Nematicidal, Acaricidal and Plant Growth-Promoting Activity of Enterobacter Endophytic Strains and Identification of Genes Associated with These Biological Activities in the Genomes

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Abstract: In the present study, the nematicidal and acaricidal activity of three Enterobacter endophytic strains isolated from Mimosa pudica nodules was evaluated. The percentages of mortality of Enterobacter NOD4 against Panagrellus redivivus was 81.2%, and against Nacobbus aberrans 70.1%, Enterobacter NOD8 72.4% and 62.5%, and Enterobacter NOD10 64.8% and 58.7%, respectively. While against the Tyrophagus putrescentiae mite, the mortality percentages were 68.2% due to Enterobacter NOD4, 64.3% due to Enterobacter NOD8 and 77.8% due to Enterobacter NOD10. On the other hand, the ability of the three Enterobacter strains to produce indole acetic acid and phosphate solubilization, characteristics related to plant growth-promoting bacteria, was detected. Bioinformatic analysis of the genomes showed the presence of genes related to IAA production, phosphate solubilization, and nitrogen fixation. Phylogenetic analyzes of the recA gene, phylogenomics, and average nucleotide identity (ANI) allowed us to identify the strain Enterobacter NOD8 related to E. mori and Enterobacter NOD10 as E. asburiae, while Enterobacter NOD4 was identified as a possible new species of this species. The plant growth-promoting, acaricidal and nematicidal activity of the three Enterobacter strains makes them a potential agent to include in biocontrol alternatives and as growth-promoting bacteria in crops of agricultural interest.

Keywords: phosphate solubilization; auxin production; plant growth promotion; biocontrol; siderophore
1. Introduction

Agricultural pests, due to phytopathogenic nematodes and dust mites, cause economic losses in crops of great importance [1]. Some species of phytopathogenic nematodes such as Pratylenchus spp., Ditylenchus spp., Psilenchus spp., and the false root-knot nematode Nacobbus aberrans, together with Meloidogyne spp., (the latter made up of 98 species, including M. chitwood, M. incognita, M. hapla, M. javanica and M. arenaria), are responsible for causing economic losses of between 12 to 20% in the crops where they occur [2]. Another organism that causes problems in agricultural production, causing considerable damage and losses in food products of plant and animal origin, is the dust mite T. putrescentiae, since they can carry fungal spores and pathogenic bacteria for humans. The T. putrescentiae mite is a vector of pathogenic bacteria of Klebsiella spp., Candida albicans and Staphylococcus which affect humans, and are present in the mucosa and parts of the skin [3].

Plants are responsible for selecting their microbiome to have beneficial bacterial colonizers designated as plant growth-promoting bacteria (PGPB). These produce different compounds such as auxins, antibiotics, and organic acids that are related to growth promotion, as well as chitinase, protease and cellulase enzymes that are related to biocontrol, health and plant development processes [4–8]. The ability of endophytic bacteria to colonize the interior of plant tissues gives them an advantage in avoiding the competition present in the rhizosphere environment, and allows them to achieve a close relationship with the plant [6,9,10]. The Enterobacter genus comprises species that have been reported to promote plant growth due to their multiple growth-promoting activities. Some strains that have been reported are: E. asburiae PDA 134 isolated from date palms [11], E. cloacae isolated from citrus and corn plants [12,13] and E. asburiae from sweet potato [14]. The strain Enterobacter sp., P23 has also been reported as a growth promoter under conditions of abiotic stress such as salinity, due to its high ACC deaminase activity, extreme pH, high temperature and in the presence of a wide variety of pesticides traditionally applied in rice, peanut and corn crops [15,16].

On the other hand, different species of the genus Enterobacter have been evaluated against phytoparasitic nematodes of agricultural pests. In this context, in a study by Zhao et al. [17], the nematicidal activity of E. ludwigii AA4 was reported in 98.3 and 98.6% against the pine nematode Bursaphelenchus xylophilus, considering E. ludwigii AA4 as a biocontrol agent of the pine nematode (Pinus sylvestris) infection. Regarding root-knot nematodes, a study by Oh et al. [18] reported the in vitro nematicidal activity of E. asburiae against eggs and J2 larvae of M. incognita. The results were obtained at a concentration of 10% of the treatment against the eggs, and 53.7% and 98.2% against J2 larvae in a time of two to seven days post-treatment.

Considering the characteristics of the species of the genus Enterobacter, the objective of this work is to analyze the genome sequences of three strains isolated from Mimosa pudica nodules to identify the genes that are associated with the characteristics that promote plant growth, nematicidal activity and acaricide, as well as its phylogenetic relationship with other species of the genus Enterobacter.

2. Results

2.1. Genome Assembly and Annotation

The analysis of the genomes by means of the average nucleotide identity (ANI) was obtained by comparing the sequences of the genomes against the genomes deposited in GenBank. The NOD4 strain was identified as Enterobacter sp. with 88.14% with a coverage of 82%, NOD8 as E. mori with 96.46% with a coverage of 87.72%, and NOD10 as E. asburiae with 97.12% with a coverage of 87.80%. The strain Enterobacter sp. NOD4 contains 242 contigs with a genome size of 4.65 Mbp, containing 53.1% GC and 4378 coding sequences, while the E. mori NOD8 strain contains 244 contigs with a genome size of 4.84 Mbp and 55.7% GC content and 4403 coding sequences. Finally, the E. asburiae NOD10 strain contains 144 contigs with a genome size of 4.51 Mbp and 56.1% GC content and 4198 coding sequences (Table 1).
Table 1. Characteristics of the genomes of *Enterobacter* endophytic strains.

