BIOSYNTHESIS AND OPTIMIZATION OF AMYLASE FROM *BACILLUS SP.* ISOLATED FROM SOIL SAMPLES USING AGRO-INDUSTRIAL WASTE AS SUBSTRATE

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Abstract. Amylase enzyme is used in various industries due to its diverse applications. In this study, bacteria from soil sample were primarily screened on starch agar medium to identify amylase producer through the detection of prominent clear zone. Total of five soil samples namely bakery points (A-1), sugar cane juice point (A-2), *Lichi chinesis* garden soil (A-3), rice field (A-4) and sugar industrial waste (A-5) were used in this study. Among the 17 strains isolated from three samples A-1, A-2 and A-3 were found positive for amylase production. The strains were further screened on the production medium. The N-1 bacterial strain revealed higher enzyme activity (92.21 ± 17 IU/ml) compared to the other strain and was thus selected for further work. The strain was identified as *Bacillus lichenoformis* from the 16S rRNA analysis. Enzyme production was enhanced by optimizing various parameters by one factor at a time technique. The agro industrial waste rice polish was used as substrate. The optimum temperature of the enzyme was 35°C, pH 5.5 and 2% (w/v) of substrate concentration. Qualitative detection by using sodium dodecyl polyacrylamide gel electrophoresis showed that molecular weight of enzyme was 35 kDa. This indicated that the enzyme requires a moderately high temperature and neutral pH to show greatest activity.
Keywords: *amylase, 16S rRNA gene, Bacillus lichenoformis, DNS, PCR*

Introduction

Enzymes are biological molecules that perform metabolic functions in living cells. The unique capacity of enzymes to perform particular biochemical processes in isolation has increased their demand in the industry (Pal and Khanum, 2010). Microbial enzymes are important in a variety of industrial activities. An enzyme's key characteristics include high substrate specificity, temperate reaction conditions and high product yield (Shah et al., 2023; Aziz et al., 2023; Ullah et al., 2023). The main barriers to using them on an industrial scale are economic issues, which may result in expensive process costs (Liu et al., 2024; Srishti et al., 2022). Amylase and protease are the most valuable microbial enzymes, with various applications and widespread use due to their capabilities in the degradation of organic wastes, as well as applications in biofuels, agricultural, pharmaceutical, chemical, and biotechnological industries (Ma et al., 2022; Ullah et al.,

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2022). Amylase is an enzyme that can act as a catalyst in the chemical degradation of starch. This enzyme is extremely important, and its manufacture is extremely profitable. α-Amylase is one of the most important amylases found in mammals, plants, and microbes. However, the biosynthesis of α-amylase by bacteria has a specified place in industry and trade. The amylase enzymes are vital commercial enzymes classified as α-amylase, β-amylase γ-amylase. The α-amylase also recognized as α-1,4-glucan-4-glucanohydrolases, are a class of amylase that degrade chains of carbohydrates such as glycogen and starch into smaller subunits with 2–3 glucose molecules. They are extracellular enzymes that randomly cleave the α-(1-4) bond between two contiguous glucose units in the linear amylose chain to form glucose, maltose, and malto-triose units (Mobini-Dehkordi and Javan, 2012; Haki and Rakshit, 2033; Tiwari et al., 2015). Amylases come from a variety of sources, including plants, animals, and microbes. Because of their great efficiency and resilience to pH and temperature, amylases derived from bacterial and fungal sources are more commonly employed in industry. Mesophilic genera include Aspergillus and certain species of penicillium among bacteria and fungi specifically Bacillus stearothermophilus, Bacillus subtilis, Bacillus amyloliquefaciens and Bacillus licheniformis can produce amylases for commercial application (Kandra et al., 2003). Various bacteria can produce α-amylase even in severe environments; for example, some thermophilic bacteria may create alpha-amylase at high temperatures. Because most starch processing steps, such as saccharification, gelatinization, and liquefaction, require high temperatures, thus the thermo-stable α-amylase is valuable for advancing the possessing steps under such severe conditions (Gazali and Suwastika, 2018). The production of amylase on industrial scale submerged fermentation SmF (submerged fermentation involves the cultivation of microorganisms in a liquid medium which has more than 95% moisture) or solid-state fermentation SSF are often applied (Khatoon et al., 2023). The SmF is used to produce any bioproducts in liquid medium. This approach necessitates a high level of moisture in the medium for the growth of microorganisms (mainly bacteria) to proliferate and manufacture alpha-amylase (Sundarram and Murthy, 2014). The high moisture also allows for simple sterilization, production, purification, regulated temperature, nutrients, pH, and other processes; for example, amylase synthesis as a microbiological source employing Bacillus sp (Vidyalakshmi et al., 2009). Because media composition has a major influence on growth and enzyme production, controlling media components and cultural parameters is critical in standardizing a bioprocess (Rajput et al., 2016). Furthermore, optimizing medium components and process parameters is critical for maximizing microbial metabolite synthesis while minimizing production costs (Bezbaruah et al., 1994). The variables relevant for microorganism growth and enzyme production were generally optimized utilizing the one factor at a time (OFAT) technique, however it requires more experimentation and is also time intensive (Elechi et al., 2022). The present study was conducted for the isolation of amylase producing strain from soil samples, the production, optimization, and purification of amylase enzymes under submerged fermentation.

