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Research Article
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Genome sequencing and analysis of plant growth-promoting attributes from Leclercia adecarboxylata

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Abstract

Plant growth-promoting bacteria are ecological alternatives for fertilization, mainly for gramineous. Since plant x bacteria interaction is genotype and strain dependent, searching for new strains may contribute to the development of new biofertilizers. We aim to characterize plant growth-promoting capacity of Leclercia adecarboxylata strain Palotina, formerly isolated by our group in corn. A single isolated colony was taken and its genome was sequenced using Illumina technology. The whole genome was compared to other Leclercia adecarboxylata strains, and their biological and growth-promoting traits, such as P solubilization and auxin production, were tested. Following that, a 4.8 Mb genome of L. adecarboxylata strain Palotina was assembled and the functional annotation was carried out. This paper is the first to report the genes associated with plant growth promotion demonstrating in vitro indole acid production by this strain. These results project the endophyte as a potential biofertilizer for further commercial exploitation.

Keywords: Endophyte, Leclercia, genome, plant growth promotion, strains.

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Introduction

Leclercia adecarboxylata, a member of the Enterobacteriaceae family, is a motile, aerobic, omnipresent Gram-negative bacterium. Infections by L. adecarboxylata in humans are scarcely reported, being considered an opportunistic pathogen (Kashani et al., 2014; Hoyos-Mallecot et al., 2017; Choudhary et al., 2018). This strain was first described and named Escherichia adecarboxylata by Leclerc (1962), and, later received the generic name Leclercia from Tamura et al., (1986). It was phenotypically differentiated by biochemical and DNA hybridization assays from other Enterobacteriaceae species (Choudhary et al., 2018). Recently, Hoyos-Mallecot et al. (2017) published the draft genome of L. adecarboxylata strain harboring an NDM1 (Multidrug-resistant New Delhi metallo-β-lactamase 1) gene.

Plants and microorganisms naturally interact in the soil, forming a narrow and complex communication network. This network operates on biochemical to molecular signals, which can be altered according to the type of association (Souza et al., 2015). The promotion of direct growth occurs through the availability of nutrients, nitrogen, phosphate, as well as the production of plant regulators as auxins, cytokinins and amino acids. These regulators mainly promote central and lateral root growth, increasing the absorption surface, which in turn increases the root’s nutrient and water uptake (Beneduzi et al., 2012; Jha and Saraf, 2015).

However, the promotion of indirect growth occurs by means of induced systemic resistance (ISR). Some biocontrol mechanisms of pathogens are antibiosis, parasitism, competition for nutrients, production of hydrogen cyanide, siderophores, including the ones involved in responses to abiotic stresses, such as drought, salinity, extreme temperatures (Moreira et al., 2016).

Although this organism has been reported globally in food, water and animals (Tamura et al., 1986; Anuradha, 2014), for instance in strawberry root (Laili et al., 2017), evidences of its efficiency as plant growth promoter bacteria is scarce. In this context, we sequenced the complete genome of Leclercia adecarboxylata strain Palotina carrying out a comparative analysis with genomes of 16 different strains. This study provides new insights into genetic determinants, and as such may clarify some reported metabolic abilities of the Palotina strain, offering basic information on genetic plant growth promotion that may be relevant for biotechnological interest.

Material and Methods

DNA extraction and sequencing

Genomic DNA was extracted from the isolated strain following the protocol by Souza et al. (1991), using as template for a PCR reaction, Y1 and Y3 primers for amplification of the 16S rRNA gene (Cruz et al., 2001). Amplicons were enzymatically treated with ExoI/SA and the sequencing was performed on BigDye® Terminator v3.1 Cycle Sequencing in an ABI3500XL. The resulting sequences were assembled with CAP3 using BLASTn for comparison at NCBI.
The gDNA of *Leclercia* was quantified with Qubit, diluted and used for the construction of genomic DNA sequencing libraries using Illumina NexteraXT kit, according to the manufacturer’s recommendations. The libraries were quantified and the quality was verified by means of Bioanalyzer. The libraries were diluted to 500 pM and pooled. This pool was quantified by qPCR using the Kapa Biosystems kit, and 17.5 pM of pooled libraries were sequenced in the Illumina MiSeq with 500V2 kit in paired-end, generating paired reads of 250 base pairs from DNA fragments.

