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Isolation and Identification of *Agrobacterium* and *Azotobacter* Species Capable of Producing Curdlan and Alginate Biopolymers

Mbahi, M. A.[1*]; Doughari, J. H.[2]

[1]Federal University, Kashere, Faculty of Life Sciences, Department of Microbiology, P.M.B, 0182 Gombe, Gombe State, Nigeria.
[2]Modibbo Adama University, Faculty of Life Sciences, Department of Microbiology, PMB 2076, Yola, Adamawa State, Nigeria.

Corresponding Author: Mbahi, M. A.[1*]

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Abstract: Curdlan is an important homopolysaccharide, while Alginate is a naturally occurring polymer. Numerous bacteria species have the potential to produce these biopolymers.In this study, *Agrobacterium* and *Azotobacter* species capable of producing these polymers were isolated and identified. Soil samples from different legume and cereal farms were inoculated unto mannitol glutamate agar containing aniline blue dyes, Nitrogen free Jensen's agar medium and Asby agar medium for isolation of the bacteria,The isolates obtained were subjected to biochemical tests and 16S rRNA sequencing for identification. Result showed that 5 species of A*grobaterium* grew on aneline blue mannitol glutamate agar (aMGA) exhibiting blue coloration; 2 *Azotobacter* spp grew on Jensen nitrogen free agar; A*grobaterium* and A*zotobacter* species appeared as Gram negative rods; A*grobaterium* spp were KOH sensitive, urease, catalase,citrate positive, hydrolyze starch while A*zotobacter* spp were positive for methly red, indole, catalase, urease, citrate utilization, starch hydrolysis, and hydrogen sulfide production. Molecular identification showed a band of 1500bp product of 16S rRNA gene of A*grobacterium* spp on gel electrophoresis. Result further showed that the *Agrobacterium* species isolates were capable of producing Curdlan while *Azotobacter vinelandii* demonstrated Alginate biopolymer production. these findings provide suitable indigenous bacterial strains that will enhance biopolymer production.

Keywords: *Agrobacterium, Alginate, Azotobacter, Biopolymers, Curdlan.*

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I. INTRODUCTION

Curdlan is an exopolysaccharide, or a homopolysaccharide, consisting solely of glucose monomers connected by β-1, 3 glycosidic bonds (Aquinas *et al*., 2021). It's molecular weight ranges from 5.3×10^4 to 2.0×10^6, which is relatively high (Aquinas *et al*., 2021). It has no taste, color, and odor, and it dissolves in alkali but not in water or alcohol when processed. In aqueous solutions, it forms thermo-reversible (low-set) and thermo-irreversible (high-set) gels (Mangolim *et al*., 2017; Aquinas *et al*., 2021). When the aqueous solution is heated to 55°C and then chilled, a thermos reversible gel is formed; when the solution is heated to about 80°C and then cooled, a thermos irreversible gel is formed (Mangolim *et al*., 2017). The first bacteria to produce curdlan by digesting glucose was *Alcaligenes faecalis* var myxogenes 10C3, which was identified from the soil (Aquinas *et al*., 2021).

Curdlan is produced through a secondary metabolic activity that takes place outside of the bacterial cell (Al-Rumaidh *et al*., 2023). Harada *et al.* (1962) had reported curdlan production by *Alcaligenes faecalis* var. The Gram negative, motile bacillus myxogenes 10C3, which is commonly found in soil, was dubbed "curdlan" in 1966 (Aquinas *et al.*, 2021). The microbial synthesis of curdlan is mostly carried out by soil bacteria (Verma *et al.*, 2020), and the main exopolysaccharide curdlan producer on an industrial scale is *Agrobacterium* sp. ATCC 31749 (Laxmi *et al*., 2018; Aquinas *et al*., 2021). In addition to *Alcaligenes faecalis*, the most frequently used type of *Agrobacterium* spp, *Agrobacterium fabrum*, a non-pathogenic, rod shaped, Gram negative bacterium found in the nodules of two leguminous plants (pea and groundnut) also produces curdlan (Laxmi *et al*., 2018). Other bacteria that produces curdlan include *Cellulomonas flavigena* KU, which was isolated from leaf litter and used extra glucose and other carbon sources, and

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Rhizobium sp, a spore forming *Bacillus* sp that was obtained from the soil (Aquinas *et al.*, 2021). Numerous sources that have yielded curdlan producing microorganisms include dirt, pea plant nodules, groundnut nodules, butterfly pea, Ipil-Ipil, moong bean, soybean, cowpea, black gram, green gram, soil, and moist soil (Wang *et al*., 2016; Laxmi *et al*., 2018; Cai *et al*., 2020).