| Features             | Enterobacter sp. NOD4 | Enterobacter sp. NOD8 | Enterobacter sp. NOD10 |
|----------------------|-----------------------|-----------------------|------------------------|
| Contigs              | 242                   | 244                   | 144                    |
| Genome size          | 4,649,192 bp          | 4,835,007 bp          | 4,517,298 bp           |
| GC content (%)       | 53.1                  | 55.7                  | 56.1                   |
| Coding gene          | 4378                  | 4403                  | 4198                   |
| tRNA                 | 77                    | 80                    | 80                     |
| rRNA                 | 17                    | 19                    | 12                     |
| tmRNA                | 1                     | 1                     | 1                      |
| Hypothetical proteins| 1064                  | 958                   | 965                    |
| Proteins with functional assignments | 3314 | 3445 | 3233 |

2.2. Phylogenomic Analysis

The sequences of the *recA* genes were analyzed using the BLASTn algorithm and showed similarity with the species of the *Enterobacter* genus. Phylogenetic trees were constructed using the *recA* genes. Phylogenomic analysis shows that the NOD4 strain may be a new species of this genus. The NOD8 strain is phylogenetically related to the *E. mori* species, while the NOD10 strain is related to the *E. asburiae* species (Figure 1). This is also observed with the BLAST results, where a 99% similarity with the aforementioned species was obtained.

**Figure 1.** Molecular Phylogenetic Analysis of *Enterobacter* with *recA* gene by Maximum Likelihood Method based on the General Time Reversible Model. The tree with the highest log likelihood (−3614.36) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1689)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 39.76% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 879 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.
2.3. Phosphate Solubilization Activity

The three strains of *Enterobacter* had the ability to solubilize phosphate, *Enterobacter* sp. NOD4 solubilizes 66.9 µg/mL, while *E. mori* NOD8, 69.2 µg/mL, and *E. asburiae* NOD10, 67.4 µg/mL at five days. The statistical analysis does not show a significant difference (ANOVA *p* < 0.05) in phosphate solubilization between strains (Figure 2A). Phosphate solubilization can also be observed through the formation of halos in Pikovskaya PVK agar culture medium (Figure 2B).

![Figure 2. Solubilization of inorganic phosphate by the three strains of *Enterobacter*. (A) Phosphate solubilization (B) Pikovskaya PVK.](image)

2.4. Determination of Auxin Production

The production of indoles by bacteria is a mechanism for promoting plant growth. This characteristic was detected in *Enterobacter* sp. NOD 4 producing 101.9 µg/mL, *E. mori* NOD8, 103.9 µg/mL and *E. asburiae* NOD10 52.1 µg/mL (Figure 3). In the statistical analysis of the indole production by the strains, a significant difference was found (ANOVA *p* < 0.05) in the NOD 4 and NOD 8 strains, with respect to the NOD 10 strain.
Figure 3. IAA quantification of the three Enterobacter strains. Media was supplemented with tryptophan. Different symbols at the top of the bars indicate a statistically significant difference ($p < 0.05$).

2.5. Siderophore Production

The Enterobacter sp. NOD4, $E. \text{mori}$ NOD8 and $E. \text{asburiae}$ NOD10 were inoculated in the CAS-CAA medium, where they formed a yellow halo around the colonies indicating the production of siderophores. The three strains were positive (Figure 4).

![Figure 4](image_url)

**Figure 4.** Determination of the production of siderophores in the three strains of Enterobacter.

2.6. In Vitro Evaluation of Enterobacter Strains against $P. \text{redivivus}$ and $N. \text{aberrans}$

The results of the evaluation of the strains of Enterobacter sp. NOD4 at a concentration of $1 \times 10^9$ cell/mL against the free-living nematode $P. \text{redivivus}$ showed a mortality of 81.2%, $E. \text{mori}$ (NOD8) of 72.4% and $E. \text{asburiae}$ (NOD10) of 64.8% at 24 h (Table 2). The strain of Enterobacter sp. NOD4 against the nematode $N. \text{aberrans}$ showed a mortality of 70.1%, $E. \text{mori}$ (NOD8) of 62.5% and $E. \text{asburiae}$ (NOD10) of 58.7% at 24 h (Table 3).
Table 2. In vitro evaluation of Enterobacter strains against Panagrellus redivivus.

| Treatment                | Concentration | % Mortality |
|--------------------------|---------------|-------------|
| Enterobacter sp. NOD 4   | $1 \times 10^9$ cell/mL | 81.2 $^b \pm 11.08$ |
| Enterobacter mori NOD8   | $1 \times 10^9$ cell/mL | 72.4 $^c \pm 10.23$ |
| Enterobacter asburiae NOD10 | $1 \times 10^9$ cell/mL | 64.8 $^b \pm 27.33$ |
| Control (Water)          | -             | 2.1 $^d \pm 3.35$ |
| Ivermectin               | 2.5 mg/mL     | 99.3 $^a \pm 4.15$ |

Means with the same letter are not statistically different in Tukey’s mean comparison test ($p \leq 0.05$).

