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Studies on Characteristic of Extracellular L–Glutaminase and Identification of L-Glutaminase Producing Bacterial Strain from Cattle Feeding Farm of Chhatrapati Sambhajinagar, Maharashtra State, India

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Abstract

L-glutaminase is gaining marked importance due to its potential clinical applications. A variety of microorganisms, including bacteria, yeast, and lamentous fungi, from soil habitat have been reported to produce L-glutaminase. The present investigation was carried out to isolate and screening of L-glutamiase producing bacteria from soil samples of cattle feeding sites. Among twenty-three soil samples of cattle feeding farms around Aurangabad, nineteen bacteria's were isolated. From that one isolate (AGT-19) that showed significant L-glutaminase activity. Morphological characteristics and 16S rRNA sequencing were used for phylogenetic analysis to identified strain AGT-19 as Kurthia gibsonii. Moreover, Kurthia gibsonii was grown in a medium containing 2.5% succinate and 2.5% L-glutamine showed significant activity of L-glutaminase (0.079 U/mg). The optimum conditions for L-glutaminase production where temperature was 39°C and pH was 7.0. The present isolation of K. gibsonii from cattle feeding site indicated that this bacterium is suitably adapted to the environment of excretion and to point of forming a microbiota in the fecal product. It revealed that soil of cattle feeding farm has diverse bacterial strains and its habitat nature allowed the bacteria expressed the protease activity.

Keywords: *L-glutaminase, Mesophilic bacteria, Kurthia gibsonii, Satara-Deolai Parisar*

1. Introduction

Glutaminases showed significant hydrolysis of L-glutamine to L-glutamic acid in various mammals and widely distributed in microorganisms in catalytic metabolism. Mammals showed glutaminases role in physiological activity in vital organs and tissues of brain, kidney, neurons, and macrophages with human immunodeficiency virus-1 infection have been studied several researchers (1,2). The *Escherichia coli* and *Rhizobium etli* was participate in nitrogen metabolism by using glutaminases in microorganisms were studied (3,4). Glutaminase showed considerable application in the food industries because it produces L-glutamic acid, along with savory flavor, from L-glutamine was explained (5). Microbial glutaminases have been studied with the aim of adapting them to food processes; most studies to date have focused on their applications to soy sauce fermentation was proved (6). In addition to catalyzing to convert from L-glutamine to L-glutamic acid, glutaminase also controls the non-enzymatic production of the tasteless amino acid, L-pyroglutamic acid from L-glutamine (7). Bacterial γ-glutamyl trans peptidase and glutaminase -asparaginase efficiently increase L-glutamic acid content in soy sauce fermentation, producing a more flavorful product (8,9). L-glutaminase play a significant activity in enzyme therapy during several cancer treatments; and would by considered to acute lymphocytic leukemia. It was found that against the human immunodeficiency virus, L-glutaminase showed significant effective functions was proved and explained the cellular nitrogen metabolism; in that the L-glutaminases act as amide enzyme in several living cells (10,11). L-Glutaminases is large superfamily

of serine-dependent β-lactamases and penicillin binding proteins has evolutionary origin, protein fold, structural motifs, and catalytic mechanism of L-glutaminases was explained (12). The various metabolism of energy metabolism, ammonia trafficking and regeneration of neurotransmitter glutamate were regulated by multifunctional enzyme glutaminase experimentally proved (13). It is a mitochondrial enzyme and localized in outer face of the inner mitochondrial membrane (14). Phosphate-activated glutaminase (PAG) exists in mitochondria in two forms, an inner membrane-bound and a soluble form. They present differential kinetic profiles and sensitivity to inhibitors and activators; the membrane-bound form seems to be the active form of the enzyme (15). There are many cattle feeding farms were found around Chhatrapati Sambhajinagar district. While various microorganisms were found in these feeding sites have been studied, to date there has been no report concerning the production and characterization of glutaminase produced by microorganisms isolated from cattle feeding farms. With the ultimate goal of improvement of information, we presently screened the glutaminase-producing bacteria from cattle feeding farms. Of the twenty-three soil samples; nineteen bacterial strains isolated; one strain (AGT-19) isolated from cattle feeding farm exhibited the highest glutaminase activity. In this paper we describe the identification of AGT-19 and the favorable conditions for L-glutaminase production. At Genus-level, the composition of microbe's community across four agricultural areas of the array was compared using the non-metric multidimensional scaling (NMDS) method. The relative average of genera found in each environment (soil, phyllosphere and milk) is given in the supplementary material. The results show a comparison of prokaryotic community composition in four agricultural areas. According to agricultural conditions, the microbial communities differ from each other. Prokaryotic soil and phyllosphere communities were distinguished from prokaryotic dairy and cattle communities along the NMDS1 axis based on centroid position. Soil and phyllosphere microbial communities along the NMDS2 axis, soil and phyllosphere prokaryotic communities were discriminated from one another whereas cow doing and milk communities were only slightly distinguishable.

