Pak. J. Bot., 56(3): 1109-1117, 2024. DOI: http://dx.doi.org/10.30848/PJB2024-3(23)

BIOSYNTHESIS OF MICROBIAL UREASE AND ITS OPTIMIZATION FOR ENHANCED PRODUCTION

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Abstract

The present study aims to produce urease enzyme from indigenously isolated microbial source. For this purpose, 55 soil samples were collected from urea-fertilized agricultural land of Punjab, Pakistan. Out of all the 42 isolated microbial strains, 35 urease positive bacteria and 7 urease positive fungal strains were screened through primary screening on Christensen medium. Secondary screening was carried out by submerged fermentation for the best urease producer. Out of all urease positive strains, S-29 strain, later identified through 16S rRNA sequencing as *Lysinibacillus xylanilyticus*, showed maximum urease production i.e., 1274±0.022 U/mL/min. Further, optimization of various cultural conditions was carried out for enhanced production. It was concluded that optimum cultural conditions were M5 medium (urea supplemented nutrient broth) of pH 6.5, with 24 hours of incubation at 35°C and 125 rpm, with 1.5 % inoculum size using 1% urea as nitrogen source and 2 % glucose as carbon source. Under optimized conditions, the urease activity was increased to1335±0.182 U/mL/min.

Key words: Urea hydrolysis, Submerged fermentation, Ureolytic, Enzyme.

Introduction

Urease (EC 3.5.1.5), also known as urea amidohydrolase, is a metalloenzyme which is ubiquitous in nature (Das & Varma, 2011). It is the most studied enzyme which functions as a catalyst during the conversion of urea into ammonia (NH_4) and carbamate which is further decomposed into another molecule of ammonia and CO_2 (Mohammed *et al*., 2022). Different plants, filamentous fungi, yeast, bacteria, and algae are capable of producing urease enzyme but animals cannot produce it (Kappaun *et al*., 2018). Biochemically, plant urease that was extracted from *Canavalia ensiformis* (jack bean) is best characterized. Different fungal species like *Aspergillus* sp., *Coprinu*s sp., *Neurospora* sp., *Pénicillium* sp. and *Ustilago* sp., are urease positive, as they utilize urea as a nitrogen source (Hasan, 2000). Other fungal sources include *Rhizopus Oryzae*, Actinomycete species, *Fusarium culmorum*, and *Penicillium spinulosum* (Aggarwal, 2016).

Ureolytic activity of bacteria is evident by a lot of work cited in the literature. *Bacillus* species i.e., *B. subtilis, B. pasteurii, B. lentus, B. sphaericus* and Urobacterium i.e., *Sarcina urea* are recognized as urease producing bacterial species (Mahmoud, 1973). Some *Lactobacillus* species like *Lactobacillus reuteri, L. fermentum, L. animalsi* also produce this enzyme. *Helicobacter* species were also observed as urease positive (Konieczna *et al*., 2012). *Streptococcus minor, S. salivarius, S. thermophilus, Staphylococcus epidermis* and *Nitrosomonas*, isolated from acidic soil are reported to have ureolytic activities. Some purple sulphur & non-sulphur bacteria are also urease positive as they breakdown urea to get nitrogen (Bast, 1986). Among *E. coli* strains (generally considered as non-ureolytic), almost 1% isolates with ureolytic activities were found. A unique bacterial class Cyanobacteria (serve as biofertilizer) is also associated with the production of urease (Hassan, 2000).

Generally, enzymes derived from microbial sources are better because they have various advantages over other sources i.e., animal and plant sources. Firstly, enzymes from microbial origin are economical as they can be produced in large quantities within limited time and space. Secondly, microorganisms has the ability to grow under extensive range of environmental conditions (Sujoy & Aparna, 2013). However, among all the microbial sources, urease enzyme derived from bacterial sources is preferred over other because growth conditions for urease producing bacteria are flexible as well as they are easy to culture. Moreover, they have comparatively less generation time than fungus so can be produced in bulk amount in less time. They can also be manipulated easily in laboratory through genetic engineering, so are linked with more variations (Mukherjee *et al*., 2023).

The bulk production of urease from bacteria can be produced through fermentation. Production of microbial enzymes by fermentation procedure involves microbial propagation to get desired product (Al-Maktari *et al*., 2019). Generally, two types of fermentation techniques are used at industrial level for the production of enzymes using microorganisms; solid state fermentation and submerged fermentation (SmF). Production of urease using SmF is encouraged in many ways as it can be monitored and controlled without much effort and it is easy to handle (Dos Reis *et al*., 2013). Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Exchange of heat and mass takes place efficiently in this fermentation as the oxygen and nutrients are dissolved in liquid easily and dispersed all over in the vessel equally. Submerged fermentation operation has three approaches i.e., Batch, Fed Batch and Continuous. Among them, Fed Batch is observed as more advantageous because in this mode, substrate and other components of medium can be added in succession at intermittent times thus, the effects of viscosity and catabolite suppression are reduced which accordingly will not delay the process of productions (Hemansi *et al*., 2019).

