Complete genome sequence of *Bacillus velezensis* YYC, a bacterium isolated from the tomato rhizosphere

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Abstract
The *Bacillus velezensis* YYC strain was isolated from the tomato rhizosphere. In a previous experiment, it increased tomato growth and induced systemic resistance against *Ralstonia solanacearum*. However, information on its genomic content is lacking. The complete genome sequence of the bacterium was described in this study. The genome size was 3,973,236 bp and consisted of 4034 genes in total, with a mean G + C content of 46.52%. In addition, 86 tRNAs and 27 ribosomal RNAs were identified. Fourteen clusters of secondary metabolites were identified. The KEGG database analysis showed that 69 genes were related to quorum sensing, which were important for microbe-plant interaction. In addition, genes involved in promoting plant growth and triggering plant immunity were identified from the genome. Based on digital DNA–DNA hybridizations (dDDH), *B. velezensis* YYC was most closely related with *B. velezensis* FZB42. The complete genome data of *B. velezensis* YYC will provide a basis for explanation of its growth-promoting mechanism and biocontrol mechanism.

Keywords *Bacillus velezensis* · PGPR · Genome · Biocontrol

Introduction

*Bacillus velezensis* is an important member of plant growth-promoting rhizobacteria (PGPR). PGPR promotes plant growth by nitrogen fixation, solubilization of phosphates or antagonists to soil-borne disease (Canellas et al. 2013). In addition, some strains of *B. velezensis* were known to have growth-promoting effects and produce a variety of secondary metabolites with antifungal or antibacterial activity (Chowdhury et al. 2015). For example, *B. velezensis* 5YN8 and DSN012 can significantly control the phytopathogenic fungus *Botrytis cinerea* by secreting some secondary metabolites and promote the pepper growth (Jiang et al. 2018). *B. velezensis* FJAT-46737 controls the tomato bacterial wilt by secreting of lipopeptides (Chen et al. 2020). *B. velezensis* can be widely isolated from diversified environments, such as plant rhizospheres, soil, rivers, human food, animal guts and seawater, and can easily be isolated and cultured (Ye et al. 2018). In our work, the rhizosphere soil of tomato was used to isolate the strain YYC in Qiqihar University Botanical Garden of Heilongjiang in China. Alexandrov medium was used to isolate bacteria from rhizosphere soil of tomato. *B. velezensis* YYC increased tomato growth and induced systemic resistance against *Ralstonia solanacearum* (unpublished data). Strain YYC is a non-pathogenic bacterium. Genome sequencing of *B. velezensis* YYC will provide basic insight into the growth-promoting and biocontrol mechanism.

Data description

Genome sequencing, assembly and annotation

*B. velezensis* YYC strain was propagated in Luria–Bertani broth with shaking at 180 r/min overnight at 30 °C. By alignments of the 16S ribosomal RNA and housekeeping genes (*dnaG*, *frr*, *infC*, *nusA*, *pgk*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rpsL*, *rpsT*, *rpmA*, *rpoB*, *rpsB*, *rpsC*, *rpsE*, *rpsL*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*).
smpB, tsf), it was identified as *Bacillus velezensis*. Bacterial genomic DNA extraction kit (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China) was used to extract genomic DNA. A TBS-380 fluorometer (Turner Bio- Systems Inc., Sunnyvale, CA) was used to quantify the purified genomic DNA. PacBio RS II Single Molecule Real Time (SMRT) and Illumina sequencing platforms were used to sequence the genomic DNA. The sequencing yielded 170,436 reads, including 1,341,760,841 bp, with 337.7 x sequence depth. A statistical technology of quality information was applied for quality trimming, by which the low-quality data could be removed to result in clean data. Using Unicycler (Version 0.4.7) with both Illumina and PacBio data (Wick et al. 2017), the reads were assembled into contigs. A complete genome was generated by inspecting and completing the last circular step. Finally, using the Illumina reads, error correction of the PacBio assembly results was performed.

The number of protein coding sequences (CDSs) in the *B. velezensis* YYC genome was predicted by GeneMarkS software (version 4.3) (Besemer et al. 2005). The transfer RNA (tRNA) gene was analyzed by tRNAscan-SE v2.0 software (Version 2.0) (http://trna.ucsc.edu/software) (Chan and Lowe 2019). Barrnap software (Version 0.8) (https://github.com/tseemann/barrnap) was utilized to predict ribosomal RNA genes. By aligning reads with the Nonredundant (NR), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2016), Gene Ontology (GO) (Ashburner et al. 2000) and Cluster of Orthologous Groups of proteins (COG) (Galperin et al. 2015) databases, all genes were annotated. The bioactive secondary metabolites were predicted by antiSMASH software (Version 6.0.1) (Blin et al. 2021) with the relaxed parameter.

