinase has recently attracted global interest (Wang *et al.*, 2006). By adjusting the fermentation conditions as well as composition of the culture medium, this production may be further improved. If we want to maximize output, productivity, and minimize production costs, we need to improve the production circumstances (Abdel-Fattah *et al.*, 2005).

This study aims to isolate and identify local soil bacterial isolates capable of producing chitinase, and to optimize environmental and nutritional conditions to enhance chitinase production by the most efficient isolate, *Bacillus licheniformis*.

Materials and Methods

Bacteria isolates sources

Nine samples of soil at the depth of approximately 9-12 cm were collected in a sterile test tube from the gardens of the houses in (Al Mansour-Al Yarmouk and Al-Jadreya in Baghdad) in October 2024.

Preparation of colloidal chitin

Ten grams of chitin powder were measured in a 250 ml glass beaker and combined with 100 ml of 37% w/w hydrochloric acid. Gradually and comprehensively mix it with a glass rod. Relocate the beaker to a plastic tray containing cooled water. Continuously agitate the slurry with the glass rod every 5 minutes for 30 minutes. Subsequent to the acid hydrolysis phase, gradually combine the acid slurry with 2 liters of cold distilled water while simultaneously mixing. Employ a magnetic stirrer for this phase. This suspension was allowed to mingle for a minimum of 15 to 20 minutes. The acidic CC suspension was gradually introduced into the Buchner funnel. This solution remained significantly acidic at this point. A vacuum pump may be utilized to accelerate the process. The whole suspension was poured into the funnel. The drainage of water was enabled from the viscous CC paste contained within the LCCM (Hisham *et al.*, 2024).

Cultural media

All media autoclaved at 121 °C and 15 pound/ inch^2 for 15 min

Chitin agar (CHIT. A)

Chitin agar consists of colloidal chitin (10.0 g/l), NH_4Cl (5 g/l), $MgSO_4 \cdot 7H_2O$ (0.5 g/l), KH_2PO_4 (2.4 g/l), K_2HPO_4 (0.6 g/l), and bacteriological agar (15 g/l) (Koteshwara *et al.*, 2021).

Nutrient agar (N.A)

Suspend 28g of nutrient agar powder in 1 litter of distilled water

Screening of chitinase producing bacteria (production media)

The screening was performed with bacterial isolates on the colloidal chitin broth mixed with 1000 ml of distilled water until the pH reached 7. Then, distributed into 250 ml Erlenmeyer flasks and autoclaved at 121 °C and 15 psi for 15 minutes.

Isolation and purification

A multitude of soil samples was gathered from several sites and transported to the laboratory in sterile tubes. A pour plate approach was employed as delineated by Al-Badran *et al.* (2019). After preparing decimal dilutions of the materials, one millilitre from every dilution has been transferred onto two plates comprising nutrition agar and chitin agar media (pH 7). Each dish had been incubating at 37 °C during 48 hrs.

Morphological, microscopic Characterization of the bacterial isolates.

Completely grown bacterial isolates have their morphological characteristics recorded, such as size, color, consistency and colony morphology. Gram staining, size of cells, form, and arrangement were some of the microscopic characteristics recorded

for the bacterial isolates (APHA, American Public Health Association 1978).

Assessment of activity of enzymes

Activity of chitinase was assessed by quantifying the reducing sugar N-acetylglucosamine (NAG) generated by the dinitrosalicylic acid (DNS) technique at 540 nm. A reaction mixture comprising 1.0% colloidal chitin (1 ml) and enzyme solution (1 ml) was sustained at pH 7.0 and 40°C for 1 hour. Subsequent to incubation, 3 ml of DNS reagent was introduced, and the reaction mixture was subjected to heating in a water bath at 100° C for 15 minutes. The mixture was subsequently centrifuged at 7500 xg for 5 minutes, and the absorbance was measured at 540 nm. The enzyme's unit can be described as the quantity of chitinase that stimulates the release of 1 millimole of the reducing sugar N-acetylglucosamine per minute under specified assay conditions (Halimahtussadiyah *et al*., 2017)

Enzyme activity calculated from the equation

$(U\backslash ml) = C^{*}V_t / t^{*}V_e$

One unit (U) of chitinase activity is defined as the quantity of enzyme necessary to liberate N-Acetyl-D-glucosamine.