Table 3. In vitro evaluation of Enterobacter strains against Nacobbus aberrans (J2).

| Treatment                | Concentration | % Mortality |
|--------------------------|---------------|-------------|
| Enterobacter sp. NOD4    | $1 \times 10^9$ cell/mL | 70.1 $^b \pm 9.2$ |
| Enterobacter mori NOD8   | $1 \times 10^9$ cell/mL | 62.5 $^b \pm 10.2$ |
| Enterobacter asburiae NOD10 | $1 \times 10^9$ cell/mL | 58.7 $^b \pm 19.4$ |
| Control (Water)          | -             | 2.1 $^d \pm 3.35$ |
| Nematrol                 | 6.0 mg/mL     | 99.3 $^a \pm 4.15$ |

Means with the same letter are not statistically different in Tukey’s mean comparison test ($p \leq 0.05$).

2.7. In Vitro Evaluation of Enterobacter against the Mite T. putrescentiae

The results of the in vitro evaluation of Enterobacter against the mite T. putrescentiae are described below. The strain of Enterobacter sp. NOD4 at a concentration of $1 \times 10^9$ cell/mL against the T. putrescentiae mite showed a mortality of 68.2%, the E. mori strain (NOD8) at the same concentration of $1 \times 10^9$ cell/mL showed a mortality of 64.3%. E. asburiae (NOD10) at the same concentration of $1 \times 10^9$ cell/mL, 79.8% was obtained, and ivermectin at a concentration of 2.5 mg/mL, 99.3% mortality at 24 h (Table 4).

Table 4. In vitro evaluation of Enterobacter strains against Tyrophagus putrescentiae.

| Treatment                | Concentration | % Mortality |
|--------------------------|---------------|-------------|
| Enterobacter sp. NOD 4   | $1 \times 10^9$ cell/mL | 68.2 $^b \pm 7.31$ |
| Enterobacter mori NOD 8  | $1 \times 10^9$ cell/mL | 64.3 $^b \pm 10.1$ |
| Enterobacter asburiae NOD10 | $1 \times 10^9$ cell/mL | 77.8 $^b \pm 15.2$ |
| Control (Water)          | -             | 2.1 $^d \pm 3.35$ |
| Ivermectin               | 2.5 mg/mL     | 99.3 $^a \pm 4.15$ |

Means with the same letter are not statistically different in Tukey’s mean comparison test ($p \leq 0.05$).

2.8. Bioinformatic Analysis of Genomes for the Detection of Genes Involved in Plant Growth-Promoting Activities and Biocontrol

In the genome of the E. asburiae NOD10 strain, the aspC gene was identified, encoding an enzyme that catalyzes the conversion of indole-3-pyruvic acid to indole-3-acetaldehyde. This is later converted into IAA by the enzyme aldehyde dehydrogenase (aldB), that was also detected in the genome of E. asburiae NOD10 (Table 5). Other genes that were detected in Enterobacter sp. NOD4, E. mori NOD 8 and E. asburiae NOD10 are nirD which codes for the minor subunit of the enzyme nitrite reductase, narI which codes for the alpha subunit of the enzyme nitrate reductase, nasR which regulates the assimilation of nitrates and nitrites, and narX that detects the presence of nitrates and nitrites, the ammonium transporter, and the nifJ gene that codes for pyruvate-flavodoxin-oxidoreductase. All of these genes are involved in nitrogen fixation processes (Table 5).
Table 5. Genes involved in possible plant growth-promoting and antagonistic activities in *Enterobacter* strains.

| Gene | Gene Locus NOD4 | Gene Locus NOD8 | Gene Locus NOD10 | Gene Product |
|------|-----------------|-----------------|------------------|--------------|
|      | JAKKOK000000000.1 | JAKNRT000000000.1 | JAKKOL000000000.1 |              |
| pstS | L2X67_10965 36964.38004 | L2X83_10375 11318.12358 | L2X78_07895 87736.88776 | Phosphate-binding protein |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| phoU | L2X67_10945 33473.34198 | L2X78_07875 84186.84911 | L2X83_10395 15184.15909 | Negative regulatory protein of pho regulon |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| aspC | L2X83_17980 1562.2752 | L2X78_22070 111.3635 | L2X83_09580 13515.17039 | Catalyzes the conversion of indole-3-pyruvic acid to indole-3-acetaldehyde |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| nifJ | L2X67_22275 1.2275 | L2X78_10325 33980..35830 | L2X83_16170 11435.13285 | Nitrogen fixation protein |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae | Fe-S protein assembly chaperone HscA |
| hscA | L2X67_06355 33733.35583 | L2X83_17325 13739..14086 | L2X83_05375 44667.45014 | Iron–sulfur cluster insertion protein ErpA |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| erpA | L2X67_09295 21597.21941 | L2X78_18585 13739..14086 | L2X83_18150 3818.4156 | P-II family nitrogen regulator |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| glnK | L2X67_18100 6179.6517 | L2X78_19110 3818.4156 | L2X83_18150 3818.4156 | Nitrate/nitrite two-component system sensor histidine kinase NarX |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae | Ammonium transporter AmtB |
| narX | L2X67_19200 1906.3702 | L2X78_20400 1883.3679 | L2X83_19625 11800.13596 | Nitrate reductase small subunit NirD |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae | Nitrate regulatory protein NasR |
| amtB | L2X67_18095 4854.6143 | L2X78_19105 2496.3782 | L2X83_18145 7443.8732 | Nitrate reductase subunit gamma |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae | Aldehyde dehydrogenase AldB |
| nirD | L2X67_10630 32781.33107 | L2X78_00765 171713.172039 | L2X83_04385 140648.140974 | Proofreading thioesterase EntH |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| nasR | L2X67_19240 15543.16727 | L2X78_20440 15561.16745 | L2X83_08015 81225.81638 | Nitrate regulatory protein NasR |
| narI | L2X83_20090 2639.3319 | L2X83_21280 5772.7310 | L2X83_08015 81225.81638 | Nitrate regulatory protein NasR |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| aldB | L2X67_04385 31416.32954 | L2X83_21280 5772.7310 | L2X83_08015 81225.81638 | Nitrate regulatory protein NasR |
|      | Enterobacter sp. | Enterobacter mori | Enterobacter asburiae |              |
| entH | L2X67_04135 81428.81841 | L2X78_06195 71878.72291 | L2X83_08015 81225.81638 | Nitrate regulatory protein NasR |