Various cultural conditions affect the growth of microorganism and hence the enzyme production significantly during the process of fermentation. Out of all the factors, temperature is considered as a very important factor as it has a huge impact on the growth of microbe and ultimately on enzyme production. To find the optimized temperature required for urease enzyme production, its

yield is checked at various temperatures. Similarly, to get the maximum yield of urease, it is necessary to know the incubation time at which it gives best results. Initial media pH and composition of fermentation medium including various carbon and nitrogen sources are other various parameters which affect the growth of microorganism and hence the product yield (He *et al*., 2022). Aeration and agitation are other important factors. These two parameters could affect the nutrient availability and distribution for microorganisms during submerged fermentations. During high-speed agitation, the developed shear forces damage the cell, while low agitation speeds may provide insufficient oxygen supply to the organisms (Mekonnen *et al*., 2021). Hence, optimizing the all these parameters can support the growth of microorganisms and greatly increase the urease production. Optimization can be done through conventional "one-factor-at-a-time" approach, where experimentally only single variable is changed at a time and keeping all the other factors constant. However, a statistical approach where multiple interacting factors with less experimental work could be used through response surface methodology (RSM). Artificial neural network (ANNs) and genetic algorithm (GA) are other approaches which could be used for optimization (Lan *et al*., 2015).

Recently, urease producing microorganisms, urease enzyme & its substrate urea have gain much attention due to their relevance/application in agricultural, ecological and medical field (Kappaun *et al*., 2018). Owing to its various applications, the present study aims to isolate an indigenous microorganism from agricultural fields of Punjab for urease production. Further, to enhance the production of urease enzyme, different cultural conditions were optimized.

Material and Methods

Sample collection: Different urea fertilized agricultural fields of Punjab, Pakistan were selected for the soil sample collection to isolate the best urease producing microorganism. Samples were collected at depth of 5-10 cm using sterile tools, placed in sterile polystyrene containers, sealed and stored in an ice box (at the sampling site) before being transported to laboratory. The samples were then preserved in the refrigerator (4°C) until further use (Fig. 1).

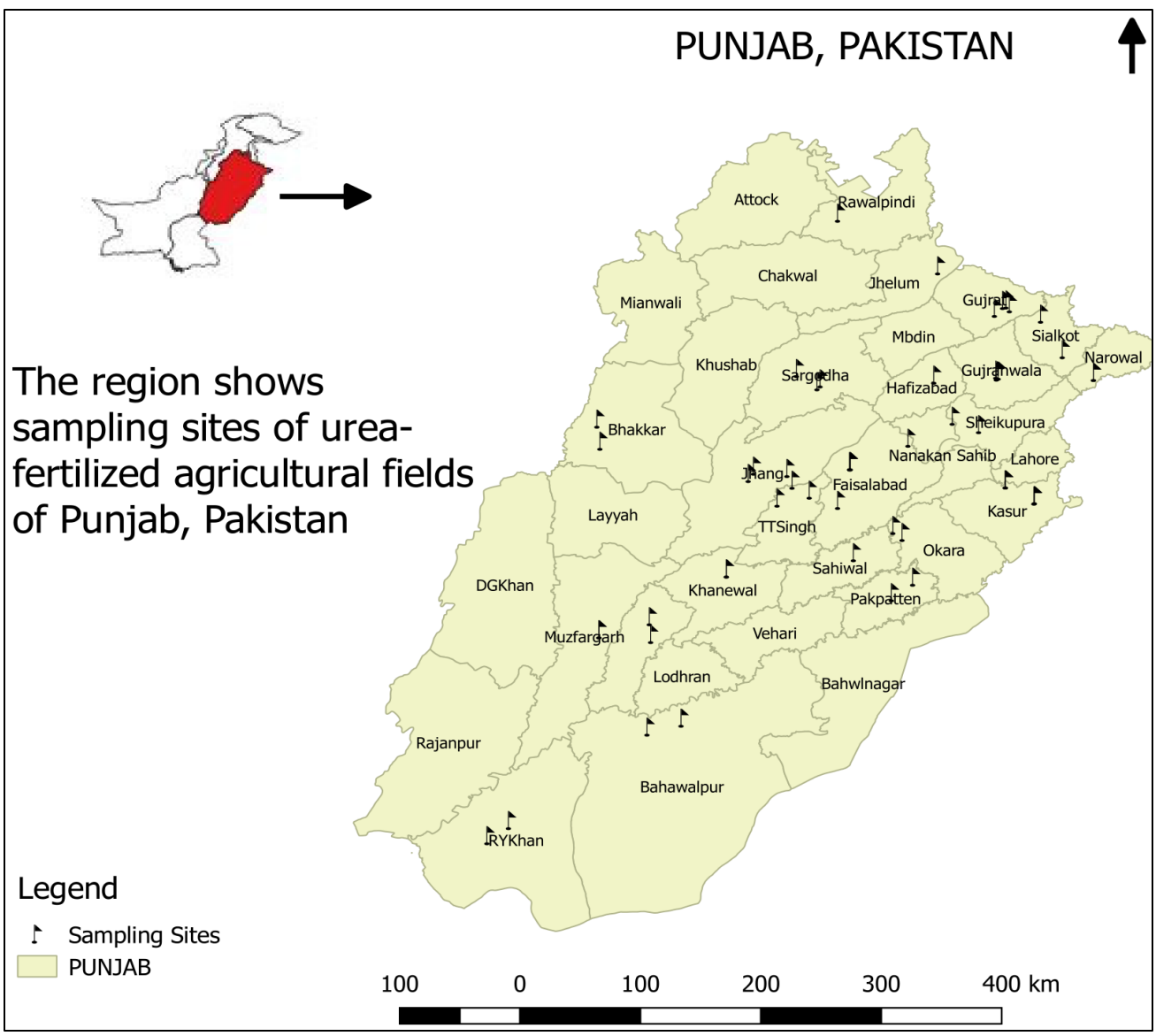

Fig. 1. Map of Punjab Province, showing different sampling sites.