2. Materials And Methods

Satara-Deolai parisar is situated at (N $75^0$18'37.28" Longitude-E $19^0$50'14.25" Latitude) in south of Chhatrapati Sambhaji nagar city shown in Figure 1. The locality Satara Deolai Parisar falls in Chhatrapati Sambhajinagar district situated in Maharashtra state, with a population 69955. The male and female populations are 36464 and 33491 respectively. The size of the area is about 10.64 square kilometer. Sampling sites were selected based on high availability of different cattle were feed continuously. There are Khillar, red Sindhhi, Sahival and Deoni type of cattles was feed in present cattle feeding farm. The feeding farm was not very well contrasted. It was an as it is natural hilly environment. Small Tiger hill and Patil Lake was situated near the present cattle feeding farm. Soil samples were collected fortnightly interval from four prefixed sampling sites near tiger hills and Patil Lake during February 2021 to May 2021 (summer season) so as the samples represent the entire cattle feeding farm (distance between two sites was forty-five meter) shown in Figure 2 as followed by standard soil sample collection method for bacterial isolation was done (16).

Figure 1. Showing sampling site in Satara-Deolai parisar, Chhatrapati Sambhajinagar.

Figure 2. The Google earth map showing four sampling sites in Satara-Deolai parisar, Chhatrapati Sambhajinagar.

About 1 gm of soil samples were collected and transferred in sterile plastic bottles as sterile condition. The samples were brought to Animal Physiology Laboratory, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar-431 004, and Maharashtra State, India for further processing as standard method (17).

Enrichment and screening of L-glutaminase isolates

Nineteen bacterial traces have been isolated from soil samples. Hundred microliters of aliquot from a hundred and five to 106 dilutions had been unfold on minimal glutamine agar (MGA) medium the use of the sterile spreder. The elements of MGA (g/l) consist of 1.0 NaCl, 0.5 KCl; 0.5 $MgSO_4$; 1.0 KH_2PO_4 0.1 $FeSO_4$; 0.1 $ZnSO_4$; 10 glutamine, 0.12 Phenol red and cycloheximide (20 µg/ml) (18). L-glutamine acts as the sole carbon and nitrogen supply. Phenol pink acts as a pH indicator. Cycloheximide retards the fungal boom. The plating turned into carried out in triplicate and all the plates were incubated at 37°C for forty-eight hours. Most effective the bacteria which synthesize L-glutaminase can develop in MGA medium and the extracellular manufacturing of L-glutaminse become detected. The formation of a crimson quarter round colonies indicated a advantageous response due to accumulation of ammonia, which has resulted in a change in pH indicator colour from yellow to crimson because of the growth in pH cost that is resulting from L-glutamine use (19). The secondary screening for the surprisingly manufacturer isolates was achieved through culturing the high-quality lines for enzyme manufacturing in liquid minimum glutamine medium. All of the flasks were incubated at 37°C for seventy-two hours in a rotary shaker at 150 ×g After centrifugation of the cultures via the use of a cooling centrifuge at 10,000 × g for half-hour at 4°C, the optical density (OD) of every supernatant was measured at 540 nm the usage of a visible spectrophotometer (20). A lifestyle, giving the very best L-glutaminase production was used further for L-glutaminase manufacturing. Regular sub-culturing of the chosen isolate became done at an interval of each 4 weeks and preserved at refrigerated conditions as slant way of life.

Phenotypic characterization of bacterial isolates

Bacterium was characterized through trendy biochemical, morphological and physiological traits in line with Bergey's guide of Systematic Bacteriology and the prokaryotes (21). Identity became specially based totally at the effects of Gram staining, catalase response, colony morphology at 45^0C, and survival at 55^0C for 20 minutes. Furthermore, all characterizations had been accomplished the usage of a reference kind pressure.