Thus, the *glnK* gene is involved in the regulation of nitrogen metabolism. In the bioinformatic analysis of the *Enterobacter* strains, the *pstS* gene that codes for the phosphate-binding protein and the *phoU* gene that is involved in the regulation of phosphate assimilation were also detected (Table 5). The *entH* gene was also identified in the bioinformatic analysis as a corrector in the synthesis of the enterobactin siderophore (Table 5).

3. Discussion

In the present study, biotechnological characteristics of interest were detected such as the production of indoles and phosphate solubilization, as well as the production of siderophores related to biocontrol activity. Nematicidal (*P. redivivis* and *N. aberrans*) and acaricidal (*T. putrecentiae*) activity was also detected in the three *Enterobacter* strains. In the bioinformatic analysis of the genomes sequenced in this work, genes involved in the solubilization processes of phosphate, indoles and siderophores were detected.

Physlogenetic analysis using the *recA* gene of the three isolates showed that they belong to the genus *Enterobacter*. Consistently, phyllogenomic inference suggests that the *Enterobacter* NOD8 strain is related to *E. mori*, while the *Enterobacter* NOD10 strain is related to *E. asburiae*. However, the *Enterobacter* NOD4 strain is grouped externally from the
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E. asburiae-sichuanensis and E. bundangensis-kobei complexes. This could be an indication that the NOD4 isolate is a new species of Enterobacter. These results were confirmed with the analysis of the genomes using average nucleotide identity (ANI), which is a similar statistic of the genomes that allows us to identify them at the species level [19,20]. Where the cutoff to consider 96% as a species is established by the ANI analysis, two strains meet this criteria Enterobacter NOD8 and Enterobacter NOD10 to be considered as Enterobacter mori and Enterobacter asburiae species, respectively, while the Enterobacter NOD4 strain has an ANI of 84%, so it can be considered a new species [21].

In the E. asburiae HK169 strain, nematicidal activity against the root-knot nematode M. incognita (eggs and J2) was reported Oh et al. [18]. In this work, the nematicidal activity of the strains E. asburiae NOD10, E. mori NOD8 and Enterobacter sp. NOD4 against the free-living nematode P. redivivus and the plant parasite N. aberrans (J2). The study carried out by Zhao et al. [17] reported that the E. ludwigii AA4 strain has activity against the pine nematode B. xylophilus and identified the sdaB gene that codes for L-serine hydrolase that is involved in nematicidal activity.

The biocontrol of the three strains of Enterobacter was not only limited to the nematode N. aberrans, it also had activity against the dust mite T. putrescentiae, being the first report of the species E. mori NOD8, E. asburiae NOD10 and Enterobacter sp. NOD4 against this nematode. Additionally, the nematode P. redivivus was used as a study model to evaluate the nematicidal activity of the species E. mori NOD8, E. asburiae NOD10, and Enterobacter sp. NOD4. These previous results can be considered for future field applications, considering the environmental impact of these organisms.

On the other hand, a study by Hall et al. [22] reported the isolation of Comamonas as endosymbionts of the Psoroptes ovis mite for biocontrol. Saccà and Lodesani [23] reported Lactobacillus kunkeei, Bacillus thuringiensis, Bifidobacterium asteroides and Acetobacteraceae isolated from healthy honey bees and dead varroa mites, which had acaricidal activity against Varroa destructor. Finally, the strain B. amyloliquefaciens W1 and Burkholderia rinojensis were reported to have acaricidal activity against Tetranychus urticae [24,25].

Other characteristics of biotechnological interest detected in these Enterobacter strains was the ability to solubilize phosphate, where the pstS gene related to this process was identified. This phosphate-solubilizing activity has been reported in the E. ludwigii GAK2 strain and in the Enterobacter sp. [8,26]. Indole Acetic Acid (IAA) production activity was also detected in E. mori NOD8, E. asburiae NOD10 and Enterobacter sp. NOD4. This activity has been reported in E. ludwigii GAK2 and Enterobacter sp. [8,26]; the aspC gene was detected by bioinformatic analysis in the genome of the strain E. asburiae NOD10. The ability to produce siderophores could be observed in the three Enterobacter strains. This ability related to biocontrol has been reported in the E. roggenkampii ED5 strain, and is related to the biocontrol of phytopathogenic fungi [27]. Another study by Solanki et al. [28] reported the production of siderophores by the Enterobacter sp. strain, which is related to the biocontrol of Rhizoctonia solani. In the genomes of the three Enterobacter strains the entH gene was identified, which is involved as a corrector in the synthesis of the siderophore enterobactin. Sánchez et al. [29] reported that Enterobacter strains detected the production of indole acetic acid, solubilization of phosphate and siderophores, as well as the detection of nifH genes.

