Journal of Biotechnology 260 (2017) 38–41

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

# Complete genome sequence of endophyte *Bacillus flexus* KLBMP 4941 reveals its plant growth promotion mechanism and genetic basis for salt tolerance

Tian-Tian Wang[a], Peng Ding[a], Pan Chen[a], Ke Xing[b], Juan-Luan Bai[a], Wen Wan[a], Ji-Hong Jiang[a], Sheng Qin[a,*]

[a] *The Key Laboratory of Biotechnology for Medicinal Plant of Jiangsu Province, Jiangsu Normal University, Xuzhou, Jiangsu, 221116, PR China*
[b] *School of Life Science, Jiangsu Normal University, Xuzhou, Jiangsu, 221116, PR China*

## ARTICLE INFO

*Chemical compounds studied in this article:*
Spermidine (PubChem CID: 1102)
Acetoin (PubChem CID: 179)
Phenazine (PubChem CID: 4757)
Cobalamin (PubChem CID: 5311498)
Arginine (PubChem CID: 6322)

*Keywords:*
Endophyte
*Bacillus flexus* KLBMP 4941
Plant growth promotion
Salt stress

## ABSTRACT

*Bacillus flexus* KLBMP 4941 is a halotolerant endophyte isolated from the halophyte *Limonium sinense*. This strain can improve host seedling growth under salt stress conditions. We here report the complete genome information of endophyte KLBMP 4941. It has a circular chromosome and two plasmids for a total genome 4,104,242 bp in size with a G + C content of 38.09%. Genes related to plant growth promotion (PGP), such as those associated with nitrogen fixation, siderophore, spermidine, and acetoin synthesis were found in the KLBMP 4941 genome. Some genes responsible for high salinity tolerance, like genes associated with the $Na^+/H^+$ antiporter, glycine betaine transporter, and betaine-aldehyde dehydrogenase were also found in the KLBMP 4941 genome. The genome analysis will provide better understanding of the mechanisms underlying the promotion of plant growth in strain KLBMP 4941 under salt stress conditions and its ability to adapt to coastal salt marsh habitats, and provide a basis for its further biotechnological applications in agriculture.

Bacteria of the genus *Bacillus* are well known for their ability to produce large ranges of bioactive secondary metabolites to be used as biocontrol agents, and they have been isolated from diverse habitats (Wu et al., 2015). Many *Bacillus* species can colonize crop endo and rhizospheres and act as plant growth-promotion bacteria (PGPB). Endophytic bacteria have enormous biodiversity and have been found to promote plant growth directly or indirectly through a combination of mechanisms, including nitrogen fixation, biosynthesis of siderophores, plant-growth hormones, hydrolytic enzymes, various antibiotics, and induced plant resistance (Qin et al., 2009; Qin et al., 2011; Afzal et al., 2014; Wang et al., 2015; Qin et al., 2017). Recently, some studies have shown that *Bacillus* species can help plants withstand abiotic stresses, such as drought, salt and heavy metals, and improve plants growth (Yaish et al., 2015; Ali et al., 2017). However, information on endophytic *Bacillus* spp. from halophytes and their capacity to enhance plant growth under salinity stress is relative scarce.

In our previous study, a Gram-positive endophytic bacterium KLBMP 4941 was isolated from the inner tissues of a halophytic traditional Chinese medicinal herb *Limonium sinense* (Girard) Kuntze, collected from coastal region in eastern China (Qin et al., 2014). It was placed in the genus *Bacillus* by morphological observation and 16S rRNA gene sequencing. The 16S rRNA gene neighbor-joining phylogenetic tree constructed with MEGA 6.0 software indicated that strain KLBMP 4941 was a member of the genus *Bacillus*. It formed a subclade with the nearest neighbor *B. flexus* NBRC $15715^T$, with 100% 16S rRNA gene similarity (Fig. 1). Strain KLBMP 4941 was observed to be able to grow on nitrogen-free agar medium, and the *nifH* gene was detected by PCR analysis. It also exhibited multiple PGP traits, including biosynthesis of siderophores, ammonia, acetoin, and tolerance to 8% NaCl, indicating its ability to tolerate salinity and to promote plants growth under salt stress condition. In the present study, strain KLBMP 4941 was used to inoculate the host plant *L. sinense* using pot experiment under salt (NaCl) stress to evaluate its PGP ability. The results showed that KLBMP 4941 significantly improved host growth under 200 mM NaCl stress conditions (Fig. 2) after the pots were inoculated with strain-suspension culture ($OD_{600}$ 0.5) directly into the soil. After 30 d of salt stress treatments, inoculation of strain KLBMP 4941 increased seedling length 1.31 fold and fresh weight increased 1.93 fold. For this reason, to fully understand the molecular mechanisms underlying the PGP and salinity tolerance, we carried out the complete genome sequencing of KLBMP 4941.

