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MOLECULAR CHARACTERIZATION OF *Xanthomonas axonopodis pv. vignicola* MORPHOTYPES ISOLATED FROM COWPEA GROWN IN THREE AGROECOLOGICAL ZONES IN NIGERIA

Oguntade O.[1,2]., D. B. Olufolaji[2], V. O. Oyetayo[2], A. A. Onasanya[3], and M. A. Ayodele[1]
[1]Germplasm Health Unit, IITA, P.M.B 5320, Oyo road. Ibadan Nigeria, [2]Department of Crop, Soil and Pest Management, Federal University of Technology Akure P.M.B. 704 Akure, Ondo State Nigeria, [3]Department of Chemical Science, Afe Babalola University Ado-Ekiti PMB 5454, Ado-Ekiti, Nigeria
Corresponding author: o.oguntade@cgiar.org

SUMMARY

Cowpea (*Vigna unguiculata* (L.) Walp) is a drought-tolerant crop and a vital food legume in the tropical Savannah zones of Africa. It is a preferred staple food due to its high protein and digestible carbohydrate content, along with essential micronutrients. However, the production of cowpea is hindered by pests and diseases, including bacterial blight caused by *Xanthomonas axonopodis pv vignicola (Xav)*. This study employed molecular characterization to confirm the presence of different morphotypes and distinguish between isolates of *Xav* collected from cowpea samples in 81 locations across 9 states in Nigeria, covering 5 agro-ecological zones. The isolates were classified into two groups based on their morphological characteristics on nutrient agar medium, varying in color from light yellow to brownish yellow. Genomic DNA extraction was carried out on all the *Xav* isolates, involving centrifugation, resuspension, incubation, phenol-chloroform-isoamyl alcohol extraction, ethanol precipitation, and resuspension. DNA concentration was measured using a DU-65UV spectrophotometer, and quality was checked via electrophoresis on a 1% agarose gel. Polymerase chain reaction (PCR) analysis using forward and reverse primers was carried out on the *Xav* isolates genomic DNA. The amplified DNA PCR products of *Xav* morphotype, was extracted from low melting agarose gel and sequenced using ABI 3130 DNA Sequencer at INQABA Biotec International. Phylogenetic analysis of the obtained sequences confirmed differences between the two morphotypes, validating the results from conventional identification methods. These findings suggest that the presence of both types of isolates, as confirmed through conventional and molecular characterization, is necessary for the manifestation of typical symptoms of bacterial blight in cowpea.

Keywords: Morphotypes, DNA extraction, Polymerase chain reaction (PCR), Bacteria, Legume

Cowpea (*Vigna unguiculata* (L.) Walp) is a tropical grain legume which plays an important nutritional role in developing countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, Central and South America (5, 15, 17). Cowpea has been referred to as "poor man's meat" because of its high protein content in leaves (23–40%), pods (18–25%), and grains (23–29%) (15, 32). Cowpea young leaves, pods and peas contain vitamins and minerals which have fueled its usage for

human consumption and animal feeding (30, 22, 17). Approximately 30 countries cultivate cowpea, Nigeria is the largest producer and consumer of cowpeas and accounts for *61% of the production in Africa* and 58% globally (12). It forms a primary source of income for small scale farmers in developing countries (5, 17, 32).

Despite its importance, the production of cowpea is low in sub-Saharan regions compared to other growing regions and does not meet the need of consumers (10, 15, 17). The low yield is the result of poor soil, high cost of chemical fertilizers and more importantly pest and diseases (**25,** 40, 1, 17). There are about 12 major diseases of cowpea (2) and two of which are bacterial diseases (bacterial blight and pustule).The most important of these two is bacterial blight caused by *Xanthomonas axonopodis pv vignicola* (Burkholder) formerly *X. campestris pv. vignicola* (28, 27) because the distribution of bacterial pustule is restricted (25).

Cowpea bacterial blight (*X. axonopodis* pv *vignicola*) is a devastating disease of cowpea in Nigeria (26, 15) resulting in 3-100% yield loss depending on the strain, cultivars and stage of growth at the time of infection (26). It is transmitted through the seeds, wind-driven rain and crop debris (26, 15). Symptoms appears as tiny water-soaked translucent spots visible from the abaxial surface of the leaves (7). These spots enlarge and coalesce into a large necrotic spot with yellow halo resulting in leaf drop prematurely (7). The pods are also attacked producing a dark-green water-soaked resulting in discolored and shriveled seeds (7), seed mortality, stem cankers, bushy and stunted growth, leaf and pod blight (25; 24).

The development of resistant cultivars to cowpea bacterial blight would be most attractive to farmers as it is cheaper and sustainable in the long run compared to other management options (3, 15). Cowpea resistance to *X. axonopodis* pv *vignicola* exist (25, 3, 24, 7) but this largely depends on the strains of the bacterium prevalent in the area (3, 26, 7). Accurate identification is the first step for an effective management option. Information on the identity of strain peculiar to an agro- ecological zone is critical to efforts aimed at screening cowpea cultivars for resistance as this depend on isolates/strains prevalent (27). Virulence (34, 24, 3, 13, 8) morphological characteristics, biochemical and antibiotic sensitivity tests (27) were used to characterize the *X. axonopodis* pv. *vignicola* strains from different agro-ecologies in Nigeria. Information on molecular characterization of *X. axonopodis* pv. *vignicola* is limited, hence the present study which was a continuation of the work of Oguntade *et al*. (27) to molecularly identify the two morpho-types of *X. axonopodis* pv. *vignicola* reported from three agro-ecological zones of Nigeria where cowpea are commonly grown.

MATERIALS AND METHODS

Sample collection site

Diseased leaves were collected from susceptible lines of cowpea with typical bacterial blight symptoms on farmers' fields in 81 locations (3 farms from each Local Government Area) from 9

states in three agro-ecological zones of Nigeria: Guinea savanna (Kano, Kaduna, Niger), humid forest (Cross River, Ebonyi, Edo state) and rain forest (Oyo, Ogun, Ondo) in Nigeria were visited (Plate 1). Three Local Government Area (LGA) per state were surveyed for bacterial blight symptomatic leaves from three farmers' fields per LGA collected randomly on 1 - 2 months old cowpea plants. Sample collected (leaves) were wrapped in used

newspapers and labelled with the farm sites, geographical coordinate, and date of sampling and placed in a box.

Isolation of bacterial blight pathogen

The leaves from each lot from the different location was surface sterilized in 5% NaOCl (20). The leaf was rinsed in three exchanges of sterile distilled water and blotted dry with sterile paper towels. Tissue segments of about $5mm^2$ were excised from advancing lesion margin on symptomatic cowpea leaves. The tissue segments were teased in a few drops of sterile distilled water (SDW) and allowed to stand for 2 mins in a lamina airflow chamber. A loopful of the resulting suspension was streaked onto plates of nutrient agar (NA), incubated for 48 – 72 h at 25 – 28oC, and observed for colony growth.

Presumptive colonies of the pathogen (pale to strongly yellow, mucoid colonies) were purified by sub-culturing single colonies following procedures described by (20). One colony of the purified presumptive pathogen from each sample was selected and retained on NA slants for further tests.

Bacterial Isolate, Morphology, and Propagation

The 162 isolates of *X. axonopodis* pv. *vignicola* (Xav) used for this study were obtained from 81 locations in nine States in Nigeria. Subsequently, the isolates were narrowed down to 40 after their identities were confirmed. The confirmation process involved grouping and selecting the isolates based on agro-ecological areas, with a focus on prioritizing locations known for substantial production (Table 1). The identities of each isolate were verified through biochemical and selective media techniques as detailed in the work of Oguntade *et al*. (27). The isolates were stored in freezer for future research use. The isolates were propagated using a modified procedure developed by Onasanya *et al*. (23). Nutrient broth (75 ml; pH 7.5) was prepared inside a 250 ml conical flask. Each isolate (200 µl) from storage was transferred into 75 ml of nutrient broth and kept under constant shaking at 30°C for 24 hours for bacterial growth. The bacterial cells were removed by centrifugation, washed with 0.1 mM Tris-EDTA (pH 8.0) and kept at -20°C for DNA extraction.

