Saudi Journal of Biological Sciences 27 (2020) 3235–3248

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

# Molecular diversity and hydrolytic enzymes production abilities of soil bacteria

Chirom Aarti [a], Ameer Khusro [a,*], Paul Agastian [a,**], Noura M. Darwish [b], Dunia A. Al Farraj [c]

[a] Research Department of Plant Biology and Biotechnology, Loyola College (Affiliated to University of Madras), Nungambakkam, Chennai 600034, Tamil Nadu, India
[b] Faculty of Science, Biochemistry Department, Ain Shams University, Cairo, Egypt
[c] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

*Article history:*
Received 15 July 2020
Revised 20 September 2020
Accepted 22 September 2020
Available online 29 September 2020

*Keywords:*
Bacterial diversity
Bioinformatics tools
Hydrolytic enzymes
Soil
Tamil Nadu

ABSTRACT

Soil is an integral part of ecosystem which is niche for varieties of microflora. The present study was investigated to isolate varied strains of bacteria from soil samples of three different geographical regions of Tamil Nadu (India) and evaluate their hydrolytic enzymes (amylase, cellulase, and inulinase) producing potentialities. Among 72 bacterial cultures isolated from Ambattur Industrial Estate, Neyveli Lignite Corporation, and Arignar Anna Zoological Park regions, 41.66, 38.88, and 36.11% of isolates were observed amylase, cellulase, and inulinase producers, respectively. On the other hand, 20.83% of total bacteria isolated from all three regions exhibited concurrent production of amylase, cellulase, and inulinase. Potent isolates depicting maximum enzyme activities were identified as *Bacillus anthracis* strain ALA1, *Bacillus cereus* strain ALA3, *Glutamicibacter arilaitensis* strain ALA4, and *Bacillus thuringiensis* strain ALA5 based on molecular characterization tools. Further, the thermodynamics parameters, open reading frames (ORFs) regions, and guanine-cytosine (GC) content were determined by distinct bioinformatics tools using 16S rRNA sequences of strains. Minimum free energy values for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 were calculated as −480.73, −478.76, −496.63, and −479.03 kcal/mol, respectively. Mountain plot and entropy predicted the hierarchical representation of RNA secondary structure. The GC content of sequence for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 was calculated as 53.06, 52.94, 56.78, and 53.06%, respectively. Nine ORFs were obtained for strain ALA1, strain ALA3, and strain ALA5 while 10 ORFs were observed for strain ALA4. Additionally, bootstrap tree demonstrated close resemblance of strains with existing bacteria of similar genus. Findings showed higher variability of bacterial diversity as hydrolytic enzymes producers in the investigated geographical regions.


## 1. Introduction

Soil is a complex biomaterial on the ecosystem that is generally composed of disparate ingredients, particularly organic matters, inorganic particles, minerals, and microflora. It is considered as the most common medium for the growth and other metabolic processes of microorganisms (Al-Dhabi et al., 2016; Talwar and Chatli, 2018). Varieties of chemical mechanisms take place in soil that ensure the sustainability of invisible life and create an ideal ecosystem for them. Soil is the most common niche for bacteria, actinomycetes, fungi, protozoa, and algae which contribute in the maintenance of diversified natural processes such as enhancement of plants' growth, decaying of organic substances, nitrogen fixation, and production of secondary metabolites (Al-Dhabi et al., 2018, 2019; Imadi et al., 2016). Among diverse soil microflora, bacteria are the predominant prokaryotes with high physiological and metabolic variations (Archana et al., 2015). Bacterial population in soil is affected by the structure and pH of soil (Talwar and Chatli, 2018).

Soil bacteria play imperative role not only in sustaining cleaner ecosystem and building the fertility of soil but also maintain bio-geochemical cycles (Imadi et al., 2016). Most importantly, bacteria autochthonous to soil are vast producers of enzymes. Enzymes are dexterous biocatalysts that play paramount role

* Corresponding author.
** Corresponding author.
*E-mail addresses:* armankhan0301@gmail.com (A. Khusro), agastian@loyolacollege.edu (P. Agastian).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.09.049
1319-562X/

in the formation of useful by-products. Bacterial enzymes are comparatively more stable than chemical catalysts and are produced rapidly with desired characteristics (Niyonzima, 2019). In addition, bacteria associated enzymes are not only thermo-alkali resistant but also its downstream bioprocess technique is inexpensive (Khusro et al., 2017). Among diversiform groups of hydrolytic enzymes, amylases, cellulases, and inulinases have created huge interest among worldwide researchers due to their pivotal roles in foods, dairy, paper and pulp, detergent, textile, and biofuel industries (Al-Dhabi et al., 2018; Alves et al., 2014; Singh et al., 2017).

Amylases (EC 3.2.1.1) are starch hydrolyzing enzymes which constitute about 65% of total enzyme market worldwide. The enzyme acts on α-1,4-glucosidic linkages of starch molecules as prime substrate and converts them into simpler monomer units (Khusro and Aarti, 2015; Al-Dhabi et al., 2019b). Bacterial amylases are often preferred over other microbial sources because of its versatility in the biochemical process, high rate of production, and easy availability (Khusro et al., 2017). Amylase has tremendous significance in varied industries for their commercial roles in the saccharification of biomasses, liquefaction of starch, desizing of fabrics, fermentation of beverages, production of sugar syrups etc. (Alves et al., 2014). Cellulase (E.C.3.2.1.4) is another crucial hydrolytic enzyme produced by bacteria that catalyzes lignocellulosic substances (eg. cellulose) into monosaccharides units. Generally, endoglucanases, exoglucanases, and β-glucosidases are three important components of cellulose which catalyze cellulose (Lugani et al., 2015). Over the past few years, cellulase ranks among third largest hydrolytic enzymes utilized commercially around the globe (Binod et al., 2013). Beside enormous applications of cellulases in biofuel production, it is being commonly used in textile, brewery, food, wool, pulp, detergent, and livestock industries (Zhou et al., 2008). Inulinases (EC 3.2.1.7) are one of the most important key industrial enzymes of glycoside hydrolase family 32 (GH32) which targets β-2,1 linkages of inulin (second most abundant polysaccharides in plants) to produce fructose or fructooligosaccharides as by-products (Chi et al., 2011). Endoinulinases and exoinulinases are two categories of inulinase which produce fructooligosaccharides and fructose units, respectively by hydrolyzing inulin as substrate (Chi et al., 2009). The versatile role of inulinases is commonly observed in the production of biofuel, acetic acid, fructooligosaccharides, fructose syrup, and sorbitol.

Despite several reports investigating the productions of hydrolytic enzymes for diversified applications, there is still a colossal demand to isolate hyper amylase, cellulase, and inulinase producing bacteria from distinct natural resources for their paramount industrial applications (Al-Dhabi et al., 2018, 2019a, 2019b). In view of the prior knowledge regarding the abundance of disparate categories of microflora in soil, this investigation was carried out to isolate hydrolytic enzymes, particularly amylase, cellulase, and inulinase producing bacteria from soil samples of varied geographical regions of Tamil Nadu, India. The promising isolates were further identified and characterized using molecular and various bioinformatics tools.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade with the highest purity, and were obtained from HiMedia, India. These chemicals and reagents were stored at specific temperature as specified for further experimental purposes.

### 2.2. Sample collection

Soil samples (at 1 cm depth) were collected from three different geographical regions of Tamil Nadu, India: i) Ambattur Industrial Estate (AIE), Ambattur, Chennai, ii) Neyveli Lignite Corporation (NLC), Neyveli, and iii) Arignar Anna Zoological Park (AAZP), Vandalur, Chengalpattu. Map, location, and coordinates of each sample collection site are shown in Fig. 1.

### 2.3. Isolation of bacteria and pure culture preparation

Bacteria were isolated from soil samples using serial dilution technique. One gram of soil sample collected from each region was mixed with 9 mL of sterile distilled water. The suspension was serially diluted ($10^{-1}$-$10^{-6}$) and 1 mL of the suspension was transferred onto sterilized nutrient agar (g/L: peptone – 10.0, beef extract – 10.0, sodium chloride – 5.0, agar – 18.0, and pH – 7.2) plates. After spreading the suspension using L-rod, plates were incubated at 37°C in incubator (Model - ORBITEK: LE0102DCBA) for 24 h under aerobic condition. After required period of incubation, the appearance of different colonies on the agar plates was observed. The morphologically dissimilar colonies of isolates were selected and streaked on the freshly prepared nutrient agar plates. Purified bacterial cultures were preserved in 50% v/v glycerol stock at −80°C for further experiments.

### 2.4. Extracellular enzymes production–

All the isolates were studied for the production of extracellular hydrolytic enzymes (amylase, cellulase, and inulinase) using standard methodologies as described below:

#### 2.4.1. Amylase production

*2.4.1.1. Plate assay (Qualitative analysis).* Purified isolates were grown in fermentation medium (% w/v peptone – 1.0, yeast extract – 0.5, potassium dihydrogen phosphate – 0.1, magnesium sulphate – 0.02, sodium carbonate – 1.0, sodium chloride – 0.3, and pH – 7.0) constituting 1% (w/v) starch and kept under shaking condition at 37°C for 24 h. After required incubation period, cultures were centrifuged at 6000 *g* for 20 min for collecting supernatants. Meanwhile, wells were created on starch agar medium (g/L: starch – 10.0, agar – 20.0, and pH – 7.0) plates. The supernatant of each isolate was loaded into the agar wells and incubated at 37°C. After 24 h, each amylum agar plate was flooded with Lugol's iodine solution for observing amylase production (Khusro et al., 2017). Amylase positive isolates were further selected for estimating amylase activity.

*2.4.1.2. Amylase activity (Quantitative analysis).* Amylase activities of selected starch hydrolyzing isolates were quantified as per the method of Miller (1959) with minor changes. Isolates were inoculated in the broth medium constituting 1% (w/v) starch as discussed in the previous section and incubated at 37°C for 24 h. Further, the culture was centrifuged at 6000 *g* for 20 min and the collected supernatant was used as crude amylase. One millilitre of crude amylase was added into 1 mL of amylum solution (1% w/v) and heated at 60°C for 10 min. One millilitre of dinitrosalicylic acid (DNS) reagent was added into the solution for stopping the reaction and then it was boiled at 100°C for 5 min. After cooling the solution, it was centrifuged at 6000 *g* for 15 min and absorbance was recorded at 540 nm using ultraviolet–visible (UV–Vis) spectrophotometer (Model – ELICO: SL 210). Amylase activity (U/mL) was calculated as the amount of enzyme releasing 1 μg of maltose per minute.