Alginate, a naturally occurring polymer that is mainly isolated from the cell walls of brown algae (Phaeophyceae), was initially discovered in 1881 by a British chemist named E.C.C. Stanford (Bouzenad *et al*., 2025). Alginate is made up of two monomeric units, α-Lguluronic acid (G) and β-D-mannuronic acid (M), which are joined by 1→4 glycosidic linkages, and brown algae species such *Ascophyllum*, *Macrocystis*, and *Laminaria* are rich in these monomers (Garcia-Vaquero *et al*., 2017). Also, apart from brown algae, several bacteria, such as *Pseudomonas* species and *Azotobacter vinelandii*, also produce alginates (Bouzenad *et al*., 2025).

Despite wide report of several bacteria species capable of producing Curdlan and Alginate Biopolymers (Laxmi *et al*., 2018; Aquinas *et al*., 2021; Bouzenad *et al.,* 2025), there is little or no information on their isolation and identification from legume and cereal farms in Nigeria, especially in Girei Local Government Area, Adamawa State. This study was therefore aimed at isolating and identifying *Agrobacterium* and *Azotobacter* species capable of producing Curdlan and Alginate biopolymers from soil using agrowaste as substrate.

II. MATERIALS AND METHODS

➢ *Samples Collection*

Samples of soil were obtained from selected legumes farm in localities within Girei LGA, Adamawa State in a sterile plastic container, kept in an icebox containing ice packs, conveyed to the Microbiology Laboratory of the Department of Microbiology in Modibbo Adama University, Yola and subsequently stored in the refrigerator until use.

➢ *Isolation of Agrobacterium and Azotobacter Species*

For isolation of *Agrobacterium* spp, after agitating 100 milliliters of sterile distilled water with 10 grams of soil samples, 0.1 millilitres of the supernatant were transferred to 0.9 milliliters of sterile distilled water, and serial dilutions (10-1 to 10-6) were made. 100µl of each dilution was added and sprayed on mannitol glutamate agar containing aniline blue dyes (Aquinas *et al.*, 2024). After five days of incubation at 30 degrees Celsius, colonies exhibiting a strong blue hue were streak purified at least three times on the same agar medium and stored at 4 degrees Celsius for additional research. To identify the most effective isolate, the pure isolates were screened (Yang *et al.*, 2016).

For isolation of *Azotobacter* spp the same procedure as in the isolation of *Agrobacterium* was followed except that Nitrogen free Jensen's agar medium consisting of : Sucrose (20.00 g/L), Dipotassium phosphate (1.00g/L), Magnesium sulphate (0.50g/L), Sodium chloride (0.50g/L), Ferrous sulphate (0.10g/L), Sodium molybdate (2.00g/L), Calcium carbonate (2.00g/L), and Agar (15.0g/L), and Asby agar medium, the medium consist of : sucrose (20.00), yeast extract (5.00 g/L), dye (0.05 g/L) and agar (20.00 g/L) at pH 7.0)for isolation. The media were prepared according to manufacturer's instructions and sterilized at 15 psi (121^0C) for 15 minutes. After the sterilization, the media was allowed to solidify and then used for cultivation of the organisms. For the inoculation, 0.1 ml (100 µl) of aliquots of 10^{-1},$10^{-6,}$ dilution was spread onto the agar plates containing nitrogen Free Jensen's medium (JM) and Asby's medium using a glass spreader by gently rotating the Petri dish clockwise. The culture plates were then inverted and incubated at 28°C for 48 hrs after which the bacterial isolates were identified using biochemical tests and molecular sequencing.

➢ *Identification of Curdlan and Alginate Producing Bacteria*

Preliminary identification of the bacterial isolates with the highest curdlan yield amongst others was carried out morphologically through Gram staining, endospore staining, and some biochemical tests such as catalase, methyl red, indole, citrate utilization, urease, oxidase, and starch hydrolysis. Additionally, KOH sensitivity test was done for alginate producing bacteria. The isolate was identified by 16S rRNA sequencing (Aquinas *et al.,* 2024).

➢ *Gram Staining Reaction*

Gram staining as described by Tripathi *et al*. (2023), was used to identify the isolate from the soil sample. Briefly, a tiny amount of a colony from the Petri dish was aseptically transferred to the slide together with a drop or a few loop full of water, and a very thin layer was smeared onto the slide. An inoculation loop was used to evenly distribute the culture. In order to prevent localized overheating, the slide was moved in a circular motion while the culture was air-dried and attached over a low flame. The fixed culture was covered with crystal violet stain, which was then let to stand for ten seconds. Iodine solution was added to the smear in sufficient amounts to cover the fixed culture after the stain was poured off and any remaining stain was gently washed with a stream of water. After then, it stood for ten to sixty seconds. After the iodine solution was removed, running water was used to rinse the slide. The slide was treated with a few drops of decolorizer. After five seconds, water was used to rinse it off. After 40 to 60 seconds of safranin counterstaining, the solution was removed with water. After shaking the slide to get rid of the majority of the water, it was allowed to air dry. After then, a microscope was used to inspect the slides. Light microscopy was used to analyse the isolates' morphological features and verify the existence of rod-shaped cells.

