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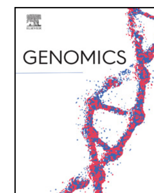

Short Communication

Genome sequence of the endophytic strain *Enterobacter* sp. J49, a potential biofertilizer for peanut and maize

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ABSTRACT

Enterobacter sp. J49 is a plant growth promoting endophytic strain that promotes the growth of peanut and maize crops. This strain promotes plant growth by different mechanisms with the supply of soluble phosphorus being one of the most important. *Enterobacter* sp. J49 not only increases the phosphorus content in the plant but also in the soil favoring the nutrition of other plants usually used in rotation with these crops. The aims of this study were to analyze the genome sequence of *Enterobacter* sp. J49 in order to deepen our knowledge regarding its plant growth promoting traits and to establish its phylogenetic relationship with other species of *Enterobacter* genus. Genome sequence of *Enterobacter* sp. J49 is a valuable source of information to continuing the research of its potential industrial production as a biofertilizer of peanut, maize and other economically important crops.

1. Introduction

Plants are responsible for the selection of their microbiome in order to have beneficial bacterial colonizers designated as plant growth promoting bacteria (PGPB). This heterogeneous group of bacteria can positively impact plant's growth and health, by providing nutrients and/or by suppressing soil-borne pathogens [1]. PGPB includes those bacteria that live in the rhizosphere and also endophytes that live inside the plant tissues [2,3]. The ability of endophytic bacteria to colonize plant tissues is considered a promising trait since it has been described that they promote more efficiently plant's growth, health and development than rhizospheric bacteria [4–7]. As well as other PGPB, endophytic bacteria can produce plant's growth regulating compounds like indole acetic acid, acetoin, 2,3-butanediol and cytokinins [4–8]. In particular in many of endophytes analyzed, the synthesis of enzyme ACC deaminase that decreases the stress ethylene precursor ACC (1-aminocyclopropane-1-carboxylic acid) has been described [5–7,9,10]. The ability to colonize the interior of plant tissues gives the advantage of evading the competition present in the rhizospheric environment and it allow to achieve a more intimate relationship with the plant [6,11,12].

Peanut (*Arachis hypogaea* L.) is a *Fabaceae* plant of great agricultural and economic significance in many countries around the world. Argentina is one of the most important world producers and 80% of its production is exported. In this country, approximately 90% is produced in Córdoba province. In this area, peanut crop cultivation is normally rotated with maize (*Zea mays* L.) crop, which production, in this region, achieves 50% of national production [13,14]. In agricultural soils of Córdoba the use of intensive practices applied along many years has decreased their nutritional quality, exhibiting low levels of important nutrients like phosphorus (P), nitrogen (N) and potassium (K) [15].

The “*Enterobacter* clade” consists of species belonging to the *Leclercia, Enterobacter* and *Lelliottia* genera [16]. *Enterobacter* genus comprises species that are difficult to identify with biochemical and phylogenetic tests [17–19], and some of them have been reported as plant-growth promoters because of their multiple growth-promoting activities. For example; *Enterobacter asburiae* PDA 134 from Date palm [9], *Enterobacter cloacae* from citrus and maize plants [20,21] and

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Table 1
Genome features of *Enterobacter* sp. J49.

	Enterobacter sp. J49
Genome size (bp)	4,969,619 bp
GC content	54.43
CDS	4620
Genes	4628
rRNA	12
tRNA	83
No of plasmid	0
No of chromosome	1

Enterobacter asburiae from sweet potato [22]. Strain *Enterobacter* sp. P23 promotes rice growth under salt stress because of its high ACC deaminase activity [23]. Besides, *Enterobacter cloacae* subsp. *dissolvens* MDSR9 has been recovered from the soybean rhizosphere and it has been reported that it can enhance significantly the growth of this legume and wheat [24]. In a recent work, Andrés-Barrao et al. [7] described an endophytic *Enterobacter* sp. SA187 capable of provide abiotic stress tolerance to *Arabidopsis thaliana*. Besides, *Enterobacter mori*, *Enterobacter asburiae* and *Enterobacter ludwigii* showed to promote wheat growth under stress conditions by lowering ethylene levels through the production of ACC deaminase enzyme [25].

Enterobacter cloacae complex (Ecc) comprises bacteria which belong to *Enterobacter* genus and includes different species; *Enterobacter soli*, *Enterobacter cancerogenus*, *Enterobacter xianfangensis*, *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii* and *E. mori* [16]. Ecc includes species that are PGPB as mentioned previously, and also members of clinical significance that are isolated as nosocomial pathogens [17,26]. The high diversity of Ecc can only be explained by the analysis of the complete genome sequence of every strain belonging to this group [7,16].