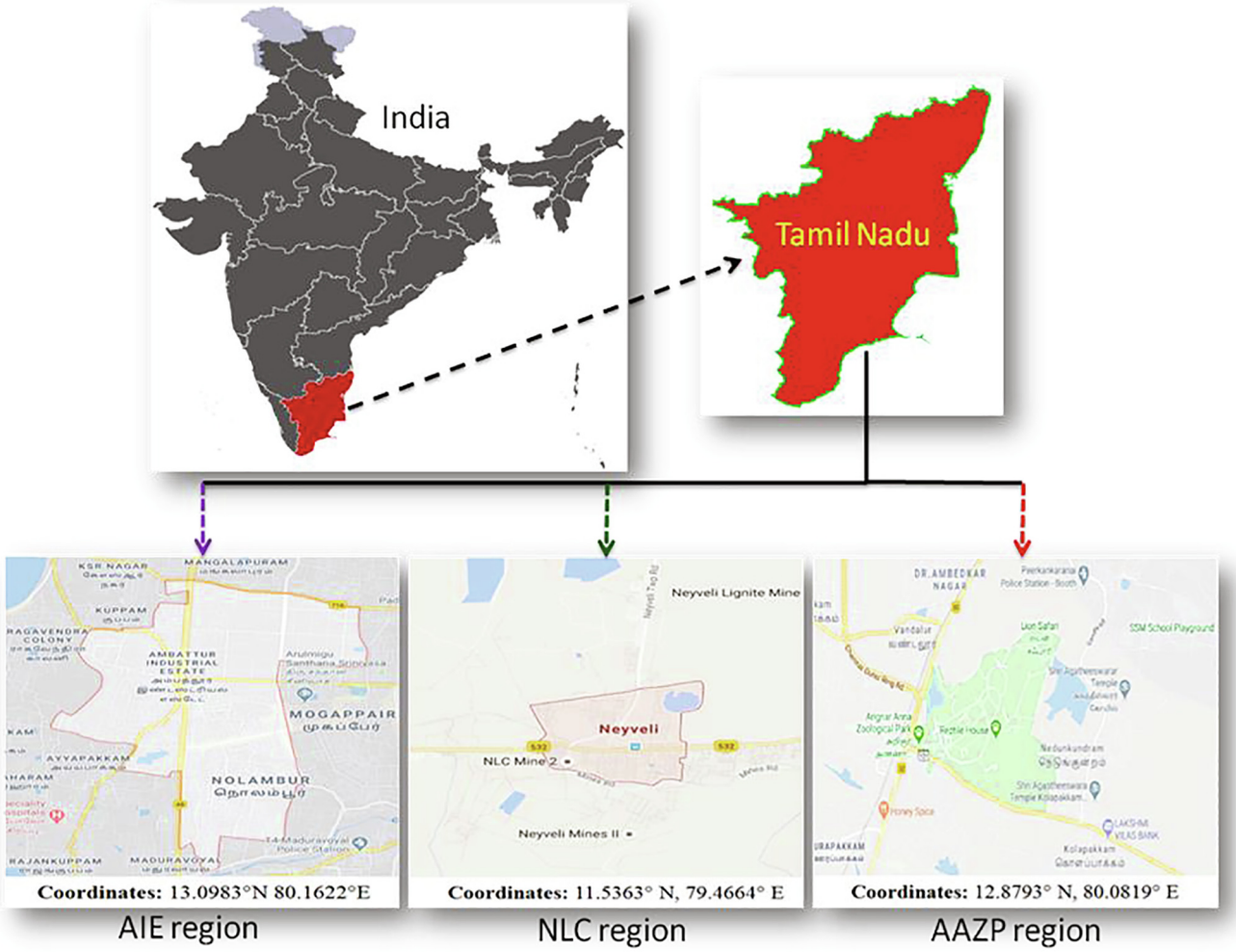


**Fig. 1.** Map, location, and coordinates of each sample collection site.

*2.4.2. Cellulase production*

*2.4.2.1. Plate assay (Qualitative analysis).* Isolates were inoculated in the fermentation medium (as discussed in the earlier section) containing 0.5% (w/v) carboxymethyl cellulose (CMC) and incubated at 37 °C. After 24 h, the culture was centrifuged at 6000 *g* for 20 min and supernatant was collected. Meanwhile, CMC agar medium (g/L: CMC – 5.0, agar – 20.0, and pH – 7.0) plates were prepared aseptically, crude cellulase was loaded into the agar wells created, and plates were incubated at 37 °C. After 24 h of incubation, agar plates were stained with 0.5% (w/v) congo red solution for 15–20 min, destained with 0.1 M sodium chloride solution for 5–10 min, and then observed for cellulase production (Azadian et al., 2017). Cellulase positive isolates were further selected for estimating cellulase activity.

*2.4.2.2. Cellulase activity (Quantitative analysis).* Cellulase activities of selected cellulose hydrolyzing isolates were estimated using 0.5% (w/v) CMC as the substrate (Ghosh, 1987). Isolates were inoculated in the broth medium (as discussed in the earlier section) containing 0.5% (w/v) CMC and incubated at 37$^{0}$C for 24 h. Further, the culture was centrifuged at 6000 *g* for 20 min and the collected supernatant was used as crude cellulase. One millilitre of crude cellulase was added into 1 mL of CMC solution. Further, 1 mL of DNS reagent was mixed into it for stopping the reaction and heated at 100$^{0}$C for 5 min. After cooling the solution, absorbance was recorded using UV–Vis spectrophotometer at 540 nm. Cellulase activity (U/mL) was calculated as the amount of enzyme that released 1 μg of reducing sugar per minute.

*2.4.3. Inulinase production*

*2.4.3.1. Plate assay (Qualitative analysis).* The extracellular inulinase or exoinulinase production from strain ALA4 was assessed as per the modified methodology of Li et al. (2011). Isolates were inoculated in the fermentation medium (as discussed in the earlier section) containing 1% (w/v) inulin and incubated at 37 °C. Further, the culture was centrifuged at 6000 *g* for 20 min and the collected supernatant was used as crude inulinase. Meanwhile, wells were created on inulin agar medium (% w/v: inulin – 1.0, agar – 1.8, and pH – 7.0) plates. The supernatant of each isolate was loaded into the agar wells and incubated at 37$^{0}$C. After 24 h, each agar plate was flooded with Lugol's iodine solution for observing inulinase production. Inulinase positive isolates were further selected for estimating inulinase activity.

*2.4.3.2. Inulinase activity (Quantitative analysis).* Inulinase activities of selected inulin hydrolyzing isolates were quantified as per the method of Miller (1959) with minor changes. Isolates were inoculated in the broth medium (as discussed in the previous section) containing inulin and incubated at 37$^{0}$C for 24 h. Further, the culture was centrifuged and the collected supernatant was used as crude inulinase. One millilitre of crude inulinase was added into 1 mL of inulin solution (1% w/v) and heated at 60$^{0}$C for 10 min.

One millilitre of DNS reagent was added into the solution for stopping the reaction and then it was boiled at 100$^0$C for 5 min. After cooling the solution, it was centrifuged at 6000 *g* for 15 min and absorbance was recorded at 540 nm using UV–Vis spectrophotometer. Fructose solution was used as standard. Inulinase activity (U/mL) was determined as the amount of enzyme released 1 μg of fructose per minute from inulin.

### 2.5. Estimation of protein

Protein content (mg/mL) of each enzyme sample was quantified by Bradford test (Bradford, 1976) using bovine serum albumin as standard. Specific activities of enzymes were calculated as unit per milligrams (U/mg).

### 2.6. Identification of potent isolates–

#### 2.6.1. Morphological and biochemical characterization

The morphological and biochemical characteristics of potent isolates exhibiting hyper amylase, cellulase, and inulinase productions were carried out using standard Bergey's Manual of Systemic Bacteriology (Sneath, 1994). Gram staining was performed for differentiating Gram positive (+) and Gram negative (-) isolates. The cellular morphology and colony characteristics of isolates were also examined on agar plates. Various biochemical properties and carbohydrates fermentation traits of isolates were tested using standard protocols.

#### 2.6.2. Molecular characterization

Genomic DNA of isolates was purified using NucleoSpin® DNA isolation kit. Polymerase chain reaction amplification was performed in thermal cycler (GeneAmp PCR System 9700) using specific forward (“CAGGCCTAACACATGCAAGTC”) and reverse (“GGGCGGWGTGTACAAGGC”) primers. The amplicon was run in 1.2% (w/v) agarose gels and observed using gel documentation system. The 16S rRNA sequence of each bacterial strain was submitted to GenBank, NCBI for obtaining accession numbers.

### 2.7. Sequence analyses using bioinformatics tools–

#### 2.7.1. Thermodynamic structure prediction

The minimum free energy (MFE), mountain plot, and entropy of sequences were predicted using RNAfold web server at 37$^0$C (Gruber et al., 2008).

#### 2.7.2. Determination of guanine cytosine (GC) content

The GC content of the sequences was calculated according to the algorithm of Rosano and Ceccarelli (2009). The GC content (%) was calculated as:

$$\%\,\text{GC content} = \frac{\text{Total number of G and C}}{\text{Total number of A, T, C, and G}} \times 100$$

#### 2.7.3. Open reading frame (ORF) determination

The ORF of sequences of strains was determined using ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). The 16S rRNA sequences of strains in the FASTA format were pasted in the box provided in the tool and ORF was determined.

#### 2.7.4. Bootstrap tree construction

The evolutionary relationship of strains was inferred by comparison with the 16S rRNA sequences of close isolates. The variable and incomplete sites at both the 5′ and 3′ ends of the 16S rRNA gene sequences were excluded from the alignment. Regions that could not be aligned unambiguously were excluded from the analysis. Bootstrap tree was inferred using Neighbor Joining (NJ) algorithm with the Kimura two-parameter model in Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Tamura et al., 2007).

### 2.8. Statistical analysis

Experimental sections of the study were carried out in triplicate and data were expressed as mean ± standard variation (SD).

## 3. Results

### 3.1. Bacteria isolation

Spread plate method showed the isolation of disparate bacterial colonies of varied shapes and sizes from these geographical regions (Figure not shown). A total of 72 bacterial cultures (24 bacterial isolates from each sample collection site) were isolated and purified for further experimental analyses.

### 3.2. Extracellular enzymes production

Fig. 2a shows some of the amylase assay plates of purified bacterial cultures isolated from AIE, NLC, and AAZP regions. Of 24 isolates purified from AIE region, isolate ALA1, ALA3, ALA4, and ALA5 exhibited maximum amylase activities of 510.37 ± 20.6, 654.43 ± 18.3, 836.46 ± 24.6, and 478.66 ± 20.6 U/mL, respectively. Other amylase positive isolates revealed comparatively lower amylase activities. Isolate ALA21 showed minimum amylase activity (190.76 ± 16.3 U/mL). Maximum and minimum amylase activities of 296.47 ± 22.3 and 120.22 ± 12.6 U/mL were estimated from isolate AMA9 and AMA23, respectively of NLC region. On the other hand, isolate APA13 of AAZP region was observed as the highest producer of amylase (420.56 ± 21.6 U/mL) while isolate APA20 showed the lowest amylase activity of 168.44 ± 14.6 U/mL (Table 1A).

Cellulase assay plates of few purified bacterial cultures isolated from AIE, NLC, and AAZP regions are shown in Fig. 2b. Among 24 isolates of AIE region, isolate ALA1, ALA3, ALA4, and ALA5 showed maximum cellulase activities of 914.34 ± 21.6, 854.33 ± 20.6, 1123.42 ± 23.3, and 810.56 ± 21.6 U/mL, respectively. Other cellulase positive isolates revealed comparatively lower cellulase activities. Minimum cellulase activity of 172.75 ± 15.6 U/mL was estimated from isolate ALA21. Of 24 isolates purified from NLC region, maximum and minimum cellulase activities of 420.25 ± 18.6 and 201.34 ± 14.3 U/mL were estimated from isolate AMA23 and AMA4, respectively. On the other hand, maximum and minimum cellulase activities of 518.64 ± 21.6 and 192.38 ± 14.6 U/mL were reported from isolate APA2 and APA15, respectively of AAZP region (Table 1B).