Isolation and primary screening of ureolytic microorganism: To isolate urease producing microorganism, 1 g of soil was taken from each sample and was serially diluted up to 10^{-6} dilution. Dilutions 10^{-4}, 10^{-5} and 10^{-6} from each sample were selected to be plated on nutrient agar and PDA plates. 0.1 mL of each dilution was aseptically transferred and spread on the plates. Inoculated plated were then incubated overnight at 30°C. The isolated bacterial and fungal strains, were screened primarily for urease production on differential medium i.e., Christensen's urea agar medium. The urease production was examined through visual observation throughout the incubation period for color changes of medium from pale yellow to pink red.

Secondary screening through submerged fermentation

Enzyme production and extraction: Secondary screening of ureolytic microorganism was carried out by quantitatively assessing the urease production through submerged fermentation. All the selected microbes were subjected to submerged fermentation by inoculating 1% of starter culture in 250 mL Erlenmeyer flask containing 50 mL of sterilized Luria-Bertani (LB) medium (tryptone 1%, yeast extract 0.5%, NaCl 1%). The flasks were incubated for 24 hours (72 hours for fungal culture) at 30°C at 120 rpm in shaking incubator. At the end of fermentation, the fermentation broth was transferred to centrifuge tubes and were centrifuged at 6000 rpm for 10 mins at 4°C. For bacterial enzyme extraction, the supernatant was discarded and the pellet was re-suspended in 15 mL 0.2 M Phosphate Buffered Saline (PBS). This was sonicated to release the intracellular contents. After sonication, the mixture is transferred to eppendorf and centrifuged at 12000 rpm for 5 minutes 4°C. The supernatant collected was used as crude enzyme. In case of fungal isolates urease was produces extracellularly and fermentation broth was used as a crude enzyme.

Urease activity analysis: To test the activity of the urease, 1 mL (0.2M) urea solution was added in a test tube containing 0.9 mL (0.5M) Tris-Cl buffer of pH 7.3 followed by 0.1 mL urease solution. The test tubes were incubated at 30°C for 10 minutes. Then, the reaction mixture was stirred before the reaction was terminated by 1 mL of 20% trichloroacetic acid (TCA). The amount of NH_3 liberated was determined by adding 1 mL of Nessler's reagent to 1 mL reaction mixture and taking the absorbance in UV spectrophotometer (TU-1810) at 405 nm. The control was also run by adding the enzyme after incubation. Urease activity was calculated by the following formula and using Ammonium chloride (NH_4Cl) standard curve.

$$\text{Enzyme activity (U/mL/min)} = \frac{\mu\text{M of ammonia liberated x Total volume of reaction mixture}}{\text{Volume of enzyme (0.1 mL) x Test volume (1 mL) x Incubation time (10 min)}}$$

One-unit of urease is defined as the amount of urease that liberates 1 µmol NH_3 per minute at the desired pH (37°C) (Kayastha, 2019).

Identification of best ureolytic strain: The ureolytic isolate which revealed high activity was then characterized biochemically and morphologically. Biochemical characteristics were studied by execution of different tests including Gram staining, endospore staining, catalase test, oxidase test, starch hydrolysis test, casein hydrolysis and gelatin hydrolysis, according to described protocol of Bergey's Manual of Determinative Bacteriology. Furthermore, molecular identification of best bacterial strain was carried out by 16S rRNA gene sequencing. For this purpose, the isolated bacterial colonies were sent to Macrogen, Korea. All the commercial services including DNA isolation and amplification using universal primers were provided by Macrogen. Afterward, phylogenetic analysis was performed.

Optimization of cultural conditions for urease production: Optimization of various cultural parameters was carried out for the production of urease enzyme using the best ureolytic isolate. Enzyme activity was compared for choosing the best production conditions. Effect of fermentation medium composition (Table 1), incubation time (12 to 72 hours), pH (4.0 to 8.0), temperature (25 to 45°C), inoculum size (1 to 3%), agitation speed (100 to 200 rpm), nitrogen sources (yeast extract, peptone, tryptone, meat extract, urea, ammonium sulphate, and ammonium chloride), concentration of nitrogen sources (0.5 to 2.5%), carbon sources (glucose, sucrose, maltose, fructose, molasses, and soybean meal), and concentration of carbon source (0.5 to 2.5%) were analyzed for this purpose, by changing one factor at a time and keeping other conditions constant.

Table 1. Composition of various fermentation media for urease production.