Enterobacter sp. J49 belongs to a bacterial collection that was isolated from peanut root nodules grown in Cordoba production area [27]. This strain exhibits a strong *in vitro* ability to solubilize inorganic and organic insoluble phosphates [15,27], it is able to synthesize siderophores [27] and promotes directly the growth of peanut and maize under controlled growth conditions [15,27,28]. *Enterobacter* sp. J49 also promotes biological nitrogen symbiosis by native rhizobium strains present in unsterile soil [28] and when it is co-inoculated with reference strain *Bradyrhizobium* sp. SEMIA 6144 [29].

In addition, it was also reported that this strain can grow well under abiotic stresses, like salinity, extreme pHs, high temperature and in the presence of a wide variety of pesticides traditionally applied in Argentina on peanut and maize crops [15]. Considering all the information previously described, the aims of this work were to analyze the genome sequence of *Enterobacter* sp. J49 to deepen our knowledge regarding its plant growth promoting traits and to establish its phylogenetic relationship with other species of *Enterobacter* genus. Genome sequence of *Enterobacter* sp. J49 is a valuable source of information to continuing the research of its potential industrial production as a biofertilizer of peanut, maize and other economically important crops.

2. Results and discussion

2.1. General characteristics of the genome sequence of Enterobacter sp. J49

The Whole Genome Shotgun project of *Enterobacter* sp. J49 has been deposited at GenBank under the accession NZ_MWPY00000000 and showed an output of 2,956,310 reads. The version described in this

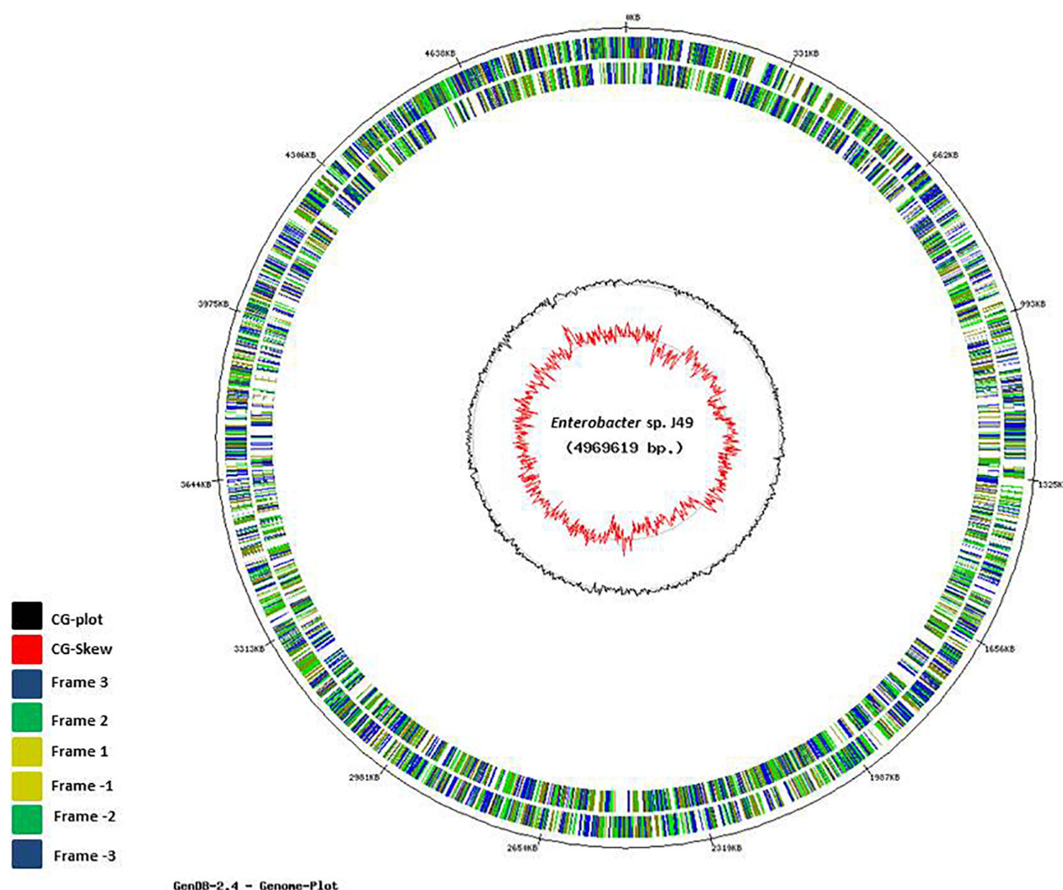

Fig. 1. Graphical circular genomic maps of *Enterobacter* sp. J49. The red and black circles show GC content (%) and GC skew, respectively. The outer circles show the predicted protein-coding sequences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