Fig. 2c shows inulinase assay plates for some of the purified bacterial cultures isolated from AIE, NLC, and AAZP regions. In AIE region, isolate ALA1, ALA3, ALA4, and ALA5 demonstrated prominent production of inulinase with enzyme activities of 820.47 ± 24.3, 786.33 ± 20.6, 985.32 ± 32.3, and 718.54 ± 22.6 U/mL, respectively. Other isolates exhibited comparatively lower inulinase activities. In contrary, maximum and minimum inulinase activities of 286.57 ± 20.3 U/mL (isolate AMA9) and 200.42 ± 15.3 U/mL (isolate AMA17), respectively were observed among NLC region isolates. Likewise, among bacterial cultures autochthonous to AAZP region, isolate APA2 and APA23 depicted maximum and minimum inulinase activities of 720.44 ± 21.3 and 201.34 ± 21.3 U/mL, respectively (Table 1C).

Of 24 bacteria purified from AIE region, 54.16% of isolates were observed as amylase producers. However, only 29.16% of bacteria isolated from NLC region were reported as amylase producers. The AAZP region was reported a niche for 41.66% of isolates as

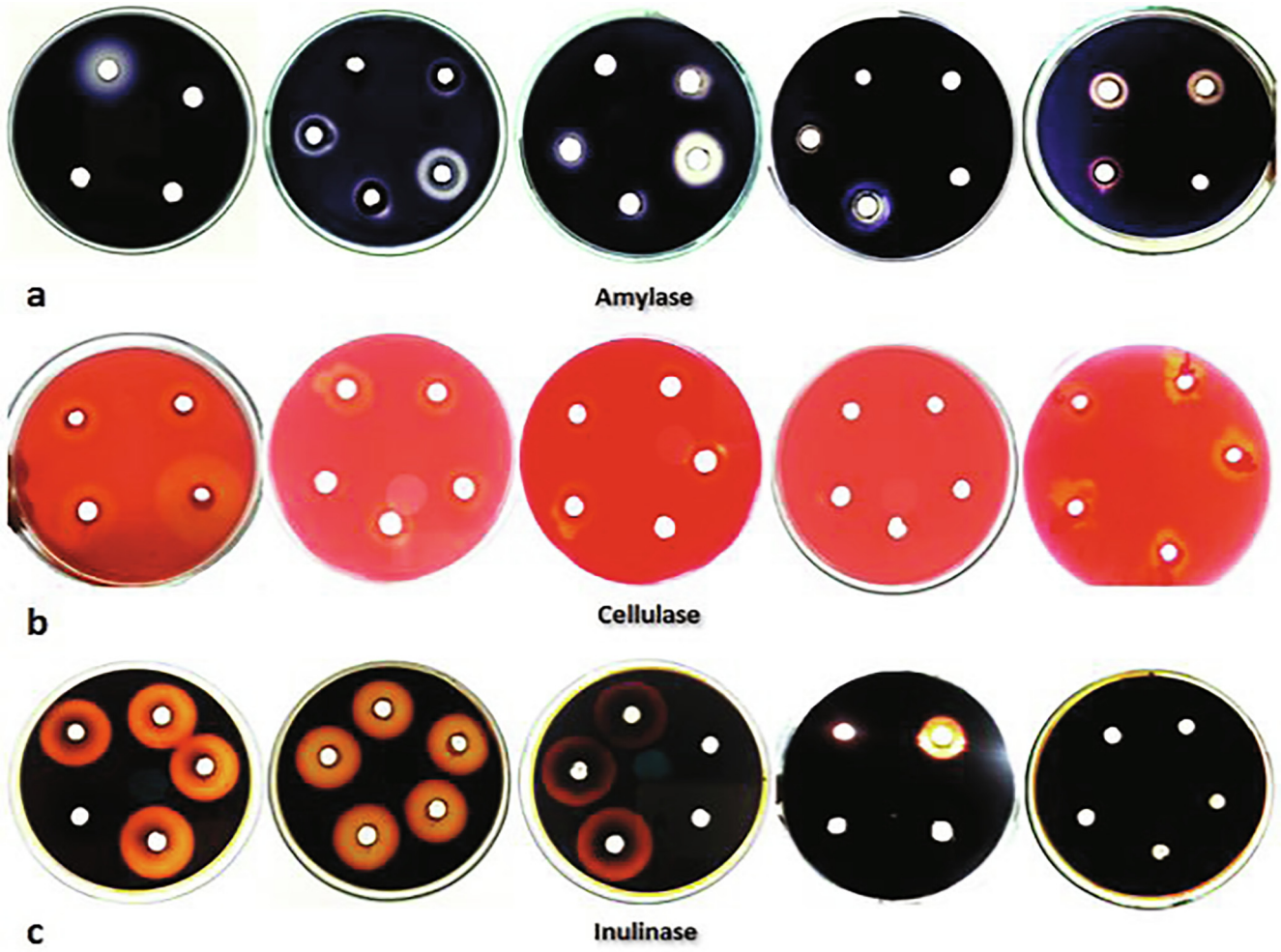


**Fig. 2.** **(a)** Amylase, **(b)** cellulase, and **(c)** inulinase assay plates of some of the purified bacterial cultures isolated from AIE, NLC, and AAZP regions. Zone of hydrolysis around the wells indicates hydrolytic enzymes production while lack of zone of hydrolysis around the wells represents lack of enzymes production.

amylase producers (Fig. 3a). Among 24 isolates of AIE region, 41.66% of them produced cellulase. Of 24 isolates purified from NLC region, 29.16% of them were reported as cellulase producers. On the other hand, 45.83% of bacterial cultures isolated from AAZP region were observed as cellulase producers (Fig. 3b). In AIE region, 45.83% of isolates were observed as inulinase producers. In contrary, only 20.83% of total bacteria isolated from NLC region revealed inulinase production. Among bacteria isolated from AAZP region, 41.66% of them showed inulinase production (Fig. 3c). Overall, among 72 bacterial cultures isolated from AIE, NLC, and AAZP regions, 41.66% of them were observed amylase producers, 38.88% of them were reported to produce cellulase, and 36.11% of isolates showed inulinase production (Fig. 3d).

On the other hand, 29.16% of bacteria isolated from AIE region showed concurrent production of amylase, cellulase, and inulinase. Likewise, 8.33 and 25.0% of bacteria isolated from NLC and AAZP regions, respectively revealed concurrent production of all three hydrolytic enzymes. Overall, 20.83% of total bacteria isolated from all three regions exhibited concurrent production of amylase, cellulase, and inulinase (Fig. 4).

### 3.3. Identification of active isolates

Since, isolate ALA1, ALA3, ALA4, and ALA5 were reported as prominent producers of extracellular amylase, cellulase, and inulinase, hence these four isolates were selected further for identification based on morphological, biochemical, and molecular characterization techniques. The morphological and biochemical tests results are summarized in Table 2. All the isolates were Gram positive in nature. Colonies of each isolate were large, rough, and round in shape. The colonies of all isolates were cream white in colour except isolate ALA4 whose colonies colour were observed pale white. Isolate ALA1, ALA3, and ALA5 showed positive results towards indole, voges-proskauer, nitrate reductase, arginine, catalase, and fermentation characteristics of carbohydrates (glucose, sucrose, trehalose, dextrose, and lactose) and negative results towards citrate utilization, ONPG, malonate, methyl red, and fermentation of few carbohydrates viz. arabinose and mannitol. On the other hand, isolate ALA4 showed negative results towards all the tests investigated except nitrate reductase, catalase, and fermentation ability of certain carbohydrates (glucose, sucrose, arabinose, dextrose, and lactose).

The amplicon of these four isolates under gel documentation system was observed as 1274 (isolate ALA1), 1273 (isolate ALA3), 1238 (isolate ALA4), and 1274 bp (isolate ALA5) (Figure not shown). Further, 16S rRNA sequencing and BLAST, NCBI similarity search results identified the isolates as *Bacillus anthracis* strain ALA1 (similarity 100%, Accession number – KY795953), *Bacillus cereus* strain ALA3 (similarity 100%, Accession number – KY795954), *Glutamicibacter arilaitensis* strain ALA4 (similarity 100%, Accession number – KY795955), and *Bacillus thuringiensis* strain ALA5 (similarity 100%, Accession number – KY795956).

**Table 1A**
Extracellular amylase production ability of bacteria isolated from soil samples of different geographical regions.