Although the nitrogen production tests were not carried out in the different strains, the bioinformatic analysis allowed the detection of genes involved in nitrogen metabolism, such as the nif1 genes involved in nitrogen fixation, the narX gene in the detection of nitrates and nitrite, the nirD gene that encodes the minor subunit of the enzyme nitrite reductase involved in the reduction of nitrates to nitrites, as well as the amtB gene related to ammonium transport (Table 5). Guo et al. [27] reported the genes involved in plant growth-promoting characteristics in the E. roggenkampii ED5 genome. Ludueña et al. [8] reported the genes involved in the production of siderophores, indole acetic acid and phosphate solubilization in the Enterobacter sp. J49.
4. Materials and Methods

4.1. Isolation of Microorganisms from Mimosa Pudica Nodules

Strains NOD4, NOD8 y NOD10 were isolated from *M. pudica* nodules collected in the Lacandon tropical rain forest in Chiapas, México (16°45'0'' N, 91°30'0'' W). Nodules were surface sterilized using 70% ethanol (10 min) and 2% sodium hypochlorite (20 min). Sterile nodules were crushed, subsequently adding 1 mL of 10 mM MgSO₄ and the resulting suspension was streaked on PY agar plates (2% peptone, 1% yeast extract, 2% bacteriological agar), which were incubated at 28 ± 1 °C for five days. Axenic cultures were obtained and conserved in 20% glycerol [29].

4.2. DNA Extraction, Library Preparation, and Sequencing

Genomic DNA was extracted from freshly cultivated cells of the strains using the ZR Fungal/Bacterial DNA Kit kitTM (Zymo Research (Tustin, California, USA)), according to the manufacturer’s instructions. The libraries for sequencing were performed with the Nextera XT® (Heslin Rothenberg Farley & Mesiti P.C. (Albany, New York, USA)) protocol following the manufacturer’s recommendations. The samples were fragmented obtaining short chains of DNA ~500 bp. The resulting fragments go through an adapter ligation process (index), and were subsequently amplified following the cycling conditions specified in the NexteraXT protocol. At the end of this process, fragment purification was performed using the Agencourt Ampure XB beads commercial system (Beckman Coulter™ (Brea, California, USA)). Once the libraries were obtained, their quality was verified and analyzed by capillary electrophoresis using the 2100 Bioanalyzer (Agilent Technologies Inc. (Santa Clara, California, USA)). Finally, the samples were analyzed on the Illumina MiSeq high-throughput sequencing platform with paired ends.

The quality analysis of the lectures was performed with FASTQC (bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 24 February 2021)) and for cleaning TRIM_GALORE (bioinformatics.babraham.ac.uk/projects/trim_galore/ (accessed on 24 February 2021). Genomes were assembled using Spades version 3.12.0 [30]. Genome sequence annotation was made by PROKKA version 1.12 [31]. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession isolate 4 JAKKOK000000000 (PRJNA798777), isolate 8 JAKNRT000000000 (PRJNA798778), and isolate 10 JAKKOL000000000 (PRJNA798780). The version described in this paper is version isolate 4 JAKKOK000000000, isolate 8 JAKNRT000000000, and isolate 10 JAKKOL000000000.

4.3. Phylogenetics and Phylogenomics

From the phylogeny carried out with the recA gene, we selected the closest and most representative genomes of the sequenced isolates to obtain a complete nucleotide level database of 8 species of *Enterobacter* and one of *Klebsiella* as an outgroup. We used GET_HOMOLOGUES and GET_PHYLOMARKERS, a pipeline designed to identify high-quality markers to estimate robust genome phylogenies from the orthologous clusters, or the pan-genome matrix (PGM). A ML species tree was estimated from the concatenated set of top-ranking alignments at the DNA or protein levels, using either FastTree or IQ-TREE [32].

4.4. Phosphate Solubilization

The ability to solubilize phosphate was qualitatively determined by inoculating the strains in PY liquid medium with 0.14 mM CaCl₂ and incubating at 28 °C for 24 h with shaking at 200 rpm to obtain a pre-inoculum. Bacterial cultures were centrifuged and adjusted to 0.2 OD 600 nm. They were seeded in triplicate in NBRIP culture medium (glucose, 1%; Ca₃(PO₄)₂, 0.5%; (NH₄)₂SO₄, 0.01%; MgSO₄ 7H₂O, 0.025%; KCl, 0.02%; MgCl₂·6H₂O, 0.5%; Congo red, 2.5 mg/mL agar, 1.8%) and incubated at 28 °C for seven days. After this period, the sizes of the halos around the colonies were measured [33]. Inorganic phosphate concentration was quantitatively measured in liquid culture using NBRIP medium without agar and Congo red. The strains were inoculated at 0.2 OD 600 nm and cultured for
ten days at 28 °C at 200 rpm. Samples were taken every 48 h, and after centrifugation of the samples, the concentration of phosphate in the supernatant was measured by the method of Rodriguez and Fraga [34]. The phosphate concentration was expressed as 50 µg/mL.

4.5. Determination of Auxin Production

To determine the production of indoleacetic acid (IAA), the strains were grown in liquid NFB medium (composition in g/L: malic acid, 5; K$_2$HPO$_4$, 0.5; MgSO$_4$·7H$_2$O, 0.2; NaCl, 0.1; CaCl$_2$, 0.02; FeSO$_4$, 0.015; Na$_2$MoO$_4$, 0.0025; MnSO$_4$, 0.01; KOH, 4.8; NH$_4$Cl, 0.2; yeast extract, 0.3; H$_3$BO$_3$, 0.01); bacterial cultures were incubated for 18 h at 28 °C at 200 rpm. Then, the cultures were adjusted to 0.2 at OD 600 nm, and 100 µL of culture was inoculated into Jain and Patriquin medium (composition in g/L: succinic acid, 2.5; fructose, 2.5; K$_2$HPO$_4$, 6; KH$_2$PO$_4$, 4; NH$_4$Cl, 1; MgSO$_4$, 0.2; NaCl, 0.1; CaCl$_2$, 0.02; FeCl$_3$, 0.01; NaMoO$_4$, 0.002 and KOH, 2.1) with and without tryptophan (0.1 g/L) and incubated at 28 °C for 24 and 48 h at 200 rpm. 1 mL aliquots of the culture were taken, centrifuged for 5 min at 5000 g and 0.5 mL of the supernatant was mixed with 0.5 mL of Salkowski reagent [35].