Standard curve of n-acetyl-d-glucoseamine

The NAG solution was formulated by dissolving 0.0221 g of NAG powder in 100 mL from D.W. The mixture was thereafter serially diluted in several test tubes by using a 0.1 M buffer. The color intensity for every optical density (OD) solution was quantified using a spectrophotometer at 540 nm.

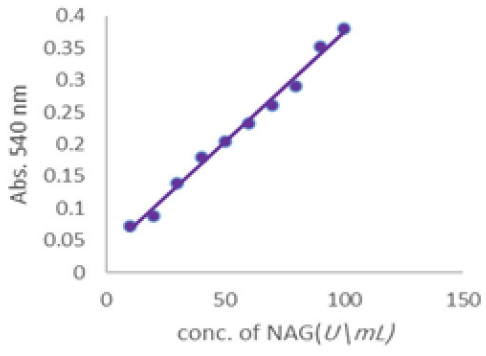


Figure 1: *standard curve of N-ACETYL-D-GLUCOSEAMINE*

Vitek 2 compact system

Bacterial isolates were diagnosed Using the Vitek system, Bacterial isolates were cultured on solid media using the streaking technique and the plates were incubated at 37°C for 24 hours to ensure the growth of bacterial colonies (Majeed *et al*., 2020).

Molecular identification

Genetic diagnosis

For bacteria, PCR amplification of 16S rRNA with 27 and 1492R primers, resulting in sequencing data of 1,300 bp or greater. To guarantee the outcome of Vitek 2 Compact, Genomic DNA was to the highest enzyme activity isolated bacteria according to the protocol of (Monreal & Reese, 1969) 1ml of culture was spun at 13,000 revolutions per minute for two minutes to get pellet cells. The remaining liquid was dumped. Before incubating the tube at 56 °C for 30 minutes, 20µl of Proteinase K solution (20 mg/ml) and 200µl of Buffer BL were added to the sample for digestion of proteins and lysis of cells. The mixture was then vortexed rapidly. The material was completely mixed by adding 200µl of 100% ethanol and then using a pulse-vortex blender. After meticulously transferring all of the mixtures to the small column, they were centrifuged at 6,000 x g above (>8,000 rpm) for 1 minute. Afterwards, a fresh collecting tube was used. A fresh collection tube was used after adding 600µl of Buffer BW to the mini column, which was centrifuged for 1 minute at 6,000 x g above (>8,000 rpm). We applied 700µl of Buffer TW. Processed in a centrifuge at 6,000 x g for 1 minute at speeds more than 8,000 rpm

Table1: *The sequencing of primers that utilize*

Primer name	Seq.	Annealing temp. (C)	Product Size (bp)
27F	5-AGAGTTTGATCCT-GGCTAG-3	60	1500
1492 R	5-TACGGTTACCTTGT TACGCTT-3		

Table 2: *the constituents in the master mixture*

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
Master Mix	2	X	1	X	12.5
Forward primer	10	µM	0.5	µM	1
Reverse primer	10	µM	0.5	µM	1
Nuclease free water					8.5
DNA		ng/µl		ng/µl	2
Total volume					25

The mini-column was reinserted into the collecting tube after the pass-through was discarded. To remove any remaining wash buffer, the mini-column was centrifuged at maximum speed (>13,000 x g) for 1 minute. After that, it was transferred to a new 1.5 ml tube. The mixture was centrifuged at 5,000 rpm for 5 minutes after 100µl of Buffer AE was added and left to incubating at ambient temperature for 1 minute.