Materials and methods

Strain isolation

The bacterial strain was isolated from different soil samples including bakery manufacturing points (A-1), sugar cane juice point (A-2), *Lichi chinesis* garden soil (A-3), rice field (A-4) and sugar industrial waste (A-5) from various localities of Lahore,

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Pakistan. A total of five samples of soil was taken by digging 5 cm below the surface. The serial dilution method was applied and allowed to heat shock at 80°C for 15 min to kill all other microorganisms except *Bacillus* spores. One ml from each dilution was poured on petri plates and 1% starch containing agar medium was added and incubated at 37°C for 24 h. The zone of hydrolysis was measured using iodine solution and stored at 4°C for further study.

Screening of amylolytic strain

The primary screening was carried out by the hydrolysis of starch medium and measured the zone of hydrolysis. The quantitative screening was estimated by the culturing each bacterial strain on the production medium having the composition % (w/v) starch 1, peptone 0.5, yeast extract 0.2, NaCl 0.1, $MgSO_4$ 0.1 under submerged fermentation and incubate for 48 h at 37°C after sterilization. The enzyme activity was measured after 48 h of incubation period by standard enzyme assay. The strain was selected on the basis of highest amylase activity for further identification and enzyme production.

Identification of amylase producing strain

Biochemically and morphologically the strain was identified by the recommended protocol of Bergey's Manual of Determinative Bacteriology (Holt, 1994) and Diagnostic Microbiology (Noble, 2002).

Molecular characterization

The 16S ribosomal gene of bacteria was sequenced in order to identify the bacterial strain. To extract the whole genome, Wizard® Genomic DNA Purification Kit was used (Promega, USA). From the extracted DNA, the 16S rRNA gene was amplified using Polymerase Chain Reaction. This was done using the forward primer 27F (AGA GTT TGA TCM TGG CTC AG) and reverse primers 1427R (GGT TAC CTT GTT ACG ACT T) (Islam et al., 2016). The PCR product was sent to the '1st BASE Laboratories' in Malaysia via Invent Technologies Ltd. where it was sequenced using the Sanger method.

Production of amylase

Inoculum preparation

The inoculum was prepared from the preserved culture in the nutrient broth and incubates at 37°C for 24 h.

Production medium

The production of amylase was carried out by using (w/v) yeast extract 0.5%, peptone 1% NaCl 0.3%, $CaCl_2$ 0.06% and 1% rice polish as substrate. The initial pH of the medium was adjusted as 7.2 of the fermentation medium. Initially the inoculums were added 2% (v/v) and incubate at 37°C for 48 h. After incubation the fermented broth was centrifuged at 9000 rpm for 10 min the supernatant used as crude enzyme.

Enzyme assay

The enzyme activity was determined according to a standard procedure recommended by Kaur et al. (2012). 1 ml of 1% starch stock solution was added into 1 ml crude enzyme

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and was incubated at room temperature for 15 min. 3 ml DNS reagent was added into the mixture and was heated at boiling temperature for 15 min. The color was developed; the absorbance was taken at 540 nm on the spectrophotometer against the blank.