Phylogenetic analysis of bacterial isolates

Bacterial isolate displaying the very best L-glutaminase manufacturing was examined for species identification the use of the 16S rRNA sequencing method (22). DNA sequences have been aligned using Gene Mapper v4.1 & statistics series v 3.1 conversations Patch-1. To extract the genomic DNA, bacterial colonies are picked with a sterilized toothpick and suspended in 0.5 ml of sterilized saline,

then centrifuged at 10,000 × gm for 10 min. After removal of supernatant, the pellet was suspended in 0.5 ml of Insta Gene Matrix (Bio-Rad, U.S.A.), incubated at 56°C for 30 min, and then heated to a 100°C for 10 min. After heating, the supernatant may be used for the PCR reaction. Cells had been re-suspended in 200 L of TE buffer and DNA become extracted consistent with the approach of (23). Primers used for the polymerase chain reaction (PCR) were 25F (5'-TGTAAATCGGCCAGTACAGTTTGATCCTGGCTC-3') and 1510R (5'-CAGGAAACAGCTATGACCGGCTACCTTGTTACGACT-3'), which corresponded to positions 10 -26 and 1495 -1510, respectively, of the 16S rRNA sequence of *Escherichia coli*. The PCR mixture (50 L) consisted of Ex Taq polymerase, 10X Taq polymerase buffer, 2.5 mM dNTPs, 50 pmol of every primer and the extracted DNA (50-100 ng). The thermal cycling program consisted of a preliminary denaturation at 95^0C for 3 minutes, 30 cycles of 95^{0C} for 0.5 minutes, 56^0C for 0.5 minute, and 72^0C for 1.5 minutes. The amplified PCR merchandise have been analyzed by agarose gel electrophoresis with a 500-bp DNA ladder marker as the molecular weight general. The PCR product was purified with a PCR-M clean-up device (VIOGENE, Sunnyvale, United states of America). The DNA sequence of the product changed into decided with the 25F and 1510R primers the usage of computerized DNA sequences, DSQ-2000L system (Shimadzu Biotech, Kyoto, Japan). The partial series (500 bp) become used for the phylogenetic analysis. Sequence evaluation was achieved with sequences inside the countrywide middle for Biotechnology facts (NCBI), U.S.A. database the use of primary neighborhood Alignment seek device for Nucleotides (24).

Bacterial Inoculum preparation

Bacterial inoculum of the tested bacterial pressure changed into prepared in 250 ml Erlenmeyer flasks containing 50 ml of nutrient broth liquid medium (pH 7.0). Prepared medium turned into autoclaved and then inoculated with a loopful of culture from 24 hours vintage nutrient agar slant. The inoculated flasks have been saved on a shaker at 150 × g for 24 hours and used as the inoculum (19).

Production and extraction of L-glutaminase

Five ml of the organized bacterial inoculum turned into transferred aseptically to 45 ml of minimal glutamine manufacturing medium. All of the flasks were incubated at 37°C in a rotary shaker at 150 × g for 72 hours. After each 24 hours, 10 ml of pattern became removed aseptically and centrifuged using cooling centrifuge at 10,000 × g for 30 min at 4°C. The clean supernatant became used for enzyme estimation (25).

L-glutaminase assay

Assay of L-glutaminase turned into performed as standard method by utilizing L-glutaminase as a substrate and the launched ammonia changed into measured the use of Nessler reagent (25). In brief, 0.5 ml of 0.05 M glutamine turned into taken in a test tube, to which 0.5 ml of 0.05 M buffer (Tris HCl, pH 7.2), 0.5 ml of enzyme, and 0.5 ml of distilled water changed into added to make up the volume up to 2.0 ml, the reaction mixture become incubated at 37°C for 30 minutes. After incubation, the response turned into stopped via adding 0.5 ml of 0.5 M TCA (Trichloro-acetic acid). The induced proteins had been eliminated by centrifugation (10,000 × g for 20 minutes). The blank turned into organized in addition without including enzyme instruction. 0.1 ml became taken from the above-referred to reaction mixture and added to 3.7 ml distilled water and to that 0.2 ml of Nessler's reagent turned into added, incubated for 10 minutes, and the absorbance (OD) became measured at 450 nm using a UV–visible spectrophotometer. Then a widespread curve turned into plotted the usage of ammonium sulfate as the same old for estimation of ammonia liberated. One unit of L-glutaminase is the amount of enzyme which liberates 1 μmol of ammonia in step with minute per ml (μmole/ml/min) under choicest assay conditions. Assays were completed in triplicate, and the mean enzyme pastime changed into expressed as unit according to ml (U/ml) (19).