4.6. Siderophore Production Assays

Siderophore production was determined by the method described by Schwyn and Neilands [36]. CAS-CAA (Chrome azurol (100 mM) and S-casamino acids) agar plates were inoculated with the isolates at 28 °C for 12 days. Orange halos formed around the colonies on blue agar, and were considered indicative of siderophore production.

4.7. Panagrellus Redivivus

A strain of the free-living nematode *P. redivivus* (mixed populations) was used by Dr. Roberto-de-Lara of the Autonomous Metropolitan University (UAM, Xochimilco, Mexico in 2009). The nematodes were grown in plastic containers using commercial oat flakes and water as a substrate. The oat flakes and water were mixed and sterilized in a microwave oven [37]. The nematodes were transferred to the substrate. The containers were covered with an aluminum lid with a mesh window of fine cloth to allow oxygenation. The cultures were maintained at room temperature (25–30 °C). After one week, the population of nematodes increased considerably [38].

4.8. Nacobbus Aberrans

The inoculum of *N. aberrans* was obtained from tomato roots (monoxenic culture) (Population of Colegio de Postgraduados, Campus Montecillo, Estado de México, México). Egg extraction was carried out following the methodology described by Vrain [39], and obtaining juveniles (J2) was carried out according to Villar et al. [40].

4.9. Tyrophagus Putrescentiae

Populations of the *T. putrescentiae* mite were used, whose breeding stock is found in the National Center for Disciplinary Research in Animal Health and Safety, INIFAP, Jiutepec, Morelos. The specimens were isolated in 2013 in the town of San Juan Tlacotenco, Morelos, Mexico. The population is maintained by feeding them the free-living nematode *P. redivivus* (Goodey) for reproduction. The mites are transferred to plates with sterile agar in order to obtain monocultures of mites, and are kept at room temperature (28 ± 2 °C) under dark conditions [38].

4.10. Experimental Design

A completely randomized design was used, which consisted of four repetitions and two replicates for each treatment. The treatments for each bioassay were *Enterobacter NOD4*, NOD8 and NOD10 at a concentration of $1 \times 10^9$ cell/mL, as a negative control distilled water was used in the tests against the mite *T. putrescentiae* and the phytoparasite *N. aberrans*. As a positive control, Nematrol plus®, a commercial nematicide at a concentration of
6 mg/mL for *N. aberrans* was used. In the case of *P. redivivus* and *T. putrescentiae*, ivermectin, a commercial anthelmintic, was used at a concentration of 5 mg/mL, and distilled water was used as a negative control in the *P. redivivus* bioassays.

For the in vitro tests with *P. redivivus* and *N. aberrans*, 96-well microtiter plates were used. In each well 50 µL of distilled water with 100 nematodes and 50 µL of *Enterobacter* (NOD4, NOD8 and NOD10) were added. They were subsequently incubated in a humid chamber at 28 °C, and the nematicidal activity was evaluated at 48 h. For the in vitro bioassays of *T. putrescentiae*, 24-well microtiter plates were used. In each well, 10 mites were added with 50 µL of *Enterobacter* (NOD4, NOD8 and NOD10). They were subsequently incubated in a humid chamber at 28 °C, and after 72 h the readings were made with a stereoscope (4× and 10×).

4.11. Statistical Analysis

The data obtained were normalized using the arcsine square root transformation and analyzed as a completely randomized design. Means were compared using Tukey’s test (using the R® environment). A value of *p* ≤ 0.05 was considered significant [41].

5. Conclusions

With the results obtained in the present study, the strains of *Enterobacter* sp. NOD4, *E. mori* NOD8 and *E. asburiae* NOD10, can be alternative biocontrols against the nematode *N. aberrans* and the mite *T. putrescentiae*. The production of IAA and phosphate solubilization were also detected, which are characteristics that promote plant growth and which can be used in soils composed of insoluble phosphate. Therefore, they are candidates for the biocontrol of nematodes and mites in crops, as well as promoting plant growth in crops of agricultural interest.

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**References**

1. Manzanilla-López, R.H.; Costilla, M.A.; Doucet, M.; Insera, R.N.; Lehman, P.S.; Cid del Prado-Vera, L.; Souza, R.; Evans, K. The genus *Nacobbus* thorne & Allen, 1944 (Nematoda: Pratylenchidae): Systematics, distribution, biology and management. *Nematropica* 2002, 32, 149–227.