| *Ambattur Industrial Estate (AIE)* | | | | | |
|---|---|---|---|---|---|
| S. No. | Isolates | Zone of hydrolysis (mm) | Amylase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
| 1. | ALA1 | 20.3 ± 0.6 | 510.37 ± 20.6 | 3.2 ± 0.03 | 159.49 |
| 2. | ALA2 | – | – | 2.8 ± 0.03 | – |
| 3. | ALA3 | 22.6 ± 0.3 | 654.43 ± 18.3 | 3.4 ± 0.06 | 192.47 |
| 4. | ALA4 | 26.6 ± 0.3 | 836.46 ± 24.6 | 4.6 ± 0.03 | 181.83 |
| 5. | ALA5 | 18.6 ± 0.6 | 478.66 ± 20.6 | 3.2 ± 0.06 | 149.58 |
| 6. | ALA6 | – | – | 2.4 ± 0.06 | – |
| 7. | ALA7 | – | – | 3.2 ± 0.03 | – |
| 8. | ALA8 | – | – | 2.6 ± 0.06 | – |
| 9. | ALA9 | 11.3 ± 0.3 | 200.45 ± 16.6 | 2.8 ± 0.06 | 71.58 |
| 10. | ALA10 | – | – | 2.2 ± 0.03 | – |
| 11. | ALA11 | 14.6 ± 0.6 | 276.63 ± 18.3 | 3.7 ± 0.06 | 74.76 |
| 12. | ALA12 | 15.3 ± 0.6 | 310.54 ± 18.6 | 3.4 ± 0.06 | 91.33 |
| 13. | ALA13 | 12.3 ± 0.3 | 220.67 ± 16.3 | 3.2 ± 0.03 | 68.95 |
| 14. | ALA14 | – | – | 2.1 ± 0.06 | – |
| 15. | ALA15 | – | – | 1.8 ± 0.03 | – |
| 16. | ALA16 | – | – | 1.6 ± 0.03 | – |
| 17. | ALA17 | – | – | 2.3 ± 0.06 | – |
| 18. | ALA18 | 16.6 ± 0.6 | 375.53 ± 21.3 | 4.1 ± 0.06 | 91.59 |
| 19. | ALA19 | – | – | 2.4 ± 0.03 | – |
| 20. | ALA20 | 14.3 ± 0.6 | 271.63 ± 16.3 | 3.1 ± 0.06 | 87.62 |
| 21. | ALA21 | 10.6 ± 0.3 | 190.76 ± 16.3 | 2.8 ± 0.03 | 68.12 |
| 22. | ALA22 | 17.3 ± 0.6 | 396.43 ± 14.6 | 3.6 ± 0.03 | 110.11 |
| 23. | ALA23 | 13.6 ± 0.6 | 248.54 ± 18.6 | 3.3 ± 0.03 | 75.31 |
| 24. | ALA24 | – | – | 1.4 ± 0.06 | – |
| ***Neyveli Lignite Corporation (NLC)*** | | | | | |
| 1. | AMA1 | 12.3 ± 0.6 | 224.35 ± 20.6 | 2.3 ± 0.03 | 97.54 |
| 2. | AMA2 | – | – | 1.6 ± 0.06 | – |
| 3. | AMA3 | – | – | 1.7 ± 0.06 | – |
| 4. | AMA4 | 12.6 ± 0.6 | 240.32 ± 18.6 | 2.5 ± 0.06 | 96.12 |
| 5. | AMA5 | – | – | 1.8 ± 0.03 | – |
| 6. | AMA6 | – | – | 3.2 ± 0.03 | – |
| 7. | AMA7 | – | – | 3.1 ± 0.03 | – |
| 8. | AMA8 | – | – | 2.2 ± 0.06 | – |
| 9. | AMA9 | 14.6 ± 0.3 | 296.47 ± 22.3 | 3.1 ± 0.03 | 95.63 |
| 10. | AMA10 | 13.6 ± 0.6 | 250.45 ± 16.6 | 2.8 ± 0.06 | 89.44 |
| 11. | AMA11 | – | – | 2.8 ± 0.06 | – |
| 12. | AMA12 | – | – | 3.2 ± 0.03 | – |
| 13. | AMA13 | – | – | 2.8 ± 0.03 | – |
| 14. | AMA14 | – | – | 2.4 ± 0.06 | – |
| 15. | AMA15 | – | – | 1.8 ± 0.03 | – |
| 16. | AMA16 | – | – | 1.4 ± 0.06 | – |
| 17. | AMA17 | 10.3 ± 0.3 | 210.32 ± 14.3 | 2.1 ± 0.03 | 100.15 |
| 18. | AMA18 | – | – | 1.6 ± 0.03 | – |
| 19. | AMA19 | 11.6 ± 0.6 | 201.22 ± 14.6 | 1.8 ± 0.03 | 111.78 |
| 20. | AMA20 | – | – | 1.8 ± 0.03 | – |
| 21. | AMA21 | – | – | 1.9 ± 0.06 | – |
| 22. | AMA22 | – | – | 1.5 ± 0.03 | – |
| 23. | AMA23 | 8.3 ± 0.3 | 120.22 ± 12.6 | 1.7 ± 0.06 | 70.71 |
| 24. | AMA24 | – | – | 1.9 ± 0.03 | – |
| ***Arignar Anna* Zoological *Park (AAZP)*** | | | | | |
| 1. | APA1 | – | – | 2.1 ± 0.06 | – |
| 2. | APA2 | 16.3 ± 0.3 | 368.54 ± 20.3 | 3.2 ± 0.06 | 115.16 |
| 3. | APA3 | 12.3 ± 0.6 | 235.63 ± 16.3 | 2.8 ± 0.06 | 84.15 |
| 4. | APA4 | – | – | 2.4 ± 0.03 | – |
| 5. | APA5 | – | – | 2.3 ± 0.06 | – |
| 6. | APA6 | – | – | 1.4 ± 0.03 | – |
| 7. | APA7 | 11.6 ± 0.6 | 200.32 ± 18.6 | 2.4 ± 0.06 | 83.46 |
| 8. | APA8 | – | – | 1.8 ± 0.03 | – |
| 9. | APA9 | 14.6 ± 0.3 | 292.37 ± 20.3 | 2.6 ± 0.03 | 112.45 |
| 10. | APA10 | – | – | 1.9 ± 0.03 | – |
| 11. | APA11 | – | – | 1.4 ± 0.06 | – |
| 12. | APA12 | 14.3 ± 0.3 | 284.45 ± 18.6 | 3.1 ± 0.06 | 91.75 |
| 13. | APA13 | 17.3 ± 0.6 | 420.56 ± 21.6 | 3.5 ± 0.03 | 120.16 |
| 14. | APA14 | – | – | 1.9 ± 0.03 | – |
| 15. | APA15 | – | – | 1.7 ± 0.06 | – |
| 16. | APA16 | – | – | 2.7 ± 0.06 | – |
| 17. | APA17 | – | – | 2.5 ± 0.03 | – |
| 18. | APA18 | 10.3 ± 0.3 | 208.28 ± 16.3 | 2.4 ± 0.03 | 86.78 |
| 19. | APA19 | – | – | 2.4 ± 0.03 | – |
| 20. | APA20 | 9.3 ± 0.3 | 168.44 ± 14.6 | 2.1 ± 0.03 | 80.2 |
| 21. | APA21 | – | – | 2.3 ± 0.06 | – |
| 22. | APA22 | – | – | 1.2 ± 0.03 | – |

**Table 1A** (*continued*)

| *Ambattur Industrial Estate (AIE)* | | | | | |
|---|---|---|---|---|---|
| S. No. | Isolates | Zone of hydrolysis (mm) | Amylase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
| 23. | APA23 | 11.6 ± 0.3 | 204.44 ± 20.3 | 2.5 ± 0.03 | 81.77 |
| 24. | APA24 | 13.6 ± 0.6 | 254.66 ± 18.6 | 2.3 ± 0.06 | 110.72 |

Note: Data represent mean ± SD; '-' = No activity

**Table 1B**
Extracellular cellulase production ability of bacteria isolated from soil samples of different geographical regions.

| *Ambattur Industrial Estate (AIA)* | | | | | |
|---|---|---|---|---|---|
| S. No. | Isolates | Zone of hydrolysis (mm) | Cellulase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
| 1. | ALA1 | 26.6 ± 0.6 | 914.34 ± 21.6 | 4.1 ± 0.06 | 223.0 |
| 2. | ALA2 | – | – | 3.8 ± 0.03 | – |
| 3. | ALA3 | 24.6 ± 0.6 | 854.33 ± 20.6 | 3.5 ± 0.06 | 244.09 |
| 4. | ALA4 | 30.3 ± 0.3 | 1123.42 ± 23.3 | 4.8 ± 0.06 | 234.04 |
| 5. | ALA5 | 23.6 ± 0.3 | 810.56 ± 21.6 | 3.8 ± 0.06 | 213.3 |
| 6. | ALA6 | – | – | 2.8 ± 0.03 | – |
| 7. | ALA7 | – | – | 3.6 ± 0.03 | – |
| 8. | ALA8 | 12.3 ± 0.6 | 210.35 ± 18.6 | 3.4 ± 0.06 | 61.86 |
| 9. | ALA9 | – | – | 2.6 ± 0.03 | – |
| 10. | ALA10 | – | – | 2.1 ± 0.03 | – |
| 11. | ALA11 | – | – | 2.6 ± 0.06 | – |
| 12. | ALA12 | – | – | 2.4 ± 0.06 | – |
| 13. | ALA13 | 10.6 ± 0.6 | 184.57 ± 14.3 | 3.4 ± 0.06 | 54.28 |
| 14. | ALA14 | | | 2.2 ± 0.06 | – |
| 15. | ALA15 | – | – | 2.1 ± 0.06 | – |
| 16. | ALA16 | | | 1.8 ± 0.03 | – |
| 17. | ALA17 | – | – | 1.6 ± 0.06 | – |
| 18. | ALA18 | 18.3 ± 0.6 | 415.63 ± 20.6 | 3.7 ± 0.03 | 112.33 |
| 19. | ALA19 | – | – | 2.1 ± 0.03 | – |
| 20. | ALA20 | 14.6 ± 0.6 | 276.53 ± 14.6 | 2.8 ± 0.03 | 98.76 |
| 21. | ALA21 | 10.3 ± 0.3 | 172.75 ± 15.6 | 2.6 ± 0.03 | 66.44 |
| 22. | ALA22 | – | – | 3.2 ± 0.06 | – |
| 23. | ALA23 | 10.3 ± 0.3 | 180.64 ± 16.3 | 2.3 ± 0.03 | 78.53 |
| 24. | ALA24 | – | – | 1.8 ± 0.06 | – |
| ***Neyveli Lignite Corporation (NLC)*** | | | | | |
| 1. | AMA1 | 10.6 ± 0.6 | 210.45 ± 18.6 | 1.8 ± 0.03 | 116.91 |
| 2. | AMA2 | – | – | 1.4 ± 0.06 | – |
| 3. | AMA3 | 12.3 ± 0.3 | 234.52 ± 16.3 | 2.6 ± 0.03 | 90.2 |
| 4. | AMA4 | 10.3 ± 0.6 | 201.34 ± 14.3 | 2.8 ± 0.06 | 71.9 |
| 5. | AMA5 | – | – | 1.6 ± 0.03 | – |
| 6. | AMA6 | – | – | 1.8 ± 0.06 | – |
| 7. | AMA7 | – | – | 2.1 ± 0.03 | – |
| 8. | AMA8 | – | – | 1.7 ± 0.06 | – |
| 9. | AMA9 | 16.6 ± 0.6 | 398.67 ± 20.6 | 3.2 ± 0.03 | 124.58 |
| 10. | AMA10 | – | – | 2.6 ± 0.03 | – |
| 11. | AMA11 | – | – | 2.1 ± 0.06 | – |
| 12. | AMA12 | – | – | 2.2 ± 0.03 | – |
| 13. | AMA13 | 15.3 ± 0.3 | 336.58 ± 20.3 | 2.7 ± 0.06 | 124.65 |
| 14. | AMA14 | – | – | 1.8 ± 0.06 | – |
| 15. | AMA15 | – | – | 1.7 ± 0.03 | – |
| 16. | AMA16 | – | – | 1.5 ± 0.06 | – |
| 17. | AMA17 | – | – | 1.1 ± 0.06 | – |
| 18. | AMA18 | – | – | 1.5 ± 0.03 | – |
| 19. | AMA19 | – | – | 2.1 ± 0.03 | – |
| 20. | AMA20 | – | – | 1.6 ± 0.06 | – |
| 21. | AMA21 | – | – | 1.7 ± 0.06 | – |
| 22. | AMA22 | – | – | 2.2 ± 0.03 | – |
| 23. | AMA23 | 18.6 ± 0.6 | 420.25 ± 18.6 | 2.7 ± 0.06 | 155.64 |
| 24. | AMA24 | 14.6 ± 0.3 | 310.28 ± 16.3 | 2.5 ± 0.06 | 124.11 |
| ***Arignar Anna* Zoological *Park (AAZP)*** | | | | | |
| 1. | APA1 | – | – | 1.5 ± 0.06 | – |
| 2. | APA2 | 20.6 ± 0.3 | 518.64 ± 21.6 | 3.4 ± 0.03 | 152.54 |
| 3. | APA3 | 14.6 ± 0.6 | 285.56 ± 14.3 | 2.5 ± 0.06 | 114.22 |
| 4. | APA4 | – | – | 2.1 ± 0.03 | – |
| 5. | APA5 | 15.6 ± 0.6 | 347.62 ± 18.6 | 1.8 ± 0.06 | 193.12 |
| 6. | APA6 | – | – | 1.5 ± 0.03 | – |
| 7. | APA7 | – | – | 2.5 ± 0.03 | – |
| 8. | APA8 | – | – | 1.7 ± 0.03 | – |
| 9. | APA9 | 14.3 ± 0.6 | 294.26 ± 15.6 | 2.8 ± 0.03 | 105.09 |
| 10. | APA10 | 12.3 ± 0.3 | 212.45 ± 14.3 | 1.8 ± 0.06 | 118.02 |

(*continued on next page*)