Genomic DNA Extraction from Bacteria Culture

Genomic DNA extraction method used was according to Onasanya *et al*. (23). The procedure was as follows: 600 µl of each bacterial isolate culture was centrifuged down at 14,000 rpm inside 1.5 ml Eppendorf tube, then re-suspended in 600 µl of 2xCTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethylammonium bromide; 0.1% 2-mercaptoethanol), and incubation at 65°C for 30 mins. DNA was purified with phenol: chloroform: isoamyl alcohol (24:25:1) and precipitated with absolute ethanol at -20°C. After which, it was washed with 70% ethanol, the DNA was dried at room temp for 30 mins and resuspended in 200 µl of sterile distilled water. After which DNA concentration was measured using DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerto CA, USA) at 260 nm. DNA degradation and quality was checked by electrophoresis on a 1% agarose gel in 1xTAE (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).

Polymerase Chain Reaction analysis

The PCR reaction mixture contained 2.5 µL of 5x reaction buffer, 1.5 mM MgCl2, 0.3 U of Taq DNA polymerase, 0.2 mM dNTPs mix (New England Biolabs, USA), 0.1 µM of each oligonucleotides targeting 16s rRNA (IDT, Belgium) and 2 µL of diluted 1:50 (v/v) (100 ng/µL) total nucleic acid extract. Oligonucleotides used for nucleic acid amplification were 27F:

AGAGTTTGATCMTGGCTCAG and 1525R: AAGGAGGTGWTCCARCC. The thermal cycling condition consisted of initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1.5 mins; and final extension at 72 °C for 3 mins. PCR product was resolved in 2% agarose gel, pre-stained with EZ-Vision Bluelight DNA Dye (VWR, USA) and visualized using GelDoc (Biorad, USA).

Electrophoresis of DNA on agarose gels

The qualities of genomic DNA were assessed on 1 % agarose gel. Agarose was melted in 1x TAE buffer in a microwave oven. It was allowed to cool to about 60 °C and then poured into already balanced gel trays with gel combs of appropriate sizes 2 x 1.5mm. After it had solidified, it was placed in the gel tank containing the same buffer as was used for preparing the gel. A 1/10 of the sample volume of 6X loading dye was added to give the sample weight and to track the movement of the loaded samples. Lambda DNA digested with Pst 1 at a concentration of 0.2ug/µl was used as molecular weight marker. The gels were run for 1 - 1.5hrs at 50 – 100 volts depending on gel size. Gels were stained in 5 ug/µl ethidium bromide solution for 20 secs; de-stained in water for about 10 - 15 mins and the gels were documented under UV light using a UVP/ white trans-illuminator.

Extraction of DNA fragment from agarose gel

A thin slice of gel harboring the amplified PCR product corresponding to the expected size was excised on UV light box and DNA was extracted from the gel using the Quiaex 11 gel extraction kit (Quiagen, USA). The procedure is as follows: The gel slice was put into 1.5ml Eppendorf tube, 300µl of QXI buffer to dissolve the gel: the tube was vortexed for 2 minutes and then incubated at 50°C for 10 minutes. The tube was centrifuged at full speed in an Eppendorf microcentrifuge model 5714 C, the supernatant was discarded after which 500µl of QXI buffer was added. The contents of the tube were vortexed and centrifuged to remove the gel. Then 50µl PE buffer was added twice and the mixture was later centrifuged at full speed. After this step, a pellet was observed and the pellets was later left to dry and afterwards dissolved in 20µl of sterile water and kept in -20°C freezer.

DNA Sequencing of Purified DNA fragment

DNA Sequencing was done at Inqaba Biotech., Ltd Ibadan using ABI 3130 sequencer. The sequencing reaction was done by using a big dye terminator ready-mix, 5X sequencing buffer, 5 pmol/µl primer and gel purified DAN in a 10ul mix. Products were later purified, denatured and put inside the genetic analyser. Authenticating Transposable Elements using database searches/sequence analysis. The sequences of the isolates were viewed using Chromas version 3.7, to assess the quality of the sequence. A good sequence was identified based on the sharpness and how distinct the peak is for each of the four nucleotides represented by color codes. The sequences were aligned using the MegAlign program of Molecular Evolution Genetic Analysis (MEGA) software (39) and QALIGN software (33) to get a consensus sequence. The nature of cloned sequences was confirmed by performing computer based similarity searches against the GenBank non redundant database (Altschul *et al.,* 1990) using Basic Local Alignment Tool (BLAST) and BLASTN, BLASTX algorithms at the National Center for Biotechnology Information (NCBI). This was done to detect the homologous sequences and thereby characterize the TEs in cowpea. Sequences from the different clones of same TEs were later compared

with each other using the programs such as QAlign and MEGA versions 4 (39*)* which made use of the relaxed dissimilarity algorithm of CLUSTAL W (16). The phylogeny of the clones and other sequences were obtained using Neighbor Joining program and Tree View (29). Estimates of divergence were also obtained from the MEGA software. Open reading frame (ORF) finder was used to identify ORFs while the BANKIT program was used to submit the sequences to the NCBI database.

Cluster analysis of *Xav* morphology data

The morphology data were converted into binary character matrix for different colours on the culture media. This was done manually. Although lots of different statistical software are available for use in cluster analysis, NTSYS-pc software was used for this genetic cluster analysis. Using the NTSYS-pc, the transformed binary character matrix data was first transferred into the software data collection module from which pairwise distance matrices was used to compile (31) the Jaccard coefficient of similarity (18). Using the output data and the graphical module of the software, a phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis (9, 38).

RESULTS

Isolation of *Xav isolates* and Morphotypes

The selected isolates of *Xav* (Table 1) from cowpea leaf samples obtained from the different locations from 9 states (Kano, Kaduna, Niger, Oyo, Ogun, Ondo, Cross River, Ebonyi and Delta states) in Nigeria produced two colors (light yellow and deep/brownish yellow) on Nutrient agar medium (Plate 1). However, cluster analysis of *Xav* isolates colour numeric data revealed two morphotypes characters among the forty *Xav* isolates (*XavM1* and *XavM2*) (Figure 1).

DNA Sequencing and Sequence Analyses

Sequencing analyses of Xav isolates produced two contingent DNA sequences, *Xav-Seq1 and Xav-Seq2, with corresponding* 859 bp and 949 bp total length respectively (Tables 2 and 3). The *Xav-Seq1* sequence formed the *Xav* isolates in *XavM1* morphotype while *Xav-Seq2* sequence constituted the *XavM2* morphotype.

BLAST homology search using nucleotide sequences of *Xav-Seq1 and Xav-Seq2* gave some significant alignments, at 80.90% and 80.81% nucleotide identity respectively with *Xanthomonas citri* pv. *vignicola* complete genome (Figures 2a and 2b). The *Xav-Seq1* nucleotide sequence was unique at 19.1 % and *Xav-Seq2 at 19.09 % when* compared with other *Xanthomonas sp* nucleotide sequences on the NCBI database (Figure 2). BLAST homology search using translated amino acid sequence from *Xav-Seq1 and Xav-Seq2* nucleotide sequence produced significant alignments at between 92.42 - 93.83% with known virulence gene proteins which include hypothetical protein, histone deacetylase, conserved protein, and conserved hypothetical protein (Figures 3a and 3b).