2. Valadez, R.R.; Siller, M.C.; Chávez, E.C.; Fuentes, Y.M.O.; Pérez, A.H.; Vargas, L.M.T. Nematodos asociados al cultivo de berenjena en Cañada Honda, México. *REMEXCA* 2021, 12, 1304–1312.
3. Erban, T.; Klimov, P.B.; Smrz, J.; Phillips, T.W.; Nesvorna, M.; Kopecky, J.; Hubert, J. Populations of Stored Product Mite Tyrophagus putrescensiae Differ in Their Bacterial Communities. *Front. Microbiol.* 2016, 7, 1046. [CrossRef] [PubMed]

4. Taghavi, S.; Van Der Leije, D.; Hoffman, A.; Zhang, Y.B.; Walla, M.D.; Vangronsveld, J.; Monchy, S. Genome sequence of the plant growth promoting endophytic bacterium *Enterobacter* sp. 638. *PLoS Genet.* 2010, 6, e1000943. [CrossRef]

5. Ali, S.; Charles, T.C.; Glick, B.R. Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. *J. Appl. Microbiol.* 2012, 113, 1139–1144. [CrossRef]

6. Santoyo, G.; Moreno-Hagelsieb, G.; Orozco-Mosqueda, C.M.; Glick, B.R. Plant growth-promoting bacterial endophytes. *Microbiol. Res.* 2016, 183, 92–99. [CrossRef]

7. Barrao, C.A.; Lafi, F.F.; Alam, I.; De Zélécourt, A.; Eida, A.A.; Bokhari, A.; Saad, M.M. Complete genome sequence analysis of *Enterobacter* sp. SA187, a plant multi-stress tolerance promoting endophytic bacterium. *Front. Microbiol.* 2017, 8, 2023. [CrossRef]

8. Ludueña, L.M.; Anzuay, M.S.; Angelini, J.G.; McIntosh, M.; Becker, A.; Rupp, O.; Goesmann, A.; Blom, J.; Fabra, A.; Taurian, T. Genome sequence of the endophytic strain *Enterobacter* sp. J49, a potential biofertilizer for peanut and maize. *Genomics* 2019, 111, 913–920. [CrossRef]

9. Naveed, M.; Mitter, B.; Reichenauer, T.G.; Wieczorek, K.; Sessitsch, A. Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. *Environ. Exp. Bot.* 2014, 97, 30–39. [CrossRef]

10. Ali, S.; Duan, J.; Charles, T.C.; Glick, B.R. A bioinformatics approach to the determination of genes involved in endophytic behavior in *Burkholderia* spp. *J. Theor. Biol.* 2014, 343, 193–198. [CrossRef]

11. Yaish, M.W. Draft genome sequence of endophytic bacterium *Enterobacter asburiae* PDA134, isolated from date palm (*Phoenix dactylifera* L.) roots. * Genome Announc.* 2016, 4, e00848-16. [CrossRef] [PubMed]

12. Hinton, D.M.; Bacon, C.W. Enterobacter cloacae is an endophytic symbiont of corn. *Mycopathologia* 1995, 129, 117–125. [CrossRef] [PubMed]

13. Araujo, W.L.; Marcon, J.; Machcheroni, W.J.; van Elsas, D.; van Vuure, J.W.L. Diversity of endophytic bacterial populations and their interaction with *Xylostepha fastidiosa* in citrus plants. *Appl. Environ. Microbiol.* 2002, 68, 4906–4914. [CrossRef] [PubMed]

14. Asis, C.A.; Adachi, K. Isolation of endophytic diazotroph *Pantoea agglomerans* and nondiazotroph *Enterobacter asburiae* from sweet potato stem in Japan. *Lett. Appl. Microbiol.* 2003, 38, 19–23. [CrossRef]

15. Anzuay, M.S.; Ruiz Ciancio, M.G.; Ludueña, L.M.; Angelini, J.G.; Barros, G.; Pastor, N.; Taurian, T. Growth promotion of peanut (*Arachis hypogaea* L.) and maize (*Zea mays* L.) plants by single and mixed cultures of efficient phosphate solubilizing bacteria that are tolerant to abiotic stress and pesticides. *Microbiol. Res.* 2017, 199, 98–109. [CrossRef]

16. Sarkar, A.; Ghosh, P.K.; Pramanik, K.; Mitra, S.; Soren, T.; Pandey, S.; Maiti, T.K. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Res Microbiol.* 2018, 169, 20–32. [CrossRef]

17. Zhao, Y.; Yuan, Z.; Wang, S.; Wang, H.; Chao, Y.; Sederoff, R.R.; Sederoff, H.; Yan, H.; Pan, J.; Peng, M.; et al. Gene sdaB Is Involved in the Nematocidal Activity of *Enterobacter ludwigii* AA4 Against the Pine Wood Nematode *Bursaphelenchus xylophilus*. *Front. Microbiol.* 2022, 113, 870519. [CrossRef]

18. Oh, M.; Han, J.W.; Lee, C.; Choi, G.J.; Kim, H. Nematicidal and Plant Growth-Promoting Activity of *Enterobacter asburiae* HK169: Genome Analysis Provides Insight into Its Biological Activities. *J. Microbiol. Biotechnol.* 2018, 28, 968–975. [CrossRef]

19. Konstantinidis, K.T.; Tiedje, J.M. Genomic insights that advance the species definition for prokaryotes. *Proc. Natl. Acad. Sci. USA* 2005, 102, 2567–2572. [CrossRef]

20. Richter, M.; Rossello-Móra, R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. USA* 2009, 106, 19126–19131. [CrossRef]

21. Ciufó, S.; Kannan, S.; Sharma, S.; Badretdin, A.; Clark, K.; Turner, S.; Brover, S.; Schoch, C.L.; Kimchi, A.; DiCuccio, M. Using average nucleotide identity to improve taxonomic assignments in prokaryotic genomes at the NCBI. *Int. J. Syst. Evol. Microbiol.* 2018, 68, 2386–2392. [CrossRef]