Protein estimation

Protein content in the crude enzyme source was estimated by Lowry's method using bovine serum albumin as the standard, and the values were expressed as mg/ml (26).

Optimization of cultivation condition for L-glutaminase production

Bacterial pressure AGT-19 became cultured for L-glutaminase production in 500 ml Sakaguchi flask with 100 ml of a pH 7.0 medium containing carbon and nitrogen sources (every 1.0%), 0.05% yeast extract, and 0.5% NaCl (w/v), at 39^0C with shaking at 100-200 rpm for 12-24 hours. The results of carbon sources (glucose, fructose, sucrose, dextrose, lactose, maltose, succinate) and nitrogen sources (peptone, yeast extract, L-arparagine, L-proline, L-glutamine, L-glutamic acid, L-methionine, L-

proline, L-lysine, succinate+glutamine) on enzyme production were investigated. After determining the most advantageous medium, composition, the effects of pH, temperature on glutaminase manufacturing were investigated (27).

3. Results and Discussion

Isolation and characterization of the bacterial isolate

Bacterial isolates (n = 19) had been isolated from livestock feeding farms. One isolate that became received from Satara parisar, Chhatrapati Sambhajinagar become specific AGT-19. The isolate exhibited the highest L-glutaminase activity and turned into selected for similarly studies of enzyme manufacturing. AGT-19 was Gram-superb and catalase-high quality with everyday un-branched rods shown in Figure 3, and did not form acid from glucose and different carbohydrates including sorbitol and mannitol in peptone-based media. AGT-19 became motile and exhibited a 'medusa-head' like appearance. The floor of colonies that developed on yeast nutrient agar tended to be rhizoid. The production of 'hen's feather' on yeast nutrient gelatin medium is a completely unique feature of *Kurthia gibsonii* (21). Furthermore, the potential of JRT-7 to grow at 45^0C and live to tell the tale at 55^0C for 20 minutes was the other phenotypic characteristics of *K. gibsonii* that had been discovered. The in part amplified 16S rRNA sequence of AGT-19 become determined (1217 bps). Comparative analyses revealed that 99% identity within the sequences of AGT-19 and the type pressure *K. gibsonii*, with most effective four nucleotide mismatches obvious.

Table 1. Showing results of morphological and cultural characteristics of L-glutaminase isolates

Colony Characterization	Observation results
Shape	Round
Size	2mm
Colour	Creamy
Margin	Entire
Opacity	Opaque
Elevation	Convex
Consistency	Butyrous
Gram Character	Gram positive rod
Growth	Rapid
Motility	Motile
Endospore stain	Spores present

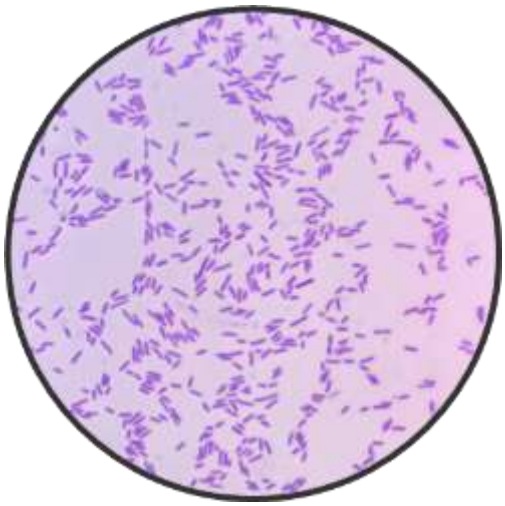

Figure 3. Showing gram characteristics of L-glutaminase producing bacterial isolates

A dendrogram constructed the usage of the neighbour-becoming a member of method for the 16S rRNA series of the strain and their closest spouse and children is depicted in Figure 4. The phylogenetic tree of the strain AGT-19 turned into placed in the genus Kurthia and showed a close relationship to *K. gibsonii* with 1000 bootstraps. As end result, AGT-19 become specified as *K. gibsonii*. within the excretion of farm animals in feeding farms have been urine, milk and cow dank, plant substances and soil containing indigenous microorganisms play an essential role in degradation. Even as the microbial flower in farm animals feeding farms has been studied (27), isolation of L-glutaminase-producing

microorganisms from livestock feeding farms has no longer been suggested till date. *Kurthia gibsonii* has previously been isolated from beef and Nham products (28).