Optimization of parameters

For maximum production of amylase one factor at a time technique (OFAT) was used for optimization of parameters. The effect of different temperature ranges (25, 35, 45, 55°C), incubation periods such as (24, 48, 72 and 96) h, fermentation medium pH (4.5, 5.5, 6.5, 7.5) and rice polish as substrate concentrations 1, 2, 3, 4, 5% (w/v) to evaluate the enzymes activity. A 24 h old culture of 2% (v/v) as inoculum was used in the production medium under submerged fermentation.

Qualitative detection of amylase

Qualitative detection of amylase was carried out by using sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). The molecular mass of crude enzymes was estimated using the above-mentioned method.

Sodium dodecyl sulphate polyacrylamide Gel electrophoresis (SDS - PAGE)

A 12% of SDS-PAGE was performed followed the method mentioned by Laemmli applying a small piece of glass plates (8 × 8 cm) gel apparatus (Laemmli, 1970). Molecular weight of crude of enzyme was obtained by using standard molecular weight marker (fermentas).

Results

Isolation and screening of bacteria

Total of 17 bacterial strains were isolated from various sources of soil sample suspension, processed on starch containing agar medium. Among these 3 strains showed clear zone on the starch media as indicated in *Figure 1* and *Table 1*. The strains were also screened on the production medium containing 1% (w/v) of starch and the unit activity were calculated.

Table 1. *Screening of bacterial strains on starch agar medium*

S.No	Bakery points (A-1)	Zone mm	Sugar cane juice point (A-2)	Zone mm	*Lichi chinesis* garden soil (A-3)	Zone mm
1	N-1	21	N-1	23	N-1	32
2	N-2	15	N-2	14	N-2	21
3	N-3	12	N-3	21	N-3	24
4	N-4	25	N-4	19	N-4	15
5	N-5	22	N-5	18	N-5	19
6	N-6	18				
7	N-7	16				

Screening of bacterial strain on production media

The bacterial strains showed prominent clear zone on starch agar medium was further screened on production medium for maximum amylase activity. The N-4 strain

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from Bakery points (A-1) and N-1, N-3 from *Lichi chinesis* garden soil (A-3) were screened. The N-1 strain from *Lichi chinesis* garden soil (A-3) showed maximum amylase activity 92.21 ± 17 IU/ml as shown in *Table 2*.

Table 2. *Screening of bacterial strains on production medium*

S.No	Strain	Enzyme activity IU/mL ± SD
1	Strain 3	92.21 ± 17^{a}
2	Strain 1	60.2 ± 12^{b}
3	Strain 5	47.79 ± 24^{cd}

Different letters superscripted represents significant differences ($p < 0.05$) among isolated strains on the basis of enzyme activity

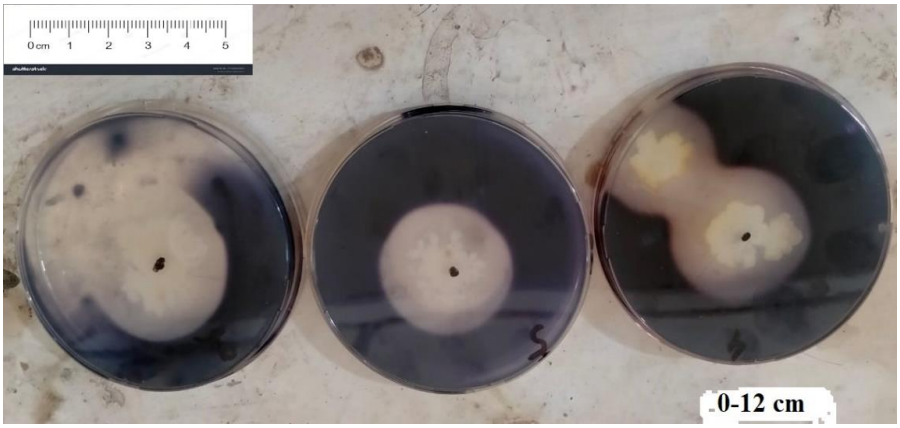

Figure 1. *Zone of hydrolysis by selected amylase producing Bacillus sp*

Identification of strain

In accordance with Bergey's Manual of Determinative Bacteriology (Holt, 1994) and Diagnostic Microbiology (Michael, 2002) the isolated strain was identified based on biochemical and phenotypic characterization shown in *Table 3*. *Bacillus* sp. was identified as the gram positive rod-shaped.