Medium	Composition of medium (g/L)
M1	Glucose: 20, Urea: 0.2, Yeast extract: 10, $NiCl_2$: 0.032, Na_2HPO_4: 15, NaCl: 5
M2	Yeast extract: 20, $(NH_4)_2SO_4$: 10
M3	Peptone: 30, Glucose: 50, Urea: 10, K_2HPO_4: 7, KH_2PO_4: 3, $MgSO_4$. $5H_2O$: 0.1, Yeast extract: 1
M4	Glucose: 20, Peptone: 10, Yeast extract: 5, KH_2PO_4: 2, NaCl: 5, NaAc: 2, Urea: 5, $MnSO_4$: 0.05, $NiSO_4$: 0.05.
M5	Nutrient broth: 8, Urea: 6
M6	Urea: 14, Sucrose: 20, Yeast extract: 34, $MgSO_4$. $7H_2O$: 0.7, $CaCl_2.2H_2O$: 0.04, $NiSO_4.7H_2O$: 0.03, KH_2PO_4: 1, Na_2HPO_4: 3
M7	Urea: 1.3, Glucose: 20, $MgSO_4.7H_2O$: 0.5, KH_2PO_4: 13.3, K_2HPO_4: 0.34, $CaCl_2$: 0.3 $NiSO_4.7H_2O$: 0.03
M8	Urea: 0.2, Glucose: 20, Yeast extract: 10, $NiSO_4.7H_2O$: 0.032, Na_2HPO_4: 15

Fig. 2. Pure culture of ureolytic bacteria S-29 on Christensen's medium showing pink colour.

Statistical analysis

The computer statistical software (SPSS 16.0) was used for the statistical analysis of the results. Significant difference among the replicates has been presented as Duncan's multiple range tests in the form of probability (*p*) values.

Results and Discussion

Isolation and primary screening: Total 55 soil samples from urea-fertilized agricultural land of different localities of Punjab Province, Pakistan were collected for the isolation of urease positive microorganisms. Urea-fertilized soil was selected for the study because the chances of finding urease producing microbes are more in urea rich environment. Different living organisms utilize urea as a source of nitrogen for their growth (Solomon *et al*., 2010). Primary screening for urease producing microbe was carried out on a differential medium i.e., Christensen's Medium. Out of these soil sample, 42 urease positive strains (35 bacterial and 7 fungal) were screened through primary screening and only those colonies were considered positive which gave positive results within 18 hours. Slow producers (showing late result) were considered negative as only the best ureolytic bacteria were required. The presence of phenol red indicator in medium turns the medium yellow to pink upon increase in pH, due to ammonia production, as a result of urea degradation. Thus, only those microorganism turn the medium pink which can produce urease enzyme and can degrade the urea present in medium (Ali *et al*., 2020). Fig. 2 shows the pure culture of urease positive bacteria on Christensen medium. Several studies have reported to use the same medium for qualitative urease assay for isolation and screening of uratolytic bacteria (Li *et al*., 2013; Mekonnen *et al*., 2021; Farajnia *et al*., 2022; Leeprasert *et al*., 2022). However, various other sources including tropical peat, mangrove forest soil and dung-rich soil has been used to isolate ureolytic microorganisms (Phang *et al*., 2018; Khambholja *et al.*, 2022; Leeprasert *et al.*, 2022).

Secondary screening: Secondary screening for urease production was carried out using submerged fermentation. Out of all the selected fungal (7) and bacterial (35) isolates, strain 29 (S-29) showed the maximum urease production i.e., 1274 U/mL. (Table 2) shows the enzyme activity of all the selected strains. Chahal *et al*., (2011) reported maximum urease activity as 598 U/mL. Various studies have reported the urease activity through conductivity method. However, this method has limitations when used in the presence of calcium ions (Maleki-Kakelar *et al*., 2022). Khambholja *et al*., (2022) has reported 25 IU/mL and 89 IU/ mL of urease activity in their study by *Quasibacillus* sp. Strain DGDK-3 and *Bacillus* sp. Strain DGDK-4, respectively using the Nessler's method. Kulanthaivel *et al*., (2020) has reported 370 U/mL of urease enzyme produced by *Lysinibacillus fusiformis*, also using the Nessler's method. In another studies, *L. boronitolerans* is reported to urease with 5028.72 U/g (Xu *et al*., 2022). In another recent studies, three different urease-producing bacteria, i.e., *Bacillus aryabhattai*C14, *Bacillus megateriumC6*, and *Bacillus* sp. C15 were screened from agricultural soil producing up to 815.4 U/mL, 876.1 U/mL, and 809.3 U/mL, respectively within 24h (Chen *et al*., 2022). The variation in the activity units can clearly attributed to the variation in bacterial strains producing urease enzyme.

Identification of bacterial strain: Isolate showing best urease activity (S-29) was subjected to morphological and biochemical characterization for its identification. Colony morphological characteristics of S-29 and results of biochemical characterization are presented in (Table 3).

In order to confirm the identification of isolated strain, it was sent for 16S rRNA gene sequencing. After sequencing, it was subjected to GenBank NCBI-BLAST, which revealed close homology with the members of the genus *Lysinibacillus*, signifying 99% sequence homology with a bacterium *Lysinibacillus xylanilyticus* strain CHO1 (GenBank Ac. No. KX371346.1). Moreover, the query sequence of isolated strain has 97.71%, 97% and 96% similarity with *Lysinibacillus cresolivorans* strain K5 (MN493618.1), *Lysinibacillus macroides* strain SAR09, *Bacillus* sp. MPB3 (GenBank Ac. No. HM629506.1), respectively. In a nutshell, the acquired results strappingly recommended that the isolated bacterial strain is *Lysinibacillus xylanilyticus* (GenBank Ac. No. OR793170). Phylogenic tree is shown in (Fig. 3).

