Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

WHOLE GENOME SEQUENCING OF ENTEROBACTER CLOACAE SM32 IN THE FIRST ISOLATION OF THE MEDICAGO SATIVA L. ROOT NODULES IN MOSUL, IRAQ CAPABLE OF PRODUCING INDOLE-3-ACETIC ACID

S. M. Mohammed [1]* R. H. Sultan [1] V. Edavana [2]

[1] Biology Department, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.
[2] Director, Clinical Development, Replimune Woburn, Massachusetts, USA.

***Correspondence to:** Sara Musbah Mohammed, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq.
Email: sara.22esp4@student.uomosul.edu.iq

Article info

Received: 2024-10-11
Accepted: 2024-12-08
Published: 2025-06-30

DOI-Crossref:
10.32649/ajas.2025.186676

Cite as:
Mohammed, S. M., Sultan, R. H., and Edavana, V. (2025). Whole genome sequencing of enterobacter cloacae sm32 in the first isolation of the medicago sativa l. root nodules in mosul, iraq capable of producing indole-3-acetic acid. Anbar Journal of Agricultural Sciences, 23(1): 391-404.

Abstract

Seven rhizobacterial isolates from the root nodules of several leguminous plants were obtained from various cultural areas in Mosul, Iraq for this study. The SM21 and SM32 isolated from *Medicago sativa* L., SM27, SM30, and SM43 from *Trifolium alexandrinum* L., and SM1 and SM33 from *Vigna unguiculata* L were used to determine which could produce indole-3-acetic acid. After 3 days of incubation in an LB liquid medium, the SM32 isolate produced a maximum output of 21.43 g mL^{-1}. It reached 72.96 g mL^{-1} with an ideal tryptophan content of 3 mg mL^{-1} supporting the process. The molecular characteristics of the SM32 rhizobacteria isolate, with a total genome length of 4,583,676 base pairs, were similar to those of *E. cloacae*. 4,348 CDSs were coded for proteins and 75 RNA genes. This first version of the full genome shotgun sequence of *E. cloacae* SM32 was deposited at the DDBJ/ENA/GenBank under accession number JBEFMZ000000000 (BioProject accession number PRJNA1121377 and BioSample accession number SAMN41748806). The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) added the annotation.

Keywords: IAA production, Whole genome sequence, Rhizobacteria, Enterobacter cloacae.

تسلسل الجينوم الكامل لبكتيريا Enterobacter cloacae SM32، أول عزل من العقيدات الجذرية Medicago sativa L. في الموصل، العراق، القادرة على إنتاج حمض الإندول-3-حامض الخليك

ساره مصباح محمد [1]* رعد حساني سلطان [1] فينيث إيدافانا [2]

[1] قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق.

[2] مدير التطوير السريري، ريبليميون، ووبورن، ماساتشوستس، الولايات المتحدة الامريكية.

***المراسلة الى:** ساره مصباح محمد، قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق.

البريد الالكتروني: sara.22esp4@student.uomosul.edu.iq

الخلاصة

تم في هذه الدراسة عزل سبعة عزلات من الرايزوبكتيريا من العقد الجذرية لنباتات بقولية مختلفة تم جمعها من مناطق زراعية مختلفة في الموصل-العراق وكالاتي: SM1 وSM33 معزولتان من *Vigna unguiculata* L., SM21 وSM32 ومعزولتان من *Medicago sativa* L., SM30 وSM27 وSM43 والمعزولة من *Trifolium alexandrinum* L.. تم تقدير أنتاج الأندول-3-حامض الخليك من العزلات السبعة المحلية، كان الحد الأقصى للإنتاج 21.43 مايكروغرام/مل بواسطة العزلة SM32 بعد 3 أيام من التحضين في الوسط L.B. السائل. وصل الانتاج الى 72.96 مايكروغرام/مل باستخدام التركيز الأمثل للتريبتوفان الداعم للإنتاج 3.0 ملغم/مل. أظهر التشخيص الجزيئي لعزلة الرايزوبكتيريا SM32 أنها تمتلك الصفات الجزيئية للنوع *Enterobacter cloacae* ولها جينوم كلي بطول 4,583,676 زوج قاعدي. مجموع التسلسلات المشفرة للبروتين كانت 4,348 زوج قاعدي. وعدد جينات الحامض النووي الرايبوزي هو 75. تم أيداع الإصدار الأول من تسلسل الجينوم الكلي المستحصل بطريقة قذف الإطلاقة للعزلة *Enterobacter cloacae* SM32 في بنك الجينات DDBJ/ENA تحت رقم الانضمام JBEFMZ000000000 ورقم الوصول للمشروع الحيوي PRJNA1121377 ورقم الوصول للنموذج الحيوي SAMN41748806 وتم أضافة الهامش من قبل المركز العالمي للمعلومات الحيوية لجينوم كائنات بدائية النواة (PGAP).