**Genome assembly, annotation and serotyping**

Overall, 5,795,728 reads were generated, representing a 31-fold coverage for the strain Palotina. FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to check the quality of the reads. SPAdes program (Bankevich et al., 2012), version 3.11.1 was used to reassemble the sequence dataset, which were deposited at NCBI site under the BioSample access number SAMN09791487. In order to identify putative coding sequences (CDS) and provide an initial automatic annotation, the genome sequences were submitted to the RAST server annotation pipeline (Aziz et al., 2008) and Artemis (Sanger Institute, Cambridge, UK) was used to curate annotations manually.

**Comparative genomics**

BLAST Ring Image Generator (BRIG) program (Alikhan et al., 2011) was used to compare the genome of *L. adecarboxylata* strain Palotina at nucleotide level against other strains available in the NCBI site (Table 1). It uses the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990), which is considered the most common tool for comparing genomes. Only 16 complete genomes found at NCBI were considered, being 9 from *L. adecarboxylata* strains (NCTC13032; Z96-1; E61; P12375; J656; 16005813; USDA-ARS-USMARC-60222; E1 and R25) and 7 from *Leclercia* sp. strains (W6; 119287; 1106151; LSNH1; LSNH3; J807 and W17), the great majority from clinical isolates (Table 1).

Also, three genes related to plant growth promotion (P metabolism and auxins biosynthesis) were selected and compared by BLASTn against all genomes. In phylogenetic analyses, a Neighbor-Joining tree (Saitou and Nei, 1987) was constructed with 98 genomes with NCBI Tree Viewer (version 1.17.5).

**Biochemical characterization of L. adecarboxylata**

At first, *L. adecarboxylata* strain Palotina was isolated in LB medium, growing well in DYGS (Dobereiner et al., 1995), following the isolation protocol by (Chaves et al., 2019).

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**Table 1 – Characteristics of Leclercia strains used in the genomic comparison.**

| Organism              | Strain       | BioSample    | Assembly                | Size (Mb) | GC% | Replicons         | Isolation source         |
|-----------------------|--------------|--------------|-------------------------|-----------|-----|-------------------|--------------------------|
| *Leclercia adecarboxylata* | NCTC13032    | SAMEA2580321 | GCA_001472455.1         | 5.06      | 55.5| Chromosome        | Drinking water            |
| *Leclercia adecarboxylata* | Z96-1        | SAMN11950933 | GCA_006171285.1         | 5.76      | 55.4| Chromosome + 7 plasmids | Human stool               |
| *Leclercia adecarboxylata* | E61          | SAMN12289350 | GCA_008931385.1         | 5.69      | 55.0| Chromosome + 5 plasmids | Shower                   |
| *Leclercia adecarboxylata* | P12375       | SAMN13341565 | GCA_009720165.1         | 4.93      | 55.6| Chromosome        | Hospital                 |
| *Leclercia adecarboxylata* | J656         | SAMN12530229 | GCA_008807335.1         | 4.84      | 55.6| Chromosome        | Human secretion           |
| *Leclercia adecarboxylata* | 16005813     | SAMN10923138 | GCA_004295325.1         | 4.82      | 55.7| Chromosome        | Sputum                   |
| *Leclercia adecarboxylata* | USDA-ARS-USMARC-60222 | SAMN04158503 | GCA_001518835.1         | 4.80      | 55.8| Chromosome        | Calf nasopharynx          |
| *Leclercia adecarboxylata* | E1           | SAMN12289304 | GCA_008931445.1         | 5.51      | 54.8| Chromosome + 6 plasmids | Shower                   |
| *Leclercia adecarboxylata* | R25          | SAMN10790257 | GCA_006874705.1         | 4.91      | 56.2| Chromosome + 2 plasmids | Rabbit                   |
| *Leclercia sp.*         | W6           | SAMN09667310 | GCA_003363435.1         | 4.95      | 55.9| Chromosome        | Human stomach             |
| *Leclercia sp.*         | 119287       | SAMN13394079 | GCA_009734485.1         | 4.87      | 55.6| Chromosome        | Hospital                 |
| *Leclercia sp.*         | 1106151      | SAMN13394552 | GCA_009740165.1         | 4.85      | 56.1| Chromosome        | Urine                    |
| *Leclercia sp.*         | LSNH1        | SAMN06040403 | GCA_002902985.1         | 5.41      | 55.5| Chromosome + 4 plasmids | Sludge                   |
| *Leclercia sp.*         | LSNH3        | SAMN06040408 | GCA_002931051.1         | 5.39      | 55.3| Chromosome + 4 plasmids | Sludge                   |
| *Leclercia sp.*         | J807         | SAMN13393390 | GCA_009734465.1         | 4.72      | 56.1| Chromosome        | Human blood               |
| *Leclercia sp.*         | W17          | SAMN09667311 | GCA_00336325.1          | 5.13      | 56.0| Chromosome + 2 plasmids | Human stomach             |
Visual assays determined bacteria morphology and colony color. Bacterial phosphate solubilization was detected in vitro by inoculation in NBRIP medium (Nautiyal et al., 2000). A bacterial colony was collected with a toothpick, and each ¼ plate of NBRIP medium plate was inoculated. A halo around the colonies was observed after 10 days in a culture incubated at 28 °C. The solubilization index (SI) was calculated as: SI=Diameter of Halo (mm) / Diameter of colony (mm) (Nautiyal, 1999).