Relationship among *Xav-Seq1 and Xav-Seq2* nucleotide sequences and other known *Xav* virulence genes has been revealed by BLAST homology nucleotide sequence alignment unweighted pair-group method arithmetic (UPGMA) analysis (Figure 4a - 4d). The *Xav-Seq1* and *Xav-Seq2* nucleotide sequence phylogenetic relationship with other known *Xav* nucleotide sequences revealed *Xav-Seq1 and Xav-Seq2* nucleotide sequences were different from each other as well as from

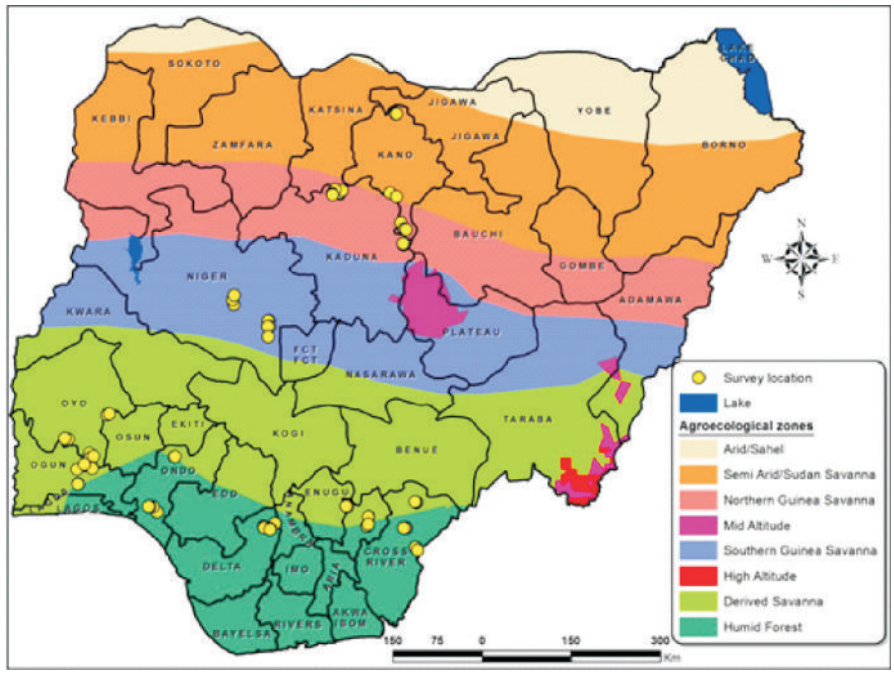

Plate 1. Map showing collection sites in different Agro-ecological zones in Nigeria.

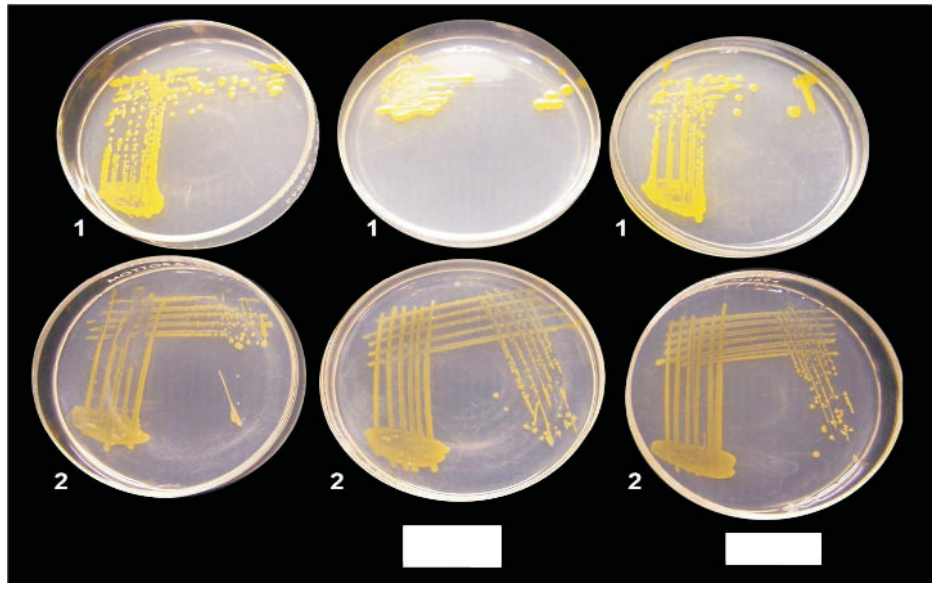

Plate 2. The **forty *Xav* isolates produced two colors (light yellow to deep/brownish yellow) on Nutrient agar medium.**

Table 1. Identity of selected *Xanthomonas axonopodis* pv. *vignicola* isolates used for the study.

S/N	Isolates Code	Host Plant	Location	Local Government	State
1	Xav-1	cowpea	Podo village Farm 1	Oluyole	Oyo
2	Xav-2	cowpea	Idi Ayunre village Farm 2	Oluyole	Oyo
3	Xav-3	cowpea	Arowojeka village Farm 3	Oluyole	Oyo
4	Xav-4	cowpea	Dagilegbo village Farm 1	Ibarapa	Oyo
5	Xav-5	cowpea	Idi- Ata village Farm 2	Ibarapa	Oyo
6	Xav-6	cowpea	Kara Ajeyo- Okolo village Farm 3	Ibarapa	Oyo
7	Xav-7	cowpea	Igbotu village Farm 1	Ese Odo	Ondo
8	Xav-8	cowpea	Igbobini village Farm 2	Ese Odo	Ondo
9	Xav-9	cowpea	Igbobini village Farm 3	Ese Odo	Ondo
10	Xav-10	cowpea	Ojokodo village Farm 1	Okitipupa	Ondo
11	Xav-11	cowpea	Okitipupa Farm 2	Okitipupa	Ondo
12	Xav-12	cowpea	Okitipupa Farm 3	Okitipupa	Ondo
13	Xav-13	cowpea	Ekonde village Farm 1	Ikom	Cross River
14	Xav-14	cowpea	Ekonde village Farm 2	Ikom	Cross River
15	Xav-15	cowpea	Ekonde village Farm 3	Ikom	Cross River
16	Xav-16	cowpea	Ajassor village Farm 1	Etung	Cross River
17	Xav-17	cowpea	Ajassor village Farm 2	Etung	Cross River
18	Xav-18	cowpea	Ajassor village Farm 3	Etung	Cross River
19	Xav-19	cowpea	Onoja village Farm 1	Ishelu	Ebonyi
20	Xav-20	cowpea	Onu- Nwafor village Farm 2	Ishelu	Ebonyi
21	Xav-21	cowpea	Ugbo - Anyim village Farm 3	Ishelu	Ebonyi
22	Xav-22	cowpea	Oroke - Onuoha village Farm 1	Ebonyi	Ebonyi
23	Xav-23	cowpea	Oroke - Onuoha village Farm 2	Ebonyi	Ebonyi
24	Xav-24	cowpea	Oroke - Onuoha village Farm 3	Ebonyi	Ebonyi
25	Xav-25	cowpea	Sasakawa-Kanti village Farm 1	Danbarta	Kano
26	Xav-26	cowpea	Sasakawa-Kanti village Farm 3	Danbarta	Kano
27	Xav-27	cowpea	Hayin yawa village Farm 2	Tudu Wada	Kano
28	Xav-28	cowpea	Hayin Kano Sabowa village Farm 1	Dogwa	Kano
29	Xav-29	cowpea	Sabon Gari Dadin Kowa village Farm 2	Dogwa	Kano
30	Xav-30	cowpea	Sabon Gar Dadin Kowa village Farm 3	Dogwa	Kano
31	Xav-31	cowpea	Huguru village Farm 3	Tudu Wada	Kano
32	Xav-32	cowpea	Sasakawa-Kanti village Farm 2	Danbarta	Kano
33	Xav-33	cowpea	Jaja village Farm 1	Kudan	Kaduna
34	Xav-34	cowpea	Jaja village Farm 2	Kudan	Kaduna
35	Xav-35	cowpea	Zabi village Farm 3	Kudan	Kaduna
36	Xav-36	cowpea	Mararaba-Guga Farm 2	Giwa	Kaduna
37	Xav-37	cowpea	Kwanan- Bakoshi- Jorde village Farm 1	Lere	Kaduna
38	Xav-38	cowpea	Girka - Kampah village Farm 2	Lere	Kaduna
39	Xav-39	cowpea	Hanyan - Jirgi village Farm 3	Lere	Kaduna
40	Xav-40	cowpea	Guda Village Farm 3	Giwa	Kaduna