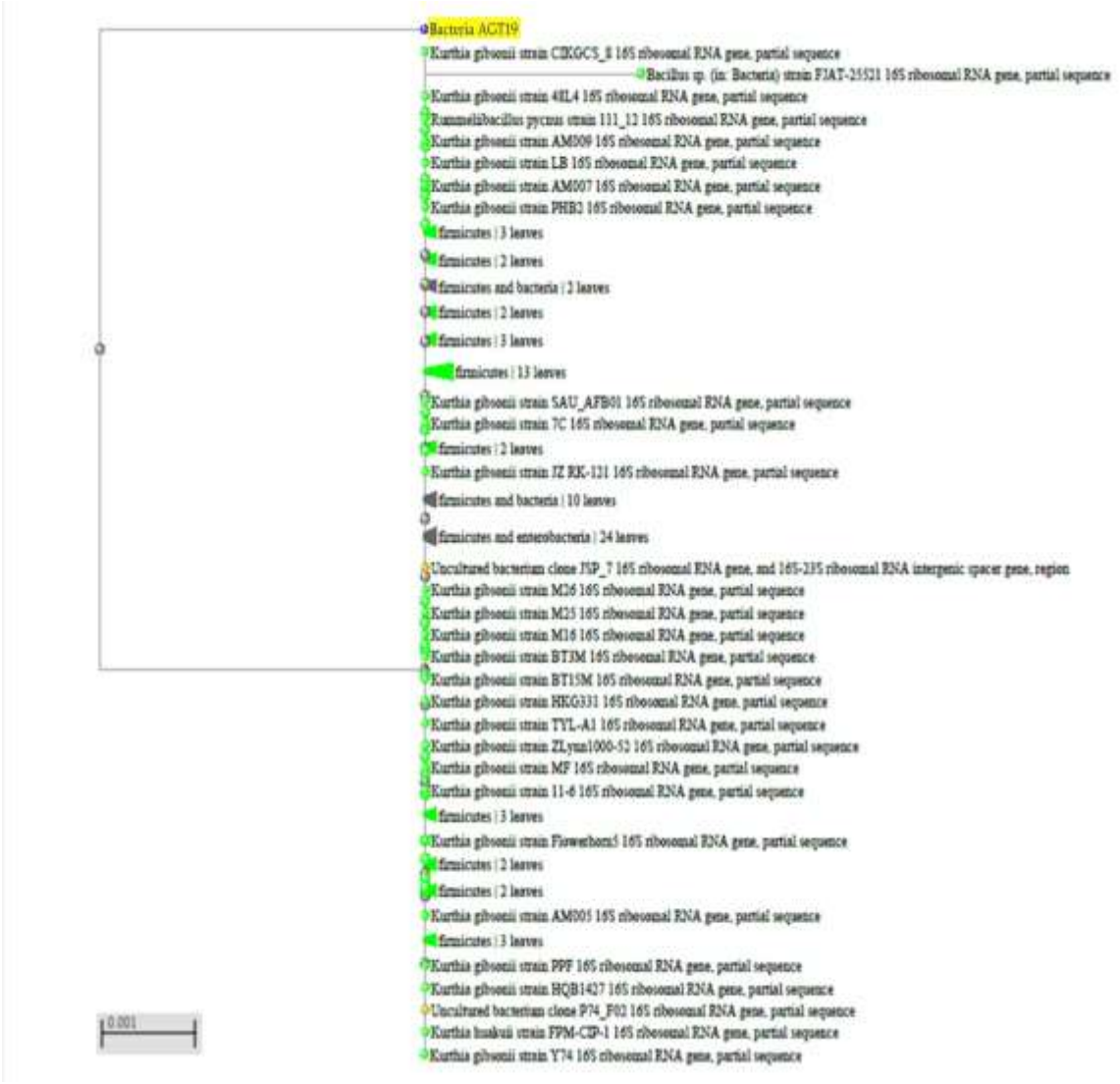

Figure 4. Showing bacterial phylogeny of AGT- 19 on basic of 16S rRNA gene sequence