كلمات مفتاحية: رايزوبكتيريا، Enterobacter cloacae، تسلسل الجينوم الكامل، أنتاج الأندول-3-حامض الخليك.

Introduction

Only a small number of nodule bacteria have been examined out of the many species and types of legumes. A distinguishing characteristic of legume plants is their capacity to form a mutualistic symbiosis for using atmospheric nitrogen as a source with bacteria known as rhizobia, which are members of the Rhizobiaceae family under Alphaproteobacteria (23). Additionally, certain Gammaproteobacteria, such as

Enterobacter, *Pseudomonas*, *Pseudoalteromonas*, *Leclercia*, and *Pantoea* have been identified from the root nodules of several legume plants (13). Therefore, the link between legumes and bacteria for nitrogen fixation may be far more ubiquitous than previously thought.

Indole-3-acetic acid (IAA) is an important plant hormone of the auxin family. It is one of the most basic and widespread hormones, naturally regulating nearly all aspects of plant growth, including cell division, elongation, development, and senescence (8).

Apart from plants, many rhizosphere bacteria species belonging to different genera can produce IAA (11, 19 and 20). They include *Acetobacter*, *Acinetobacter*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Herbaspirillum*, *Mesorhizobium*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, *Rouxiella*, *Serratia*, *Streptomyces*, *Stenotrophomonas*, and *Klebsiella*.

It is believed that bacteria that produce IAA lengthen and develop roots, thereby increasing their surface area and allowing plants to absorb greater nutrients in culture (5). A crucial mechanism for plant growth in rhizobacteria is the synthesis of IAA and the solubility of iron and phosphate (7).

Little is known about the bacterial endophytes found in the root nodules of Medicago sativa plants grown in Mosul, Iraq, that do not modulate. This study aimed to isolate and characterize the rhizobacterial endophyte extracted from the alfalfa root nodules by molecular means. In addition to genes and characteristics linked to biotic and antibiotic resistance, the genome sequence of Enterobacter cloacae SM32 is reported in this work.

Materials and Methods

Isolating the Rhizobacterial Strains: Several leguminous plants were collected from Mosul City's cultural districts and their rhizosphere and transported to the Molecular Biology Laboratory of the Department of Biology at the College of Pure Sciences. Isolation of the rhizobacterial strains was done according to (27).

Test for synthesizing indole-3-acetic acid (IAA) by Rhizobacterium SM32: The pure rhizobacterial isolates were cultivated in liquid cultures of the YEMB medium and then incubated for 24 hours at 28°C. Subsequently, 0.1 ml of the liquid culture was transferred to a conical flask holding 20 ml of liquid LB media and incubated at 150 rpm for 1, 2, 3, and 4 days. The liquid cultures that had undergone fermentation were centrifuged at 3000 rpm for thirty minutes. Two to three drops of *O*-phosphoric acid were added to two milliliters of the suspension.

Four milliliters of culture suspension were mixed with 2 ml of Salkowski solution and incubated for 30 minutes. A spectrophotometer set at 353 nm was used to measure the amount of IAA created when the color changed to red. The medium that was not rhizobacteria-inoculated was utilized as a control group. Concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µL ml^{-1} slandered IAA were prepared for the standard curve. A spectrophotometer at a wavelength of 535 nm was used to record optical densities (14).

DNA isolation: DNA isolation and the following molecular steps were conducted at the Molecular Lab Company in Mosul using a genomic DNA isolation kit from GeneEd Company. Genomic DNA was directly isolated from the root nodules of the

Rhizobacteria (SM32) which were isolated from the root nodules of leguminous plants according to the manufacturer's directions. The DNA was measured for concentration and purity and kept at -20°C until needed.

Polymerase chain reaction: PCR was carried out in a 20 µL reaction volume with Promega USA's GoTaq G2 Green Master Mix. The entire 16S rRNA gene region was amplified using the universal primers 27F AGAGTTTGATCMTGGCTCAG and 1522R AAGGAGGTGATCCARCCGCA (22). As instructed by the manufacturer, 100 ng of template DNA and 1 µM of primer concentration were added.

The 16S rRNA gene PCR program was set up as follows: 1 minute of denaturation at 95°C followed by 30 cycles of amplification, which included 30 seconds of denaturation at 95°C, 30 seconds of annealing at 55°C, and 1 minute of extension at 72°C. At 72°C, the last extension stage was chosen. On a 1% agarose gel, the PCR products were separated and stained with Midori Green Advance DNA stain. As a molecular weight marker, a 100 bp DNA marker from New England Biolabs, UK was employed.

Sequencing DNA and searching for homology: The 16S rRNA gene PCR products were purified and forwarded to the Psomagen sequencing firm (USA). Using the NCBI BLAST program, the recovered sequences were compared for similarity to published genes that had been added to GenBank.

Submissions of genomes to NCBI GenBank: Under the accession number JBEFMZ000000000, the genomic sequence of *E. cloacae* SM32 was made available at DDBJ/ENA/GenBank.