➢ *KOH Sensitivity Test*

KOH sensitivity test was carried out as described by Prescott *et al.* (2011) and Aquinas *et al.* (2024). On a spotless, grease-free glass slide, a drop of a 3% potassium hydroxide (KOH) solution was applied. After that, a sizable portion of a 24-hour colony of the test isolates was removed from the agar plate using a plastic loop and emulsified in the KOH drop on the slide. After vigorously stirring the mixture for 60 seconds, the loop was carefully raised straight out of the suspension and checked for any stringy mucoid threads extending

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between the slide and the loop, which would indicate a positive test.

➢ *Catalase Test*

Catalase test was performed by as described by Jimenez *et al.* (2011) and Sandeep *et al.* (2015). On a spotless slide, a few drops of hydrogen peroxide were applied. Using a wooden stick, fresh cultures of the isolates were emulsified on the slide, and the formation of bubbles was monitored.

➢ *Oxidase Test*

Oxidase test was done as described by Oksel *et al.* (2024). Three drops of freshly made oxidase reagent were applied to a piece of filter paper in a sterile petri dish. A colony of the 24-hour-old test organisms was then removed using a glass rod, smeared on filter paper, and monitored for the formation of a purple hue.

➢ *Indole Test*

Indole test was carried out as described by Jimenez *et al*. (2011). The test organisms were placed in a test tube with five millilitres of sterile tryptone broth and cultured for forty-eight hours at 370 degrees Celsius. After adding 0.5 ml of Kovac's reagent to the culture, it was gently agitated to test for indole. Within 10 seconds, the surface layer was checked for redness and the formation of a red ring layer.

➢ *Methyl Red Test*

Methyl red (MR) test was performed as described by Ijah and Antai (2003) and Kouadjo *et al*. (2025). A sterile loop was used to inoculate a tube of MR-VP broth with a pure culture of the test isolates. The culture was then cultured aerobically at 36°C for 48 hours. Five drops of methyl red indicator were then applied to a clean, empty test tube along with a 1 mL sample of the soup. The colour was noticed right away.

➢ *Voges Proskeur's Test*

The test isolates were inoculated into glucose phosphate broth and incubated at 37ºC for 5 days. At the end of the incubation period, 0.6 ml of 5% solution of alpha Naphthol and 0.2ml of 40% potassium hydroxide (KOH) were added to the culture tubes. The entire solution was mixed properly by swirling, for a few seconds, and at the end of incubation period, the broth was observed for colour change (Prescott *et al.*, 2011).

➢ *Citrate Utilization Test*

The citrate test was carried out using Simmon's citrate agar medium (Cappuccino and Welsh, 2017). Simmon's citrate agar was made and sterilized for 20 minutes at 121°C and 15 pressure. Each tube was filled with 15 ml of sterile medium. After being slanted, the tubes were allowed to harden. Each isolate was streaked on the medium upon solidification. After that, the tubes were incubated at 30 and 28°C for 48 hours. Lastly, each isolate's colour variations were noted. The test isolates' use of citrate was shown by a blue colouration, but their incapacity to use citrate was demonstrated by the preservation of the original green colour.

➢ *Urease Test*

Urease test was performed according to Owuama (2019). A limited number of the well-isolated test organisms from a pure culture were selected using the inoculating loop, which was sterilized in a flame. The bacteria were then suspended by dipping the loop into the broth and giving it a gentle shake. After that, the tube's cap was removed to provide aeration, and it was incubated at 36°C. After then, the tubes were routinely examined.

➢ *Starch Hydrolysis Test*

The starch hydrolysis test used to determine the capability of bacteria to produce amylase and utilize it as carbon source was carried out as described by Ijah and Antai (2003). Sterile petri dishes were filled with starch agar (or nutrient agar enriched with 0.2% to 1% starch) and allowed to harden. The test isolates were streaked across the agar surface in a single, short, straight line using a sterile inoculating loop. After that, the plates were inverted and incubated for 48 hours at 36°C. Gram's Iodine solution was then applied to the agar's surface, and the plate was examined right away.

➢ *Hydrogen Sulphide Production*

The hydrogen sulfide test was carried out as described by Acharya (2021). The sulphur source (sodium thiosulphate) and indicator (ferrous ammonium sulphate) were both present in the sulfur-containing medium (Sulfide-Indole-Motility). A colony of the test isolate was selected and inserted deeply into the "butt" (bottom) of the agar tube using a sterile inoculating needle. After that, the infected tube was kept in an incubator for 48 hours at 36°C. After that, the tube was routinely examined to see if ferrous sulphide, a black precipitate, had spread throughout the agar or along the stab line.

➢ *Molecular Characterization of Bacterial Isolates*

The bacterial isolates were obtained in preserved form on agar slants from the Microbiology Laboratory. Each isolate was resuscitated by inoculating into Luria-Bertani (LB) broth and incubated at 30°C for 18–24 hours to allow for active growth and recovery. The revived cultures were subsequently maintained under appropriate storage conditions for downstream molecular analyses.