Table 3. *Morphological and biochemical identification of amylase producing strain*

Morphological features	
Color	White
Shape	Rods
Gram staining	Positive
Biochemical features	
Urease	Positive
Catalase	Positive
Oxidase	Positive
Indole test	Negative
Casein hydrolysis test	Positive
Methyl red test	Positive
Citrate utilization test	Positive

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Molecular characterization

On the basis of 16S rRNA sequence analysis the strain was identified as Bacillus licheniformis as shown in *Figure 2*.

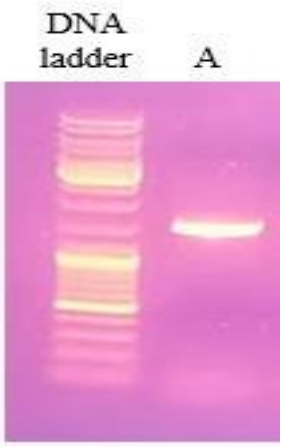

Figure 2. *DNA from a bacterial strain was isolated using a DNA isolation kit and loaded onto an agarose gel containing 0.8%*

Primer sequence

CCAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCC
GCGATTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCG
AACTGAGAACAGATTTGTGGGATTGGCTTAGCCTCGCGGCTTCGCTGCCCTT
TGTTCTGCCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGA
TTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAG
TGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGA
CTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGT
CACTCTGCCCCCGAAGGGGAAGCCCTATCTCTAGGGTTGTCAGAGGATGTC
AAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACC
GCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACT
CCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTAAAGGGCGGAAACC
CTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAAT
CCTGTTCGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAGAG
TCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACG
TGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCT
CCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGCG
CGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCG
GCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTAC
CGCCCTATTCGAACGGTACTTGTTCTTCCCTAACAACAGAGTTTTACGATCC
GAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGC
GGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCC
CAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAG
CCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTAAGTG.

The 16S rRNA gene sequence of isolated strain is shown in *Figure 3*.

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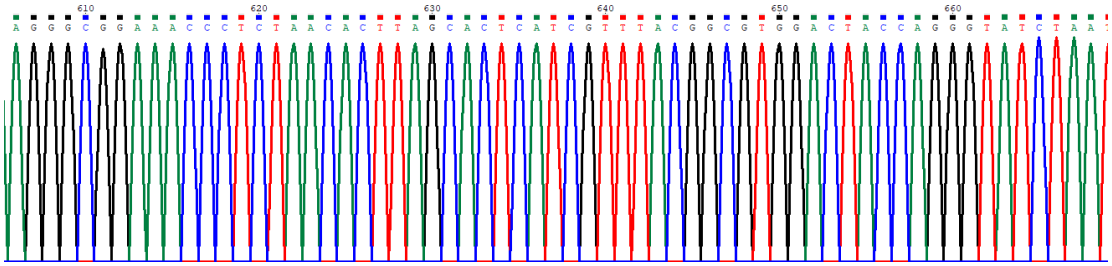

Figure 3. *16S rRNA gene sequence of isolated strain (Bacillus licheniformis)*

Production of amylase

The amylase enzyme was produced by *Bacillus licheniformis* as identified above. The enzyme was produced under submerged fermentation and the starch was used as substrate. The composition of medium (w/v %) peptone 0.5, $MgSO_4$ 0.1, NaCl 0.1, $CaCl_2$ 0.02, soluble starch 1 at the initial condition were used for the production of amylase.

Optimization of parameters

One factor at a time technique (OFAT) was used for optimization of amylase. Various parameters were optimized to achieve maximum activity of amylase.