**Table 1B** (*continued*)

| *Ambattur Industrial Estate (AIA)* | | | | | |
|---|---|---|---|---|---|
| S. No. | Isolates | Zone of hydrolysis (mm) | Cellulase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
| 11. | APA11 | – | – | 1.5 ± 0.06 | – |
| 12. | APA12 | – | – | 2.1 ± 0.06 | – |
| 13. | APA13 | – | – | 1.5 ± 0.03 | – |
| 14. | APA14 | – | – | 1.8 ± 0.03 | – |
| 15. | APA15 | 10.6 ± 0.3 | 192.38 ± 14.6 | 2.7 ± 0.03 | 71.25 |
| 16. | APA16 | 12.3 ± 0.6 | 208.18 ± 14.6 | 2.6 ± 0.06 | 80.06 |
| 17. | APA17 | – | – | 2.4 ± 0.03 | – |
| 18. | APA18 | 16.6 ± 0.3 | 412.38 ± 18.3 | 1.9 ± 0.03 | 217.04 |
| 19. | APA19 | – | – | 1.9 ± 0.06 | – |
| 20. | APA20 | 18.6 ± 0.6 | 478.34 ± 18.3 | 2.3 ± 0.03 | 207.97 |
| 21. | APA21 | – | – | 2.4 ± 0.03 | – |
| 22. | APA22 | 14.3 ± 0.6 | 274.76 ± 16.3 | 1.9 ± 0.06 | 144.61 |
| 23. | APA23 | 12.6 ± 0.3 | 211.34 ± 18.6 | 2.9 ± 0.03 | 72.87 |
| 24. | APA24 | – | – | 1.8 ± 0.06 | – |

Note: Data represent mean ± SD; '-' = No activity

**Table 1C**
Extracellular inulinase production ability of bacteria isolated from soil samples of different geographical regions.

| ***Ambattur Industrial Estate (AIE)*** | | | | | |
|---|---|---|---|---|---|
| S. No. | Isolates | Zone of hydrolysis (mm) | Inulinase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
| 1. | ALA1 | 26.6 ± 0.3 | 820.47 ± 24.3 | 3.8 ± 0.03 | 215.91 |
| 2. | ALA2 | – | – | 2.4 ± 0.06 | – |
| 3. | ALA3 | 25.6 ± 0.6 | 786.33 ± 20.6 | 3.6 ± 0.06 | 218.42 |
| 4. | ALA4 | 28.3 ± 0.6 | 985.32 ± 32.3 | 4.8 ± 0.06 | 205.27 |
| 5. | ALA5 | 24.3 ± 0.6 | 718.54 ± 22.6 | 3.7 ± 0.06 | 194.2 |
| 6. | ALA6 | – | – | 1.8 ± 0.06 | – |
| 7. | ALA7 | 18.6 ± 0.3 | 510.55 ± 18.6 | 2.8 ± 0.03 | 182.33 |
| 8. | ALA8 | – | – | 2.1 ± 0.03 | – |
| 9. | ALA9 | 18.6 ± 0.3 | 514.35 ± 17.3 | 3.7 ± 0.06 | 139.01 |
| 10. | ALA10 | – | – | 2.1 ± 0.03 | – |
| 11. | ALA11 | 16.6 ± 0.3 | 430.53 ± 16.6 | 3.3 ± 0.06 | 130.46 |
| 12. | ALA12 | – | – | 2.4 ± 0.06 | – |
| 13. | ALA13 | – | – | 2.2 ± 0.03 | – |
| 14. | ALA14 | – | – | 2.4 ± 0.03 | – |
| 15. | ALA15 | – | – | 2.8 ± 0.03 | – |
| 16. | ALA16 | – | – | 1.8 ± 0.03 | – |
| 17. | ALA17 | – | – | 2.6 ± 0.03 | – |
| 18. | ALA18 | – | – | 3.1 ± 0.06 | – |
| 19. | ALA19 | – | – | 1.4 ± 0.03 | – |
| 20. | ALA20 | 15.6 ± 0.6 | 375.53 ± 18.3 | 3.4 ± 0.06 | 110.45 |
| 21. | ALA21 | 12.3 ± 0.6 | 230.56 ± 17.3 | 2.8 ± 0.06 | 82.34 |
| 22. | ALA22 | 16.6 ± 0.3 | 395.33 ± 18.6 | 3.3 ± 0.03 | 119.79 |
| 23. | ALA23 | 18.3 ± 0.6 | 465.44 ± 20.3 | 2.3 ± 0.03 | 202.36 |
| 24. | ALA24 | – | – | 1.4 ± 0.06 | – |
| ***Neyveli Lignite Corporation (NLC)*** | | | | | |
| 1. | AMA1 | – | – | 2.3 ± 0.03 | – |
| 2. | AMA2 | – | – | 1.8 ± 0.03 | – |
| 3. | AMA3 | – | – | 1.7 ± 0.06 | – |
| 4. | AMA4 | 11.6 ± 0.3 | 210.42 ± 14.6 | 2.7 ± 0.06 | 77.93 |
| 5. | AMA5 | – | – | 1.7 ± 0.06 | – |
| 6. | AMA6 | – | – | 3.1 ± 0.03 | – |
| 7. | AMA7 | – | – | 3.2 ± 0.03 | – |
| 8. | AMA8 | – | – | 1.9 ± 0.06 | – |
| 9. | AMA9 | 13.6 ± 0.3 | 286.57 ± 20.3 | 2.9 ± 0.03 | 98.81 |
| 10. | AMA10 | – | – | 2.7 ± 0.06 | – |
| 11. | AMA11 | – | – | 2.9 ± 0.03 | – |
| 12. | AMA12 | – | – | 1.7 ± 0.03 | – |
| 13. | AMA13 | 12.3 ± 0.6 | 240.35 ± 14.6 | 2.6 ± 0.03 | 92.44 |
| 14. | AMA14 | – | – | 2.1 ± 0.06 | – |
| 15. | AMA15 | – | – | 1.9 ± 0.06 | – |
| 16. | AMA16 | – | – | 1.7 ± 0.06 | – |
| 17. | AMA17 | 10.3 ± 0.3 | 200.42 ± 15.3 | 2.2 ± 0.03 | 91.1 |
| 18. | AMA18 | – | – | 1.7 ± 0.06 | – |
| 19. | AMA19 | 12.6 ± 0.3 | 245.32 ± 16.6 | 1.9 ± 0.06 | 129.11 |
| 20. | AMA20 | – | – | 1.3 ± 0.03 | – |
| 21. | AMA21 | – | – | 1.4 ± 0.06 | – |
| 22. | AMA22 | – | – | 1.5 ± 0.03 | – |
| 23. | AMA23 | – | – | 1.6 ± 0.06 | – |

**Table 1C** (*continued*)

| S. No. | Isolates | Zone of hydrolysis (mm) | Inulinase activity (U/mL) | Total protein content (mg/mL) | Specific activity (U/mg) |
|---|---|---|---|---|---|
| ***Ambattur Industrial Estate (AIE)*** | | | | | |
| 24. | AMA24 | – | – | 1.9 ± 0.06 | – |
| ***Arignar Anna* Zoological *Park (AAZP)*** | | | | | |
| 1. | APA1 | – | – | 1.9 ± 0.06 | – |
| 2. | APA2 | 24.3 ± 0.6 | 720.44 ± 21.3 | 3.3 ± 0.03 | 218.31 |
| 3. | APA3 | 22.6 ± 0.6 | 615.73 ± 20.6 | 2.7 ± 0.06 | 228.04 |
| 4. | APA4 | – | – | 2.9 ± 0.03 | – |
| 5. | APA5 | – | – | 2.4 ± 0.06 | – |
| 6. | APA6 | – | – | 1.9 ± 0.06 | – |
| 7. | APA7 | 18.3 ± 0.6 | 410.42 ± 16.3 | 2.5 ± 0.06 | 164.16 |
| 8. | APA8 | – | – | 1.5 ± 0.03 | – |
| 9. | APA9 | 20.3 ± 0.3 | 512.47 ± 21.6 | 2.5 ± 0.06 | 204.98 |
| 10. | APA10 | – | – | 1.5 ± 0.03 | – |
| 11. | APA11 | – | – | 1.9 ± 0.06 | – |
| 12. | APA12 | 16.3 ± 0.6 | 324.35 ± 20.3 | 3.3 ± 0.06 | 98.28 |
| 13. | APA13 | 17.6 ± 0.6 | 380.66 ± 18.3 | 3.4 ± 0.06 | 111.95 |
| 14. | APA14 | – | – | 1.8 ± 0.03 | – |
| 15. | APA15 | – | – | 1.9 ± 0.03 | – |
| 16. | APA16 | – | – | 2.1 ± 0.06 | – |
| 17. | APA17 | – | – | 2.1 ± 0.03 | – |
| 18. | APA18 | 14.6 ± 0.6 | 288.68 ± 18.6 | 2.8 ± 0.03 | 103.1 |
| 19. | APA19 | – | – | 1.8 ± 0.06 | – |
| 20. | APA20 | 18.6 ± 0.3 | 428.34 ± 18.3 | 2.5 ± 0.03 | 171.33 |
| 21. | APA21 | – | – | 2.2 ± 0.06 | – |
| 22. | APA22 | – | – | 1.8 ± 0.03 | – |
| 23. | APA23 | 13.3 ± 0.6 | 201.34 ± 21.3 | 2.6 ± 0.06 | 77.43 |
| 24. | APA24 | 14.6 ± 0.3 | 290.43 ± 17.6 | 2.2 ± 0.06 | 132.01 |

Note: Data represent mean ± SD; '-' = No activity.

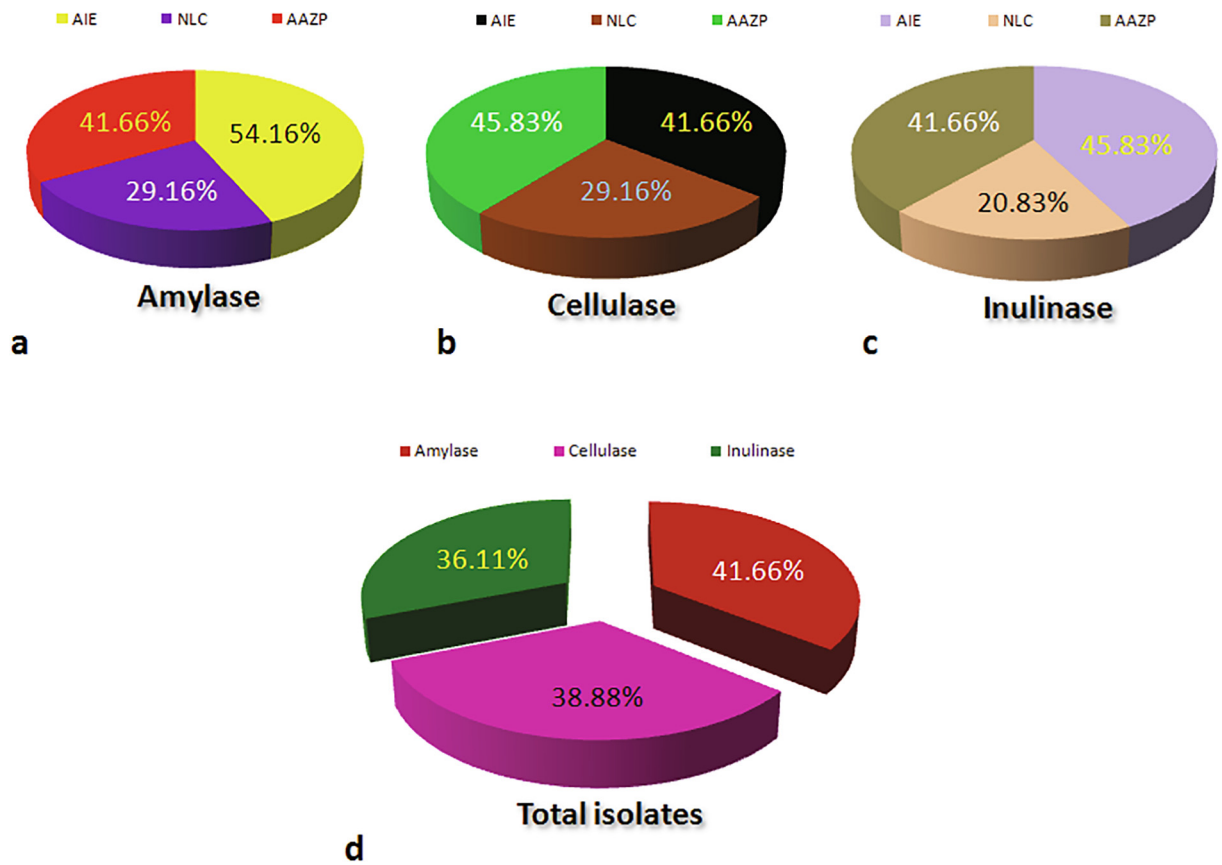


**Fig. 3.** Isolates of AIE, NLC, and AAZP regions as **(a)** amylase, **(b)** cellulase, and **(c)** inulinase producers. Total isolates of AIE, NLC, and AAZP regions producing amylase, cellulase, and inulinase **(d)**.