Indole-3-acetic acid (IAA) production by bacteria was based on the Glickmann and Dessaux (1995) protocol. Isolates were inoculated into glass vials (penicillin-type) containing 4 mL of medium with tryptophan (5.0 g.L⁻¹ glucose, 0.025 g.L⁻¹ yeast extract and 0.204 g.L⁻¹ L-TRP) and no tryptophan supplementation (Sarwar and Kremer, 1995). Triplicate vials were incubated in a shaker cooled at 28 °C in the dark at 120 rpm. After the growth, which occurred in 48 h, 2 mL of the culture medium was centrifuged at 10000 g for 10 min at 4 °C. Next, 1 mL of the bacterial suspension supernatant was transferred to a 15 mL Falcon-type tube with the addition of 1 mL of Salkowski reagent. The standard curve was assayed for final concentrations of 0 to 0.03 mg mL⁻¹. Samples were left in the dark for 30 min and the AIA quantification was performed by spectrophotometer reading at 535 nm.

Liquid-4-acetic acid (IAA) production by L. adecarboxylata was primarily screened and further grown on LB plate containing 1%, 2%, 5% and 10% NaCl separately for 48 h at 30 °C. In addition, the optimum pH was checked using LB liquid medium with different pH (4; 5; 5.5; 6; 6.5; 7; 7.5 and 8). The growth temperatures assessed were 25 and 37 °C. The presence of oxidase was tested using TEMED 1 % (N-N-dimetil-p-phenilenediamine) (Kovacks,1956). The presence of catalase was verified by the presence of bubbles when hydrogen peroxide was deposited in a colony (Yano et al., 1991). All assays were made in triplicate.

Blood agar plates (5 % (v/v) sheep blood) were used for biosafety test (Russell et al., 2006; Suleman et al., 2018). The hemolytic capacity was evaluated after 48 h from fresh culture of L. adecarboxylata streaked onto blood agar plate and incubated at 37 ± 2 °C.

Results

Genome assembly, annotation and comparative genomics

After de novo assembly, the genome of Leclercia adecarboxylata strain Palotina was represented in 20 contigs, sized 4,801,735 bp, with GC content of 55.7%, 4,379 coding sequences and no plasmid were observed. The comparison showed differences in the genome of L. adecarboxylata strain Palotina (Figure 1, Table 1). The size of L. adecarboxylata strains ranged from 4.72 to 5.76 Mb, their CG content between 55-56% CG content. Some contained plasmids (up to 7).

Figure 1 – Genomic comparison among Leclercia strains. Each ring represents the genome of one strain. Leclercia adecarboxylata strain Palotina (NCBI BioSample SAMN09791487) was used as genome of reference. Gaps in the rings mean absence of the region in the target genome.
The BRIG genomic analyses showed CRISPR system and mobile elements as phages were absent in some strains. One interesting data is that no *Leclercia* sp contained the indole acetamide hydrolase gene. However, group genes related to bacterial systems, such as several hypothetical proteins, Type I restriction and cobalt/cadmium/zinc RND efflux, were absent in all strains used in the comparison (Figure 1, Figures S1 to S3).

Of all identified coding sequences, Rast server classified 2597 genes (60%) in categories (Subsystems) and 1782 (40%) were grouped as not classified (Not in Subsystems). The categories with the highest number of genes were carbohydrates metabolism (613 genes), followed by amino acids and derivatives (470 genes), and protein metabolism with 302 genes (Figure 2). Dormancy, sporulation and secondary metabolism showed the lowest gene number (only 5).