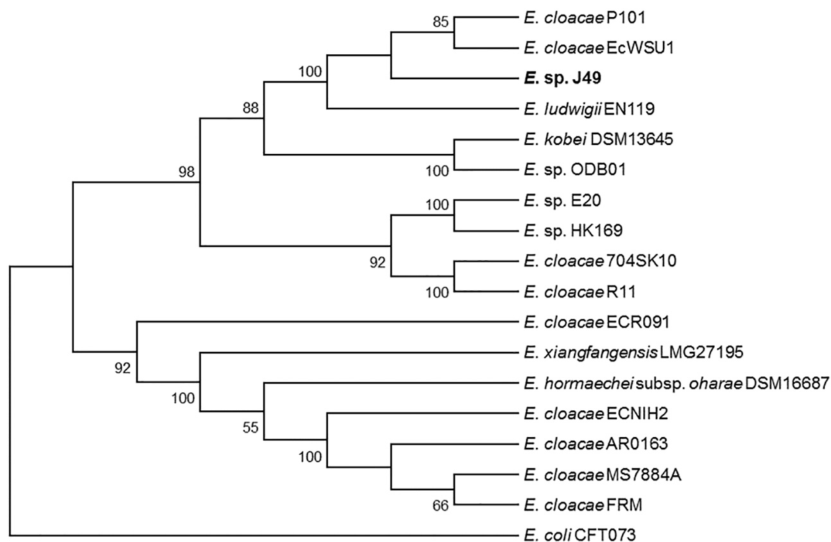

Fig. 2. Phylogenetic tree highlighting the position of *Enterobacter* sp. J49 with respect to other closely related species within the genus of *Enterobacter*. The phylogenetic tree was constructed based on concatenated sequences of 16S rRNA, *gyr*B and *rpo*B genes aligned with ClustalW2 using Maximum Likelihood method and rooted with *Escherichia coli* CFT073 in MEGA5 workbench [32].

Table 2
Phylogenomic overview using average nucleotide identity analysis (ANI) data calculated from whole genome sequences compared to *Enterobacter* sp. J49 strain by using the online calculator www.ezbiocloud.net/tools/ani.

Bacterial strains	Accession number	Total lenght (bp)	GC%	ANI value (%) respect to *Enterobacter* sp. J49
Enterobacter sp. J49	NZ_MWPY00000000.1	4.956.522	54.44	100
Enterobacter cloacae EcWSU1	CP002886.1	4.734.438	54.61	98.92
Enterobacter cloacae P101	CP006580.1	5.369.929	54.38	98.87
Enterobacter ludwigii EN119	CP017279.1	4.574.439	54.60	98.77
Enterobacter sp. E20	CP012999.1	4.763.114	55.75	88.21
Enterobacter sp. HK169	CP017087.1	4.551.186	56.15	88.20
Enterobacter cloacae 704SK10	CP022148.1	4.876.946	55.86	88.90
Enterobacter cloacae R11	CP019839.1	4.812.230	55.92	87.88
Enterobacter cloacae GGT036	CP009756.1	4.848.754	55.03	87.48
Enterobacter kobei DSM13645	CP017181.1	4.880.257	54.95	87.46
Enterobacter sp. ODB01	CP015227.1	4.534.036	54.81	87.30
Enterobacter mori	NZ_NFZM00000000.1	4.960.127	55.24	87.23
Enterobacter cloacae ECNIH2	CP008823.1	4.852.980	55.46	86.24
Enterobacter hormaechei DSM16687	CP017180.1	4.724.316	55.58	86.17
Enterobacter cloacae MS7884A	CP022532.1	4.810.853	55.44	86.11
Enterobacter cloacae AR0163	CP021749.1	5.172.197	54.95	86.09
Enterobacter cloacae FMR	CP019889.1	4.899.400	55.49	86.09
Enterobacter xiangfangensis LMG27195	CP017183.1	4.661.849	55.28	86.03
Enterobacter cancerogenus	FYBA000000000.1	4.879.939	55.63	86.00
Enterobacter soli	FYBB000000000.1	5.020.403	53.76	85.92
Enterobacter asburiae LF7a	NC-015968.1	4.812.833	53.85	85.37
Enterobacter sp. 638	NC-009436.1	4.518.712	52.98	82.06
Enterobacter lignolyticus SCF1	NC-014618.1	4.814.049	56.20	79.40
Enterobacter sp. R4-368	CP005991.1	5.039.027	54.03	78.39