Assembly and annotation of the genome: Using the SPAdes 3.5 bioinformatics program (4), the raw reads were de novo assembled to contigs with k-mer lengths of 21, 33, 55, and 77. Assembly data were produced using the QUAST program (9). The RAST server was used to annotate the assembled genome (3). The functional genes in the subsystem categories were predicted using the SEED program (21).

Phylogenetic tree based on the entire genome: Using the Type Strain Genome Server (TYGS) (15), the phylogenetic tree of *E. cloacae* SM32 and its closest relatives was inferred based on their whole genomes. With the default parameters, the genome was uploaded to the server in FASTA format. The TYGS incorporates the FastME 2.0 tool (12) for inferring the tree.

Analysis of DNA-DNA hybridization in silico (isDDH): Based on whole genome sequence data, the isDDH values between *E. cloacae* SM32 and the most closely related strains were calculated using the GGDH bioinformatics tool (16).

Phylogenetic tree study of the 16S rRNA gene: The NCBI GenBank database's sequences and the *E. cloacae* SM32 sequence were compared for homology using the nucleotide Basic Local Alignment Search Tool (BLASTn) tool. The phylogenetic tree was created based on the MEGA-11 program and bootstrap (100X) analysis (26).

Identification of antibiotic resistance genes in *E. cloacae* SM32 genome: The Comprehensive Antibiotic Resistance Database (CARD) tool version 3.2.6 was utilized to identify the antibiotic resistance genes present in the genome of *E. cloacae* SM32 (2).

Comparison of genomes: *E. cloacae* SM32 was aligned with the closest species using the GView-GenoCat tools (25).

Results and Discussion

Local rhizobacterial isolates are isolated: Seven distinct rhizobacterial strains were successfully recovered from the root nodules of several leguminous plants after the nodules were crushed and sterilized, as indicated in Table 1. *Rhizobium* sp. Was isolated from *Mimosa pudica* root nodules by earlier researchers (24). Additionally, endophytic bacteria were recovered by researchers from peanuts (10) and soybeans (1).

Table 1: Plant hosts and isolated rhizobacteria.

Isolate No.	Plant hosts
SM1	***Vigna unguiclata* L.**
SM21	***Medicago sativa* L.**
SM27	***Trifolium alexandrinum* L.**
SM30	***Trifolium alexandrinum* L.**
SM32	***Medicago sativa* L.**
SM33	***Vigna unguiclata* L.**
SM43	***Trifolium alexandrinum* L.**

Testing isolated rhizobacteria for the synthesis of IAA: The ability to produce IAA was demonstrated by all seven isolates (Table 2) which reached their maximum production levels after three days of incubation. Isolate SM32 produced the maximum in the LB medium achieving 21.43 µg mL^{-1} and an intense pink color with the highest OD value at 535 nm. It was further molecularly characterized by sequencing its entire genome. After 4-days incubation, isolate SM21 produced a minimum of 5.39 µg mL^{-1}. (18) isolated ten rhizospheric soil bacteria after two days of incubation and discovered that the mr3 isolate produced the most IAA (22 µg mL^{-1}) in the LB medium. (17) found that poor IAA generation was the outcome of incubation durations exceeding 72 hours.

Table 2: Local rhizobacterial isolates producing IAA µ/mL in LB medium.

Isolate No.	Incubation period/Day			
	1	2	3	4
SM1	5.88*±0.53§	10.33±0.27	14.51±0.31	6.03±1.75
SM21	3.81±1.60	7.29±0.04	10.10±0.45	5.39±0.65
SM27	6.22±1.43	10.11±1.08	13.93±0.22	6.71±0.90
SM30	6.19±1.00	10.58±1.10	14.77±0.98	8.50±1.18
SM32	9.15±0.72	16.99±0.16	21.43±0.17	10.90±1.13
SM33	3.77±0.80	5.98±0.09	11.46±0.14	7.48±0.36
SM43	5.5±1.11	9.47±0.41	16.31±0.08	9.12±1.66

*: Mean value of triplicates; §: SD of triplicates.

Adding tryptophan to the LB medium produced a positive influence on IAA synthesis (Table 3). When 3.0 g mL^{-1} of tryptophan was added to the LB medium and incubated for 3 days, the maximum production was 72.96 µg mL^{-1}. After 3-days incubation, the mean value dropped to 69.73 mg mL^{-1} due to production inhibition from the increase in tryptophan content. L-tryptophan is referred to as an IAA precursor as it improves the production of IAA when added to a medium. According to (18), tryptophan was the preferred amino acid for IAA production in all five isolates. The medium supplemented with 0.1, 1.5, and 0.05% of IAA produced the highest amounts of IAA.

Table 3: Tryptophan concentration-dependent production of IAA (µg mL^{-1}) in LB medium supplemented by the SM32 rhizobacteria isolate.