Polymerase chain reaction (PCR) to the 16 sRNA

We used PCR primers (27F, 1492R) provided by Macrogen Company to amplify the 16S RNA gene.

Table 3: *Conditions of the PCR master mixture*

Steps	C°	m:s	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	01:00	
Final extension	72	07:00	1
Hold	10	10:00	

The DNA amplification products electrophoresed on an agarose gel

Following the procedure outlined in, PCR products were identified by electrophoresis in an agarose gel. 10 microliters of PCR products were loaded into the designated wells and the DNA ladder (100-3000 base pairs). The device was operated by passing an electric current (100 volts and 70 milliamps) for one hour, and the dye was transferred to the other side of the gel. The gel was then transferred to be examined using a device using ultraviolet light at a wavelength of 312 nanometres, and the results were photographed using a digital camera included in the device.

Identification of the sequencing for nitrogenous bases

The PCR results of the bacterial isolates were sent for identification using the XL3730ABI automated DNA sequencing machine. Using the nucleotides BLAST program, the results were compared with information available in the GenBank database of the National Center for Biotechnology Information (NCBI) to confirm the identity of the bacterial isolates previously identified using the Vitek2 system.

Optimization production of enzyme

Zahraa & Jasim (2020) have investigated the impact of various environmental factors on enzyme production from a specific bacterial isolate. These factors involve the following: concentration of colloidal chitin, nitrogen source in the production medium, main pH, temperatures, period of incubation, as well as ventilation.

Influence of temperature on chitinase synthesis

The influence of temperature upon enzyme synthesis had been assessed by incubated inoculation media at various temperatures (25, 30, 35, 40, and 45 °C) for 72 hrs.

Influence of pH on chitinase synthesis

The pH levels for enzyme synthesis were adjusted to 4, 5, 6, 7, 8, and 9 utilizing 1N HCl and 1N NaOH. The medium was injected then incubated for 72 hrs.

Impact of colloidal chitin focus on chitinase synthesis

Varied concentrations of colloidal chitin (1%–5%) were utilized in optimum medium and conditions to ascertain the optimal concentration. At optimal temperature and pH.

Impact of nitrogen sources on chitinase synthesis

The impact of many organic and inorganic nitrogen sources, including yeast extract, beef extract, peptone, ammonium sulfate, and potassium nitrate. One percent was utilized as an extra supplement in the medium to optimize enzyme synthesis. The additional medium was inoculated with 1% inoculum and fermented under optimum conditions.

Table 4: *Morphological characteristics of the bacterial isolates*

Sample	Isolate code	Colony colour	Colony shape	Colony texture	Colony size
1	A	Fuzzy white	Irregular	Rough	Medium
2	B	Opaque	Irregular	Rough and wrinkled	Large
3	C	Creamy	Round	Wavy margin	Medium
4	D	Opaque	Sightly curved	Rough matted	Small
5	E	Fluo-rescent greenish	Circular	Flat and smooth	Medium
6	F	Red	Circular	Smooth	Medium
7	G	Opaque	Circular	Mucold	Small
8	H	Green	Elongat-ed round	Rough	Small
9	I	Creemy	Irregular	Ground glass	Medium

Impact of incubation duration on chitinase synthesis

To achieve optimal incubation duration, the culture of bacteria was cultivated for 5 days, with chitinase output assessed daily.

Impact of ventilation

For optimal incubation, the culture of bacteria was cultivated in a stable incubator and a rotary shaking incubator for 48 hours.

Results and Discussion

Isolation of chitinase-producing bacterium

Nine bacterial isolates were procured from various soil types in Baghdad. Three isolates were classified as Bacillus spp. based on their cultural and physical features.