Effect of nitrogen source on amylase production

At a concentration of 0.5% w/v, beef extract, yeast extract, tryptone, casein, and inorganic sources like $NaNO_3$, KNO_3, and NH_4Cl were studied. In *Table 4*, yeast extract was shown to be the best source of nitrogen for amylase production among all organic and inorganic nitrogen sources.

Table 4. *Source of nitrogen*

S. No	Nitrogen source	Enzyme activity IU/mL
1	Beef extract	100.2 ± 10.2^{fg}
2	Yeast extract	231.25 ± 20.23^{a}
3	Tryptone	121.09 ± 12.3^{e}
4	Casein	163.21 ± 13^{b}
5	$NaNO_3$	135.09 ± 13.89^{d}
6	KNO_3	108.39 ± 12.32^{f}
7	NH_4Cl	153.91 ± 15.22^{bc}

Mean values are presented with ± standard deviation that were taken in triplicates. Different letters superscripted represents significant differences ($p < 0.05$) of isolated strain on the basis of nitrogen source

Effect of temperature

Effect of various temperature range (25, 35, 45, 55°C) were studied. The incubation temperature considerably affected the enzyme production and also the growth of bacteria. The highest enzyme activity was achieved at 35°C of temperature 341.34 ± 16.31 IU/mL as represented in *Table 5*. As the temperature increases, the enzyme activity decreases.

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Table 5. *Effect of different incubation temperature on amylase production*

S.No	Incubation temperature (°C)	Enzyme activity IU/mL
1	25	178.32 ± 10.2^{d}
2	35	341.34 ± 16.31^{a}
3	45	210.12 ± 12.22^{b}
4	55	171.32 ± 15.3^{c}

Mean values are presented with ± standard deviation that were taken in triplicates. Different letters superscripted represents significant differences ($p < 0.05$) of isolated strain on the basis of incubation temperature

Effect of incubation period

The fermentation medium was inoculated with 2% (v/v) of 24 h old *Bacillus licheniformis* culture and incubated at 35°C for various times in order to achieve maximum enzyme production. Maximum enzyme activity 441.95 ± 20.4 IU/mL was observed at 48 h of incubation period as shown in *Table 6*.

Table 6. *Effect of incubation period*

S.No	Incubation period (h)	Enzyme activity IU/mL
1	24	318.12 ± 11^{d}
2	48	441.95 ± 20.4^{a}
3	72	410.31 ± 13.52^{b}
4	96	371.08 ± 15.5^{c}

Mean values are presented with ± standard deviation that were taken in triplicates. Different letters superscripted represents significant differences ($p < 0.05$) of isolated strain on the basis of incubation period

Effect of pH on enzyme production

The effects of different pH values were studied. The initial pH of the fermentation medium was adjusted 6 but further optimization the values were kept varied. The pH 5.5 was found to be the optimal pH for enzyme activity (578.87 ± 19.39 IU/ml) as shown in *Table 7*. Further increase in pH value the enzyme activity becomes lowered down.

Table 7. *Effect of pH on enzyme production*

S.No	pH of fermentation medium	Enzyme activity IU/mL
1	4.5	367.61 ± 13.2^{d}
2	5.5	578.87 ± 19.39^{a}
3	6.5	417.09 ± 14.91^{b}
4	7.5	394.51 ± 16^{c}

Mean values are presented with ± standard deviation that were taken in triplicates. Different letters superscripted represents significant differences ($p < 0.05$) of isolated strain on the basis of pH

Effect of substrate concentration

Substrate concentrations significantly affect the production of enzymes. Different concentrations of substrate 1, 2, 3, 4 and 5 were applied (w/v %) in the fermentation

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medium for maximal amylase production. Highest amylase activity was obtained at 2% (w/v) of substrate concentration in the basal medium. At 2% (w/v) of substrate 757.67 ± 15.22 of enzyme activity was achieved as shown in *Table 8*.