Optimization of culture condition for L-glutaminase production

Table 1 show the impact of various carbon and nitrogen resources on the manufacturing of L-glutaminase through *K. gibsonii*. An excessive unique pastime (0.046 U/mg protein) became acquired when the medium contained 1% succinate and 1% L-glutamine as carbon and nitrogen sources, respectively. The premiere concentrations of succinate and glutamine for enzyme production were observed to be 2.5 percent shown in Table 1. Furthermore, the most reliable pH and temperature of L-glutaminase production changed into decided to be pH 7.0 and at 39^0C, respectively. The effect of aeration on glutaminase production of *K. gibsonii* AGT-19 changed into additionally tested. Maximum L-glutaminase production with the highest particular pastime of 0.064 U/mg protein changed into received whilst the bacteria have been cultured within the medium containing 2.5% succinate and L-glutamine at pH 7.0, at 39^0C with reciprocal shaking at 200 rpm for 18 hours. A time direction of bacterial growth and L-glutaminase manufacturing changed into obtained under these optimized culture situations. L-glutaminase production elevated concurrently together with increase in bacterial cell increase, accomplishing a maximum during the past due logarithmic section of growth at 18 hours. As the lifestyle entered desk bound segment, enzyme pastime gradually reduced. The observations suggested that the *K. gibsonii*. L-glutaminase features as a boom-associate enzyme, just like different microbial glutaminases (29). In *K. gibsonii*, its miles considered that L-glutaminase performs an essential role in nitrogen metabolism. Further, the manufacturing of L-glutaminase from *Bacillus licheniformis* reaches a most in the direction of the end of exponential phase and returns to a low stage of activity when the way of life enters desk bound phase (30). Succinate and L-glutamine have been the most effective carbon and nitrogen assets, respectively, for L-glutaminase manufacturing in *K. gibsonii* AGT-19. It is widely recognized that succinic acid stimulates power production through the citric acid cycle, and that glutaminase catalysis. The conversion of glutamine to glutamic acid, with the latter being

in flip converted to the other compounds, inclusive of α-ketoglutaric acid, that is an essential intermediate within the citric acid cycle. Similar effects have been reported for the manufacturing of L-glutaminases with the aid of *Vibrio costicola* and *Beauveria sp*. below solid-kingdom fermentation. Both these microorganisms showcase maximal enzyme production whilst cultured in a medium containing 0.25-1% L-glutamine (29,31). Different L-glutaminase-generating microorganisms along with *Escherichia coli, Pseudomonas spp., Micrococcus luteus, Bacillus spp., Vibrio spp., Rhizobium etli, Aspergillus oryzae* and *Saccharomyces cerevisiae* were removed from soil or marine environments (6).

Table 2. Showing effects of various factors on L-glutaminase production

Factors	Enzyme Activity (U/ml)
Control	5.89±0.17
Glucose	19.77±1.1
Fructose	7.69±0.27
Sucrose	16.84±1.2
Dextrose	18.78±0.56
Lactose	12.78±0.32
Maltose	12.98±0.71
Peptone	37.04±0.01
Yeast Extract	33.79±0.97
Urea	17.34±0.54
L-gltamine	37.71±1.71
L-aspargine	25.91±0.57
L-glutamic acid	11.34±1.1
L-methionine	19.71±0.8
L-proline	11.97±1.0
L-lysine	12.43±0.38
Succinate+glutamine	49.77±0.96
NaCl	33.2±0.77
Ph	22.91±1.6
Temperature	24.67±1.9

Data represent mean value ± Standard deviation

The production of pink shade zone around the bacterial colony grown on minimum glutamine agar (MGA) medium is an indication of L-glutaminase production (32). Earlier method entails the isolation of microorganisms from sure environments by way of recurring isolation approaches and then screened for enzymatic activity. However, the usage of selective media and the presence of antibiotics, NaCl, and pH indicators make MGA medium appropriate for direct and selective isolation of L-glutaminase generating marine isolates (33).

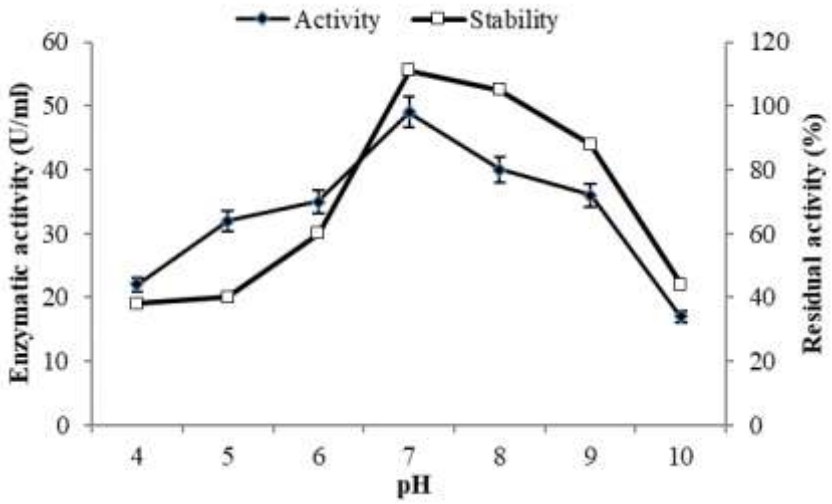

Figure 5. Showing enzymatic activity and stability L-glutaminase produce by AGT- 19