Ali *et al*., (2020) also isolated urease positive bacterial strains and all of them were gram positive rods with spore forming ability and were identified as *Bacillus* species. Mekonnen *et al*., (2021) also reported *Bacillus paramycoides* as one of the best urease producer along with *Citrobacter sedlakii*, and *Enterobacter bugandensis*. Imran *et al*., (2019) also isolated a *Lysinibacillus* sp. from coastal areas which were gram-positive, rod-shaped, and round-spore-forming bacterial genus in the family Bacillaceae. Bacteria belonging to this family and specifically *Lysinibacillus* genus can survive in harsh conditions and are able to survive within a pH range of 6-10 (Seifan *et al*., 2017; Leeprasert *et al*., 2022).

Table 2. Secondary screening of urease positive isolates through submerged fermentation.

Sr. No.	Train No.	Type of microorganism	Enzyme activity (U/mL/min)	Sr. No.	Type of microorganism	Strain No.	Enzyme activity (U/mL/min)
1.	S-1	Bacteria	987 ± 0.012	22.	Fungus	S-22	395 ± 0.121
2.	S-2	Bacteria	872 ± 0.134	23.	Bacteria	S-23	536 ± 0.107
3.	S-3	Bacteria	1023 ± 0.031	24.	Bacteria	S-24	977 ± 0.134
4.	S-4	Fungus	332 ± 0.034	25.	Bacteria	S-25	1034 ± 0.152
5.	S-5	Bacteria	728 ± 0.013	26.	Fungus	S-26	241 ± 0.013
6.	S-6	Bacteria	349 ± 0.062	27.	Bacteria	S-27	760 ± 0.022
7.	S-7	Bacteria	946 ± 0.013	28.	Bacteria	S-28	940 ± 0.130
8.	S-8	Bacteria	1074 ± 0.141	**29.**	**Bacteria**	**S-29**	**1274 ± 0.022**
9.	S-9	Bacteria	685 ± 0.022	30.	Bacteria	S-30	1058 ± 0.013
10.	S-10	Bacteria	532 ± 0.013	31.	Bacteria	S-31	875 ± 0.105
11.	S-11	Fungus	392 ± 0.105	32.	Bacteria	S-32	947 ± 0.024
12.	S-12	Fungus	236 ± 0.134	33.	Fungus	S-33	381 ± 0.013
13.	S-13	Bacteria	712 ± 0.181	34.	Bacteria	S-34	473 ± 0.134
14.	S-14	Bacteria	1087 ± 0.173	35.	Bacteria	S-35	785 ± 0.131
15.	S-15	Bacteria	978 ± 0.015	36.	Bacteria	S-36	1131 ± 0.012
16.	S-16	Bacteria	1094 ± 0.106	37.	Fungus	S-37	130 ± 0.013
17.	S-17	Bacteria	983 ± 0.134	38.	Bacteria	S-38	586 ± 0.121
18.	S-18	Bacteria	892 ± 0.024	39.	Bacteria	S-39	1064 ± 0.032
19.	S-19	Bacteria	1006 ± 0.105	40.	Bacteria	S-40	1187 ± 0.014
20.	S-20	Bacteria	1100 ± 0.120	41.	Bacteria	S-41	947 ± 0.105
21.	S-21	Bacteria	914 ± 0.013	42.	Bacteria	S-42	1042 ± 0.134

Table 3. Morphological and biochemical characterization of urease positive strain showing maximum urease production.

Colony characteristics	
Colour	Pink
Shape	Rounded
Size	1 mm
Transparency	Opaque
Margin	Entire
Elevation	Raised
Biochemical characteristics	
Gram's reaction	positive
Bacterial morphology	Rods
Endospore formation	+
Catalase test	+
Oxidase test	+
Starch hydrolysis test	+
Gelatin hydrolysis test	+
Casein hydrolysis test	+

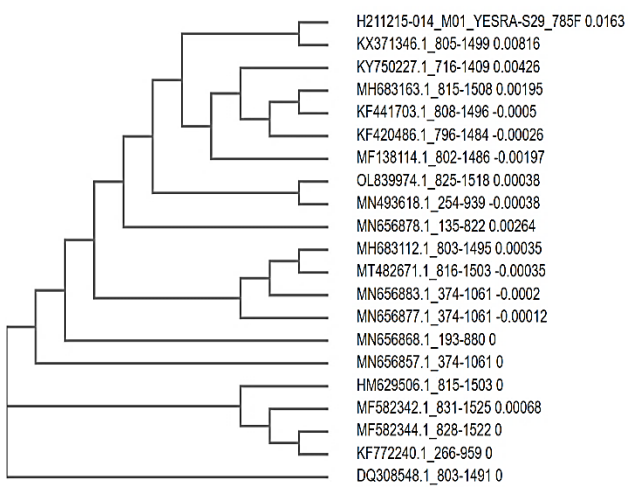

Fig. 3. Dendogram of urease positive S-29 strain with other closely related species.