➢ *DNA Extraction*

The extraction of genomic DNA was carried out as describe by sambrook and Russell (2001), genomic DNA was extracted from overnight bacterial cultures using the Genomic DNA Isolation Kit manufactured by Norgen Biotek Corp. strictly according to the manufacturer's protocol for Gram-negative/Gram-positive bacteria. Briefly, 1.5mL of overnight broth culture was transferred into a sterile 1.5 mL microcentrifuge tube and centrifuged at 14,000 × g (approximately 13,000–14,000rpm) for 2 minutes to pellet the cells. The supernatant was carefully discarded, and the pellet was re-suspended in 200µL of Lysis Buffer. Subsequently, 12µL of Proteinase K was added, and the mixture was vortexed thoroughly and incubated at 55 °C for 60 minutes to ensure complete cell lysis. After incubation, 200µL of 96–100% ethanol and buffer SK each were added to the lysate and mixed by vortexing for 10 seconds.

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The mixture (600µL) was transferred to the provided spin column assembled with a collection tube and centrifuged at 10,000 × g (approximately 10,000rpm) for 1 minute to allow DNA binding to the silica membrane. The flow-through was discarded, and the column was reinserted into the collection tube. The column was washed by adding 500µL of Wash Buffer I, followed by centrifugation at 10,000 × g for 1 minute. The flow-through was discarded, and 500µL of Wash Buffer II was added to the column and centrifuged at 14,000 × g for 1 minute. To ensure complete removal of residual ethanol, an additional dry spin was performed at 14,000 × g for 2 minutes.

The column was then transferred to a clean 1.5 mL elution tube, and genomic DNA was eluted by adding 50µL of Elution Buffer directly onto the center of the membrane. After incubation at room temperature for 2 minutes, the column was centrifuged at 10,000 × g for 2 minutes to obtain the purified DNA. The extracted genomic DNA was stored at −20°C until further molecular analysis. DNA concentration and purity were determined spectrophotometrically at 260/280 nm, and integrity was verified by electrophoresis on 0.8% agarose gel

➢ *Gel Electrophoresis for Genomic DNA Confirmation*

The presence and integrity of the extracted genomic DNA were confirmed using 0.8% agarose gel electrophoresis following the procedure described by Odiba *et al.* (2022). After weighing the proper quantity of agarose powder, it was added to 1X TAE buffer. With caution not to let it boil over, the mixture was microwaved until it was totally dissolved. After allowing the mixture to cool significantly (to roughly 550C), ethidium bromide, a nucleic acid stain, was added. After pouring the molten gel into a casting tray with a comb, it was left to solidify for 28 minutes at room temperature. After the gel had set, it was put into the electrophoresis tank, immersed in 1X running buffer, and the comb was gently taken out. To make the test isolate gDNA samples dense and guarantee that they sink to the bottom of the wells, they were combined with a 6X DNA loading dye that contains glycerol. To precisely measure size later, a DNA ladder (molecular weight marker) was added to the first well before gDNA samples were pipetted into the wells. Since DNA is negatively charged and migrates towards the red (positive) anode, the gel was set up with the wells closest to the black (negative) cathode. After that, a lesser voltage (75V) of electrical current was delivered for two hours.

➢ *PCR Amplification of 16S rRNA Gene*

The amplification of the 16S rRNA gene was carried out using the polymerase chain reaction (PCR) method as described by Aquinas *et al.* (2024). PCR reagents (a master mix comprising Taq DNA polymerase, dNTPs, primers, buffer, and nuclease-free water) were mixed with the extracted DNA template. The following cycles were performed 35 times after the prepared mixture was put in a thermal cycler: To fully separate the double-stranded template DNA, the initial denaturation was carried out for five minutes at 95^0C. To keep the DNA strands apart, denaturation was carried out at 94^0C for 40 seconds in each cycle. In order to enable the forward and reverse primers to adhere (anneal) to the precise target locations on the 16S gene, annealing involved chilling to 57^0C for 40 seconds. In order to allow the Taq polymerase to create the new DNA strand by adding dNTPs, beginning with the bound primers, extension involved heating to 72^0C for 60 seconds. To make sure that any partially synthesized strands were finished, a last extension was performed, which involved holding at 72^0C for five to ten minutes.

➢ *Gel Electrophoresis of 16S rRNA PCR Products*

The presence and integrity of the extracted genomic DNA were confirmed using 0.8% agarose gel electrophoresis following the procedure described by Lee *et al.* (2012). The agarose gel was prepared in 1× TBE buffer and stained with ethidium bromide (0.5 µg/mL). Three microliters of each DNA sample were mixed with loading dye and carefully loaded into the gel wells alongside a 100 p DNA ladder serving as a molecular size marker. Electrophoresis was carried out at 100 V for 30 minutes**.** After electrophoresis, the gel was visualized under a UV transilluminator**,** and the appearance of distinct, intact DNA bands indicated successful DNA extraction and good DNA quality.