Tryptophan mg mL^{-1}	Incubation period/ Day		
	1	2	3
0.0	9.09*±0.18§	17.01±0.32	21.38±0.26
1.0	23.76±1.03	41.55±0.09	45.33±0.56
2.0	35.00±0.79	37.90±0.72	56.42±0.41
3.0	45.11±0.85	63.01±1.15	72.96±1.01
4.0	43.58±0.42	60.99±1.06	69.28±0.73

*: Mean value of triplicates; §: SD of triplicates.

Whole-genome assembly and sequencing: This resulted in an image that displays the differences and similarities between the genome sequences of other bacteria and *E. cloacae* SM32 as a set of concentric rings. The genome of the SM32 local isolate underwent hole-genome sequencing. Its estimated length was 4,583,676 base pairs (bp) (Table 4). The length of the smallest number of contigs, or 501 bps, was 50% of the genome. The local isolate SM32 belongs to the Enterobacteriaceae family, with a genome size comparable to other members of the same family, according to phylogenetic analysis comparing protein coding genes. The SM32 isolate has a GC content of 55.48%. Within the order Enterobacterales of the Enterobacteriaceae family, the local isolate *E. cloacae* SM32 is classified as a member of the phylum Proteobacteria of the gamma-proteobacteria class.

Specialty genes were also present in the SM32 isolate's genome. Several antibiotic genes were included in the whole-genome sequencing of *E. cloacae* SM32, according to results acquired from the CARD database. The antibiotic resistance genes (ARGs) found in the SM32 genome of the *E. cloacae* isolate are listed in Table 4. A chromosomal *blaCMH-3* gene with a 98.69% identity is noteworthy. Thus, this is the first instance of multidrug-resistant genes in a single narrow spectrum *blaCMH-3* beta-lactamase in an Iraqi *E. cloacae* strain, SM32. Additionally, the FosA2 gene for fosfomycin resistance was found. This gene was also identified by (6) in an *E. cloacae* ST473 strain from clinical infections linked to a multidrug resistance (MDR) phenotype in Nigeria.

Table 4: Antibiotic resistance genes in the genomic sequence of E. cloacae SM32.

Resistance gene	Drug class	Resistance mechanism	Predicted phenotype	Percentage identity
baeR	Aminoglycoside antibiotic, Aminocoumarin antibiotic	Antibiotic efflux	Neomycin, Amikacin, Kanamycin A, Tobramycin, Novobiocin, Gentamicin	100
ramA	Fluoroquinolone antibiotic, Monobactam, Carbapenem, Cephalosporin, Glycylcycline, Cephamycin, Penam, Tetracycline antibiotic, Rifamycin antibiotic, Phenicol antibiotic, Penem, Disinfecting agents and antiseptics	Antibiotic efflux, Reduced permeability to antibiotics	Tigecycline, Tetracycline, Rifampin, Chloramphenicol, Ampicillin, Cefalotin, Triclosan	99.12
CRP	Macrolide antibiotic, Fluoroquinolone antibiotic, Penam	Antibiotic efflux	Erythromycin, Cloxacillin, Oxacillin, Norfloxacin	99.05
blaCMH-3	Cephalosporin	Antibiotic inactivation	Unknown	98.69
FosA2	Phosphonic acid antibiotic	Antibiotic inactivation	Fosfomycin	95.74
oqxA	Fluoroquinolone antibiotic, Glycylcycline, Tetracycline antibiotic, Diaminopyrimidine antibiotic, Nitrofuran antibiotic	Antibiotic efflux	Tigecycline, Ciprofloxacin, Nitrofurantoin, Trimethoprim	90.79
rsmA	Fluoroquinolone antibiotic, Diaminopyrimidine antibiotic, Phenicol antibiotic	Antibiotic efflux	Trimethoprim, Chloramphenicol	85.25
leuO	Nucleoside antibiotic, Disinfecting agents and antiseptics	Antibiotic efflux	Acriflavine, Puromycin	81.12

Gene annotation and prediction: Whole genome shotgun (WGS) isolation of *E. cloacae* SM32 isolate. With the accession number JBEFMZ010000000, this version of the project (01) included the sequences JBEFMZ010000001–JBEFMZ010000298. The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) contributed to the annotation. (see https://www.ncbi.nlm.nih.gov/genome/annotation_prok/.) According to the unique genome identifier, this genome has 4,348 CDSs (protein-coding sequences) and 75 RNA genes (Table 5, Fig. 1).

Through comparative genome analysis, the genome sequence of *E. cloacae* SM32 offers a chance to learn more about the distinctions between the several strains of *E. cloacae* that involve endophytic plants and human pathogenic strains. Furthermore, the SM32 strain carries genes whose roles and pathogenic potential are still unknown. The

draft genome sequence of the plant growth-promoting rhizospheric bacteria *E. cloacae* SBP-8 was disclosed by Singh et al. (2017) who further demonstrated that the isolate SBP-8's genome was made up of one plasmid with 85,398 bp and a chromosome with 48,54,065 bp.

Table 5: General genomic properties of E. cloacae SM32 produced using the RAST server and QUAST software.