Optimization of cultural parameters for enhanced urease production: To evaluate the effect of fermentation medium on urease production through *Lysinibacillus xylanilyticus*, 8 different types of fermentation media (M1-M8) were utilized. Out of all the 8-fermentation media, M5 gave the best results by producing 1298±0.013 U/mL/min of urease enzyme (Fig. 4a). The fermentation medium i.e., M8 and M2, also showed significant results, after M5. Fermentation medium M5 was nutrient broth supplemented with urea, the main substrate of urease. As the organism is ureaolytic in nature the presence of urea in medium may have induced the increased production of urease enzyme. Most bacteria hydrolyze urea to utilize nitrogen, to produce energy, and to increase ambient pH (Achal & Pan, 2011). The enhancement bacterial growth and urease activity by urea supplementation in medium is confirmed by other studies as well (Kenny & Frank, 1977; Khambholja *et al.*, 2022). Optimization of Incubation time showed that there is a significant increase in urease production from 12 to 24 hours. However, beyond 24 hours, there was a gradual decrease in enzyme activity with the increase in time of incubation as shown in (Fig. 4b). Enzyme activity started to decrease which might be due to nutrient depletion and accumulation of by-products in the growth medium causing cells lysis. On the other hand, during lower time of incubation, microorganism may not grow well and results in less enzyme production (Omoregie *et al*., 2023). Liu *et al*., (2012) has reported 2504 U/L (2.5 u/mL) of urease from *Enterobacter* sp., under optimized conditions of 16 hours incubation time. Senthil *et al.*, (2012), however, found out optimal culturing time for urease production from *Klebseilla* sp., as 48 hours. Both studies are contradictory to our findings, may be due to different genera of bacterial species used for study.

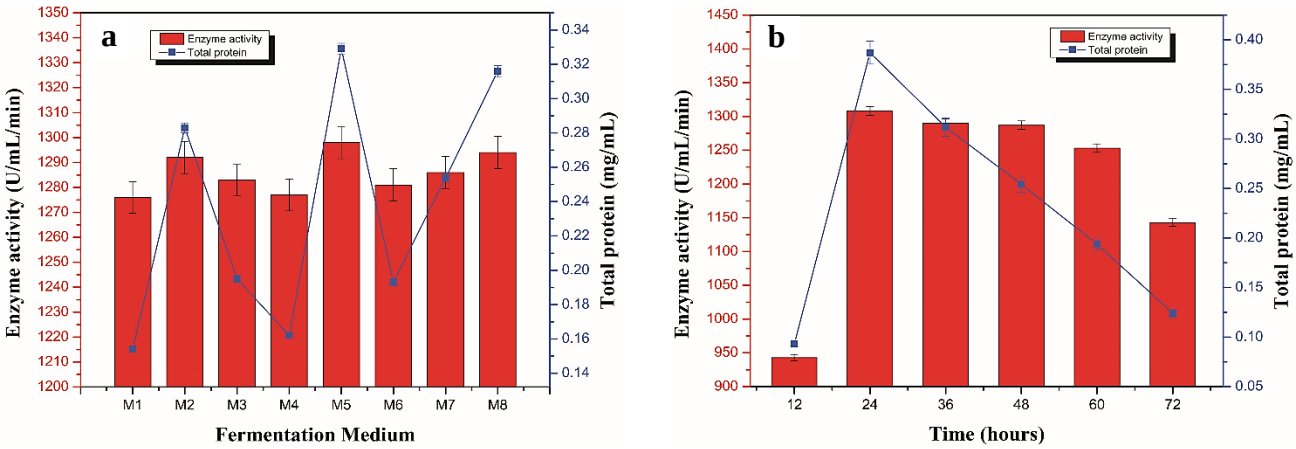

Fig. 4. Effect of (a) different fermentation medium and (b) incubation time on urease production through submerged fermentation using *Lysinibacillus xylanilyticus.*

Maximum enzyme activity of urease from *Lysinibacillus xylanilyticus* was obtained when the initial pH of the fermentation medium was set to 6.5. However, at initial pH 7, there was a minor decrease in activity, which continued to decrease up to pH 8 as shown in (Fig. 5). pH being a critical physical parameter, plays significant role in enzyme production and slight disturbance in the optimum pH of media affects the protein structure, shape and its ionic interactions (Phang *et al*., 2018). Imran *et al*., (2019) reported pH 7 as optimum for urease production through *Lysinibacillus* sp. Although they have not tested pH 6.5, the results are still close to our findings. Similar findings of 7 as optimal pH are reported by Senthil *et al*., (2012) and Nathan *et al*., (2020) from *Klebseilla* sp. and *bacillus halodurans*, respectively. However, Chen *et al*., (2022) reported pH 8 as optimal for urease production through *Sporosarcina pasteurii*.

It was observed that with the increase in temperature the urease activity increased up till 35°C. Further increase in temperature resulted in decreased urease activity. This may be due the fact that higher thermal energy leads to denaturation of protein including different enzymes, which can ultimately results in cell death (Imran *et al*., 2019; Omoregie *et al*., 2023). Senthil *et al*., (2012) and Nathan *et al*., (2020) also reported 35°C as optimum temperature for urease production from *Klebseilla sp. bacillus halodurans*, respectively. Similarly, He *et al*., (2022) optimized the urease production using *Staphylococcusxylosus* sp. and report 36°C as optimum incubation temperature. Chen *et al*., (2022), however, determined the optimal thermal condition for enhanced urease activity of *Sporosarcina pasteurii* as 30ºC.

Inoculum size and agitation speed were also optimized for enhancing urease production. Maximum production of urease was obtained using 1.5% of inoculum and at 125 rpm. There was a slight decrease in the urease activity above this concentration and below these values. He *et al*., (2022) reported 6% inoculum size for optimized urease production through *Staphylococcusxylosus* sp. On the other hand, Chen *et al*., (2022) reported 0.5% inoculum size as optimum using *Sporosarcina pasteurii*.