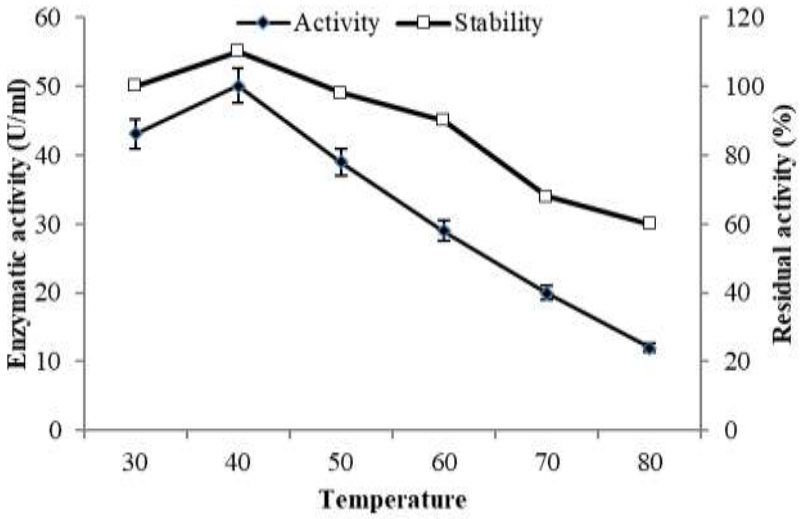

Figure 6. Showing enzymatic activity and stability L-glutaminase produce by AGT- 19

Carbon supply represents the strength source required for the increase of microorganisms. Carbohydrates and related compounds are considered as the favorable carbon sources for lots genera of microbes. The improved production of L-glutaminase by way of incorporation of carbon sources can be attributed to the wonderful impact of extra carbon assets along with glutamine for boosting the enzyme biosynthesis (34). Inside the gift have a look at, glucose turned into used as a further carbon source for yielding the maximum L-glutaminase by means of *Kurthia gibsonii*. Further, glucose become the best carbon source for L-glutaminase production through *Pseudomonas aurignosa* (35). Then again, rhamnose prompted the best L-glutaminase production of the marine bacterial isolate *Bacillus subtilis* OHEM11 (36). Nitrogen source has been given a profound influence on enzyme production as it's miles the remaining precursor for protein biosynthesis. Except, the nitrogen supply can also have an effect on the pH of the medium, which in turn can also affect the hobby and balance of the enzyme. The outcomes of the existing have a look at discovered that peptone more suitable the enzyme yield. At the contrary, said that glutaminase production by means of marine *Bacillus subtilis* JK-79 was more suitable with the aid of using yeast extract (37). Furthermore, amino acids had been stated to be a commonplace increase element required for the synthesis of enzyme because the most important nitrogen supply (38); for this reason, the yield of l-glutaminase produced became numerous when the amino acid become modified. In the present observe, L-glutamine was found to beautify L-glutaminase synthesis by *Kurthia gibsonii*. This remark suggests that L-glutamine acts as an inducer for the production of extracellular L-glutaminase enzyme.