22. Hall, S.A.; Mack, K.; Blackwell, A.; Evans, K.A. Identification and disruption of bacteria associated with sheep scab mites-novel means of control? *Exp. Parasitol.* 2015, 150, 261–264. [CrossRef] [PubMed]

23. Saccà, M.L.; Lodesani, M. Isolation of bacterial microbiota associated to honey bees and evaluation of potential biocontrol agents of *Varroa destructor*. *Benef. Microbes.* 2015, 10, 641–654. [CrossRef] [PubMed]

24. Cordova-Kreylos, A.L.; Fernandez, L.E.; Koivunen, M.; Yang, A.; Flor-Weiler, L.; Marrone, P.G. Isolation and characterization of *Burkholderia rhizoxinica* sp. nov., a non-*Burkholderia cepacia* complex soil bacterium with insecticidal and miticidal activities. *Appl. Environ. Microbiol.* 2013, 79, 7669–7678. [CrossRef]

25. Li, X.Y.; Wang, Y.H.; Yang, J.; Cui, W.Y.; He, P.F.; Munir, S.; He, Y.Q. Acaricidal Activity of Cyclodipeptides from *Bacillus amylovora* W1 Against *Tetranychus urticae*. *J. Agric. Food Chem.* 2018, 66, 10163–10168. [CrossRef] [PubMed]

26. El-Sayed, W.S.; El-Naggar, M.Y.; Elbadry, M. In vitro antagonistic activity, plant growth promoting traits and stress Tolerance Properties, Isolated from Sugarcane Root. *Front. Microbiol.* 2020, 11, 580081. [CrossRef]

27. Solanki, M.K.; Singh, R.K.; Srivastava, S.; Kumar, S.; Kashyap, P.L.; Srivastava, A.K.; Arora, D.K. Isolation and characterization of siderophore producing antagonistic rhizobacteria associated with *Rhizoctonia solani*. *J. Basic. Microbiol.* 2014, 54, 585–597. [CrossRef]
29. Sánchez-Cruz, R.; Tapia Vázquez, I.; Batista-García, R.A.; Méndez-Santiago, E.W.; Sánchez-Carbente, M.D.R.; Leija, A.; Lira-Ruan, V.; Hernández, G.; Wong-Villarreal, A.; Folch-Mallol, J.L. Isolation and characterization of endophytes from nodules of *Mimosa pudica* with biotechnological potential. *Microb. Res.* **2019**, *218*, 76–86. [CrossRef]

30. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Pyrievsky, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]

31. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef] [PubMed]

32. Vinuesa, P.; Ochoa-Sánchez, L.E.; Contreras-Moreira, B. GET_PHYLOMARKERS, a Software Package to Select Optimal Orthologous Clusters for Phylogenetics and Inferring Pan-Genome Phylogenies, Used for a Critical Geno-Taxonomic Revision of the Genus *Stenotrophomonas*. *Front. Microbiol.* **2018**, *9*, 771. [CrossRef] [PubMed]

33. Caballero, M.J.; Ondre, L.J.; Estrada De Los, S.P.; Martínez, A.L. The tomato rhizosphere, an environment rich in nitrogen-fixing *Burkholderia* species with capabilities of interest for agriculture and bioremediation. *Appl. Environ. Microbiol.* **2007**, *73*, 5308–5319. [CrossRef]

34. Rodríguez, H.; Fraga, R. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* **1999**, *17*, 319–339. [CrossRef]

35. Glickmann, E.; Dessaux, Y. A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl. Environ. Microbiol.* **1995**, *61*, 793–796. [CrossRef] [PubMed]

36. Schwyn, B.; Neilands, J.B. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **1987**, *160*, 47–56. [CrossRef]

37. De Lara, R.; Castro, T.; Castro, J.; Castro, G. Nematode culture of *Panagrellus redivivus* (Goodey, 1945) with *Spirulina* sp., enriched oatmeal. *Rev. Biol. Mar Oceanogr.* **2007**, *42*, 29–36. [CrossRef]

38. Quintero-Elena, Z.J.; Aguilar-Marcelino, L.; Castañeda-Ramírez, G.S.; Gómez-Rodríguez, O.; Villar-Luna, E.; López-Guillén, G.; Ramirez-Rojas, S.G. In vitro and micro-plot predatory activity of the mite *Caloglyphus mycophagus* against populations of nematode larvae of agricultural importance. *Biol. Control* **2022**, *165*, 104813. [CrossRef]

39. Vrain, T.C. A technique for the collection of larvae of *Meloidogyne* spp., and a comparison of eggs and larvae as inocula. *J. Nematol.* **1977**, *9*, 239–249.

40. Villar, L.E.; Reyes, T.B.; Rojas, M.R.; Gómez, R.O.; Hernández, A.A.; Zavaleta, M.E. Respuesta hipersensitiva en el follaje de chile CM.334 resistente a *Phytophthora capsici* infectado con *Nacobbus aberrans*. *Nematropica* **2009**, *39*, 143–153.

41. García Ortiz, N.; Aguilar Marcelino, L.; Mendoza de Gives, P.; López Arellano, M.E.; Bautista Garfias, C.R.; González Garduño, R. In vitro predatory activity of *Lasioseius penicilliger* (Arachnida: Mesostigmata) against three nematode species: *Teladorsagia circumcincta*, *Meloidogyne* sp. and *Caenorhabditis elegans*. *Vet. México OA* **2015**, *2*, 01–09.