Among the various organic and inorganic nitrogen sources tested, urea in 1% concentration as nitrogen source proved to be best for urease production through *Lysinibacillus xylanilyticus* as shown in (Fig. 6). Urea can be easily utilized by various organisms as an organic nitrogen source (Solomon *et al*., 2010). Inorganic nitrogen on the other hand may not be readily utilized as a nitrogen source due to the low conversion rate of inorganic nitrogen. This could lead to reduced metabolic capacity and hence urease production capacity (He *et al*., 2022). A study by Li *et al*., (2021) also supports our finding as they have reported urea (50 g/L) as best nitrogen source for urease production. Senthil *et al*., (2012), however, reported peptone as best nitrogen source in a concentration of 0.7%. He *et al*., (2022) found yeast extract as a best nitrogen source. The variation in results is because of the fact the each study has used different bacterial genera which many have different nutritional preferences.

Various organic and inorganic carbon sources were also utilized to find out the best for urease production. Glucose at 2 % was found out to be the best carbon source giving maximum activity. It was observed that with the increase in glucose concentration, the enzyme activity has increased, however, beyond this concentration there was a decrease observed. These result may be due to some high glucose concentration-induced metabolic changes that inhibited strain growth and hence urease production (He *et al*., 2022). Liu *et al*., (2012) also reported glucose as optimized nitrogen source. In another study by He *et al*., (2022), 0.7% of glucose was reported to be best. However, Ruth *et al*., (1998) and Yang *et al*., (2008) also used 2% of glucose in their studies, supporting our results.

It was therefore concluded that optimum cultural conditions for urease production using *Lysinibacillus xylanilyticus* were M5 medium (urea supplemented nutrient broth) of pH 6.5, with 24 hours of incubation at 35°C and 125 rpm, with 1.5% inoculum size using 1% urea as nitrogen source and 2% glucose as carbon source. However, it can be observed from the literature that very few parameters for urease production from different bacterial sources were optimized by each study and no study was found on optimization of urease production using *Lysinibacillus* sp. To the best of our knowledge the current studies could be the first to optimize multiple production parameters from urease production using *Lysinibacillus xylanilyticus.* However, it can be observed that very few parameters for urease production from bacterial sources were optimized by each study and no study was found on optimization of urease production using *Lysinibacillus* sp. To the best of our knowledge the current studies could be the first to optimize multiple production parameters from urease production using *Lysinibacillus xylanilyticus.*