Table 8. *Effect of substrate concentration*

S.No	Substrate concentration % (w/v)	Enzyme activity IU/mL
1	1	478.74 ± 14.3^{e}
2	2	757.67 ± 15.22^{a}
3	3	706.16 ± 15.42^{b}
4	4	674.23 ± 13.1^{d}
5	5	690.56 ± 14.34^{bc}

Mean values are presented with ± standard deviation that were taken in triplicates. Different letters superscripted represents significant differences ($p < 0.05$) of isolated strain on the basis of substrate concentration

SDS-PAGE chromatography

In order to analyze the crude enzyme samples, 12% SDS PAGE was run under denaturing conditions. After staining the crude enzyme with commissive blue R-250, a linear logarithmic plot of relative molecular mass was used to determine the molecular weight of amylase. It was found that the amylase band weighs 35 kDa as shown in *Figure 4*.

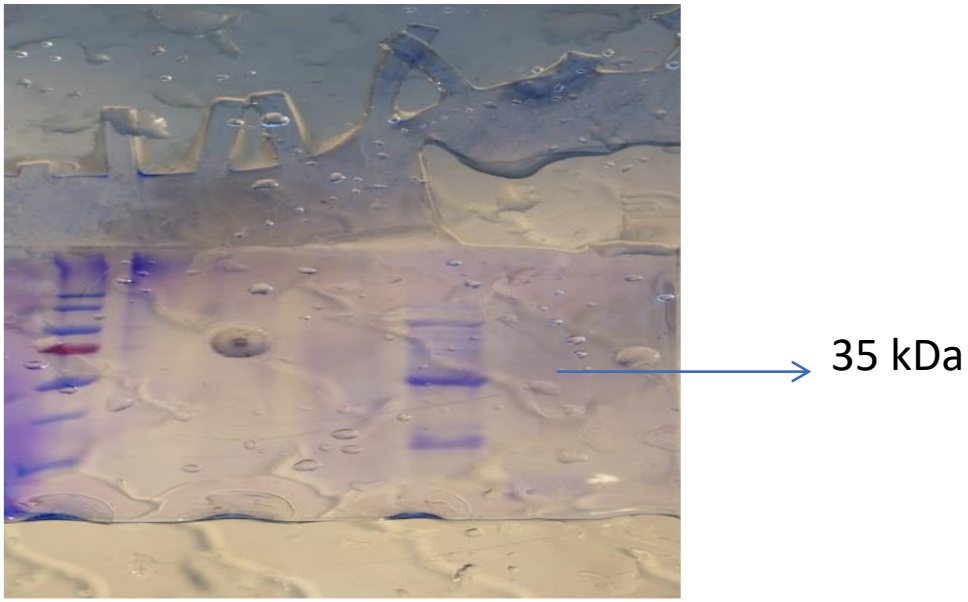

Figure 4. *SDS PAGE of produced amylase enzyme band*

Discussion

Amylase is a hydrolytic enzyme responsible for the breakdown of starch into simple sugars. This enzyme is used as a digest aid in starch manufacture, brewing, detergent formulation, paper making, and medicines (Niyomukiza et al., 2022). The major goal of this study to used presumptive and genotypic approaches to isolate a bacterial strain

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from soil samples and identify it as an amylase-producing strain. This study isolated an amylase-producing strain from soil samples and screened it for amylase production. Gram staining revealed the strain was gram positive rod-shaped. It was identified as *Bacillus* species the strain was further characterized by 16S DNA analysis and identified as *B. licheniformis* that produces maximum amylase under submerged fermentation. Since bacteria are more bountiful in the soil and it was selected as the bacterial source for the isolation. Burhan et al. (2003) study showed the production of amylase from microorganisms to be more effectual than other sources of amylase production as the method of consistent, fast and economical. Similarly, production of amylase at commercial level by microorganisms like bacteria and fungi characterizes about 30% of the global enzymes market (Niyomukiza et al., 2020). Likewise, there are several species of bacteria capable of producing amylase for commercial purposes. *Bacillus* species are most likely to produce amylase, including *B. amyloliuequefaciens, B. subtilis, B. stearothermophilus, B. licheniformis, B. coagulans, B. vulgaris, B. megaterium, B. stearothermophilus, B. coagulans, B. cereus, B. dipsosauri, and B. Halodurans* (Ling et al., 201; Far et al., 2020). In the previous studies many amylase producing microorganisms from the soil samples and marine were reported (Lakshmi et al., 2020). The present study used one factor at a time technique (OFAT) to optimize the parameters for maximum amylase enzyme production by *B. licheniformis* under submerged fermentation. Various nitrogen sources were screened for maximum activity of enzymes. Among all organic and inorganic sources yeast extract showed highest amylolytic activity as compared to other origin.