paper is version NZ_MWPY00000000.1 (Table 1, Fig. 1) and, comprises 4,956,522 bp, 832 contigs and 80 Scaffolds (N50: 403.18 kb).

2.2. Taxonomic classification of Enterobacter sp. J49

To define taxonomic classification of the strain *Enterobacter* sp. J49 three bioinformatic approaches were performed: (i) multilocus sequence alignment (MLSA) analysis based on three core housekeeping genes highly conserved among bacteria; 16S rRNA, *gyr*B and *rpo*D, (ii) an average nucleotide identity analysis (ANI) was done by using the web page EzBiocloud, and (iii) whole-genome phylogenetic analysis using 24 complete genomes of closely related species. The phylogenetic tree constructed using the concatenated gene sequences of 16S rRNA, *gyr*B and *rpo*B showed that *Enterobacter* sp. J49 is closely related to *Enterobacter cloacae* P101, *Enterobacter cloacae* EcWSU1 and *Enterobacter ludwigii* EN119 strains (Fig. 2), all of them members of *Enterobacter cloacae* complex [7,16,19]. The ANI analysis also indicated a close relationship of J49 with these strains since it showed an ANI value of 98.87%, 98.92% and 98.77% with respect to *Enterobacter cloacae* P101, *Enterobacter cloacae* EcWSU1 and *Enterobacter ludwigii* EN119, respectively (Table 2). The first two strains were isolated from switchgrass [18] and onion tissues [30], meanwhile strain EN119 (which presents the lowest ANI value among the three strains compared) was recovered from a clinical sample [26]. The whole-genome phylogenetic tree obtained from the analysis of the core genes of 24 complete genomes belonging to *Enterobacter* species confirms the previously described results (Fig. 3). According to these analyses, it is reasonable to suggest that *Enterobacter* sp. J49 could be included in the *Enterobacter cloacae* complex.

A further analysis of orthologous genes within the four bacteria (*Enterobacter* sp. J49, *Enterobacter cloacae* P101, *Enterobacter cloacae* EcWSU1 and *Enterobacter ludwigii* EN119) revealed that J49 shares 4135 genes with EcWSU1, 4103 genes with P101, and 3985 genes with EN119 (Fig. 4). Considering that strains P101 and EcWSU1 were isolated from plant tissues, it is reasonable to hypothesize that those genes shared by J49 and these bacteria that are not present in EN119 could be

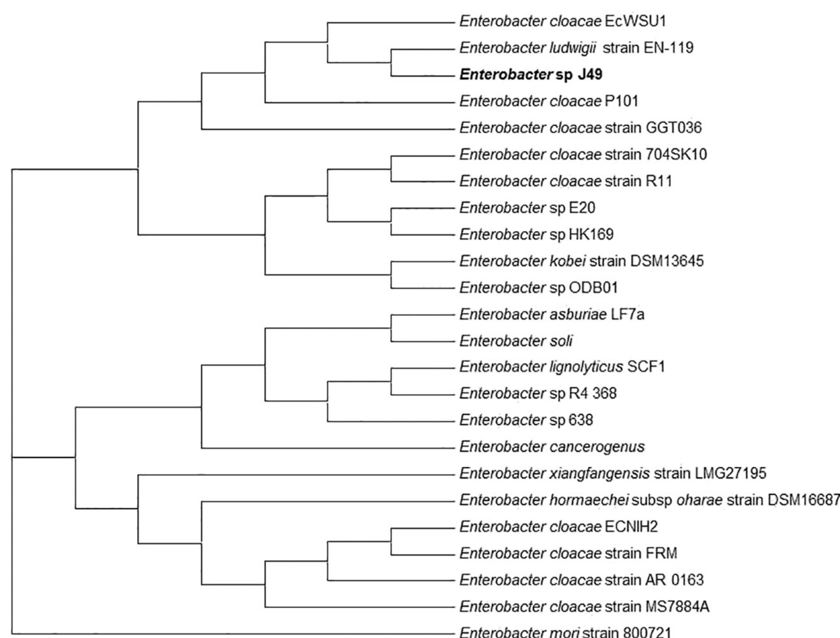

Fig. 3. Phylogenetic tree highlighting the position of *Enterobacter* sp. J49 with respect to other closely related species within the genus of *Enterobacter*. The phylogenetic tree was constructed based on 24 genomes, build out of a core of 2110 genes per genome, 50,640 in total using EDGAR platform for phylogenetic tree construction.