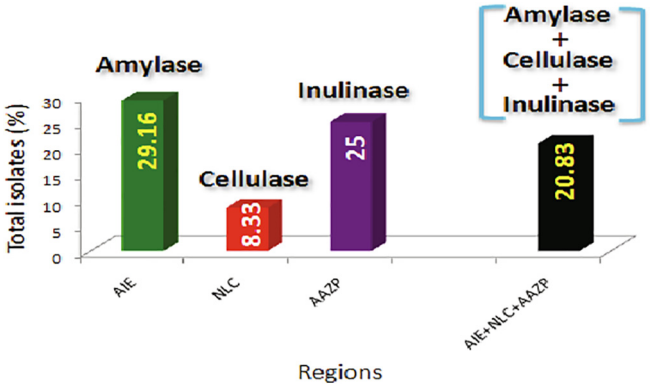


**Fig. 4.** Concurrent production of amylase, cellulase, and inulinase from isolates of each region, followed by concurrent production of all three hydrolytic enzymes from isolates of total regions.

### 3.4. Sequence analyses using bioinformatics tools

The 16S rRNA sequences of strains were used to predict the secondary structure of ribonucleic acid (RNA) using RNAfold web server. The thermodynamic parameter i.e. MFE for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 was calculated as −480.73, −478.76, −496.63, and −479.03 kcal/mol, respectively (Fig. 5a). Mountain plot and entropy depicted the hierarchical representation of RNA secondary structure (Fig. 5b). Further, the GC content of sequence for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 was calculated as 53.06, 52.94, 56.78, and 53.06%, respectively (Fig. 6). Fig. 7 shows 9 ORFs for strain ALA1, strain ALA3, and strain ALA5 while 10 ORFs were observed for strain ALA4. The evolutionary history of strains was inferred using NJ method which showed close resemblance with other bacilli and *G. arilaitensis* strains. There were a total of 1517 positions in the final dataset of bootstrap tree (Fig. 8).

## 4. Discussion

Plethora of studies has been investigated in the past to discover novel microflora present in different types of soils of distinct geographical regions worldwide. However, bacteria are predominantly present in the living soil as natural microflora that contributes in the maintenance of varied ecological processes (Jacoby et al., 2017). Those bacteria reside most commonly between the pores of soil and depict effectual roles in the sustainability of ecosystem through organic or natural ways. Soil bacteria convert certain toxic components into the non-toxic forms which are later utilized by plants for diverse metabolic purposes (Lladó et al., 2017, Arasu et al., 2013, Arasu et al., 2017, Arasu et al., 2019). On the other hand, bacteria maintain the nature of the soil by modifying the physio-chemical and biological properties of soil (Lladó et al., 2017). In addition to the effectual bio-geochemical roles, soil bacteria are being exploited in diversified industries (food, chemical, biofuel etc.) for their irrefutable applications in the production of disparate enzymes (Sankari and Khusro, 2014; Khusro et al., 2014).

In order to fulfil the vast demand of enzymes, industries are recurrently in quest of isolating hyper enzymes producing bacteria with desirable characteristics. In this context, we isolated several bacterial cultures from soil samples of AIE, NLC, and AAZP regions of Tamil Nadu (India) and evaluated their potentialities to produce industrially important three prime hydrolytic enzymes (amylase, cellulase, and inulinase). Of 72 isolates, strain ALA1, ALA3, ALA4, and ALA5 exhibited maximum production of extracellular amylase, cellulase, and inulinase. In accordance with the previous reports (Singh and Kumari, 2016; Hussain et al., 2017; Guttikonda et al., 2017; Dida, 2018; Manzum and Al Mamun, 2018; Ramapriya et al., 2018; Lasa et al., 2019) who demonstrated soil bacteria as ideal producers of enzymes, our findings revealed comparatively higher production of amylase, cellulase, and inulinase from *Bacillus* spp. and *Glutamicibacter* sp.

Bacterial taxonomy is generally influenced by the utilization of various molecular tools (Khusro, 2015; Arokiyaraj et al., 2015; Franco-Duarte et al., 2019). The identification and classification of microbiota through molecular techniques depend particularly on the sequence analysis of 16S rRNA gene. In fact, targeting 16S rRNA gene of bacteria for sequence analyses is a pivotal genetic marker because 16S rRNA gene is not only universal among bacteria but also conserved and exhibit variations for distinguishing between taxa (Ntushelo et al., 2012). The 16S rRNA gene sequence analyses using different bioinformatics tools help determine comparative findings for classifying particular bacterial group (Aarti

**Table 2**
Morphological and biochemical properties of potent isolates.

| Tests | Isolates | | | |
|---|---|---|---|---|
| | ALA1 | ALA3 | ALA4 | ALA5 |
| Colony colour | Cream white | Cream white | Pale white | Cream white |
| Colony shape | Large, rough, and round | Large, rough, and round | Large, rough, and round | Large, rough, and round |
| Gram staining | Gram (+) | Gram (+) | Gram (+) | Gram (+) |
| Indole | + | + | – | + |
| Voges-Proskauer | + | + | – | + |
| Citrate utilization | – | – | – | – |
| ONPG | – | – | – | – |
| Nitrate reductase | + | + | + | + |
| Arginine | + | + | – | + |
| Malonate | – | – | – | – |
| Catalase | + | + | + | + |
| Methyl red | – | – | – | – |
| ***Carbohydrate fermentation*** | | | | |
| Glucose | + | + | + | + |
| Sucrose | + | + | + | + |
| Arabinose | – | – | + | – |
| Mannitol | – | – | – | – |
| Trehalose | + | + | – | + |
| Lactose | + | + | + | + |
| Dextrose | + | + | + | + |

Note: '+' = Positive; '-' = Negative

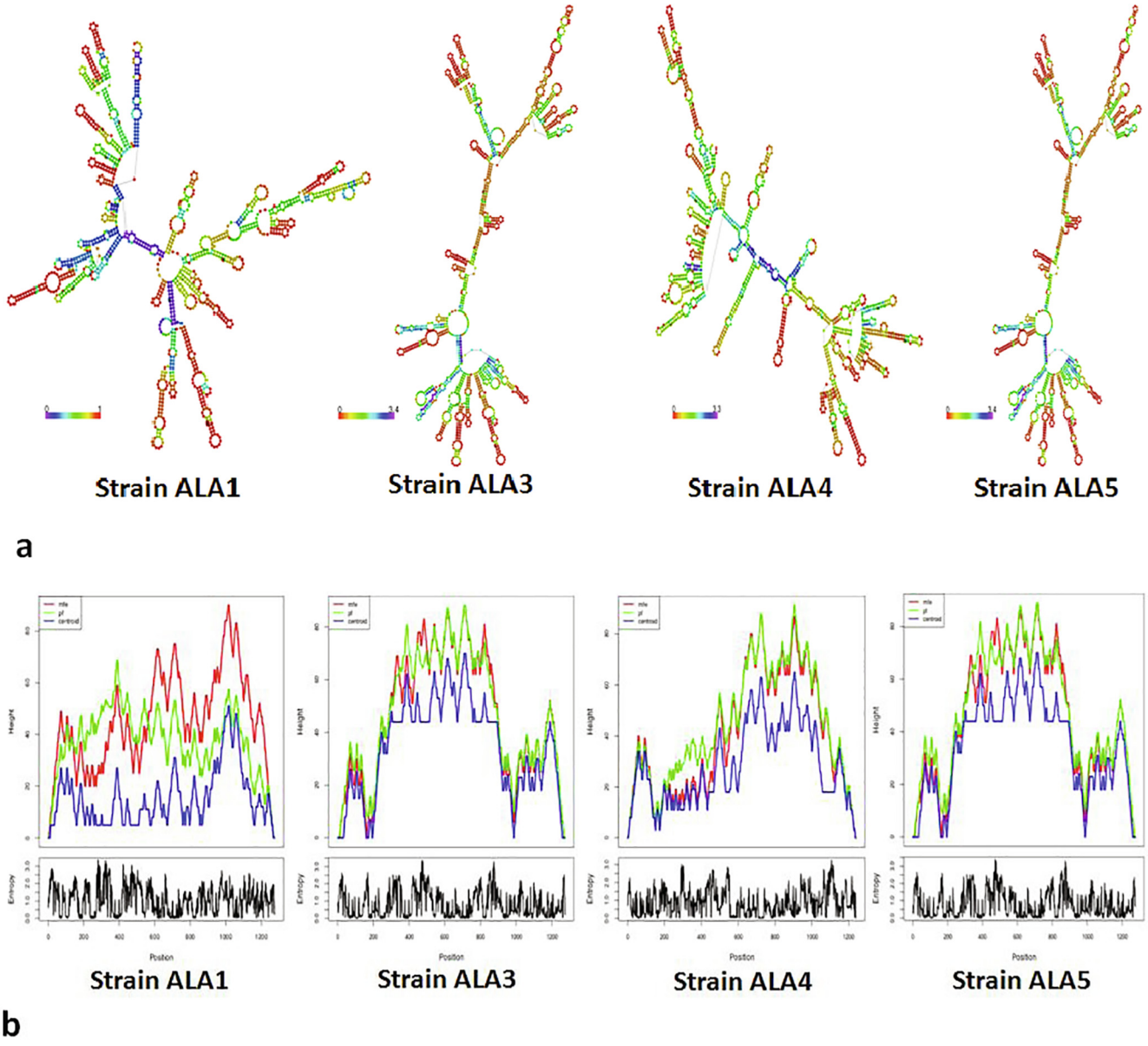


**Fig. 5.** **(a)** Secondary structure of RNA for strain ALA1, ALA3, ALA4, and ALA5. The MFE for strain ALA1, ALA3, ALA4, and ALA5 was calculated as −480.73, −478.76, −496.63, and −479.03 kcal/mol, respectively. **(b)** Mountain plot and entropy showing hierarchical representation of RNA secondary structure for strain ALA1, ALA3, ALA4, and ALA5.