Table 5: *Microscopic characteristics of the bacterial isolates.*

Sample	Isolate code	Gram staining	Cell shape	Cell arrangement
1	A	+	Rod	Single
2	B	+	Rod	Chain
3	C	+	Rod	Single
4	D	+	Rod	Chain
5	E	-	Rod	Single
6	F	-	Rod	Chain
7	G	-	Rod	Chain
8	H	+	Rod	Chain
9	I	-	Rod	Chain

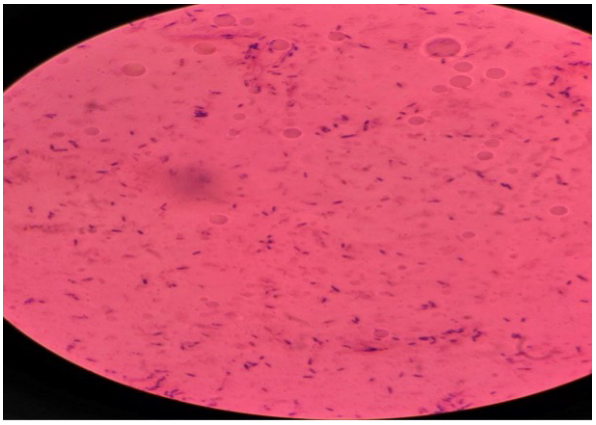


Figure 2: *A-Gram staining of the isolate*

Table 6: *Enzyme activity of isolates*

Isolate code	Enzyme activity
A	5.85 U\ml
B	3.20 U\ml
C	2.32 U\ml

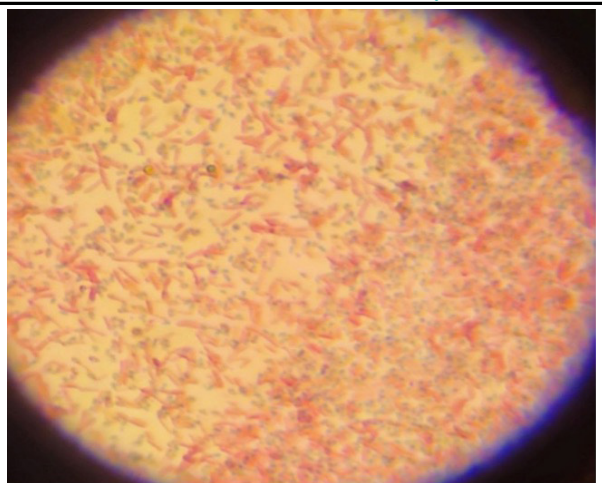


Figure 3: *B- Spore stain*

Quantitative screening

It's characterized as a supplementary confirmatory assessment to ascertain the competencies necessary for acquiring insights into the microorganisms identified in this investigation (Sedrah *et al.*, 2021). It has supplied diverse information essential for evaluating the capacity of microorganisms in industrial applications (Fierer *et al.*, 2005) Three pure isolates were selected as they had the highest enzyme activity according to Table 6.

From the table above the isolate (A) was selected to examine in the vitek 2 compact system to confirm the initial diagnosis (Figures 2 and 3).

bioMérieux Customer: Microbiology Chart Report Printed January 16, 2025 1:04:37 PM CST

Patient Name: Patient ID:
Location: Physician:
Lab ID: D Isolate Number: 1

Organism Quantity:
Selected Organism : Bacillus licheniformis

Source: **Collected:**

Comments:

Identification Information	**Analysis Time:** 13.88 hours	**Status:** Final
Selected Organism	88% Probability **Bacillus licheniformis** **Bionumber:** 5377271515167271	
ID Analysis Messages		

Biochemical Details

1	BXYL	+	3	LysA	-	4	AspA	+	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	+	10	PyrA	+	11	AGAL	+	12	AlaA	+	13	TyrA	+	14	BNAG	(+)
15	APPA	-	18	CDEX	+	19	dGAL	-	21	GLYG	+	22	INO	+	24	MdG	+
25	ELLM	+	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	(-)	31	dMAN	+
32	dMNE	+	34	dMLZ	(-)	36	NAG	(-)	37	PLE	+	39	IRHA	-	41	BGLU	+
43	BMAN	(+)	44	PHC	-	45	PVATE	-	46	AGLU	-	47	dTAG	+	48	dTRE	+
50	INU	+	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	-
60	OLD	+	61	ESC	+	62	TTZ	+	63	POLYB_B	+						