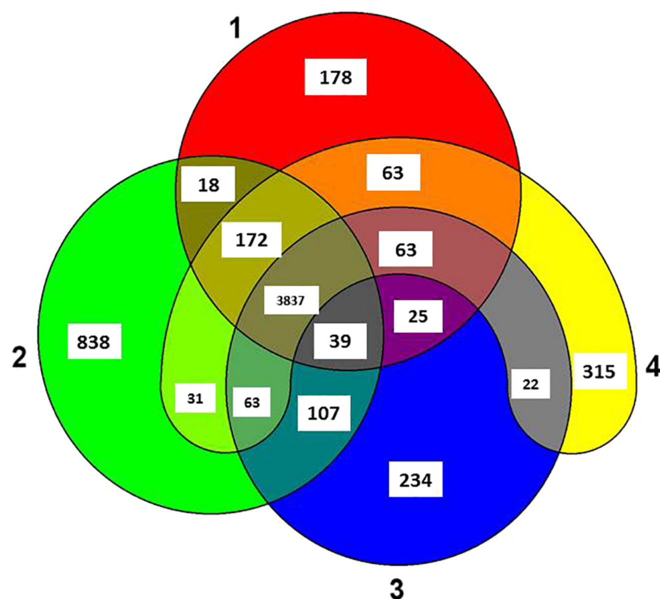

Fig. 4. Venn diagram showing the genes encoded by three *Enterobacter cloacae* strains and *Enterobacter* sp. J49. The core genes are those located at the intersection of the four colored figures.

involved in bacteria-plant interaction. This hypothesis can be supported by the fact that even though many *Enterobacter* species studied have been isolated from clinical samples and present multi-antibiotic resistances phenotypes [26,31], peanut native strain J49 lacks resistance to the tested antibiotics (Table 4) (data not shown). Nevertheless the genome of this strain contains genes related to multi-drug resistance proteins and the gene *amp*C (which codifies a B-lactamase enzyme, responsible for penicillin resistance).

2.3. Genes involved in plant growth promotion traits present in the genome of Enterobacter sp. J49

2.3.1. Phosphorus supply

Plant growth promotion of *Enterobacter* sp. J49 has been related to its phosphate solubilization and mineralization phenotype [15]. Gluconic acid, the major organic acid described for the most widely phosphate solubilization mechanism used by soil bacteria was detected in this bacterial supernatant [32]. The holoenzyme glucose dehydrogenase (GDH)-PQQ oxidizes glucose to gluconic acid [33,34]. Even though the gene responsible for GDH synthesis (gcd) was detected in the genome of strain J49, the *pqq* gene cluster (*pqqABCDE*) necessary for PQQ cofactor biosynthesis was not found (Table 3). It was possible to detect *pqq*F gene in the genome of J49 which codifies a protease enzyme generally associated to the solubilization phenotype but not essential for PQQ biosynthesis [35]. Regarding phosphate mineralization mechanism, two genes coding for a class B acid phosphatase and an alkaline phosphatase were found in its genome (Table 3).

2.3.2. Siderophores production

Low level of iron usually represents a limitation to microbial growth. Soil bacteria are able to synthetize a group of very heterogeneous molecules called siderophores that sequestrate iron from insoluble complexes. Simultaneously, these molecules present an antagonistic effect on plant pathogens by depriving them of this vital element. *Enterobacter* sp. J49 has shown siderophore production in *in vitro* assays [27] and in this study it was possible to find, in its genome sequence, genes related to the synthesis of the siderophores enterobactin (genes *entFCEB* and *entS*) and bacterioferritin (*bfr*) (Table 3). In addition, three iron ABC transporters were detected in J49 genome's sequence (Table 3).

2.3.3. Indole acetic acid synthesis and acetoin production

Soil beneficial bacteria can promote plant growth through the synthesis of molecules similar to plant hormones [36–38]. Auxin like indole acetic acid (IAA) is quantitatively the most abundant phytohormone secreted by plant associated rhizobacteria [39], even though it is not yet elucidated how bacteria obtain benefits from auxin production [40]. In some reports, authors have suggested that auxin is a signaling molecule in microorganisms [41,42].