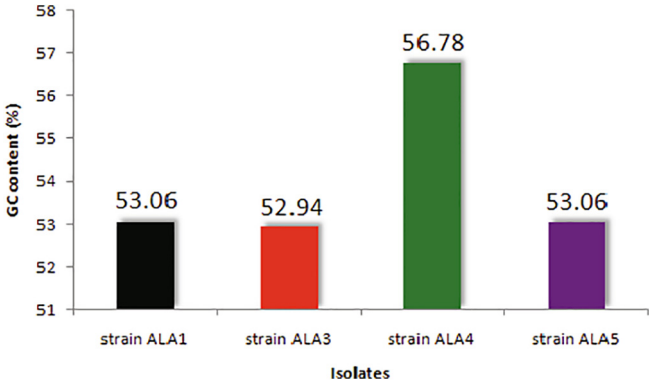


**Fig. 6.** GC content of 16S rRNA sequences for strain ALA1, strain ALA3, strain ALA4, and strain ALA5.

and Khusro, 2015). In this chapter, RNA secondary structure, thermodynamics parameters, ORF regions, GC content, and phylogenetic tree were deduced using 16S rRNA sequences of bacteria.

Free energy minimization decreases total Gibbs free energy and ascertains stability to the sequences, thereby predicting RNA secondary structure (Zhang et al., 2019). Dynamic programs are used through thermodynamic approaches to compute the most favourable secondary structure with MFE (Zuker and Stiegler, 1981). In the present investigation, the energy structure for four selected strains was different. Shape of the secondary structure, energy value, and plots among strains were determined by the nucleotides colour. The MFE illustrates the appropriate structure with maximum stable energy. Generally, Gibbs free energy of RNA secondary structure indicates evolutionary stability of the given sequences (Sankari and Khusro, 2014). Mountain plot and entropy depict the hierarchical representation of RNA secondary structure of each strain. In each plot, helices indicated mountains while terminal loops gave rise to peaks (Chandra and Shilpa, 2018). The GC con-

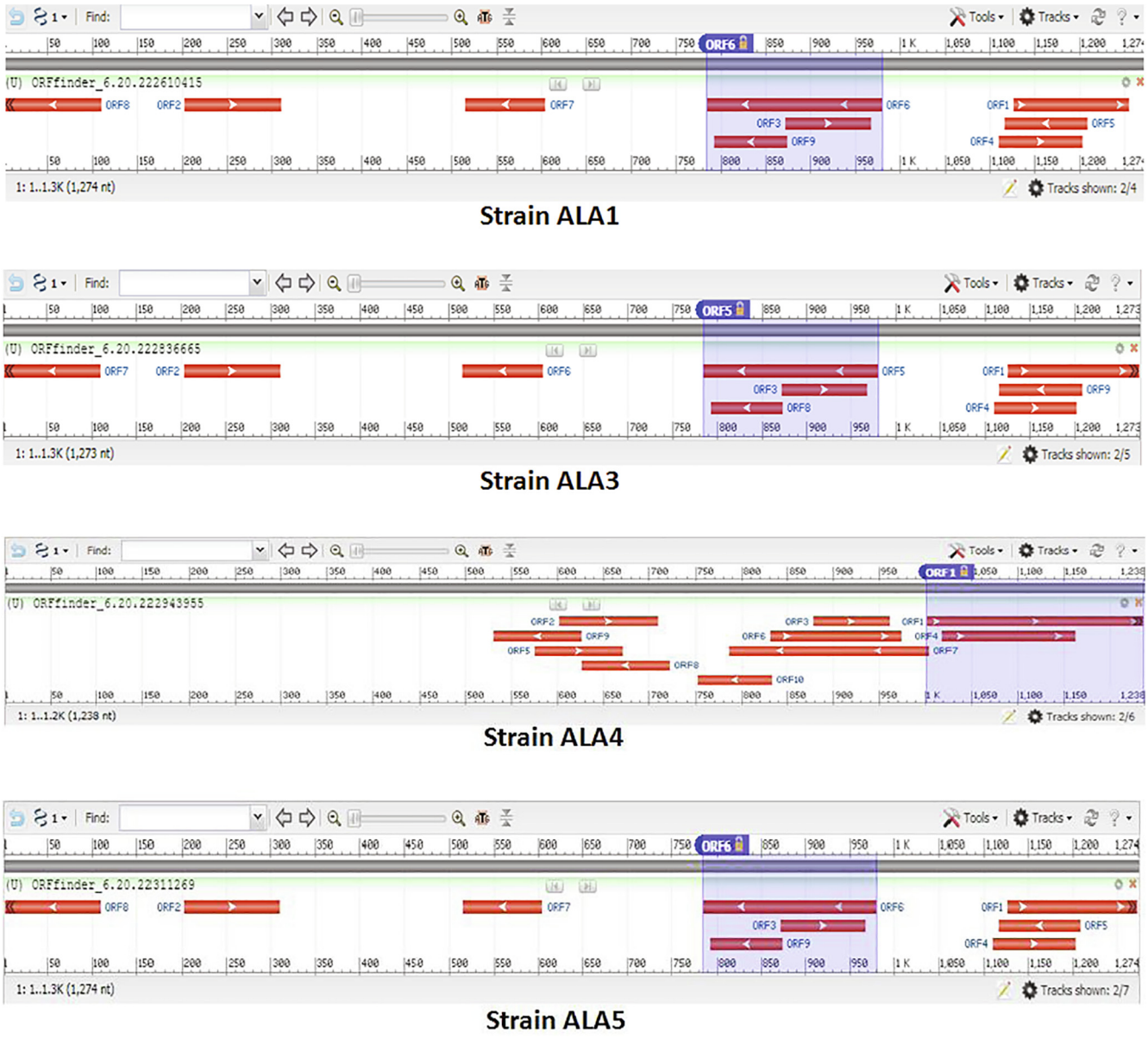


**Fig. 7.** Distinct ORF regions for each strain.

tents of sequences affect the secondary structure (Sankari and Khusro, 2014). In this study, the GC content for each bacterial sequences was calculated as > 50%, thereby indicating the stability of secondary structures. The ORFs for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 showed possible coding regions for proteins in the given sequences. The evolutionary relationships of strains based on 16S rRNA gene sequences have been implemented to categorize isolates into varied species (Chandra and Shilpa, 2018). In this investigation, the bootstrap tree of strains showed close resemblance with the existing distinct strains of similar groups of bacteria.

## 5. Conclusions

In a nutshell, a total of 72 bacteria were isolated from the soil samples of AIE, NLC, and AAZP regions of Tamil Nadu, India. Among them, 41.66% of total isolates were observed amylase producers, 38.88% of them produced cellulase, and 36.11% of isolates showed inulinase production. In addition, 20.83% of total isolates were observed co-producing all three hydrolytic enzymes. Isolates estimating maximum production of all three hydrolytic enzymes were identified as *B. anthracis* strain ALA1, *B. cereus* strain ALA3, *G. arilaitensis* strain ALA4, and *B. thuringiensis* strain ALA5 based on biochemical and molecular characterization tools. Further, the determination of RNA secondary structure, thermodynamics parameters, ORF regions, GC content, and bootstrap tree construction using 16S rRNA sequences classified the isolates among particular bacterial species. Findings suggested the prominent role of *Bacillus* spp. and *G. arilaitensis* for large scale productions of amylase, cellulase, and inulinase at industrial level.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

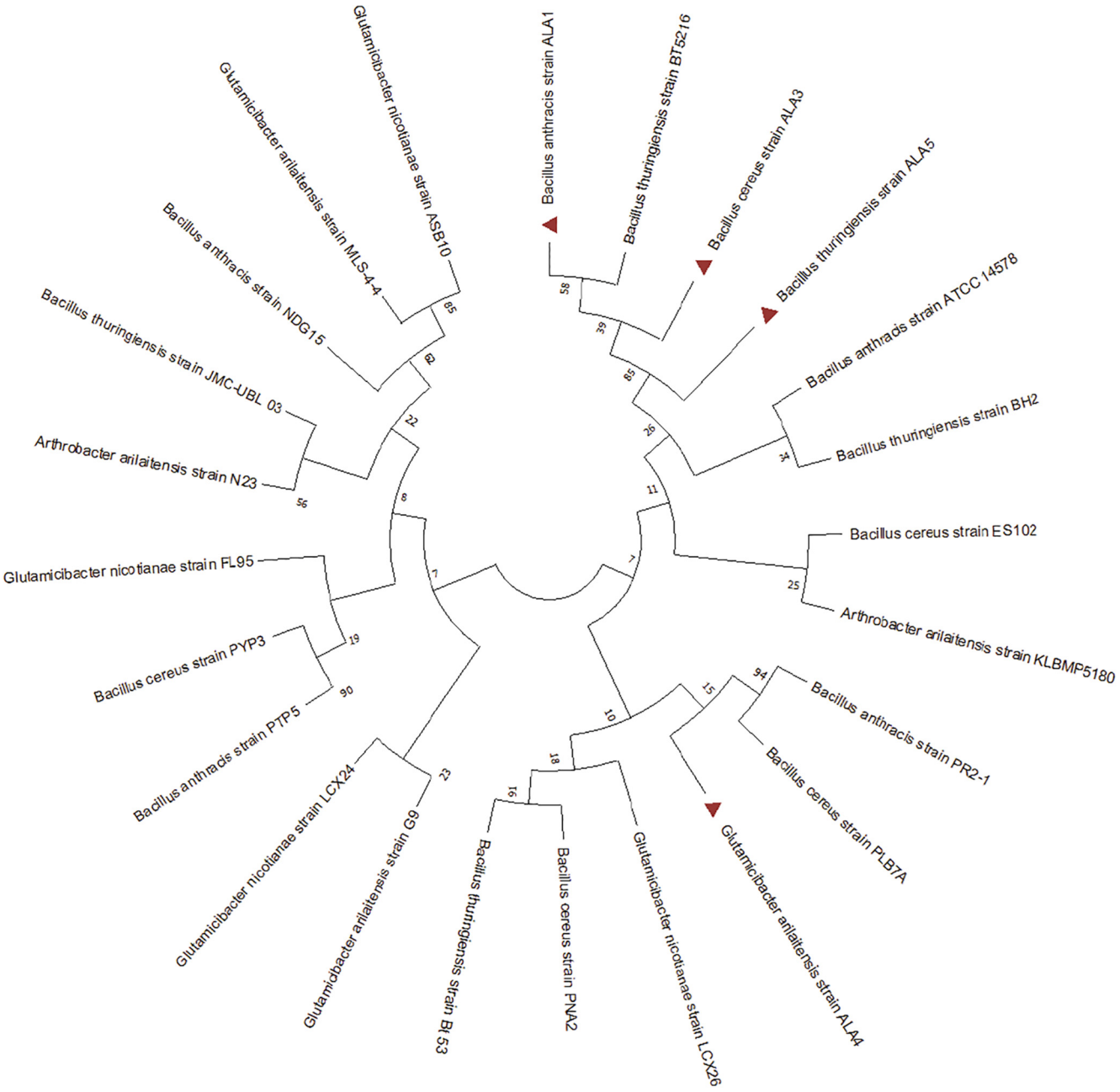


**Fig. 8.** Bootstrap tree construction for strain ALA1, strain ALA3, strain ALA4, and strain ALA5 with respect to other closely related strains.