In addition, in the nitrogen metabolism category 49 genes were identified and then grouped in four subcategories: nitrosative stress (6 genes), nitrate and nitrite ammonification (22 genes), Ammonia assimilation (13 genes) and denitrifying reductase gene clusters with 8 genes. Phosphorus metabolism (47 genes) had 8 genes related to PHO regulon and high affinity phosphate transporter, Phosphate metabolism (22), Polyphosphate (3) and Alkylphosphonate utilization (14). Finally, in secondary metabolism 5 genes were related to Aixin biosynthesis (Table S1).

BLASTp comparison revealed 14 genes related to plant growth promotion that showed high identity (> 97%) and high e-value (Table S2). The phylogenetic analysis of all the 98 genomes found at NCBI, belonging to “*Leclercia*”, showed a higher similarity between *L. adecarboxylata* strain Palotina and USDA-ARS-USMARC-60222, isolated from calf nasopharynx, an indicative that these bacteria can be associated to agricultural area, unlike clinical strains (Figure 3).

**Biochemical and molecular characterization of *L. adecarboxylata***

The biological and plant growth promotion traits are summarized in Table 2. *L. adecarboxylata* strain Palotina is a cream rod-shaped, non-spor-forming, motile, Gram-negative bacillus of family Enterobacteriaceae, having an optimum pH growing range between 5.0-8.0, 25-37 °C for growth temperature and a low salinity tolerance (below 5%).

The strain also presented oxidative and negative catalase response (Table 2). In addition, genes for chitinase production were found in the genome. Antifungal resistance was not tested in *L. adecarboxylata* strain Palotina by the inoculation with *Aspergillus flavus*.

Halo zone formation on blood agar medium was observed *in vitro*, which points to hemolysin gene expression, confirming the opportunistic pathogen trait. A lipase gene was annotated in the genome, demonstrates a potential use of this strain for biotechnological purposes. Moreover, we identified genes that can be related to the improvement of nutrient availability to plants (Tables 3 and 4), which is consistent with many plant growth promoting bacteria (PGPB). The genome of *L. adecarboxylata* strain Palotina possesses genes encoding glucose dehydrogenase (*gcd*), the major enzyme responsible for the production of gluconic acid. Palotina strain showed a medium capacity of P solubilization (2 < PSI > 4) (Table 2). UDP-glucose dehydrogenase gene was present in all 16 *Leclercia* genomes (Table 3).

*Trp* cluster (trpC, D and F), tryptophan-permease, tryptophan-synthase (a) and (b) genes, and indole acetamide hydrolase gene involved in tryptophan biosynthesis were found in the genome (Table 4). We observed an increase of 2.3-fold in IAA production when tryptophan was added to the culture medium (Table 2). When we compared all 16 *Leclercia* genomes, the phosphoribosyl anthranilate isomerase gene was found in all strains (Table 3).
Ammonia assimilation genes, among others, seem to be the main N metabolism pathway, confirmed by the presence of several genes, such as GS type I (Glutamine synthase); NADPH-GOGAT; Amt (ammonia transporter); NRI (protein regulator) and PKII (Table 4).

The Ferric hydroxamate ABC transporter Fhu genes Fhu, ViuB, TonB, TonB3, FiU were verified, although we did not evaluate the siderophore production (Table 4). Genes coding for antioxidant enzymes as peroxidases, catalases, superoxide dismutase, among others, were found at L. adecarboxylata genome (Table 4). Genes that enable bacteria to survive at harsh conditions were also detected: heat shock tolerance genes (groE, YciM, hslJ, FtsJ/RrmJ), cold shock tolerance (cspA, C, D, E, G), and glycine betaine (Gupta et al., 2014).

Some genes related to cell-cell communication via quorum sensing (QS) were found in the L. adecarboxylata genome: N-3-oxohexanoyl-L-homoserine lactone quorum-sensing transcriptional activator, Autoinducer 2 (AI-2) transport and processing (lsrACDBFGE) operon (Table 4).