There are different pathways to produce IAA reported in bacteria;

Table 3
Accession number and product of the genes involved in the most important plant growth promotion traits present in the genome of *Enterobacter* sp. J49 strain.

Gene	Accession number	Gene product	Activity
*ipd*C	OUC35045.1	indole-3-pyruvate decarboxylase	synthesis of the phytohormone
iaaH	OUC36706.1	Indole-3-acetaldehyde dehydrogenase	indole acetic acid (IAA)
*ent*B	OUC37387.1	Isochorismate synthase B	Siderophore production
entE	OUC37388.1	Enterobactin synthase subunit E	
*ent*F	OUC37391.1	Enterobactin synthase subunit F	
*ent*S	OUC37395.1	Enterobactin synthase subunit S	
entC	OUC37389.1	Isochorismate synthase	
menF	OUC35126.1	Isochorismate synthase	
bfr	OUC37395.1	Bacterioferritin	
afuA	OUC38168.1	iron ABC transporter	Iron transporters
znuB	OUC38311.1	iron ABC transporter	
feCD	OUC36188.1	iron ABC transporter	
–	OUC37776.1	Cellulase	plant polymer degradation
bcsZ	OUC39182.1/39174.1	Endoglucanase	enzymes
budB	OUC37407.1	Acetola ctate synthase	Acetoin synthesis (volatile
budA	OUC37408.1	Acetolactate decarboxylase	compound)
*flh*A-E	OUC35452.1/35451.1/35434.1/35433.1/35453.1	FlhA-FlhE Flagellar protein	
fliD-H	OUC35388.1/35383.1/35382.1/35381.1/35380.1	FliD-H/FliJ/FliL-N/FliP/FliR-T	
fliJ, fliL-N, fliP,fliR-T	OUC35378.1/35376.1/35375.1/35374.1 OUC35372.1/35370.1/35387.1/35386.1	Flagellar biosynthetic protein	
–	OUC35377.1	Flagellar hook length control protein	
flgMNABCDEFGHIJKL	OUC36417.1/36418.1/36419.1/36420.1/36421.1/36422.1/ OUC36423.1/36424.1/36425.1/36426.1/36427.1/36428.1/ OUC36429.1/36430.1	Flagellar proteins	Flagellar assembly
flhABE	OUC35452.1/35451.1/35453.1	Flagellar proteins	
flhC	OUC35434.1		
flhD	OUC35433.1		
fliACDEFGHIJLMNOPRST	OUC35396.1/35389.1/35388.1/35383.1/35382.1/35381.1/35380.1/ 35379.1/35378.1/35376.1/35375.1/35374.1/35373.1/35372.1/ 35370.1/35387.1/35386.1	Flagellar proteins	
motA	35435.1	Flagellar proteins	
motB	35436.1		
–	OUC39002.1	Type IV secretion system protein	Type 4 secretion system proteins
–	OUC39375.1	Type IV secretion system protein	
–	OUC38986.1	Type IV secretion system protein	
–	OUC36316.1	Type IV secretion system protein	
–	OUC36315.1	Type IV secretion system protein	
–	OUC35581.1	Type IV secretion system protein	
–	OUC35602.1	Type IV secretion system protein	
–	OUC35618.1	Type IV secretion system protein	
–	OUC35610.1	Type IV secretion system protein	
–	OUC38987.1	Type VI secretion system protein	Type 6 secretion system
tssG	OUC38988.1	Type VI secretion system protein	
tssF	OUC38989.1	Type VI secretion protein	
vasJ	OUC38990.1	Type VI secretion protein	
hcp	OUC39004.1	Hcp family type VI secretion system effector	
–	OUC36320.1	Type VI secretion protein	
impL	OUC35558.1	Type VI secretion protein ImpL	
–	OUC35560.1	Type VI secretion protein ImpA	
–	OUC35562.1	EvpB family type VI	
tssF	OUC35573.1	Type VI secretion protein	
tssG	OUC35574.1	Type VI secretion protein	
-	OUC39008.1	EvpB Type VI secretion protein	
clpV1	OUC39003.1	ClpV1 required for Hcp traslocation	
-	OUC39006.1	Type VI secretion protein	
-	OUC39116.1	Type II secretion system protein	Type II secretion system
outF	OUC39117.1	Type II secretion system protein OutF	
gspE	OUC39118.1	Type II secretion system protein GspE	
gspD	OUC39119.1	Type II secretion system protein GspD	
outG	OUC39116.1	Type II secretion system protein OutG	
pulD	OUC37750.1	Type II secretion system protein	
gspL	OUC39111.1	Type II secretion system protein GspL	
gspK	OUC39112.1	Type II secretion system protein GspK	
gspC	OUC39120.1	Type II secretion system protein GspC	
-	OUC37483.1	Type I secretion system protein ATPase	Type I secretion
-	OUC36904.1	Type I secretion system protein	
hlyD	OUC37482.1	Type I secretion system membrane fusion protein	
tolC	OUC36179.1	Type I secretion protein TolC	system