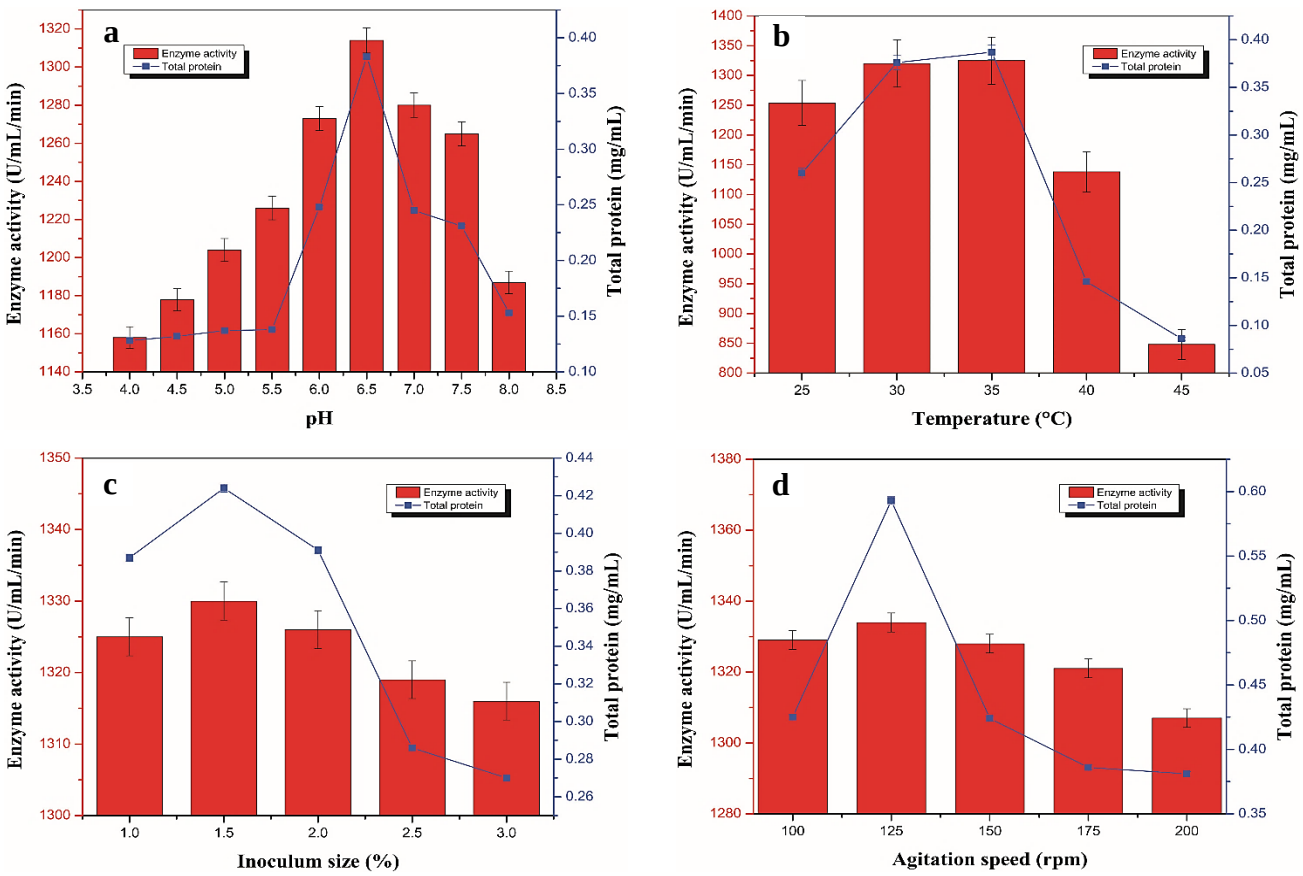

Fig. 5. Effect of (a) pH (b) incubation temperature (c) inoculum size and (d) agitation speed on urease production through submerged fermentation using *Lysinibacillus xylanilyticus.*

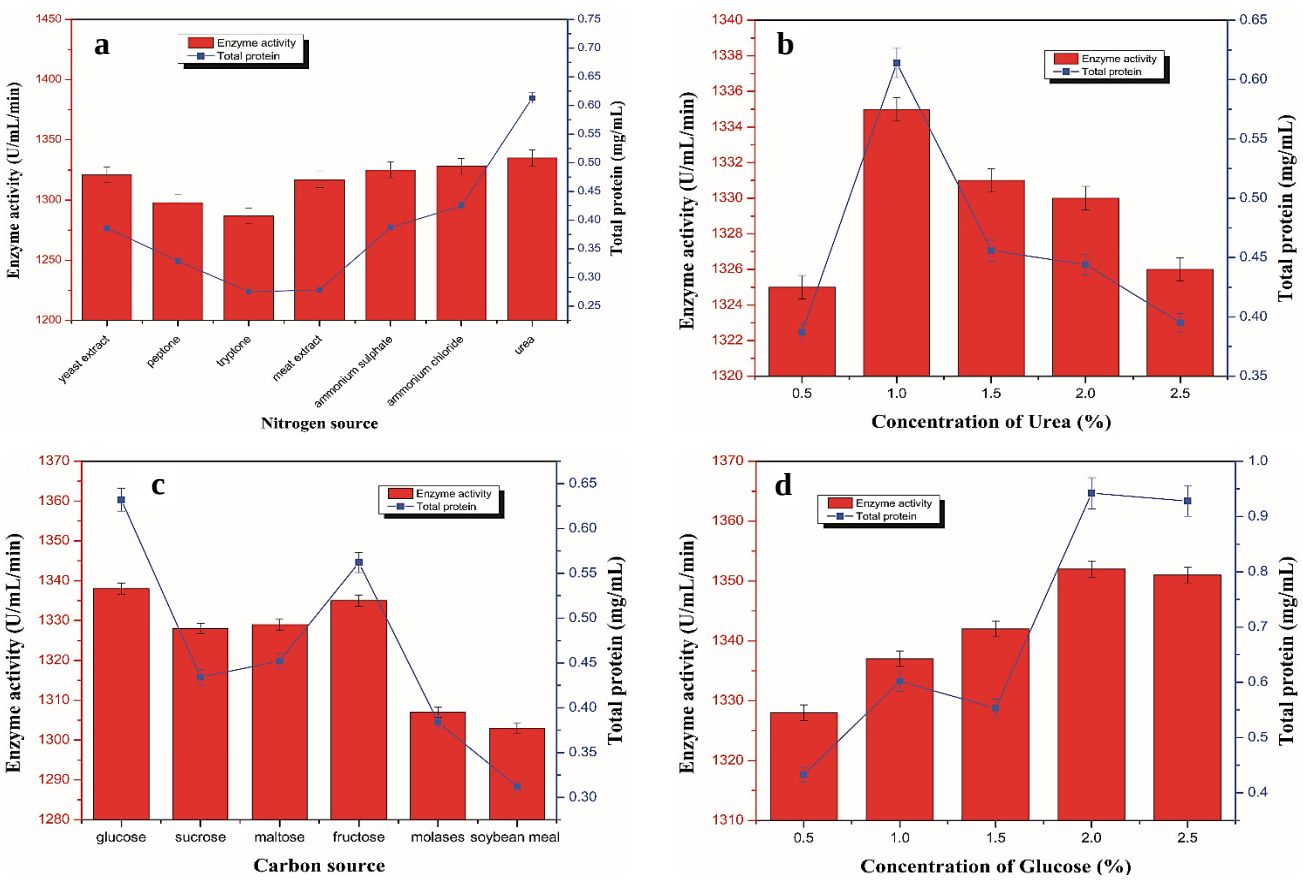

Fig. 6. Effect of (a) different nitrogen sources (b) concentration of nitrogen source (urea) (c) different carbon sources and (d) concentration of carbon source (glucose) on urease production through submerged fermentation using *Lysinibacillus xylanilyticus.*

Conclusion

Lysinibacillus xylanilyticus was identified as a potential source of urease through submerged fermentation. Optimization of various cultural conditions resulted in increased production of the enzyme. The current study will help in cost effective production of urease by indigenously isolated bacterial strain for various industrial application.

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(Received for publication 30 May 2023)