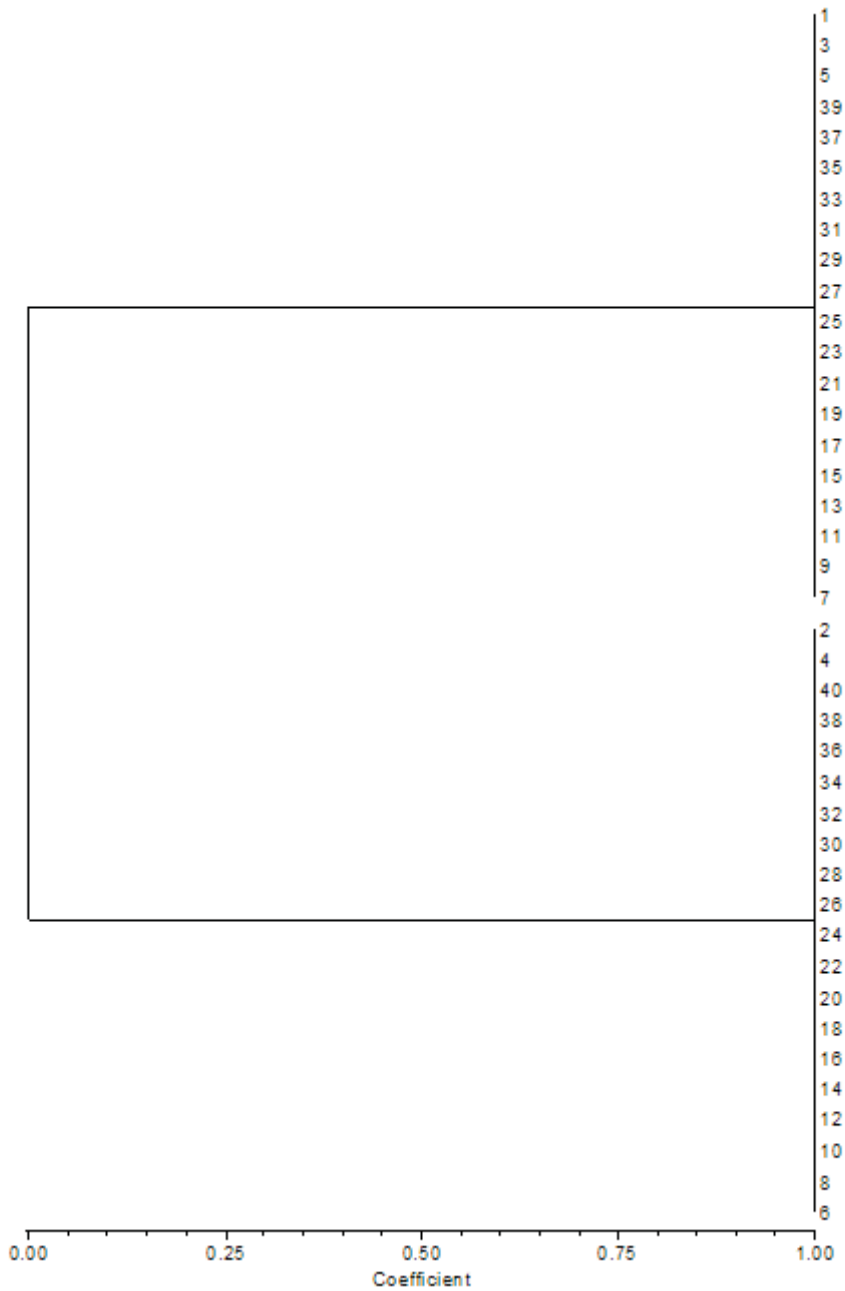

Figure 1. Cluster showing two morphotypes (*XavM1* and *XavM2*) among the forty *Xav* isolates.

Figure 2. *Xanthomonas axonopodis* pv. *vignicola* isolate *Xav-Seq1* nucleotide sequence and features.

Sequence type: DNA
Length: 859bp circular
Organism: *Xanthomonas axonopodis* pv. *vignicola*
Name: Xav-Seq1
Weight (single-stranded): 267.256 kDa
Weight (double-stranded): 530.807 kDa
LOCUS: Xav-Seq1 859 bp DNA linear

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   1 AATATGCCCT TCTCTACGGA AT  AGTCCTGG GAAACTGGGG GTAATACCGT ATACGCCCTT
  61 TGGGGGAAAG ATTTATCGGG GAAGGATTAG CCCGCGTTGG ATTAGGTAGT TGGTGGGGTA
 121 ATGGCCTACC AAGCCGACGA TCCATAGCTG GTTTGAGAGG ATGATCAGCC ACACTGGGAC
 181 TGAGACACGG CCCAGACTCC TACGGGAGGC AGCAGTGGGG AATCTTA  GAC AATGGGGGCA
 241 ACCCTGATCT AGCCATGCCG CGTGAGTGAT GAAGGCCTTA GGGTTGTAAA GCTCTTTCAG
 301 CTGGGAAGAT AATGACGGTA CCAGCAGAAG AAGCCCCGGC TAACTCCGTG CCAGCAGCCG
 361 CGGTAATACG GAGGGGGCTA GCGTTGTTCG GAATTACTGG GCGTAAAGCG CACGTAGGCG
 421 GATCGGAAAG TTGGGGGTGA AATCCCGGGG CTCAACCTCG GAACTGCCTT CAAAACTACT
 481 GGTCTTGAGT TCGAGAGAGG TGAGTGGAAT TCCGAGTGTA GAGGTGAAAT TCGTAGATAT
 541 TCGGAGGAAC ACCAGTGGCG AAGGCGGCTC ACTGGCTCGA TACTGACGCT GAGGTGCGAA
 601 AGCGTGGGGA GCAAACAGGA TTAGATACC C TGGTAGTCCA CGCCGTAAAC GATGAATGCC
 661 AGTCGTCGGG TTGCATGCAA TTCGGTGACA CACCTAACGG ATTAAGCATT CCGCCTGGGG
 721 AGTACGGTCG CAAGATTAAA ACTCAAAGGA ATTGACGGGG GGCCCGCACA AAGCGGTGGA
 781 GCATGTGGTT TAATTCGAAG CAACGCGCAG AACCTTACCA ACCCTTTGAC ATCC  CAGGAC
 841 CGCCCGAGAG ATCGGGTTT
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Figure 3. *Xanthomonas axonopodis* pv. *vignicola* isolate *Xav-Seq2* nucleotide sequence and features.

Sequence type: DNA
Length: 949bp circular
Organism: *Xanthomonas axonopodis* pv. *vignicola*
Weight (single-stranded): 294.508 kDa
Weight (double-stranded): 586.44 kDa
LOCUS: Xav-Seq2 949 bp DNA circular