Similarly, Almanaa et al. (2020) carried out the production of amylase by using *B. subtilis* D19 under solid state fermentation and applying different source of nitrogen, among this yeast extract found best source for amylase production as compared to other. The production of amylase was assessed in the presence of various inorganic and organic sources of nitrogen. On the other hand, maximum enzyme activity was observed by supplementation of $NaNO_3$ an inorganic source of nitrogen in the fermentation medium by locally isolated *Bacillus* strain (Ullah et al., 2021). The effects of different parameters were studied such as temperature, incubation period, pH, substrate concentration age and size of inoculum. Similar study was reported by Bandal et al. (2021) by optimizing various physiochemical parameters with the help of above-mentioned method. Elechi et al. (2022) used one variable at a time technique for the optimization of α-amylase production by *B. cereus* PW4. The parameters were temperature, pH, inoculum size, incubation period, nitrogen source and metal ions as standard factors. The factors responsible for the maximum production of enzymes by microorganisms were mostly optimized by applying one variable at a time (OFAT) strategy (Saha and Ghosh, 2014). The optimal temperature for the production of amylase was 35°C in the present study. Ali et al. (2023) carried out the production of amylase from *Streptomyces* sp using submerged fermentation at neutral pH the optimal temperature was indicated at 35℃. The production of enzymes decreases as the temperature increases or decreases. A mesophilic alkaline *Bacillus megaterium* strain was found to produce highest amylase at 37℃ (Fazil et al., 2023). Maximum enzyme was produced at optimum pH 5.5 with 2% (w/v) op substrate concentration on the production medium.

Similar study was reported by Trabelsi et al. (2019) that maximum amylase activity achieved at the pH 5.5 as the optimal range. In earlier studies indicated that maximum amylase production was achieved at pH 6 and the activity decreased gradually above 6

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(Ahmed et al., 2020). The results can also be compared with the study reported by Oyeleke et al. (2010), Pasin et al. (2014) and Ali et al. (2017) they perceived that maximum production of amylase ranged from 5 to 6 by *A. niger*, *A. japonicus* and *A. flavus* AUMC 11685, respectively. Zusfahair et al. (2017) stated that the optimal concentration of substrate was 1.5 to 2.5% for maximum enzymatic activity under submerged fermentation. A significant enhancement of 48% was achieved in acidic amylase production by an isolated strain of fungi. The medium supplemented with starch 3% with pH 5 at 31℃ of temperature (Ali et al., 2017). Under denaturing conditions, 12% SDS PAGE was used to analyze crude enzyme samples. After staining with commissive blue R-250, the crude enzyme showed bands. A linear logarithmic plot of relative molecular mass was used to interpolate the molecular weight of amylase. The molecular weight of amylase band was found 35 kDa. Maalej et al. (2021) estimated the molecular weight of amylase enzyme by SDS-PAGE as approximately 45 kDa. Moreover, an amylase with a low molecular mass of 28 kDa has been isolated from the distal intestine of *Anabas testudineus* (Banerjee et al., 2016). Similar study was conducted by Ullah et al. (2022) and reported same results in case of alkaline protease (Ullah et al., 2022). The present study was in good agreement with the above mentioned previous reported research work.

Conclusion

The present study demonstrated that soil is a rich source and natural habitat for all types of microorganisms especially spores forming bacteria such as *Bacillus*. The genus *Bacillus* is a significant candidate for various types of secondary metabolites. The production and extraction of enzymes are easy and safe under submerged fermentation. The extracted enzymes had a remarkable ability to resist a broad range of temperatures, pH, salt concentrations and could be used in starch liquefaction also in detergent industries due to their outstanding stability.

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Competing interests. All the authors declare no conflict of interest.

Availability of data and materials. All the data generated in this research work has been included in this manuscript.

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