(*continued on next page*)

Table 3 (*continued*)

Gene	Accession number	Gene product	Activity
mcp	OUC38867.1	Methyl-accepting chemotaxis sensory transducer	Chemotaxis
tcp	OUC36709.1	Methyl-accepting chemotaxis citrate transducer	
tsr1	OUC38889.1	Methyl-accepting chemotaxis protein I	
tsr2	OUC37821.1	Methyl-accepting chemotaxis protein I	
tarA	OUC35445.1	Methyl-accepting chemotaxis protein II	
trg	OUC36913.1	Methyl-accepting chemotaxis protein III	
tap	OUC35446.1	Methyl-accepting chemotaxis protein IV	
tarH	OUC36218.1	Methyl-accepting chemotaxis sensory transducer with TarH sensor	
mcp	OUC35914.1	Methyl-accepting chemotaxis sensory transducer with TarH sensor	
mcp	OUC38915.1	Chemotaxis protein	
mcp	OUC35640.1	Chemotaxis protein	
cp	OUC36469.1	Chemotaxis protein	
cp	OUC36972.1	Chemotaxis protein	
tsr	OUC36133.1	Chemotaxis protein	
cp	OUC35855.1	Chemotaxis protein	
cheY	OUC35449.1	Chemotaxis protein CheY	
cheA	OUC35437.1	Chemotaxis protein CheA	
cheW	OUC35438.1	Chemotaxis protein adaptor CheW	
cheV	OUC35122.1	Chemotaxis protein	
cheZ	OUC35450.1		
cheB	OUC35448.1		
cheR	OUC35447.1		
gcd	OUC35748.1	Glucose dehydrogenase	Putative Gluconic acid (Gluconate) synthesis
pqqF	OUC34875.1	Cofactor PQQ biosynthesis protein	
aphA	OUC38471.1	classB acid phosphatase alkaline phosphatase	Phosphate mineralization
phoA	OUC37670.1		

Table 4
Concentration of antibiotics used in the sensitivity test for *Enterobacter* sp. J49.

Antibiotic	Concentration used in culture media (μg/ml)
Chloramphenicol	30
Nalidixic acid	1000
Streptomycin	30
Kanamycin	50
Rifampicin	200
Neomycin	200
Ampicillin	100
Gentamicin	10
Spectinomycin	200
Tetracicline	20

those more frequently described are: (i) indolepyruvate, (ii) tryptamine, and (iii) indole-3-acetamide [4,42–44]. The analysis of the genome of strain J49 indicated that only the first one is present. The responsible genes for synthesis of indolpyruvate decarboxylase and indole-3-acetaldehyde dehydrogenase enzymes were found in J49 genome sequence (Table 3) while no other IAA pathway related genes were detected [4,42–44].

Some rhizobacteria promote plant growth by releasing volatile signals [45,46]. In particular, volatile organic compounds like acetoin have been described as an important mechanism for the elicitation of plant growth [4,46]. In the genome of strain J49 it was possible to identify two genes that code to enzymes involved in acetoin synthesis: *budA* and *budB* genes, (Table 3). The acetolactate synthase (BudB) converts pyruvate to acetolactate, which is subsequently converted to acetoin by acetoin decarboxylase enzyme (BudA).