## Acknowledgement

Authors acknowledge Maulana Azad National Fellowship (F1-17.1/2015-16/MANF-2015-17-BIH-60730), University Grants Commission, Delhi, India for the partial support. The authors extend their appreciation to the Researchers supporting project number (RSP-2020/190) King Saud University, Riyadh, Saudi Arabia.

## References

Aarti, C., Khusro, A., 2015. Discovery of polygalacturonase producing *Bacillus tequilensis* strain ARMATI using 16S rRNA gene sequencing. Asian J. Pharm. Clin. Res. 8, 58–62.

Al-Dhabi, N.A., Esmail, G.A., Duraipandiyan, V., Valan, Arasu M., 2019a. Chemical profiling of *Streptomyces* sp. Al-Dhabi-2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications. Saudi. J. Biol. Sci. 26, 758–766.

Al-Dhabi, N.A., Esmail, G.A., Duraipandiyan, V., Valan Arasu, M., Salem-Bekhit, M.M., 2016. Isolation, identification and screening of antimicrobial thermophilic *Streptomyces* sp. Al-Dhabi-1 isolated from Tharban hot spring, Saudi Arabia. Extremophiles 20, 79–90.

Al-Dhabi, N.A., Ghilan, A.K.M., Arasu, M.V., Duraipandiyan, V., Ponmurugan, K., 2018a. Environmental friendly synthesis of silver nanomaterials from the promising *Streptomyces parvus* strain Al-Dhabi-91 recovered from the Saudi Arabian marine regions for antimicrobial and antioxidant properties. J. Photochem. Photobiol. B 189, 176–184.

Al-Dhabi, N.A., Ghilan, A.-K.M., Arasu, M.V., 2018b. Characterization of Silver Nanomaterials Derived from Marine *Streptomyces* sp. Al-Dhabi-87 and Its *In Vitro* Application against Multidrug Resistant and Extended-Spectrum Beta-Lactamase Clinical Pathogens. Nanomaterials 8 (5).

Al-Dhabi, N.A., Ghilan, A.K.M., Arasu, M.V., Duraipandiyan, V., 2019:b. Green biosynthesis of silver nanoparticles produced from marine *Streptomyces* sp. Al-Dhabi-89 and their potential applications against wound infection and drug resistant clinical pathogens. J. Photochem. Photobiol. B 111529.

Al-Dhabi, N.A., Mohammed Ghilan, A.K., Esmail, G.A., Valan Arasu, M., Duraipandiyan, V., Ponmurugan, K., 2019c. Bioactivity assessment of the Saudi Arabian Marine *Streptomyces* sp. Al-Dhabi-90, metabolic profiling and its in vitro inhibitory property against multidrug resistant and extended-

spectrum beta-lactamase clinical bacterial pathogens. J. Infect. Public Health 12, 549–556.
Alves, P.D.D., Siqueira, F.F., Facchin, S., Horta, C.C.R., Victória, J.M.N., Kalapothakis, E., 2014. Survey of microbial enzymes in soil, water, and plant microenvironments. Open Microbiol. J. 8, 25–31.
Arasu, M.V., Thirumamagal, R., Srinivasan, M.P., Al-Dhabi, N.A., Ayeshamariam, A., Saravana Kumar, D., Punithavel, N., Jayachandran, M., 2017. Green chemical approach towards the synthesis of $CeO_2$ doped with seashell and its bacterial applications intermediated with fruit extracts. J. Photochem. Photobiol. B 172, 50–60.
Arasu, M.V., Arokiyaraj, S., Viayaraghavan, P., Kumar, T.S.J., Duraipandiyan, V., Al-Dhabi, N.A., Kaviyarasu, K., 2019. One step green synthesis of larvicidal, and azo dye degrading antibacterial nanoparticles by response surface methodology. J. Photochem. Photobiol. B 190, 154–162.
Arasu, M.V., Duraipandiyan, V., Ignacimuthu, S., 2013. Antibacterial and antifungal activities of polyketide metabolite from marine *Streptomyces* sp. AP-123 and its cytotoxic effect. Chemosphere 90 (2), 479–487.
Archana, J., Thilo, E., Ruth, F., Philippe, B., Wilfred, O., 2015. Quantification of spatial distribution and spread of bacteria in soil at microscale. EGU General Assembly, Vienna, Austria. Id: 15420.
Arokiyaraj, S., Saravanan, M., Badathala, V., 2015. Green synthesis of Silver nanoparticles using aqueous extract of *Taraxacum officinale* and its antimicrobial activity. South Indian J. Biol. Sci. 2, 115–118.
Azadian, F., Badoei-dalfard, A., Shoushtari, A.N., Karami, Z., Hassanshahian, M., 2017. Production and characterization of an acido-thermophilic, organic solvent stable cellulase from *Bacillus sonorensis* HSC7 by conversion of lignocellulosic wastes. J. Genet. Eng. Biotechnol. 15, 187–196.
Binod, P., Palkhiwala, P., Gaikaiwari, R., Nampoothiri, K.M., Duggal, A., Dey, K., et al., 2013. Industrial enzymes – present status and future perspectives for India. J. Sci. Ind. Res. 72, 271–286.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Chandra, P., Shilpa, V.S., 2018. Molecular characterization and identification of bioactive peptides producing *Lactobacillus* sps. based on 16S rRNA gene sequencing. Food Biotechnol. 32, 1–14.
Chi, Z., Chi, Z., Zhang, T., Liu, G., Yue, L., 2009. Inulinase-expressing microorganisms and applications of inulinases. Appl. Microbiol. Biotechnol. 82, 211–220.
Chi, Z.M., Zhang, T., Cao, T.S., Liu, X.Y., Cui, W., Zhao, C.H., 2011. Biotechnological potential of inulin for bioprocesses. Bioresour. Technol. 102, 4295–4303.
Dida, G., 2018. Isolation and characterization of starch degrading rhizobacteria from soil of Jimma University Main Campus. Ethiopia. Afr. J. Microbiol. Res. 12, 788–795.
Franco-Duarte, R., Černáková, L., Kadam, S., Kaushik, K.S., Salehi, B., Bevilacqua, A., et al., 2019. Advances in chemical and biological methods to identify microorganisms-From past to present. Microorganisms 7, 130.
Ghosh, T.K., 1987. Measurement of cellulase activities. Pure Appl. Chem. 59, 257–268.
Gruber, A.R., Lorenz, R., Bernhart, S.H., Neuböck, R., Hofacker, I.L., 2008. The Vienna RNA websuite. Nucleic Acids Res. 36, 70–74.
Guttikonda, S.T.K., Vanitha, S., Anuradha, K., 2017. Screening of inulinase producing bacteria from diverse sources. Biosci. Discov. 8, 695–701.
Hussain, A.A., Abdel-Salam, M.S., Abo-Ghalia, H.H., Hegazy, W.K., Hafez, S.S., 2017. Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environment. J. Genet. Eng. Biotechnol. 15, 77–85.
Imadi, S.R., Babar, M.M., Hasan, H., Gul, A., 2016. Soil Microflora – An Extensive Research. In: Hakeem, K., Akhtar, J., Sabir, M. (Eds.), Soil Science: Agricultural and Environmental Prospectives. Springer, Cham, pp. 303–316.
Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., Kopriva, S., 2017. The role of soil microorganisms in plant mineral nutrition- Current knowledge and future directions. Front. Plant. Sci. 8, 1617.
Khusro, A., 2015. DNA sequencing by targeting 16S rRNA gene for novel strain identification of *Bacillus* sp. Int. J. MediPharm Res. 1, 27–36.
Khusro, A., Aarti, C., 2015. Molecular identification of newly isolated *Bacillus* strains from poultry farm and optimization of process parameters for enhanced production of extracellular amylase using OFAT method. Res. J. Microbiol. 10, 393–420.
Khusro, A., Preetamraj, J.P., Panicker, S.G., 2014. Multiple heavy metals response and antibiotic sensitivity pattern of *Bacillus subtilis* strain KPA. J. Chem. Pharm. Res. 6, 532–538.
Khusro, A., Barathikannan, K., Aarti, C., Agastian, P., 2017. Optimization of thermo-alkali stable amylase production and biomass yield from *Bacillus* sp. under submerged cultivation. Fermentation. 3, 7.
Lasa, A.V., Mašínová, T., Baldrian, P., Fernández-López, M., 2019. Bacteria from the endosphere and rhizosphere of *Quercus* spp. use mainly cell wall-associated enzymes to decompose organic matter. PLoS ONE 14, e0214422.
Li, A.X., Guo, L.Z., Fu, Q., Lu, W.D., 2011. A simple and rapid plate assay for screening of inulin-degrading microorganisms using Lugol's iodine solution. Afr. J. Biotechnol. 10, 9518–9521.
Lladó, S., López-Mondéjar, R., Baldrian, P., 2017. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiol. Mol. Biol. Rev. 81, e00063–e116.
Lugani, Y., Singla, R., Sooch, B.S., 2015. Optimization of cellulase production from newly isolated *Bacillus* sp. Y3. J Bioprocess Biotech. 5, 264.
Manzum, A.A., Al Mamun, M.A., 2018. Isolation of *Bacillus* spp. bacteria from soil for production of cellulase. Nepal J. Biotechnol. 6, 57–61.
Miller, G.L., 1959. Use of dinitrosalisylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Niyonzima, F.N., 2019. Production of microbial industrial enzymes. Acta Sci. Microbiol. 12, 75–89.
Ntushelo, K., Harrison, N.A., Elliott, M.L., 2012. Comparison of the ribosomal RNA operon from Texas Phoenix decline and lethal yellowing phytoplasmas. Eur. J. Plant Pathol. 33, 779–782.
Ramapriya, R., Thirumurugan, A., Sathishkumar, T., Manimaran, D.R., 2018. Partial purification and characterization of exoinulinase produced from *Bacillus* sp. J. Genet. Eng. Biotechnol. 16, 363–367.
Rosano, G.L., Ceccarelli, E.A., 2009. Rare codon content affects the solubility of recombinant proteins in a codon bias-adjusted *Escherichia coli* strain. Microb. Cell Fact. 8, 41.
Sankari, D., Khusro, A., 2014. Biochemical, molecular characterization and sequence analysis of keratinase producing novel strain of *Bacillus licheniformis* isolated from poultry farm. Int. J. Pharm. Pharm. Sci. 6, 457–461.
Singh, P., Kumari, P., 2016. Isolation and characterization of amylase producing *Bacillus* spp. from selected soil sample. Int. J. Res. Biosci. 5, 24–29.
Singh, R.S., Chauhan, K., Kennedy, J.F., 2017. A panorama of bacterial inulinases: Production, purification, characterization and industrial applications. Int. J. Biol. Macromol. 96, 312–322.
Sneath, P.H.A., 1994. Gram positive rods. In Bergeys Manual of Systematic Bacteriology, 9th ed.; Hensyl, W.M., Ed.; Williams and Wilkins: Philadelphia, PA, USA. 2106-2111.
Talwar, H.K., Chatli, A.S., 2018. Microflora of soil: A review. Int. J. Adv. Res. 6, 1502–1520.
Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
Zhang, H., Zhang, C., Li, Z., Li, C., Wei, X., Zhang, B., et al., 2019. A new method of RNA secondary structure prediction based on convolutional neural network and dynamic programming. Front Genet. 10, 467.
Zhou, J., Wang, Y.H., Chu, J., Zhuang, Y.P., Zhang, S.L., Yin, P., 2008. Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100–14. Bioresour. Technol. 99, 6826–6833.
Zuker, M., Stiegler, P., 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Res. 9, 133–148.