Figure 3 – Phylogenetic relationship among Leclercia strains. In red L. adecarboxylata strain Palotina. The sequences were aligned using the Neighbor-joining method (Saitou and Nei, 1987). The 1,000 resampling bootstrap values are shown.
Table 2 – Biological and plant growth promotional properties of *L. adecarboxylata* strain Palotina.

| Attributes                  | Value       |
|-----------------------------|-------------|
| pH tolerance levels         | 5.0-8.0     |
| Optimum pH for growth       | 6.0-7.0     |
| NaCl tolerance              | < 5%        |
| Optimum temperature for growth | 25-37°C    |
| IAA production              | Positive (2.6 ± 0.3 µg mL⁻¹) |
| Phosphate solubilization    | Positive (PSI>2) |
| Oxidase                     | Negative    |
| Catalase                    | Negative    |
| Chitinase                   | Negative    |
| Hemolysis                   | Positive    |

Table 3 – Plant growth promotion genes in all strains compared.

| Organism          | Strain          | Indoleacetamide hydrolase | UDP-glucose dehydrogenase | Phosphoribosylanthranilate isomerase | Isolation source       |
|-------------------|-----------------|----------------------------|---------------------------|--------------------------------------|------------------------|
| *L. adecarboxylata* | NCTC13032       | 98                         | 98                        | 99                                   | Drinking water         |
| *L. adecarboxylata* | Z96-1           | 79                         | 88                        | 90                                   | Human stool            |
| *L. adecarboxylata* | E61             | 98                         | 98                        | 98                                   | Shower                 |
| *L. adecarboxylata* | P12375          | 98                         | 98                        | 98                                   | Hospital               |
| *L. adecarboxylata* | J656            | 98                         | 97                        | 99                                   | Human secretion        |
| *L. adecarboxylata* | USDA-ARS-USMARC-60222 | 98                  | 98                        | 98                                   | Sputum                 |
| *L. adecarboxylata* | E1              | 98                         | 98                        | 98                                   | Shower                 |
| *L. adecarboxylata* | R25             | 87                         | 92                        | 91                                   | Rabbit                 |
| Leclercia sp.      | W6              | Not match                  | 88                        | 90                                   | Human stomach          |
| Leclercia sp.      | 119287          | 87                         | 92                        | 91                                   | Hospital               |
| Leclercia sp.      | 1106151         | Not match                  | 89                        | 90                                   | Urine                  |
| Leclercia sp.      | LSNIH1          | Not match                  | 89                        | 90                                   | Sludge                 |
| Leclercia sp.      | LSNIH3          | 98                         | 99                        | 99                                   | Sludge                 |
| Leclercia sp.      | J807            | Not match                  | 89                        | 90                                   | Human blood            |
| Leclercia sp.      | W17             | Not match                  | 89                        | 90                                   | Human stomach          |

Table 4 – List of genes attributable to plant growth promotion traits in *L. adecarboxylata* genome.

| Plant growth promotion traits | Genes with potential for conferring PGP traits |
|------------------------------|---------------------------------------------|
| Phosphate solubilization     | Glucose dehydrogenase gene                  |
| IAA production               | *TrpD, TrpF*, tryptophan-permease, tryptophan-synthase (a) and (b), indole acetamide (Indole acetamide hydrolase) |
| N assimilation               | *GS* type I, *NaDPH-GOGAT*, *Amt*, *NRI*, *PIIK* |
| Siderophore production       | Ferric hydroxamate ABC transporter (*Fhu* genes), *ViaB, TonB, TonB3, FiU* |
| Acetoin & butanediol synthesis | Acetolactate synthase large subunit, Acetolactate synthase small subunit |
| Phenezine production         | *phzF*                                      |
| Chitinase production         | Chitinase gene                              |
| Trehalose metabolism         | Trehalose -6-phosphate synthase gene        |
| Quorum sensing               | Autoinducer 2 (AI-2) transport and processing (*ksrACDBFGE* operon) |
| Heat shock proteins          | *groE, YciM, kslJ, FisJ/RrmJ*               |
| Cold shock proteins          | *cspA, C, D, E, G*                         |
| Glycine-betaine production   | *proX*                                      |
| Peroxidases                  | *oomC*, glutathione peroxidase genes similar to *Enterobacter asburiae* |
| Catalases                    | Catalase gene                               |
| Superoxide dismutase         | superoxide dismutase gene                   |
| Auxins production            | Monoamine oxidase, Phosphoribosylanthranilate isomerase, Tryptophan synthase alpha and beta chain |
**Discussion**