Figure 4: *Vitek 2 compact system result*

Molecular detection

Extraction of DNA

The DNA was isolated from Bacillus licheniformis (A), and its purity was assessed using a Nano Drop, yielding a purity of 1.8, that's sufficient for the PCR method. It has been shown that PCR does not require a substantial amount of DNA, that can instead

yield infinite amplification products. Conversely, an insufficient quantity of DNA can decrease accuracy.

Polymerase chain reaction (PCR)

The amplification results for 16s RNA gene from an unidentified species of bacteria were separated using 1.5% agarose gel electrophoresis and stained with ethidium bromide. Br. M: 100 base pair ladder markers. Lanes A-P exhibit 1500 bp PCR products (Figure 5).

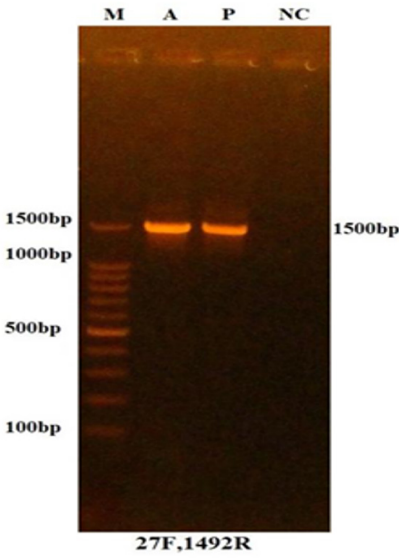


Figure 5: *Electrophoresis for the Bacillus licheiformis*

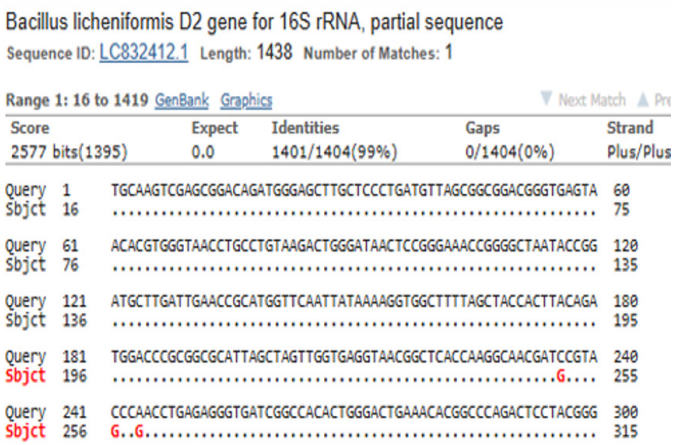
Analysis of amplification product sequences

The nitrogen base sequencing in the internal transcribed spacer ITS1 of the local bacterial isolate (*Bacillus licheniformis*) was examined by submitting the amplification products to the Korean company Macrogen (Figure 6). The arrangement of nitrogenous bases comprises 240 base pairs. The PLAST program has been employed to determine the genetic similarity using the database information (NCBI). The findings indicate that the following isolates from the bacterium Bacillus licheniformis are identical to one another to a 99.79 percent degree: PV346695, which is recorded on the NCBI website and is located in the USA.

Optimization of enzyme production

Influence of temperature on chitinase synthesis

Temperature influences several biological processes; hence, bacterial growth and enzyme synthesis are impacted by variations in incubation temperature. To assess the optimal growth temperatures for chitinase synthesis, cultures of Bacillus licheniformis were incubated at temperatures ranging from 25 to 45 °C. Chitinase synthesis peaked at 40 °C, yielding 12.3 U/ml, while the lowest production occurred at 25 °C, with an activity of 5.26 U/ml. This illustrates the adverse effects of both high and low temperatures on the proliferation of B. licheniformis cells and product generation (Figure 7). Bhargav *et al.* (2008) have reported maximal chitinase synthesis at 50 °C. Additional research has found that optimal enzyme synthesis from Bacillus licheniformis occurs at 30 °C (Jholapara, *et al.*, 2013; Narasimhan *et al.*, 2012).