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   1 AGATAATTGA CCGGTTACCC AGCCAGAAGA AAGCCCCCGG GCTAAACTCC CGTGCCAGCA
  61 GCCGCGGTAA TACGGAGGGG GGCTAGCGTT GTTCGGGAAT TACTGGGCGT AAAGCGCACG
 121 TAGGCGGATC GGAAAGTTGG GGGGTGAAAT CCCGGGGCTC AACCTCGGAA CTGCCTTCAA
 181 AACTACTGGT CTTGAGTTCG AGAGAGGTGA GTGGAATTCC GAGTGTAGAG GTGAAATTCG
 241 TAGATATTCG GAGGAACACC CAGTGGCGAA GGCGGCTCAC TGGCTCGATA CTGACGCTGA
 301 GGTGCGAAAG CGTGGGGAGC AAACAGGATT AGATACCCTG GTAGTCCACG CCGTAAACGA
 361 TGAATGCCAG TCGTCGGGTT GCATGCAATT CGGTGACACA CCTAACGGAT TAAGCATTCC
 421 GCCTGGGGAG TACGGTCGCA AGATTAAAAC TCAAAGGAAT TGACGGGGGC CCGCACAAGC
 481 GGTGGAGCAT GTGGTTTAAT TCGAAGCAAC GCGCAGAACC TTACCAACCC  TTGACATCCC
 541 AGGACCGCCC GAGAGATCGG GTTTCCACTT CGGTGGCCTG GAGACAGGTG CTGCATGGCT
 601 GTCGTCAGCT CGTGTCGTGA GATGTTCGGT TAAGTCCGGC AACGAGCGCA ACCCACGTCC
 661 CCAGTTGCCA GCATTCAGTT GGGCACTCTG TGGAAACTGC CGGTGATAAG CCGGAGGAAG
 721 GTGTGGATGA CGTCAAGTCC TCATGGCCCT TACGGGTTGG GCTACACACG TGCTACAATG
 781 GTGGTGACAG TGGGTTAATC CCCAAAAGCC ATCTCAGTTC GGATTGGGGT CTGCAACTCG
 841 ACCCCATGAA GTTGGAATCG CTAGTAATCG CGGAACAGCA CGCCGCGGTG AATACGTTCC
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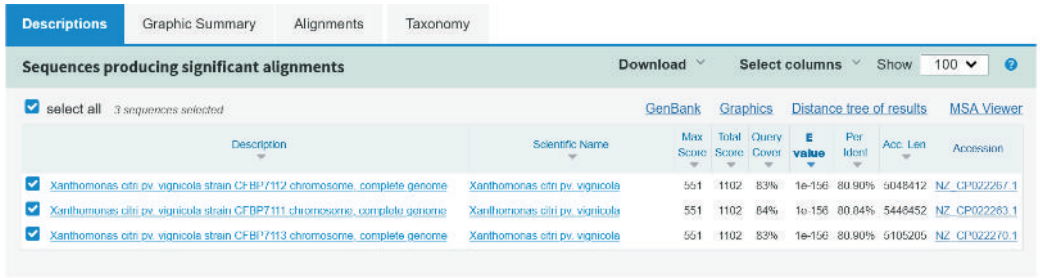


Figure 4a. BLAST analysis of *Xanthomonas axonopodis* pv. *vignicola (Xav)* isolate *Xav-Seq1* nucleotide sequence produced significant alignment with other *Xav*.

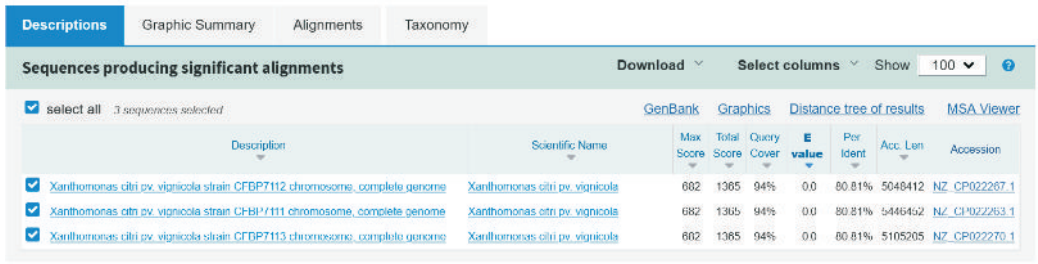
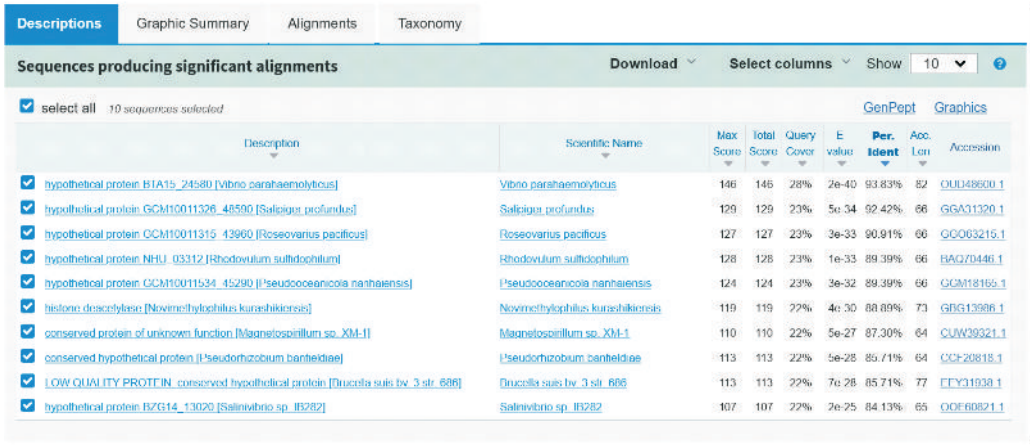


Figure 4b. BLAST analysis of *Xanthomonas axonopodis* pv. *vignicola (Xav)* isolate *Xav-Seq2* nucleotide sequence produced significant alignment with other *Xav*.



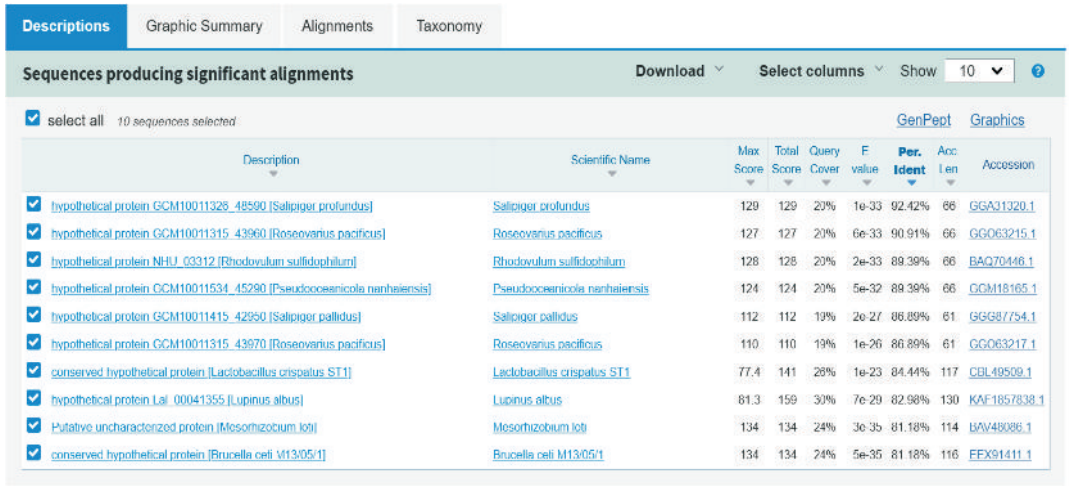
Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 10

select all 10 sequences selected — GenPept — Graphics

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
hypothetical protein BTA15_24580 [Vibrio parahaemolyticus]	Vibrio parahaemolyticus	146	146	28%	2e-40	93.83%	82	OUD48600.1
hypothetical protein GCM10011326_48590 [Saliniger profundus]	Saliniger profundus	129	129	23%	5e-34	92.42%	66	GGA31320.1
hypothetical protein GCM10011315_43960 [Roseovarius pacificus]	Roseovarius pacificus	127	127	23%	3e-33	90.91%	66	GGO63215.1
hypothetical protein NHU_03312 [Rhodovulum sulfidophilum]	Rhodovulum sulfidophilum	128	128	23%	1e-33	89.39%	66	BAQ70446.1
hypothetical protein GCM10011534_45290 [Pseudooceanicola nanhaiensis]	Pseudooceanicola nanhaiensis	124	124	23%	3e-32	89.39%	66	GGM18165.1
histone deacetylase [Novimethylophilus kurashikiensis]	Novimethylophilus kurashikiensis	119	119	22%	4e-30	88.89%	73	GBG13986.1
conserved protein of unknown function [Magnetospirillum sp. XM-1]	Magnetospirillum sp. XM-1	110	110	22%	5e-27	87.30%	64	CUW39321.1
conserved hypothetical protein [Pseudorhizobium banfieldiae]	Pseudorhizobium banfieldiae	113	113	22%	5e-28	85.71%	64	CCF20818.1
LOW QUALITY PROTEIN: conserved hypothetical protein [Brucella suis bv. 3 str. 686]	Brucella suis bv. 3 str. 686	113	113	22%	7e-28	85.71%	77	EEY31938.1
hypothetical protein BZG14_13020 [Salinivibrio sp. IB282]	Salinivibrio sp. IB282	107	107	22%	2e-25	84.13%	65	OOF60821.1