Our findings indicate the complete absence of the RND protein family, which was reported as a group of bacterial transport proteins involved in cell division, nodulation and heavy metal resistance (Nies, 2003). Another gene sequence that appeared to be distinct among *Leclercia* genomes is the clustered regularly interspaced short palindromic repeats (CRISPR), which is related to the microbial immune system. It contains a family of proteins whose functional domains are related to polynucleotide-binding proteins, polymerases, nucleases, and helicases (Horvath and Barrangou, 2010; Ishino *et al.*, 2018). This region was observed in only three of *Leclercia* strains, including strain Palotina, which shows a horizontal gene transfer promoting a genomic differentiation among strains (Portillo and Gonzalez, 2009). A Type I restriction system or Restriction modification system (R-M system) was absent in all compared genomes. R-M system has large pentameric proteins with separate restriction, methylation and DNA sequence-recognition subunits (Loenen *et al.*, 2014), which grants to the host bacterium a selective advantage (Sitaraman, 2016).

Carbohydrate metabolism genes were present in *L. adecarboxylata* strain Palotina enabling this bacterium to grow in different media using different carbohydrate/energy sources, including root exudates and other organic compounds. Moreover, this strain would interact positively with plants under harsh soil conditions.

Although our strain was able to carry out an alpha hemolysis, Muratoglu *et al.* (2009) and Anuradha (2014), who tested *L. adecarboxylata* Ld1 and human isolates respectively, found a negative response to blood hemolysis. The contrasting results could possibly be explained by the presence of the hemolysin gene set found in the genome of our strain.

Strain Palotina showed a P solubilization capacity, probably explained by the presence of the *gcd* gene. Glucose dehydrogenase is the key enzyme in the biosynthesis of gluconic acid in the direct oxidation pathway of glucose, responsible for P solubilization (Chen *et al.*, 2016; Suleman *et al.*, 2018). The amount of gluconic acid released would control the availability of soluble phosphates (De Werra *et al.*, 2006). Also, UDP-glucose dehydrogenase found in all compared strains (Table 3) catalyzes a NAD+-dependent two-fold oxidation of UDP-glucose to generate UDP-glucuronic acid (Chen *et al.*, 2019). This acid is also a precursor to UDP-xylolose component of the cell wall polysaccharides in plants (Gibeaut and Carpita, 1994).

Another important PGP feature is the auxins/cytokinin metabolism. The biosynthesis of IAA occurs from tryptophan (Patten *et al.*, 2013) and a higher IAA production can be induced by addition of tryptophan to culture media. Biosynthesis of tryptophan encoded by *trp* genes occurs in five-step reactions from chorismate (Spaepen and Vanderleyden, 2011). Five different pathways were described to the IAA production in bacteria: the indole-3-acetamide (IAM), indole-3-pyruvic acid (IPyA), indole-3-acetonitrile (IAn), tryptamine (TAM), and tryptophan side-chain oxidase (TSO) pathways (Kocher *et al.*, 2013; Li *et al.*, 2018).

Genomic analyses identified the indole acetamide hydrolase gene, which explains the IAA production mainly by IAM pathway suggesting the tryptophan-dependent IAM pathway function in strain Palotina. The main pathway to IAA production in PGPB is via indole-3-pyruvic acid, dependent on L-tryptophan (Souza *et al.*, 2015). Not all *Leclercia adecarboxylata* and no *Leclercia* sp. strains present the indole acetamide hydrolase gene, which suggests that this gene has been acquired. This fact explains the association between bacteria and corn plants.

In addition, we identified the sequence of phosphoribosyl anthranilate isomerase (PRAI) encoded by *trpC* (Table 4). This enzyme is responsible for the conversion of N-(5′-phosphoribosyl)-anthranilate (PRA) to 1-(o-carboxyphenylamino)-1-deoxyribose 5-phosphate (CDRP), the fourth step in tryptophan biosynthesis (Thoma *et al.*, 2000). Moreover, monoamine oxidase plays an important role in tryptamine biosynthesis, whose oxidative deamination of tryptamine to indole acetaldehyde is known to be the main course for IAA formation, despite the fact that the role of monoamine oxidase has not been completely characterized (Ueno *et al.*, 2003). The presence of these genes suggests that the tryptophan-dependent IAM and TAM pathways function in *L. adecarboxylata*.