Feature	Value
Genome total length (bp)	4,583,676
Number of contigs	298
Largest contig (bp)	188,733
Smallest contig (bp)	501
GC content (%)	55.48
Total of protein-coding sequences (CDSs)	4,348
Number of RNA genes	75
N50	46,588

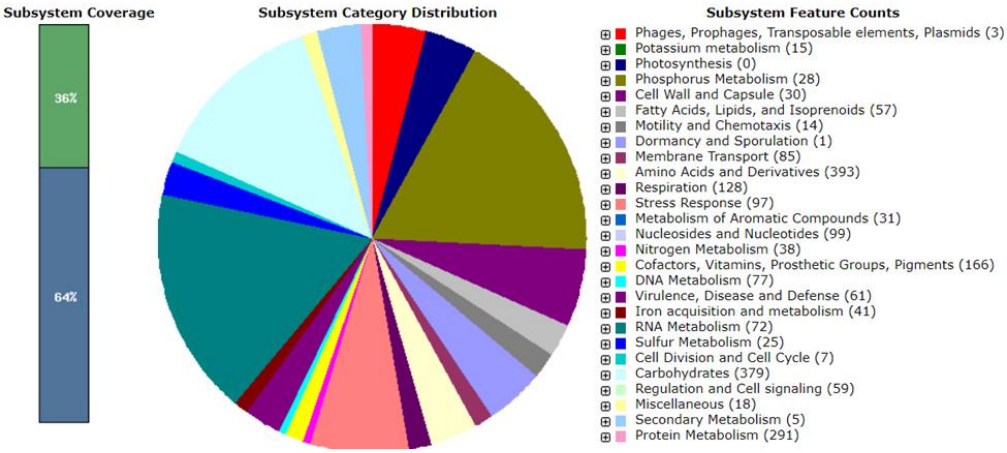

Fig. 1: Distribution statistics of the E. cloacae SM32 subsystem. The Rapid Annotation System Technology (RAST) server was used to annotate the genome.

Comparing genomes: For the comparative genomic study, the hole-sequenced genomes of ten *Enterobacter* strains isolated from various behaviors were selected (Table 6). The global alignment of genome rearrangement, performed using the software Mauve, shows that in terms of genome size (bp) and percentage of G+C content, the *E. cloacae* ATCC 13047 strain and the *E. cloacae* SM32 local isolate of the species are 100% attached (Table 6, Fig. 2).

Table 6: Pairwise comparisons of the isDDH, GC content, δ-value, genome size, and protein count of the E. cloacae SM32 genome with other closely related genomes.

E. cloacae SM32 vs. closely related genomes	Digital *is*DDH value (%)	Percent G+C (%)	δ-value	Genome Size (bp)	Number of proteins
E. cloacae ATCC 13047	91.1	55.48	0.131	4,583,676	4315
E. cloacae subsp. *dissolvens* ATCC 23373	85.3	54.58	0.132	5,598,795	5518
E. sichuanensis WCHECL1597	37.0	55.24	0.318	4,869,039	4620
E. roggenkampii DSM 16690	36.5	55.54	0.21	4,899,997	4474
E. quasiroggenkampii WCHECL1060	36.4	55.69	0.20	4,805,203	4452
E. vonholyi E13	35.8	55.58	0.257	4,579,976	4240
E. dyke sii E1	35.3	55.85	0.196	4,509,323	4161
E. asburiae ATCC 35953	35.0	55.47	0.204	4,806,219	4426
E. chuandaensis 090028T	34.7	55.68	0.214	4,629,218	4359
E. bugandensis EB-247	34.5	56	0.19	4,717,613	4332

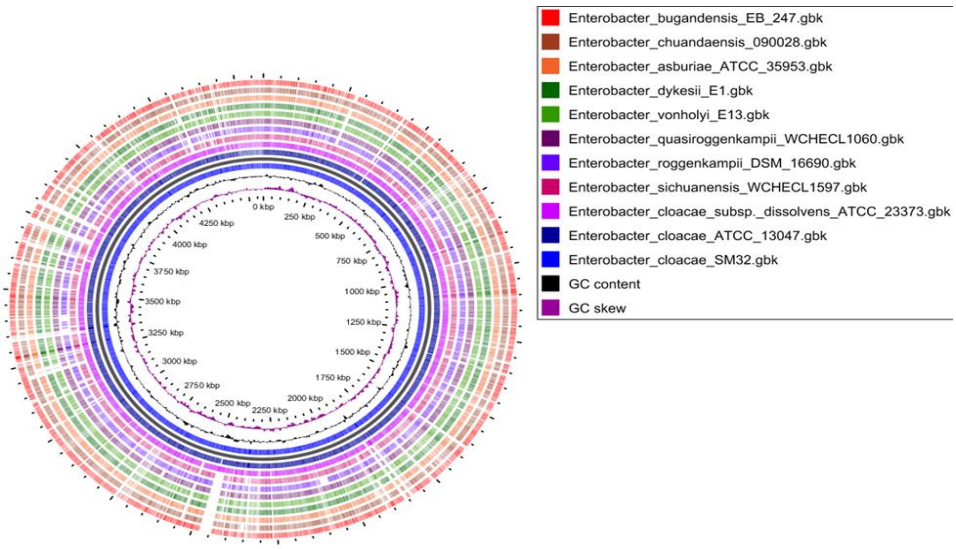

Fig. 2: Comparing genomes of the ten most closely related bacterial species with *E. cloacae* SM32. The *E. cloacae* SM32 genome is represented by the innermost red circle.