The genomic DNA of KLBMP 4941 was extracted using our previous

---

* Corresponding author.
*E-mail address:* shengqin@jsnu.edu.cn (S. Qin).

http://dx.doi.org/10.1016/j.jbiotec.2017.09.001
Received 20 June 2017; Received in revised form 1 September 2017; Accepted 2 September 2017

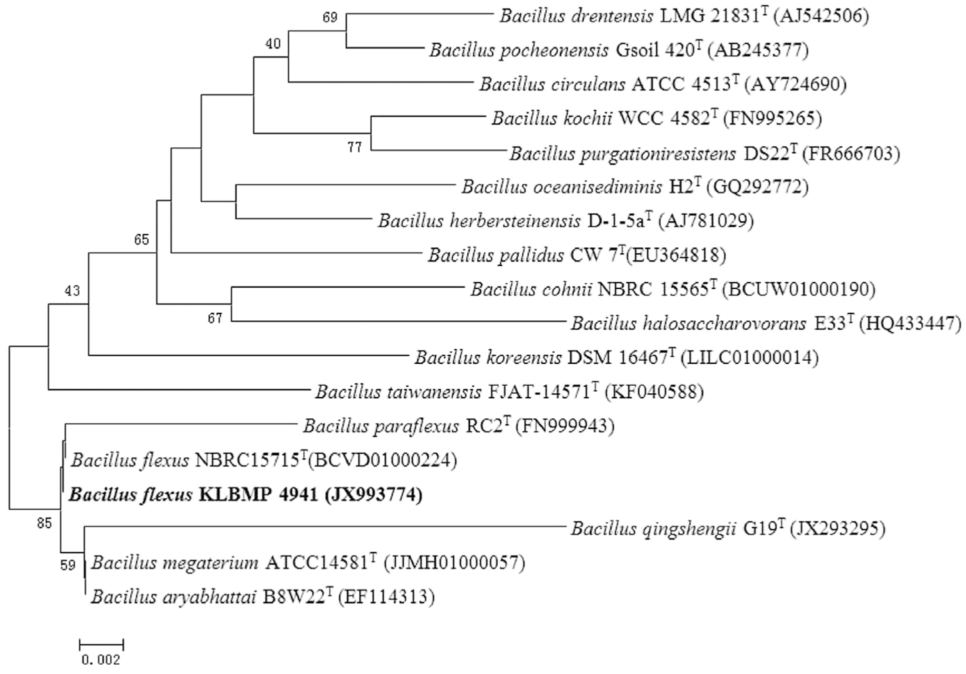

**Fig. 1.** Unrooted neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain KLBMP 4941 and members of the genus *Bacillus*. The tree was constructed using the MEGA 6.0 program based on average nucleotide identity. Bootstrap values (expressed as percentages of 1000 replications) greater than 40% are shown at branch points. Bar, 0.002 substitutions per nucleotide position.