Bacillus licheniformis D2 gene for 16S rRNA, partial sequence

Sequence ID: LC832412.1 Length: 1438 Number of Matches: 1

Range 1: 16 to 1419 GenBank Graphics

Score	Expect	Identities	Gaps	Strand
2577 bits(1395)	0.0	1401/1404(99%)	0/1404(0%)	Plus/Plus

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Query  1    TGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTA  60
Sbjct  16   ............................................................  75

Query  61   ACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGG  120
Sbjct  76   ............................................................  135

Query  121  ATGCTTGATTGAACCGCATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGA  180
Sbjct  136  ............................................................  195

Query  181  TGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATCCGTA  240
Sbjct  196  ..........................................................G...  255

Query  241  CCCAACCTGAGAGGGTGATCGGCCACACTGGGACTGAAACACGGCCCAGACTCCTACGGG  300
Sbjct  256  G..G........................................................  315
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Figure 6: *Sequence of nitrogen bases*

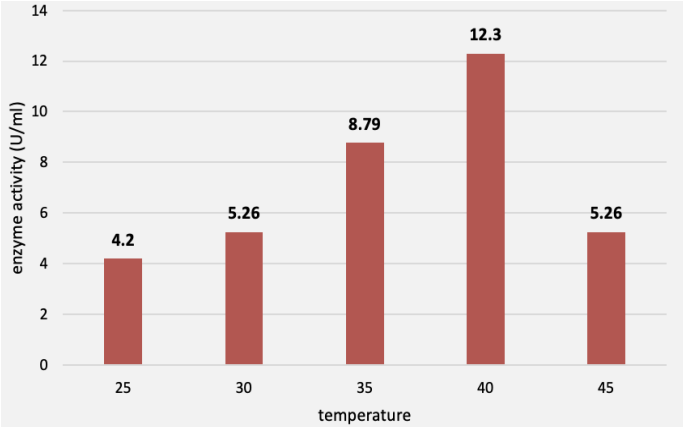

Figure 7: *Influence temperatures have on chitinase activities derived from B. licheniformis*

Influence of pH on chitinase synthesis

The impact of varying starting pH levels of the fermentation medium on the quantity of chitinase generated by *B. licheniformis* was evaluated (Figure 8). The peak chitinase synthesis occurred at pH 8, with an activity of 14.08 U/ml, whereas the lowest chitinase activity was recorded at pH 5, with a measurement of 0.8 U/ml. The pH of culture media significantly influences microbial growth and metabolism; hence, pH becomes crucial. has to be modified while making the fermentation mixture. The stability of the extracellular enzyme values may

be affected by changes in pH, which might lead to rapid denaturation at lower or higher pH levels. Similarly, studies have shown that the ideal pH for Bacillus chitinase synthesis varies. As an illustration, B. licheniformis B307 was able to produce the most effective amount of chitinase (8.56 U\ml) at a pH of 6. (Akeed *et al.*, 2020).

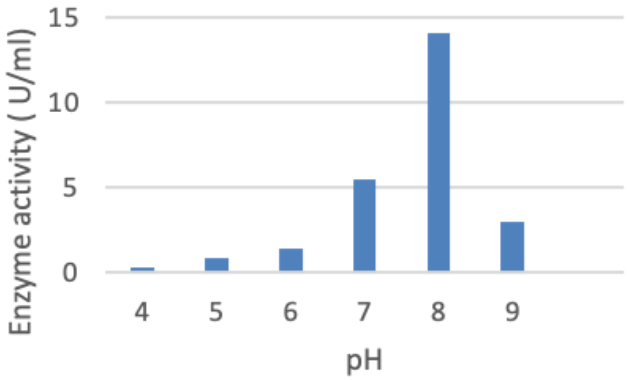

Figure 8: *Influence of pH on chitinase activities derived from B. licheniformis*