Figure 5a. BLAST analysis of *Xanthomonas axonopodis* pv. *vignicola (Xav)* isolate *Xav-Seq1* translated amino acid sequence produced significant alignment with majorly hypothetical proteins.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Select columns — Show 10

select all 10 sequences selected — GenPept — Graphics

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc Len	Accession
hypothetical protein GCM10011326_48590 [Saliniger profundus]	Saliniger profundus	129	129	20%	1e-33	92.42%	66	GGA31320.1
hypothetical protein GCM10011315_43960 [Roseovarius pacificus]	Roseovarius pacificus	127	127	20%	6e-33	90.91%	66	GGO63215.1
hypothetical protein NHU_03312 [Rhodovulum sulfidophilum]	Rhodovulum sulfidophilum	128	128	20%	2e-33	89.39%	66	BAQ70446.1
hypothetical protein GCM10011534_45290 [Pseudooceanicola nanhaiensis]	Pseudooceanicola nanhaiensis	124	124	20%	5e-32	89.39%	66	GGM18165.1
hypothetical protein GCM10011415_42950 [Saliniger pallidus]	Saliniger pallidus	112	112	19%	2e-27	86.89%	61	GGG87754.1
hypothetical protein GCM10011315_43970 [Roseovarius pacificus]	Roseovarius pacificus	110	110	19%	1e-26	86.89%	61	GGO63217.1
conserved hypothetical protein [Lactobacillus crispatus ST1]	Lactobacillus crispatus ST1	77.4	141	26%	1e-23	84.44%	117	CBL49509.1
hypothetical protein Lal_00041355 [Lupinus albus]	Lupinus albus	81.3	159	30%	7e-29	82.98%	130	KAF1857838.1
Putative uncharacterized protein [Mesorhizobium loti]	Mesorhizobium loti	134	134	24%	3e-35	81.18%	114	BAV48086.1
conserved hypothetical protein [Brucella ceti M13/05/1]	Brucella ceti M13/05/1	134	134	24%	5e-35	81.18%	116	EFX91411.1

Figure 5b. BLAST analysis of *Xanthomonas axonopodis* pv. *vignicola (Xav)* isolate *Xav-Seq2* translated amino acid sequence produced significant alignment with majorly hypothetical proteins.

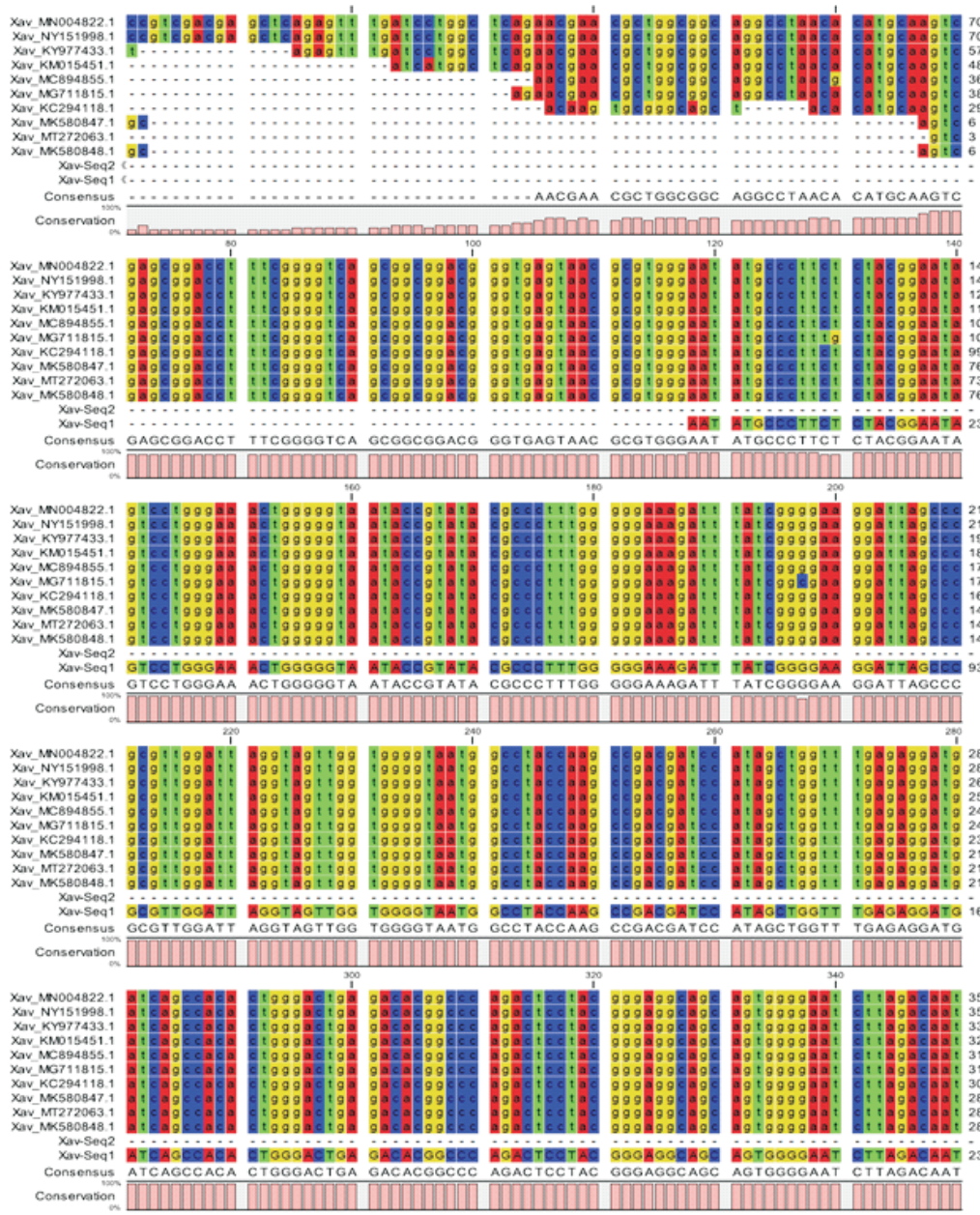


Figure 6a. *Xav-Seq1 and Xav-Seq2* isolates nucleotide sequence multiple alignments analysis.