Phylogenetic analysis: The local SM32 isolate is shown to form a cluster with *E. cloacae* ATCC 13047 and *E. cloacae* subsp. *dissolvens* ATCC23373 according to the phylogenetic tree. *E. cloacae* is polyphyletic, according to the phylogenetic tree, and is grouped with other *Enterobacter* members at a different position (Fig. 3, Fig. 4 and Table 7).

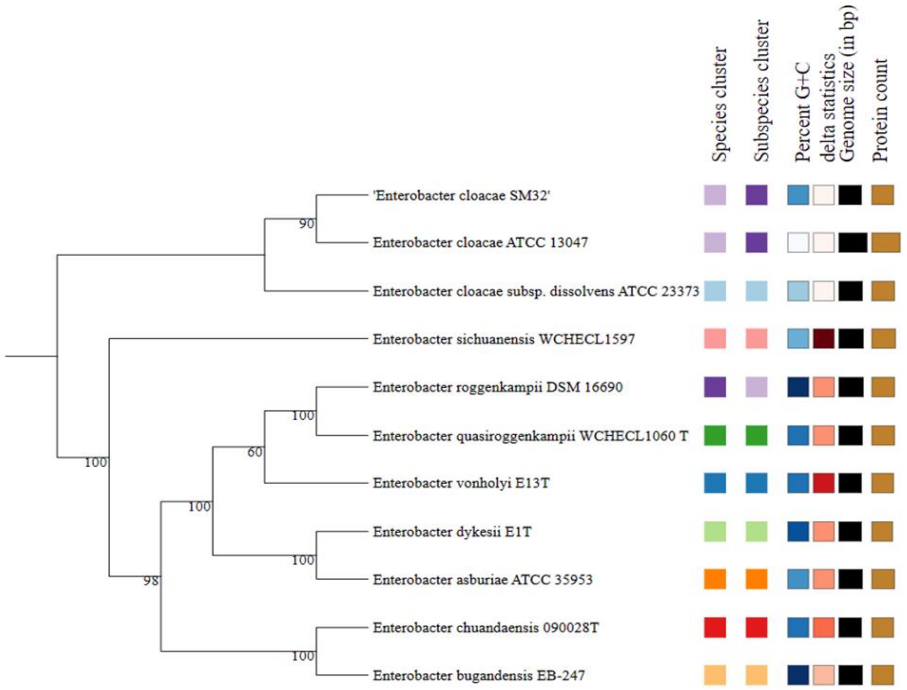

Fig. 3: *E. cloacae* SM32's phylogenetic taxonomy tree created with the TYGS service. The final tree was built using the FastME 2.0 technique and the balanced minimum evolution method (100X pseudo-bootstrap support values).

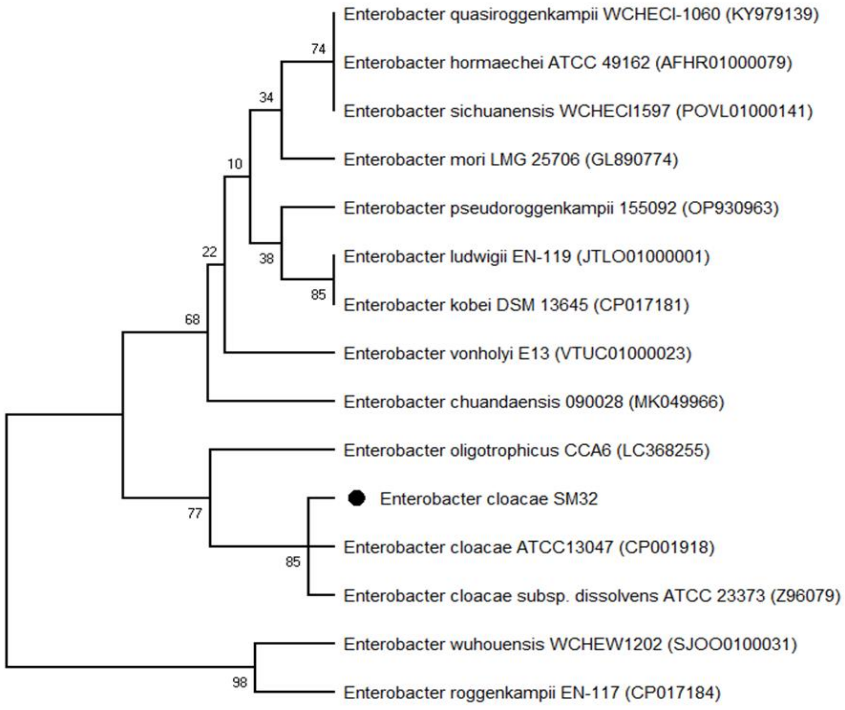

Fig. 4: Neighbor-joining phylogenetic trees created with MEGA-11 software showing the link between the closely related strains of E. cloacae SM32 (black circle) and 16S rRNA sequences.