Impact of colloidal chitin concentration on chitinase synthesis

Optimizing production media is crucial for enhancing yield and productivity while simultaneously reducing production costs (Singh *et al.*, 2017). Consequently, several amounts of colloidal chitin ranging from 1% to 5% were evaluated. The results indicated that the concentration of colloidal chitin affected the synthesis of chitinase by B. licheniformis. Figure 9 illustrates a clear disparity among both enzyme production of enzyme with colloidal chitin concentration, with peak enzyme synthesis of 15.5 U/ml seen at 1% concentration. Nonetheless, there was no substantial enhancement in enzyme output beyond a 2% - 5% concentration of colloidal chitin, with activity ranging from 10.6 U/ml to 5.33 U/ml. Findings corroborate previous findings that chitin is a crucial element in stimulating elevated chitinase synthesis from microorganisms (Soiuza *et al.*, 2005). Furthermore, Abirami *et al.* (2016) indicated that a colloidal chitin concentration of 0.5-1% significantly augmented chitinase synthesis by B. licheniformis SSCL10.

Impact of various nitrogen sources

The influence of several organic (yeast extract, beef extract, and peptone) and inorganic nitrogen sources (ammonium sulfate, potassium nitrate) on chitinase production were tested. As shown in Figure 10, it was noticed that the organic nitrogen source yeast extract was the highest level of chitinase activity by *B. licheniformis* (16.44 U\ml) and the lowest activity in peptone (5.26 U\ml) while the highest activity in inorganic nitrogen source was in ammonium sulfate with activity (10.5 U\ml).

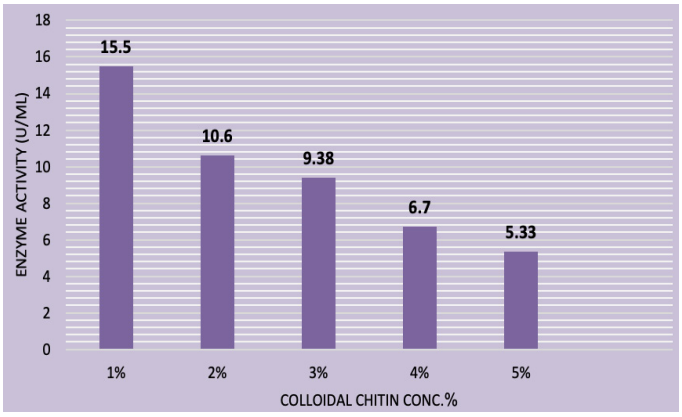

Figure 9: *Impact of colloidal chitin concentration on chitinase activities derived from B. licheniformis*

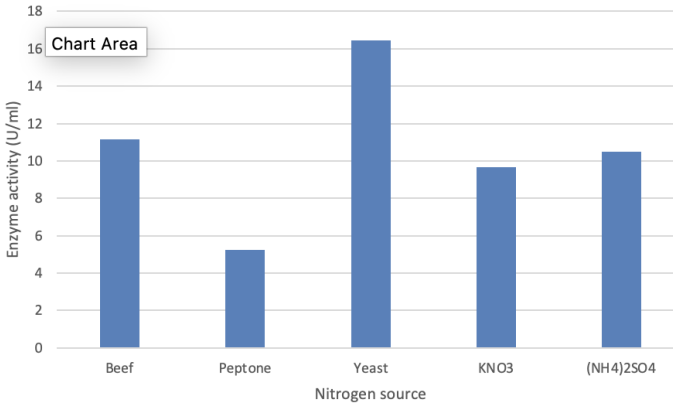

Figure 10: *Effect of different nitrogen sources on chitinase activity from B. licheniformis*

Impact of incubation duration on chitinase synthesis

The optimal chitinase production was observed following 48 hrs of incubation. (19.38 U\ml) as shown in (Figure 11) after that, the production began to decline in 72 hours (16.14 U\ml), at 96 hours the activity was (10.2 U\ml) further increase in incubation time as in 120 hours (5.18 U\ml) may diminish the yield owing to the breakdown of metabolites (Hao *et al.*, 2012).