III. RESULTS

➢ *Isolation and Identification of Agrobacterium and Azotobacter Species*

Result of the isolation of A*grobacterium* and A*zotobacter spp* (Table 1) showed that a total of 5 species of A*grobaterium* grew on aneline blue mannitol glutamate agar (aMGA) exhibiting blue coloration. Result also showed that 2 *Azotobacter* spp grew on Jensen nitrogen free agar. Furthermore, *Agrobacterium* spp cf6 yielded the highest intense blue color (+++), A*grobacterium* spp sf1 and A*grobacterium* spp gf5 showed the less + colour intensity.

Table 1 Screening of A*grobacterium* Spp from Different Soil Samples for Curdlan Production

Isolate	Source	Intensity of blue colour
A*grobacterium* spp sf1	Soya beans farm	+
A*grobacterium* spp sf2	Soya beans farm	++
A*grobacterium* spp cf3	Cow pea farm	++
A*grobacterium* spp gf4	Groundnut farm	++
A*grobacterium* spp gf5	Groundnut farm	+
A*grobacterium* spp cf6	Cow pea farm	+++

Key: + = Low Intensity Blue Colour, ++ = Moderate Intensity Blue Colour, +++ = High Intensity Blue Colour

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Result also showed A*grobaterium* and A*zotobacter* species appeared as Gram negative rods, biochemical test results showed A*grobaterium* spp were KOH sensitive, urease, catalase, and citrate positive while A*zotobacter spp.* were positive for Methly red, indole, Catalase, Urease, Citrate utilization, Starch hydrolysis Hydrogen Sulfide (Table 2).

Table 2 Microscopic and Biochemical Characteristics of Isolates

Isolate	Gram ±	App	MR	I	C	Urease	CU	SH	H_2S	KOH
A*grobacterium* spp. sf2		Mucoid	-	-	+	+	+	-	-	+
A*grobacterium* spp. cf3	-	Viscous	-	-	+	+	-	-	-	+
A*grobacterium* spp. gf4	-	Mocoid	-	-	+	+	+	-	-	+
A*grobacterium* spp. cf6	-	Viscous	-	-	+	+	+	-	-	+
A*zotobacter* spp. rf1	-	Pale colour slimy	+	+	+	+	+	+	+	+
A*zotobacter* spp. rf2	-	Watery transparent	+	+	+	+	+	+	+	+

Key: U=Urease, MR=Methyl Red, I= Indole, C=Catalase Test, CU=Citrate Utilization, SH=Starch Hydrolysis

Result of molecular identification showed a band of 1500bp product of 16S rRNA gene of A*grobacterium* spp on gel electrophoresis image (Plate 1).

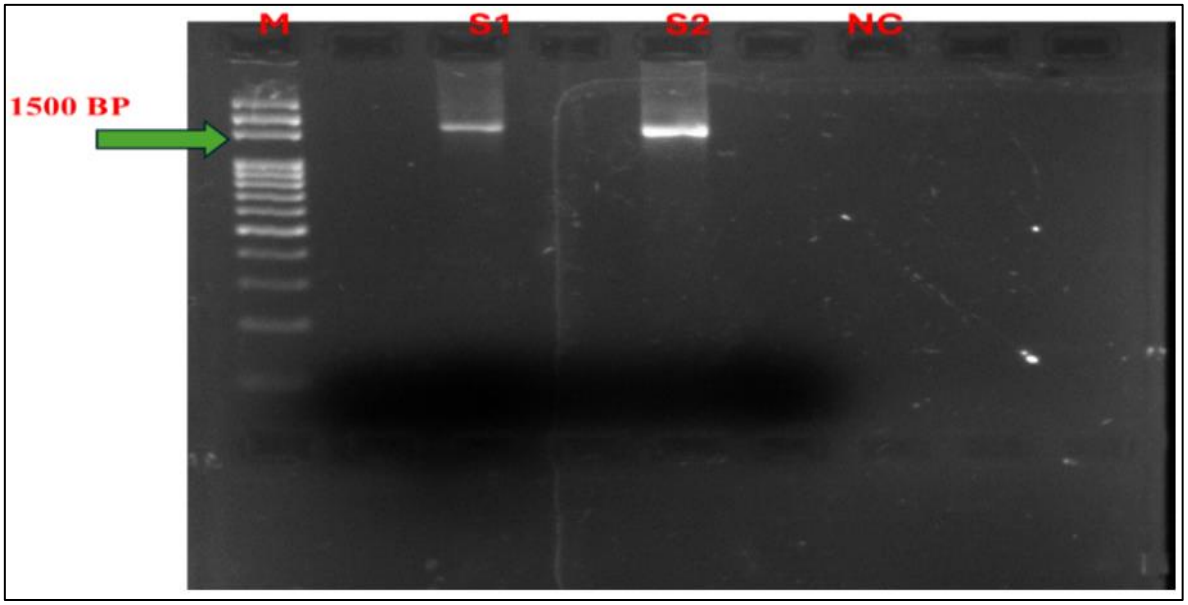

Plate 1 Gel Electrophoresis Image Showing a Positive Amplification of 1500bp Product of 16S rRNA Gene. M: 100bp Ladder, S1: Cf6 Sample, S2: rf1 and NC: Negative Control.