Table 7: Similarity of E. cloacae SM32 with bacterial species submitted to NCBI.

Species	Strain	Accession No.	Similarity (%)
E. cloacae	**ATCC 13047**	**CP001918**	**99.84**
E. cloacae* subsp. *Dissolvens	**ATCC 23373**	**Z96079**	**99.84**
E. oligotrophicus	**CCA6**	**LC368255**	**99.37**
E. kobei	**DSM 13645**	**CP017181**	**98.89**
E. ludwigii	**EN-119**	**JTLO01000001**	**98.89**
E. hormaechei	**ATCC 49162**	**AFHR0100079**	**98.73**
E. sichuanensis	**WCHECl1597**	**POVL01000141**	**98.73**
E. quasiroggenkampii	**WCHECl-1060**	**KY979139**	**98.73**
E. vonholyi	**E13**	**VTUC0100023**	**98.73**
E. pseudoroggenkampii	**155092**	**OP930963**	**98.57**
E. mori	**LMG 25706**	**GL890774**	**98.42**
E. chuandaensis	**090028**	**MK049966**	**98.27**
E. roggenkampii	**EN-117**	**CP017184**	**98.25**
E. wuhouensis	**WCHEW1202**	**SJOO0100031**	**98.10**

Conclusions

The primary finding of this study is that, to the extent of our knowledge, no previous report has been made on the Enterobacter cloacae found in the roots of *Medicago sativa* L. plants growing in Mosul, Iraq. Additionally, this is the first research that addressed the entire genome sequence of a local isolate of *E. cloacae* that shows promise in the ability to produce large amounts of the indole-3-acetic acid.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Sara, M. M.: Responsible for the practical part and writing the original draft of the research; Sultan, R. H. and Edavana, V.: Reviewing and editing the research. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by the College of Education for Pure Sciences, University of Mosul which provided the research materials, laboratories, and used research equipment.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Ministry of Higher Education, University of Mosul College of Education for Pure Sciences, Department of Biology, Iraq.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflicts of interest associated with the publication of this research.

Acknowledgments:

The authors are grateful to the University of Mosul's College of Education for Pure Sciences which provided the materials and facilities for this project.

References

1. Abdulilah, H. A. Q., Al-Asafi, E. A. A., and Hameed, A. T. (2019). Role of rhizobia (Rhizobium meliloti) of alfalfa in the bioremediation of contaminated soil with hydrocarbons. Plant Archives, 19(1): 146-152.
2. Alcock, B. P., Raphenya, A. R., Lau, T. T., Tsang, K. K., Bouchard, M., Edalatmand, A., ... and McArthur, A. G. (2020). CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic acids research, 48(D1): D517-D525. https://doi.org/10.1093/nar/gkz935.
3. Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., ... and Zagnitko, O. (2008). The RAST Server: rapid annotations using subsystems technology. BMC genomics, 9: 1-15. https://doi.org/10.1186/1471-2164-9-75.
4. Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., ... and Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, 19(5): 455-477. https://doi.org/10.1089%2Fcmb.2012.0021.
5. Boiero, L., Perrig, D., Masciarelli, O., Penna, C., Cassán, F., and Luna, V. (2007). Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Applied Microbiology and Biotechnology, 74: 874-880. https://doi.org/10.1007/s00253-006-0731-9.
6. David, E. E., Igwenyi, I. O., Iroha, I. R., Martins, L. F., Uceda-Campos, G., and Da Silva, A. M. (2023). Draft genome sequence of Enterobacter cloacae ST473 harbouring bla CMH-3 isolated from a human patient diagnosed with recurrent bacteriuria in Nigeria. Access Microbiology, 5(7): 000565-v3. https://doi.org/10.1099/acmi.0.000565.v1.
7. Deshwal, V. K., Dubey, R. C., and Maheshwari, D. K. (2003). Isolation of plant growth-promoting strains of Bradyrhizobium (Arachis) sp. with biocontrol potential against Macrophomina phaseolina causing charcoal rot of peanut. Current Science, 443-448.
8. Grossmann, K. (2010). Auxin herbicides: current status of mechanism and mode of action. Pest Management Science: formerly Pesticide Science, 66(2): 113-120. https://doi.org/10.1002/ps.1860.
9. Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. Bioinformatics, 29(8): 1072-1075. https://doi.org/10.1093/bioinformatics/btt086.
10. Hossain, M. S., Frith, C., Bhattacharyya, S. S., DeLaune, P. B., and Gentry, T. J. (2023). Isolation and characterization of bacterial endophytes from small nodules of field-grown peanut. Microorganisms, 11(8): 1941. http://dx.doi.org/10.3390/microorganisms11081941.