Effect of ventilation

Aeration and agitation processes are necessary for microorganisms to obtain dissolved oxygen, homogenize the components of the medium, and distribute the substrate in the growth medium (Sedrah *et al.*, 2021) (Figure 12).

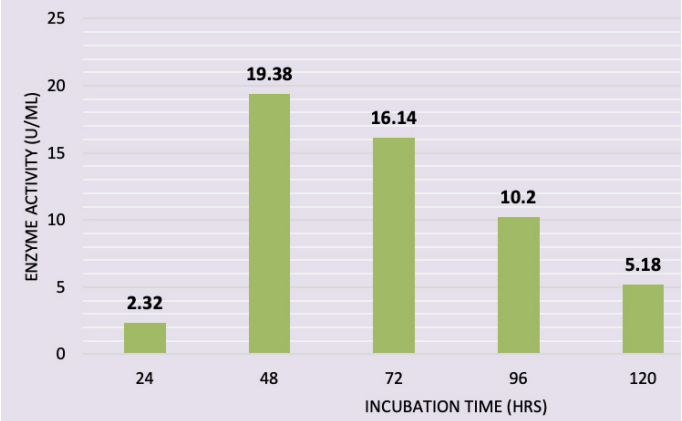

Figure 11: *Influence of incubation duration on chitinase activities from B. licheniformis*

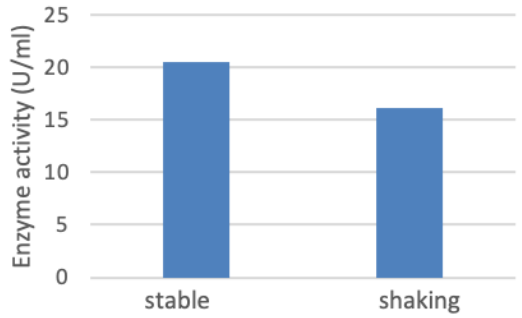

Figure 12: *Effect of ventilation on chitinase activity from B. licheniformis*

The maximum chitinase yield after incubation in stable incubator for 48 hours (20.5 U\ml) while the production of chitinase activity in shaking incubator was (16.14 U\ml)

Conclusions

This study utilized isolates of the Bacillus genus as chitinase producers. The greatest chitinase enzyme activities were recorded from isolates of Bacillus sp. The isolate was identified as Bacillus licheniformis using 16s rRNA gene sequencing, with an accuracy of 99.79%. The research demonstrated that the local isolate Bacillus licheniformis serves as essential for enhancing chitinase synthesis utilizing colloidal chitin as a substrate. The findings indicate that B. licheniformis generated a significant quantity of chitinase. Under optimum conditions, incubation time 48 hours, temperature of 40, PH 8, colloidal chitin (1%) and yeast extract as a nitrogen source (1%) in stable incubator (20.5 U\ml).

Declarations

Funding
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Ethical approval
Ethical approval for the conduction of this experiment was acquired from the Department of Food Science, collage of Agriculture Engenieering science, university of Baghdad.

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Novelty Statement

This study presents the isolation and identification of local bacterial isolates from soil with chitinase-producing capability, which have not been previously reported. The novelty of this work lies in screening indigenous soil bacteria as potential chitinase producers and systematically optimizing the conditions for chitinase production to enhance enzyme yield. This approach provides new insights into the biotechnological potential of local microbial resources for efficient chitinase production.

Author's Contribution

Both authors conducted all experimental work including isolation and identification of bacterial isolates, optimization of chitinase production, data analysis, and manuscript preparation.

Generative AI or AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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