➢ *Isolate 1 (PZ528902) Cf6*

Result confirmed that isolate 1 genome matches closely to *Agrobacterium tumefaciens* Strain SJ001 partial sequence with prominent uniqueness of 100 percent.

Molecular confirmation through 16S rRNA gene sequencing identified the most productive isolate (cf6) as *Agrobacterium tumefaciens* strain SJ001, with 100% sequence identity match on BLAST (accession PZ528902), while the *Azotobacter* isolate (rf1) matched *Azotobacter vinelandii* JCM 21475 with the same level of certainty (accession PZ528903).

Table 3 Isolate 1 (PZ528902) Cf6

Isolate	FASTA Sequences	Maximum score	Total score	Query cover	Value	Percentage identity	Acc. Length	Accession number
Agrobacterium tumefaciens	AACGAACGCTGGCGGC AGGCTTAACACATGCA AGTCGAACGCCCCGCA AGGGGAGTGGCAGACG GGTGAGTAACGCGTGG GAACATACCCTTTCCTG CGGAATAGCTCCGGGA AACTGGAATTAATACC GCATACGCCCTACGGG GGAAAGATTTATCGGG	1962	1962	100	0.00	99.91	1407	AB749219.1

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	GAAGGATTGGCCCGCG TTGGATTAGCTAGTTGG TGGGGTAAAGGCCTAC CAAGGCGACGATCCAT AGCTGGTCTGAGAGGA TGATCAGCCACATTGGG ACTGAGACACGGCCCA AACTCCTACGGGAGGC AGCAGTGGGGAATATT GGACAATGGGCGCAAG CCTGATCCAGCCATGCC GCGTGAGTGATGAAGG CCTTAGGGTTGTAAAGC TCTTTCACCGGAGAAGA TAATGACGGTATCCGG AGAAGAAGCCCCGGCT AACTTCGTGCCAGCAGC CGCGGTAATACGAAGG GGGCTAGCGTTGTTCGG AATTACTGGGGTAAAG CGCACGTAGGCGGATA TTTAAGTCAGGGGTGA AATCCCAGAGCTCAACT CTGGAACTGCCTTTGAT ACTGGGTATCTTGAGTA TGGAAGAGGTAAGTGG AATTCCGAGTGTAGAG GTGAAATTCGTAGATAT TCGGAGGAACACCAGT GGCGAAGGCGGCTTAC TGGTCCATTACTGACGC TGAGGTGCGAAAGCGT GGGGAGCAAACAGGAT TAGATACCCTGGTAGTC CACGCCGTAAACGATG AATGTTAGCCGTCGGGC AGTATACTGTTCGGTGG CGCAGCTAACGCATTA AACATTCCGCCTGGGG AGTACGGTCGCAAGAT TAAAACTCAAAGGAAT TGACGGGGGCCCGCAC AAGCGGTGGAGCATGT GGTTTAATTCGAAGCAA CGCGCAGAACCTTACC AGCTCTTGACATTCGGG GTTTGGGCAGTGGAGA CATTGTCCTTCAGTTAG GCTGGCCCCAGAACAG GTGCTGCATGGCTGTCG TCAGCTCGTGTCGTGAG ATGTTGGGTTAAGTCCC GCAACGAGCGCAACCC TCGCCCTTAGTTGCCAG CATTTAGTTGGGCACTC TAAGGGGACTGCCGGT GATAAGCCGAGAGGAA GGTGGGGATGACGTCA AGTCCTCATGGCCCTTA CGGGCTGGGCTACACA CGTGCTACAATGGTGGT							