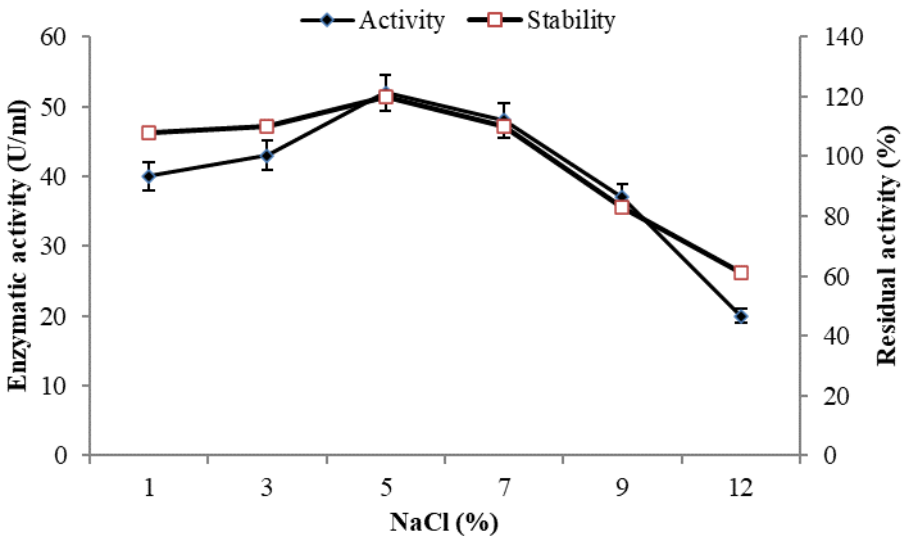

Figure 7. Showing enzymatic activity and stability L-glutaminase produce by AGT- 19

Comparable effects were stated by way of who indicated that L-glutamine and L-asparagine resulted in a excessive yield of l-glutaminase (39). Moreover, it was confirmed that the most L-glutaminase pastime by way of *Pseudomonas aurignosa* become done with glutamine out of diverse nitrogen assets (35). It's far that the pH of the way of life medium impacts the provision of certain metabolic ions and

permeability of bacterial cell membranes, which in turn helps cell boom and enzyme manufacturing (40,41). In general, the pH variety 6.0 to 8.0 changed into pronounced to be the maximum favorable range for L-glutaminase manufacturing by way of the majority of microbial organisms [42]. Within the present have a look at, it turned into observed that pH 7.0 is top-quality for the L-glutaminase production with the aid of *Kurthia gibsonii*. In the identical line, stated that the top-rated pH for L-glutaminase production by way of *Aspergillus oryzae* NCIM 1212 was at pH 7.0 (43). Similarly, the quality L-glutaminase production was located at pH 7.0 for the wooded area soil isolated bacterial pressure of *Bacillus* spp. (44). But, marine *Vibrio azureus* JK-79 bacterial pressure exhibited the most glutaminase production at pH 8 (45). Furthermore, increase temperature motivated the microbial metabolism both with respect to the rates of mobile procedures run and the enzymatic reactions arise. It mentioned that any temperature beyond the premier variety is found to have some unfavorable results on the metabolic activities of the microorganisms (46). Within the present study, the very best L-glutaminase production through *Kurthia gibsonii* turned into noticed at a temperature of 37°C. Again, within the equal line, confirmed that the most glutaminase activity through *Vibrio azureus* JK-79 isolated from marine surroundings was at 37°C. On the alternative aspect, said that a temperature of 35°C is the nice one for glutaminase production via *Pseuedomonas* NS16.

4. Conclusion

In this examine we remote bacterial pressure AGT-19. 16S rRNA collection based totally phylogenetic evaluation recognized stress AGT-19 as *Kurthia gibsonii*. moreover, the unique interest of L-glutaminase (0.046 U/mg) became received while *K. gibsonii* was grown in a medium containing 1% succinate and 1% L-glutamine. The highest quality situations for L-glutaminase production had been temperature become 38°C and pH become 7.0. L-glutaminase production changed into analyzed with unique process parameters and medium ingredients. most manufacturing become noticed in a medium supplemented with 1% (w/v) glucose as carbon supply, 1% (w/v) peptone as nitrogen supply, 5 % (w/v) NaCl, the initial pH of 7.0, at 38°C, the usage of 20% (v/v) inoculum length after 72 hours of incubation. under optimum conditions, the glutaminase manufacturing improved to 49.77 U/ml. The produced L-glutaminase holds right features as active and steady over a substantial range of pH and temperatures. On the basis of statistics received in present work it can be concluded that *Kurthia gibsonii* can be hired in the manufacturing of L-glutaminase; is an essential enzyme inside the medical, industrial, and financial factors. but, a better expertise of *K. gibsonii* L-glutaminase is essential, and purification and characterisation are in development. It discovered that soil of farm animals feeding farm has diverse bacterial traces and its habitat nature allowed the microorganism expressed the protease activity. The present isolation of *K. gibsonii* from cattle feeding website online indicated that this bacterium is definitely adapted to the surroundings of excretion and to point of forming a microbiota in the fecal product.

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