protocol (Qin et al., 2015). The genome sequencing was performed using single-molecule real time (SMRT) sequencing on a Pacific Bioscience (PacBio) RS II system (Biomarker Technologies Corporation, Beijing, China). The filtered subreads (1,644,599,241 bp) with the genome coverage of 268 folds were then *de novo* assembled using the PacBio hierarchical genome assembly process (HGAP) algorithm version 2.0 (Chin et al., 2013). Prodigal version 2.6.3 (Hyatt et al., 2010) was used to predict the open reading frames (ORFs). Software packages tRNAscan-SE v1.31 and Barrnap 0.4.2 were used to predict tRNA and rRNA, respectively. The gene functions were predicted by BLASTP using four different function databases: the non-redundant GenBank protein database (www.ncbi.nlm.nih.gov/protein), the clusters of orthologous groups database (COG) (http://www.ncbi.nlm.nih.gov/COG/), GO database (http://www.geneontology.org/), and KEGG database (http://www.genome.jp/kegg/).

The genome of strain KLBMP 4941 contained one 3,960,308 bp circular chromosome and two circular plasmids. The G + C contents of the chromosome and plasmids were 38.09%, 34.94%, and 32.95%, respectively. A total of 4197 CDS, 141 rRNA and 75 tRNA genes were predicted in chromosome sequence (Table 1). Among these CDSs, 3426 (81.63%) genes were classified into 20 clusters of orthologous groups (COG) functional categories. Most of the genes were associated with functions such as amino acid transport and metabolism, carbohydrate transport and metabolism, transcription, DNA replication, recombination and repair, and inorganic ion transport and metabolism (Table 2). In order to investigate the genomic features related to the antimicrobial activity of strain KLBMP 4941, we also identified 16 putative gene clusters responsible for secondary metabolite biosynthesis by antiSMASH database (Medema et al., 2011). Among the clusters, two are related to the biosynthesis of terpene, one is responsible for siderophore biosynthesis, one encodes lassopeptide, one encodes T3pKS, one is related to bacteriocin biosynthesis, and ten encode microcins.

The genome of KLBMP 4941 was found to contain a series of genes associated with plant growth promotion and stress tolerance, including nitrogen fixation, production of phenazine, spermidine and acetoin (Table 3). The genes *RhbCDEF* and *CobW* responsible for siderophore and cobalamin synthesis respectively, are found on the chromosome. Genes encoding acetolactate synthase (*ilvB* and *ilvH*) and acetolactate decarboxylase (*alsD*), which are involved in volatile plant growth promotion signaling molecule acetoin synthesis, were detected in the genome. In addition, arginine decarboxylase (*speA*), agmatinase (*speB*), *S*-adenosylmethionine decarboxylase (*speH*) and spermidine synthase encoding genes, related to spermidine biosynthesis are present in KLBMP 4941. Spermidine has been found to play important roles in plant growth, development, and abiotic stress responses (Zhou et al., 2016). The genome of endophyte *Klebsiella* sp. LTGPAF-6F, which has been reported to improve growth and drought tolerance in wheat, was found to have the spermidine synthesis genes cited above (Zhang et al., 2017). Recently, the beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 was found to secrete spermidine and enhance plant salt tolerance (Chen et al., 2017). Previous works have demonstrated that PGP bacteria can produce antibacterial compounds, like phenazine, to inhibit pathogenic microbes and promote plant growth (Chen et al., 2015). We also found the genes *PhzF* which are responsible for the phenazine synthesis in the genome.

Many genes involved in salinity resistance were detected in the genome. The genes responsible for synthesis and transportation of compatible solutes were predicted. These included as genes encoding glycine betaine transporter, betaine-aldehyde dehydrogenase and betaine/carnitine/choline transporter (Table 3). Genes encoding $Na^+/H^+$ antiporters and $K^+$ transporter, which are responsible for pumping out $Na^+$ and $K^+$ accumulation to help cells resist hyperosmotic stress, were found in the genome (Epstein, 2003; Hunte et al., 2005). The KLBMP 4941 genome also has a series of genes encoding cold shock and heat shock proteins, peroxidases, superoxide dismutases, glutathione peroxidases, and catalases, which could protect the cell from oxidative stress.

In conclusion, the complete genome of KLBMP 4941 provides useful clues to clarify its molecular mechanisms underlying the promotion of plant growth and salt tolerance, and they may be useful for developing