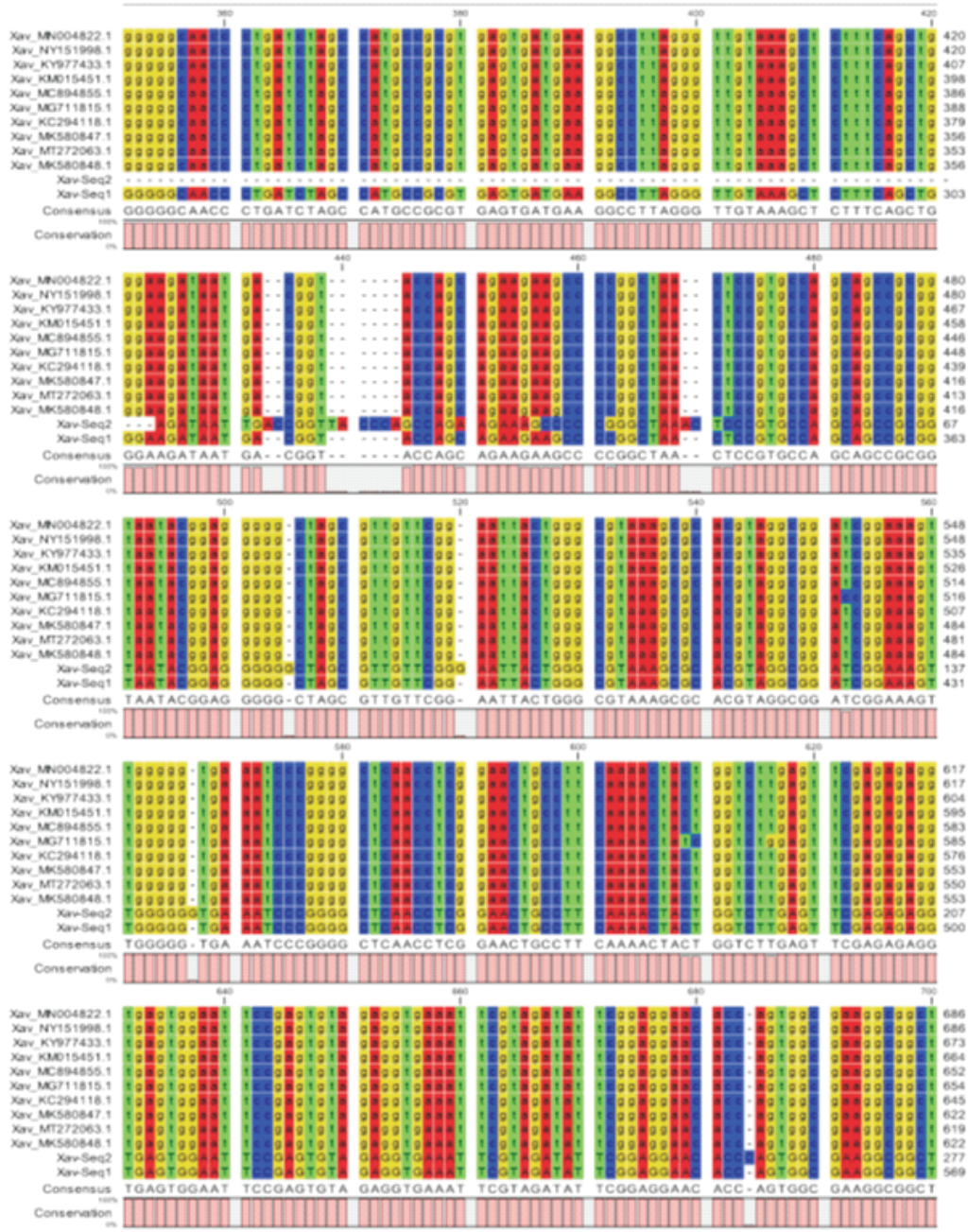


Figure 6b. *Xav-Seq1 and Xav-Seq2* isolates nucleotide sequence multiple alignments analysis.

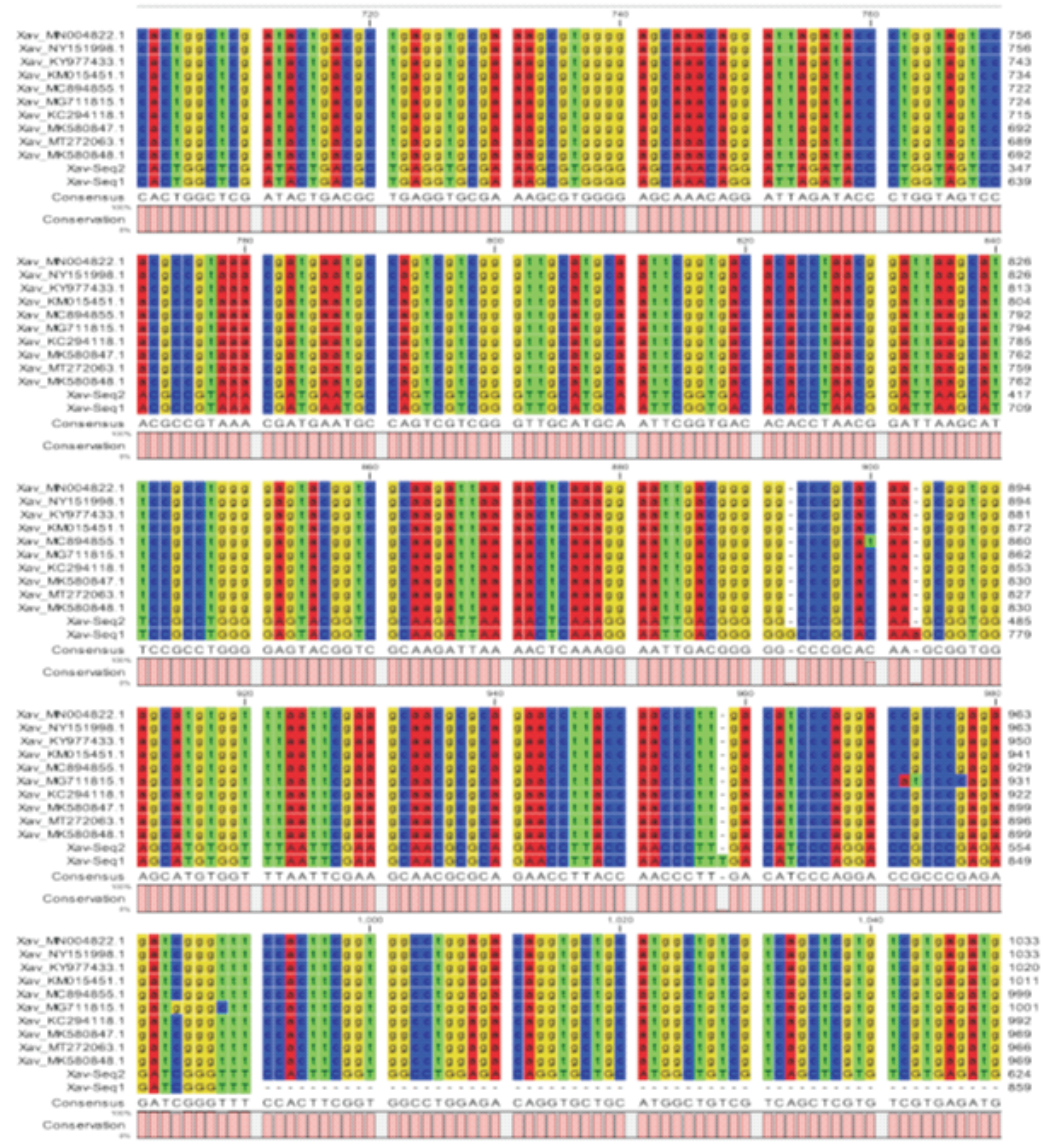


Figure 6c. *Xav-Seq1 and Xav-Seq2* isolates nucleotide sequence multiple alignments analysis.