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	GACAGTGGGCAGCGAG ACAGCGATGTCGAGCT AATCTCCAAAAGCCATC TCAGTTCGGATTGCACT CTGCAACTCGAGTGCAT GAAGTTGGAATCGCTA GTAATCGCAGATCAGC ATGCTGCGGTGAATAC GTTCCCGGGCCTTGTAC ACACCGCCCGTCACACC ATGGGAGTTGGTTTTAC CCGAAGGTAGTGCGCT AACCGCAAGGAGGCAG CTAACCACGGTAGGGT CAGCGACTGGGGTGAA G							
Azotobacter vinelandii	GGCTCAGATTGAACGCT GGCGGCAGGCCTAACA CATGCAAGTCGAGCGG CAGCGGGACCTTCGGG TTGCCGGCGAGCGGCG GACGGGTGAGTAATGC CTAGGAATCTGCCTGTT AGTGGGGGATAACGCG GGGAAACTCGCGCTAA TACCGCATACGTCCTAC GGGAGAAAGTGGGGGA CCCTCGGGCCTCACGCT AACAGATGAGCCTAGG TCGGATTAGCTAGTTGG TGGGGTAAAGGCCCAC CAAGGCGACGATCCGT AACTGGTCTGAGAGGA TGATCAGTCACACTGGA ACTGAGACACGGTCCA GACTCCTACGGGAGGC AGCAGTGGGGAATATT GGACAATGGGCGAAAG CCTGATCCAGCCATGCC GCGTGTGTGAAGAAGG TCTTCGGATTGTAAAGC ACTTTAAGTTGGGAGG AAGGGCGCTCGGTGAA TACCCAAGCGTCTTGAC GTTACCGACAGAATAA GCACCGGCTAACTTCGT GCCAGCAGCCGCGGTA ATACGAAGGGTGCAAG CGTTAATCGGAATTACT GGGCGTAAAGCGCGCG TAGGTGGTTCGGCAAGT TGGATGTGAAAGCCCC GGGCTCAACCTGGGAA CCGCATCCAAAACTACT GGGCTAGAGTACGGTA GAGGGTGGTGGAATTT CCTGTGTAGCGGTGAA ATGCGTAGATATAGGA AGGAACACCAGTGGCG AAGGCGACCACCTGGA CCGATACTGACACTGA	1855	1855	100	0.00	100	1293	MT658736.1

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	GGTGCGAAAGCGTGGG GAGCAAACAGGATTAG ATACCCTGGTAGTCCAC GCCGTAAACGATGTCG ACTAGCCGTTGGGCTCC TTGAGAGCTTAGTGGCG CAGCTAACGCATTAAGT CGACCGCCTGGGGAGT ACGGCCGCAAGGTTAA AACTCAAATGAATTGA CGGGGGCCCGCACAAG CGGTGGAGCATGTGGTT TAATTCGAAGCAACGC GAAGAACCTTACCTGG CCTTGACATCCTGCGAA CTTTCAAGAGATTGATG GGTGCCTTCGGGAGCG CAGAGACAGGTGCTGC ATGGCTGTCGTCAGCTC GTGTCGTGAGATGTTGG GTTAAGTCCCGTAACGA GCGCAACCCTTGTCCTT AGTTACCAGCGATTCGG TCGGGCACTCTAAGGA GACTGCCGGTGACAAA CCGGAGGAAGGTGGGG ATGACGTCAAGTCATCA TGGCCCTTACGGCCAGG GCTACACACGTGCTACA ATGGTCGGTACAAAGG GTTGCCAA							

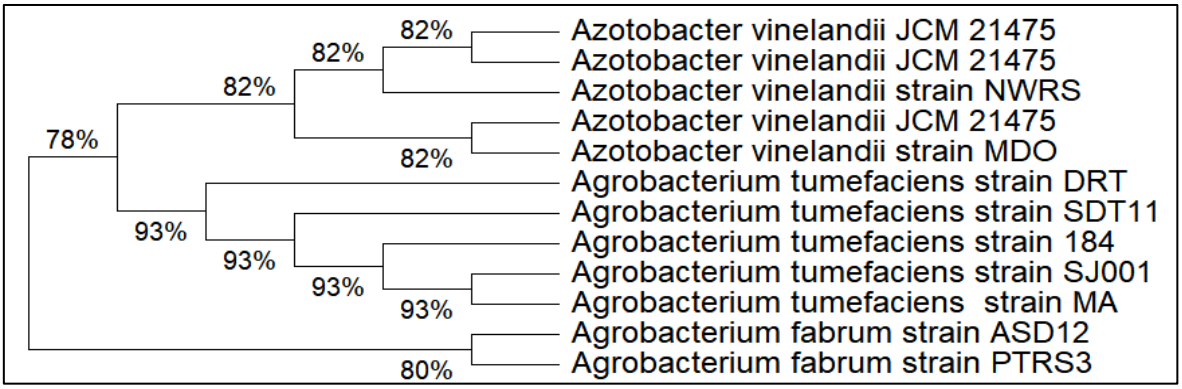

Fig 1 Phylogenetic Tree Showing the Evolutionary Relationship Between the Isolated Bacteria with Other Bacterial Sequences Retrieved from NCBI Data Base. The Tree was Constructed Using the Maximum Likelihood Method with 100 Bootstrap Replications.

IV. DISCUSSION

This study examined the isolation and identification of *Azotobacter* and *Agrobacterium* species from legume and cereal farm soils, given the significant role of soil bacteria in the microbial production of curdlan (Verma *et al*., 2020) and alginates (Bouzenad *et al*., 2025). *Agrobacterium* sp. ATCC 31749 is the main producer of exopolysaccharide curdlan on a large scale (Laxmi *et al*., 2018), and curdlan is produced by a secondary metabolic process that takes place outside of the bacterial cell (Al-Rumaidh *et al*., 2023). Similarly, alginate, is mainly isolated from the cell walls of brown algae (Phaeophyceae) (Bouzenad *et al*., 2025).