*L. adecarboxylata* produced 2.6 µg mL⁻¹ of IAA (Table 2). Albeit the variable levels, Gupta *et al.*, (2014) related IAA production of 1.2-2.5 µg mL⁻¹ to candidate PGPB strains isolated from coconut, cocoa and arecanut plants, while Moreira *et al.*, (2016) found strains that could produce more than 80 µg mL⁻¹ of indolic compounds. We did not identify an *acdS* gene coding for ACC deaminase enzyme in our strain, which demonstrates the absence of this enzyme among PGP traits. However, Kang *et al.* (2019) suggested that the IAA and ACC deaminase helped tomato (*Solanum lycopersicum*) plants to tolerate salt stress, despite having found *acdS* gene in *L. adecarboxylata* strain MO1.

Ammonia assimilation, among others, seems to be the main N metabolism pathway from nitrate. In addition, this strain exhibits the genes for denitrification used as energy source. These genes indicated that *L. adecarboxylata* has an important role in soil N cycling system. The results agree with Muratoglu *et al.* (2009) who observed an absence of nitrogen fixation capacity as well as a presence of NO, metabolism in Ld1 strain. From these data, *L. adecarboxylata* can be used as a model for PGP bacteria exclusively by auxins production.

Peroxidases, catalases, superoxide dismutase, and glutathione transferases genes found at *L. adecarboxylata* genome could help plants to overcome oxidative stress. Also, heat and cold shock genes could support bacteria to survive during abiotic or biotic stress (Gupta *et al.*, 2014), which enable bacteria to adapt to adverse growth conditions.

Another strategy to cope with abiotic stresses is the accumulation of compatible solutes, such as trehalose, proline and glycine betaine, among others, by some soil bacteria (Suarez *et al.*, 2019). The strain Palotina genome contains trehalose-6-phosphate synthase involved in GDP- or UDP-glucose conversion to trehalose (Avonce *et al.*, 2006). Also, glycine betaine/proline betaine-binding periplasmic protein (*ProX*) is one of three genes from operon *VWX* involved in binding compatible solutes with high affinity and specificity (Schiefner *et al.*, 2004).
We also found genes related to acetoin and 2,3 butanediol production, which are volatile compounds (VOCs) involved in plant growth bacteria/fungi interaction as acetolactate synthase large and small subunit (Yi et al., 2016; Fincheira and Quiroz, 2018). VOCs are synthesized by the condensation of two pyruvate molecules into acetolactate by acetolactate synthase, which forms acetoin by acetolactate decarboxylase decarboxylation. The reduction of acetoin by acetoin reductase results in 2,3-butanediol (Suarez et al., 2019).

The strain Palotina contains phzF encoding phenazine biosynthesis. Phenazines can modify the cellular redox state by electron transport, acting in the cell signaling regulating gene expression. By contributing to biofilm formation and architecture, it can enhance bacterial viability in the rhizosphere (Pierson and Pierson, 2010).

Autoinducer 2 (AI-2) transport and processing (lsrACDBFGE) operon (Table 4) codifies molecules related to motility, biofilm formation and production of virulence factors (Reading and Sperandio, 2006). AI-2 has been suggested to act directly through quorum sensing while (lsrACDBFGE) operon encodes an ATP-binding cassette transporter (ABC transporter) that internalizes AI-2 in gram-negative bacteria (Pappenfort and Bassler, 2014). In the marine bacterium Vibrio fischeri, N-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C6-HSL) acts as autoinducer in the quorum-sensing system (Yan et al., 2007).

Genome sequencing of a strain might provide more abundant screening tools for the PGBP, which could be readily detected in genomes (Finkel et al., 2017). The authors mentioned that the presence of minimal Nif cluster and genes required for indole acetic acid production are potent markers, albeit at variable levels, for screening potential strains, making the process faster and less labor extensive. Scagnoli et al. (2016) affirmed that a potential PGBP candidate must have the ability to solubilize phosphate and iron (siderophores) and IAA. The data pointed to a PGP strain candidate and further studies should be conducted to reveal the full genetic mechanisms of plant interaction.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

ECGV conceived the project and wrote the manuscript. AS and AF conducted the laboratory experiments. DM performed the genomics data analysis. MFS did the corrections.

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Supplementary Material
The following online material is available for this article:
Table S1 – List of genes and their function in L. adecarboxylata 
strain Palotina.

Table S2 – List of genes related to plant growth promotion 
in L. adecarboxylata strain Palotina.
Figure S1 – Genomic map of CRISPR system.
Figure S2 – Genomic map of RND efflux system.
Figure S3 – Genomic map of Type I restriction-modification 
system.

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