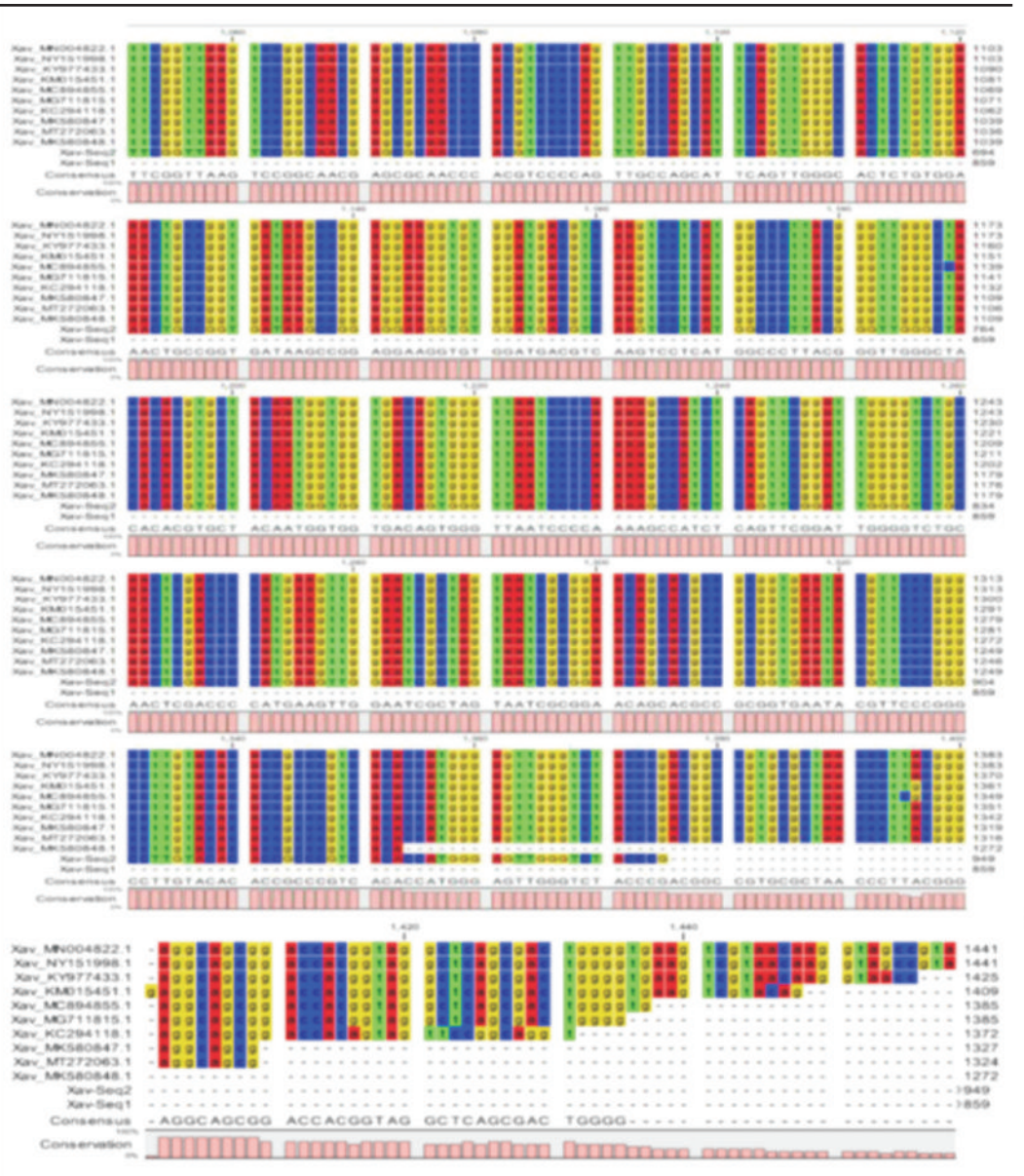


Figure 6d. *Xav-Seq1 and Xav-Seq2* isolates nucleotide sequence multiple alignments analysis.

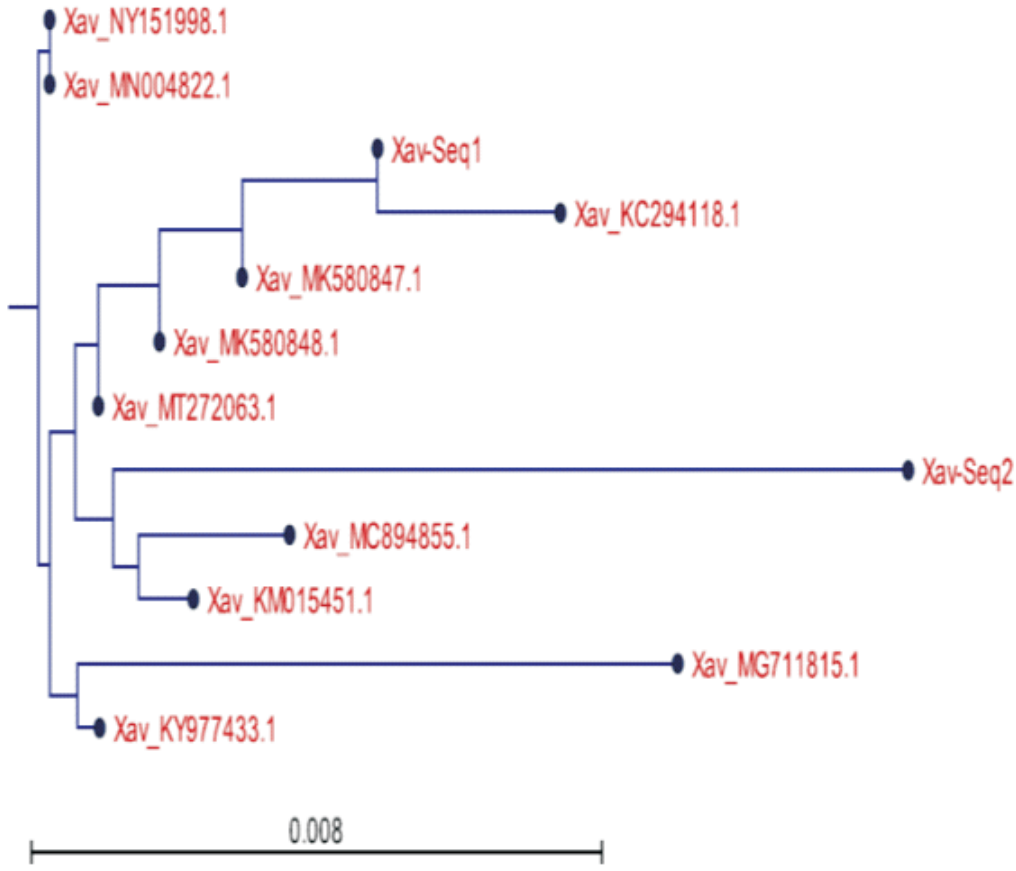


Figure 7. *Xanthomonas axonopodis* pv. *vignicola* (Xav) isolate *Xav-Seq1* and *Xav Seq2* nucleotide sequence phylogenetic relationship with other known *Xav* nucleotide sequences.

other *Xav* virulence genes obtained from NCBI database (Figure 5).

DISCUSSION

Previous study (27) has identified using morphological characteristics, biochemical and antibiotic sensitivity tests, two morphotypes of Xav which act synergistically to cause infection. These isolates need to be characterized further using molecular techniques to confirm the existence of these morphotypes. This is significant from the epidemiology point of view in order to determine the causal organism (s) of Xav in Nigeria. The DNA of the isolates were extracted and sequenced (37). The resulting nucleotide sequence was then subjected to BLAST analysis (4), which is a commonly used tool for comparing nucleotide sequences to those in public databases (19). This analysis produced significant alignment with other *Xav* isolates, indicating that the isolate was indeed *Xanthomonas axonopodis pv. vignicola* (6).

However, to gain a deeper understanding of the isolate's genetic makeup, the translated amino acid sequence was also analyzed. This analysis revealed that significant alignment was primarily found with hypothetical proteins (14). Further analysis of the Xav-Seq2 nucleotide sequence revealed circular open reading frames and multiple alignment characteristics specific to *Xanthomonas axonopodis pv. vignicola* (37).To determine the relationship between Xav-Seq2 and other Xav isolates, phylogenetic analysis was conducted (11). This involves constructing a phylogenetic tree based on the genetic differences between the isolates (21). The resulting tree can provide insights into the evolutionary relationships between the isolates and help to identify genetic markers that are unique to specific groups of isolates. By comparing the *Xav*-Seq2 sequence to other known *Xav* sequences, the researchers were able to determine its relationship with other isolates and potentially identify unique genetic markers (37, 19).

In summary, the molecular characterization of *Xanthomonas axonopodis pv. vignicola* isolate *Xav*-Seq2 involved several steps, including DNA extraction and sequencing, BLAST analysis of nucleotide and amino acid sequences, identification of circular open reading frames and multiple alignments, and phylogenetic analysis. This analysis confirmed that there are two morphotypes of *Xanthomonas axonopodis pv vignicola* combine in synergy to produce a typical symptom of bacterial blight of cowpea as established by biochemical and antibiotic sensitivity test earlier conducted (28).

CONCLUSION AND RECOMMENDATION

The study concludes that there are two distinct morphotypes of *Xanthomonas axonopodis pv vignicola* that work together to cause bacterial blight in cowpea, which is a new finding. This discovery has important implications for the control and management of bacterial blight in cowpea and other crops. The study suggests that DNA fingerprinting can be used to identify new virulent strains of the pathogen through epidemiological surveys, which can help in designing effective control measures. The recommendation is to conduct complete genome sequencing of symptomatic and asymptomatic cowpea leaves to identify the location of the morphotypes. This can enable gene manipulation techniques to control the infection, potentially using one morphotype as an endophyte while silencing the other. Overall, the study provides insights into the biology and genetics of *Xanthomonas axonopodis pv*

vignicola and suggests new strategies for managing bacterial blight in cowpea and other crops, potentially improving food security and sustainability.

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