11. Keswani, C., Singh, S. P., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., Borriss, R., ... and Sansinenea, E. (2022). Biosynthesis and beneficial effects of microbial gibberellins on crops for sustainable agriculture. Journal of Applied Microbiology, 132(3): 1597-1615. https://doi.org/10.1111/jam.15348.
12. Lefort, V., Desper, R., and Gascuel, O. (2015). FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Molecular Biology and Evolution, 32(10): 2798-2800. https://doi.org/10.1093/molbev/msv150.
13. Mahdhi, M., Fterich, A., Rejili, M., Rodriguez-Llorente, I. D., and Mars, M. (2012). Legume-nodulating bacteria (LNB) from three pasture legumes (Vicia sativa, Trigonella maritima and Hedysarum spinosissimum) in Tunisia. Annals of microbiology, 62: 61-68. https://doi.org/10.1007/s13213-011-0227-4.
14. Malik, D. K., and Sindhu, S. S. (2011). Production of indole acetic acid by Pseudomonas sp.: effect of coinoculation with Mesorhizobium sp. Cicer on nodulation and plant growth of chickpea (Cicer arietinum). Physiology and Molecular Biology of Plants, 17: 25-32. https://doi.org/10.1007/s12298-010-0041-7.
15. Meier-Kolthoff, J. P., and Göker, M. (2019). TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nature Communications, 10(1): 2182. https://doi.org/10.1038/s41467-019-10210-3.
16. Meier-Kolthoff, J. P., Carbasse, J. S., Peinado-Olarte, R. L., and Göker, M. (2022). TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Research, 50(D1): 801-807. https://doi.org/10.1093/nar/gkab902.
17. Meza, B., de-Bashan, L. E., and Bashan, Y. (2015). Involvement of indole-3-acetic acid produced by Azospirillum brasilense in accumulating intracellular ammonium in Chlorella vulgaris. Research in Microbiology, 166(2): 72-83. https://doi.org/10.1016/j.resmic.2014.12.010.
18. Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of soil science and plant nutrition, 13(3): 638-649. http://dx.doi.org/10.4067/S0718-95162013005000051.
19. Morales-Cedeño, L. R., del Carmen Orozco-Mosqueda, M., Loeza-Lara, P. D., Parra-Cota, F. I., de Los Santos-Villalobos, S., and Santoyo, G. (2021). Plant growth-promoting bacterial endophytes as biocontrol agents of pre-and post-harvest diseases: Fundamentals, methods of application and future perspectives. Microbiological Research, 242: 126612. https://doi.org/10.1016/j.micres.2020.126612.
20. Orozco-Mosqueda, M. D. C., Santoyo, G., and Glick, B. R. (2023). Recent advances in the bacterial phytohormone modulation of plant growth. Plants, 12(3): 606. https://doi.org/10.3390/plants12030606.
21. Qasim, M. A., Al-Azzami, A. A., Khaleel, N. T., Yaseen, A. A., and Fahad, M. M. (2024). Investigating the active chemicals in bay leaves in suppressing microbes and improving the sensory properties of chilled chicken meat. Anbar Journal of

Agricultural Sciences, 22(2): 1228-1239. https://doi.org/10.32649/ajas.2024.150015.1268.

22. Rasheed, H. M., and Abed, I. A. (2013). Recycling of wastes from cities and fields by preparing a substrate for *mushroom Agaricus* bisporus production. Int J Enviro Global Clim Chan, 1(1): 62-71.
23. Rivas, R., Martens, M., De Lajudie, P., and Willems, A. (2009). Multilocus sequence analysis of the genus Bradyrhizobium. Systematic and Applied Microbiology, 32(2): 101-110. https://doi.org/10.1016/j.syapm.2008.12.005.
24. Soundarya, K. R., Bhavana, D., Harsha, T. S., and Prasad, N. (2022). Isolation, identification and biochemical characterization of *Rhizobium* sp. from *Mimosa pudica*. World Wide Journal of Multidisciplinary Research, 8(05): 4-9. http://doi.org/10.17605/OSF.IO/AJBX2.
25. Stothard, P., Grant, J. R., and Van Domselaar, G. (2019). Visualizing and comparing circular genomes using the CGView family of tools. Briefings in bioinformatics, 20(4): 1576-1582. https://doi.org/10.1093/bib/bbx081.
26. Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120.
27. Youseif, S. H., Abd El-Megeed, F. H., Abdelaal, A. S., Ageez, A., and Martínez-Romero, E. (2021). Plant–microbe–microbe interactions influence the faba bean nodule colonization by diverse endophytic bacteria. FEMS microbiology ecology, 97(11): fiab138. https://doi.org/10.1093/femsec/fiab138.