The *Agrobacterium* species in this study were isolated from soybean, cowpea, and groundnut fields and screened using aneline blue mannitol glutamate ragar (aMGA), or glutamate agar (Table 1). Laxmi *et al.* (2018) previously used this strategy to isolate fifty *Agrobacterium* spp. from root nodules that were collected from various locations in Coimbatore and Palakkad and screened using the Aniline Blue technique for the production of curdlan. Curdlan producing microbes have also been isolated from a range of

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sources, including soil, pea plant nodules, groundnut nodules, butterfly pea, Ipil-Ipil, moong bean, soybean, cowpea, black gram, green gram, soil, and wet soil (Wang *et al*., 2016; Cai *et al.,* 2020). These sources are consistent with the sources of *Azotobacter* spp. and *Agrobacterium* spp. in this study. Additionally, *Agrobacterium* showed blue coloration with varied intensities on aneline blue mannitol glutamate agar (aMGA) (Table 1), which is consistent with the results of Laxmi *et al*. (2018). Arguably, the differences in the intensity of the blue coloration observed on aniline blue mannitol glutamate agar (aMGA), as well as the growth characteristics of the five *Agrobacterium* species on aMGA and the two *Azotobacter* species on Jensen nitrogen-free agar (Table 4.1), may be attributed to their distinct sources of isolation.

Results of biochemical tests revealed that *Agrobaterium* and *Azotobacter* spp. appeared as Gram negative rods. *Agrobaterium* spp were KOH sensitive, urease, catalase, and citrate positive, whereas *Azotobacter* spp were positive for Methly red, indole, Catalase, urease, citrate utilization, starch hydrolysis, and hydrogen sulphide (Table 2). These results are consistent and comparable with the findings published by Al-Rumaidh *et al*. (2023) and Vatankhah *et al.* (2022). Ferdous *et al.* (2021) reported that *Agrobacterium tumefaciens* isolates were discovered to be Gram negative, motile, and to have spherical, viscous, creamy white colonies with regular borders. They also showed that these bacteria were negative for H_2S gas production but positive for catalase and oxidase, 3-ketolactose synthesis, and ferments carbohydrates which aligns with findings of this study.

Furthermore, the gel electrophoresis image of the molecular identification result based on 16SrRNA sequencing revealed a band of 1500 bp product of the 16SrRNA gene of *Agrobacterium* spp (Plate 1). The use of 16SrRNA sequencing for the identification of isolates of bacteria that produce curdlan and alginate in this study is supported by Laxmi *et al.* (2018), Moselhy *et al.* (2020), Aquinas *et al*. (2021), and Bouzenad *et al.* (2025). The use of 16SrRNA sequencing in this study confirmed the presence of *Agrobacterium tumefaciens*, and *Azotobacter vinelandii* (Figure 1). In line with this finding, in addition to *Alcaligenes faecalis*, the most frequently used type of *Agrobacterium* sp., a non-pathogenic, rod-shaped, gram negative bacterium found in the nodules of two leguminous plants (pea and groundnut) was reported to also produces curdlan (Laxmi *et al*. 2018). Other bacteria that have been reported to produce curdlan include *Cellulomonas flavigena* KU, which was isolated from leaf litter and used extra glucose and other carbon sources, and *Rhizobium sp*., a spore forming *Bacillus* sp. that was obtained from the soil (Aquinas *et al*., 2021). Additionally, while *Macrocystis pyrifera* is one of the most important species of brown algae from which alginate is derived, from an industrial perspective, *Ascophyllum nodosum*, *Macrocystis pyrifera*, and *Laminaria hyperborea* are the most significant alginate-producing species (Peteiro, 2018). The most important bacterial species for the synthesis of alginate are *Pseudomonas aeruginosa* and *Azotobacter vinelandii* because they can produce enormous quantities of this biopolymer as an excretion polysaccharide in their biofilm (Hurtado *et al.*, 2022). Notably, alginate extraction from brown algae has historically been linked to industrial production; however, microbial alginate production using *A. vinelandii* and *Pseudomonas* species is also attracting industrial interest, and the capacity of this microbial production to produce alginates with stable and particular properties while utilizing inexpensive carbon sources, such as carbon rich industrial waste, is making it more and more appealing (Wang *et al.,* 2023).

V. CONCLUSION

This study adds to growing literature on the isolation and identification of *Agrobacterium* and *Azotobacter* species capable of producing Curdlan and Alginate biopolymers in Nigeria. The study confirmed the isolation of *Agrobacterium* and *Azotobacter* species from different legumes and cereal farm in localities within Girie LGA, Adamawa State. The identified species capable of producing Curdlan and Alginate biopolymers include *Agrobacterium tumefaciens*, and *Azotobacter vinelandii*.

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- *Authors' Contributions*

Doughari, J.H. designed the study. Mbahi, M.A carried out the laboratory work and analyzed the data. All authors wrote, read and approved the final version of the manuscript.

- *Conflict of Interest*

The authors declare that there is no conflict of interests.

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