2.4. Endophytic colonization

Several *Enterobacter* strains have been reported as plant endophytes [6,7,9,12,18,47]. The initial step in colonization involves bacteria moving towards the plant root actively *via* induction of flagellar activity by plant-released compound (chemotaxis) [4,6,7]. Motility is an important characteristic for endophytes since they need to be able to move to the selected root area and reach the inside the plant. The genome sequence of *Enterobacter* sp. J49 contains 38 genes involved in the biosynthesis and assembly of flagella, and 22 genes involved in chemotaxis signaling pathway that could be involved in this first step in plant-bacterium interaction (Table 3). In addition, the existence of flagella was confirmed by electron microcopy (unpublished data). The next step of endophytic colonization is the entry of bacterium to the plant's inner tissues. Endophytic bacteria can enter the plant root at sites of tissues damage (as the result of plant growth), by natural openings [4,6] or by releasing cellulase enzymes (which produce the breakdown of plant cell walls) [7]. The finding of genes coding cellulase enzymes in the genome sequence of strain indicates that this strain could use all the mechanisms previously described to enter the root tissues (Table 3). Other genes considered as potentially related to the endophytic behavior are those codifying for secretion systems [6]. Secretion systems type I, II and V are the most redundant in plant growth promoting endophytes [7,12]. Secretion system type III and IV are mainly present in pathogenic bacteria [12,48]. Meanwhile secretion system type VI has been found in pathogenic as well in non-pathogenic bacteria [49]. In the genome sequence of strain J49, we identified 4, 9 and 14 genes coding type I, type II and type VI secretion systems, respectively. Genes related to secretion system type V were not detected. In relation to type IV secretion system, 9 genes were identified.

All the results previously presented, together with the fact that *Enterobacter* sp. J49 contributes to phosphorus (P) content in plant tissues and soil, and promotes the growth of peanut and maize, indicate that this strain is a potential biofertilizer for both crops. Thus, the genome sequence of *Enterobacter* sp. J49 is a valuable source of information to understand this strain's plant growth promotion properties

and to study its interaction with these and other agronomic important crops.

3. Materials and methods

3.1. Bacterial growth and DNA extraction

Enterobacter sp. J49 (available in the Deutsche Sammlung von Mikroorganismen und Zelkulturen GmbH, deposit No. DSM 105031) was grown and maintained on Luria-Bertani (LB) agar medium or LB broth at 28 °C. Total DNA was isolated using phenolic extraction method described by Ausubel et al. [50] and re-extracted using DNeasy^R Blood and Tissue kit (QIAGEN). The DNA concentration was checked on Nanodrop spectrophotometer (ThermoFisher) and by Qubit Fluorometer (Invitrogen). The sample was diluted to 0.2 ng μl^{-1} concentration.

3.2. Genome sequencing, assembly and annotation

For whole-genome sequencing, the Illumina MiSeq System (Illumina, Inc.) was used. Libraries were generated using Illumina's Nextera XT V2-kit sequencing preparation kit, PCR clean-up kit (Illumina, Inc) was used to clean the fragments and the library was validated using the Bioanalyzer (Agilent). The quantification of library previously obtained was done by qRT-PCR (Peqlab) performing dilution of the purified library until 10^{-5}. Finally, library sequencing was done at the Loewe Center for Synthetic Microbiology, (Marburg, Germany) using an Illumina MiSeq Diagnostics. Data obtained from sequencing were *de novo* assembled using SPAdes assembler version 3.5.0 [51]. For genome annotation, GenDB platform was used [52]. All the bio-informatics procedures were performed at Justus-Liebig-Universität Giessen, in Bioinformatics and Systems Biology lab (Giessen, Germany).

3.3. Antibiotic sensitivity test

The susceptibilities of J49 to antimicrobial agents were determined by growing the bacterium in LB solid plates or LB broth supplemented with different antibiotics individually (Table 4). Each antibiotic was added to the media before adding the bacteria and then they were grown at 28 °C for 48 h.

3.4. Phylogenetic analysis and average nucleotide identity test

For the comparative phylogenetic analysis, the sequences of three core housekeeping loci 16S rRNA, *gyr*B and *rpo*D of different *Enterobacter* species and *Escherichia coli* CFT073 (as outgroup) were retrieved from NCBI. A phylogenetic tree was constructed based on the concatenated sequences of the three housekeeping genes using the Maximum Likelihood method in MEGA5 workbench [53]. The consensus tree was inferred using 100 bootstrap replicates.

An average nucleotide identity (ANI) analysis was performed using all complete genome sequences of the *Enterobacter* genus available in the EzBioCloud database [54] (http://www.ezbiocloud.net/eztaxon). Core genome analysis for Venn diagram and phylogenetic tree was performed using EDGAR [55] (http://edgar.computational.bio.uni-giessen.de/cgi-bin/edgar_login.cgi) among multiple *Enterobacter* species.

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