**General genome features of *B. velezensis* YYC**

Whole-genome sequencing showed that the *B. velezensis* YYC strain contained a genome size of approximately 3,973,236 bp, with an average G + C content of 46.52%. The GeneMarkS program predicted that the number of protein coding sequences (CDSs) was 3779. Furthermore, a total of 86 tRNA and 27 ribosomal RNA genes were identified in the genome. By aligning the genome to sequences from diverse databases, including the NR, KEGG, GO and COG databases, the numbers of identified genes were 4034, 2163, 2668 and 3013, respectively.

Sixty-one genes were associated with chemotaxis by querying the NR database, which plays a significant role during the rhizosphere colonization by rhizobacteria (Feng et al. 2021). And the KEGG database analysis showed a great number of two-component systems (113 genes) and ABC transporters (117 genes). At the same time, 69 genes were related to quorum sensing, which were important for microbe-plant interactions (Kan et al. 2017). The GO database analysis showed that 1483 genes were associated with molecular function of binding, which may be helpful to its plant colonization (Zeng et al. 2020). Furthermore, the COG database showed a great number of cell wall and membrane biosynthesis (180 genes), bacterial mobility (38 genes) and secondary metabolites biosynthesis (79 genes), which were important for the strain’s biocontrol, colonization, and stimulation of plant growth (Guo et al. 2015) (Fig. 1).

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**Fig. 1** Circular representation of *Bacillus velezensis* YYC genome. Outmost circle represents the genome size of *B. velezensis* YYC, from the outside to inside; Second and third circles are positive and negative chain genes, respectively, all genes are color coded according to their COG annotation functions; Fourth circle indicates nomenclature and locations of predictive secondary metabolite clusters; Fifth circle is GC content (red indicates greater than average value and blue indicates less than average)
Table 1  Pairwise comparisons of *B. velezensis* YYC with type strain genomes

| Subject strain                  | dDDH (d0, in %) | C.I (d0, in %) | dDDH (d4, in %) | C.I (d4, in %) | dDDH (d6, in %) | C.I (d6, in %) | G+C content difference (in %) |
|--------------------------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|-----------------------------|
| *Bacillus velezensis* FZB42    | 96.8            | [95.1–98.0]    | 90.8            | [88.5–92.6]    | 97.5            | [96.3–98.4]    | 0.05                        |
| *Bacillus velezensis* NRRL B-41580 | 91.8          | [88.8–94.0]    | 85.4            | [82.7–87.7]    | 93.3            | [91.0–95.0]    | 0.21                        |
| *Bacillus methylotrophicus* KACC 13105 | 95         | [92.7–96.6]    | 84.6            | [81.9–87.0]    | 95.5            | [93.7–96.8]    | 0.09                        |
| *Bacillus siamensis* KCTC 13613  | 89.1            | [85.8–91.8]    | 56.9            | [54.2–59.7]    | 85.4            | [82.3–88.1]    | 0.19                        |
| *Bacillus vanillae* XY18       | 89              | [85.6–91.7]    | 56.9            | [54.2–59.7]    | 85.3            | [82.2–88.0]    | 0.2                         |
| *Bacillus amylophiliquecens* DSM 7  | 82.7         | [78.8–86.0]    | 56              | [53.2–58.7]    | 79.8            | [76.3–82.8]    | 0.44                        |
| *Bacillus nakamurai* NRRL B-41091 | 73.4            | [69.5–77.1]    | 31              | [28.6–33.5]    | 61.2            | [57.9–64.4]    | 1.26                        |
| *Bacillus tequilensis* NCTC13306 | 30.9           | [27.5–34.5]    | 21.3            | [19.0–23.7]    | 27.5            | [24.6–30.6]    | 2.54                        |
| *Bacillus spizizenii* TU-B-10   | 33.6            | [30.2–37.2]    | 21              | [18.8–23.4]    | 29.2            | [26.3–32.3]    | 2.7                         |
| *Bacillus subtilis* NCIB 3610   | 32.5            | [29.1–36.0]    | 20.9            | [18.7–23.3]    | 28.4            | [25.5–31.5]    | 3.13                        |

The dDDH values were provided along with their confidence intervals (C.I.)

Table 2  Representative genes of *B. velezensis* YYC probably involved in plant bacterium interactions

| Gene     | Position     | Protein Description | Description                        |
|----------|--------------|---------------------|------------------------------------|
| tasA     | 2466662–2467447 | Spore coat protein  | Root colonization                   |
| sacB     | 3926734–3928215 | Levan sucrase       | Root adhesion                       |
| spo0A    | 2432108–2432908 | Sporulation transcription factor Spo0A | Biofilm formation                   |
| sinR     | 2466273–2466614 | Transcriptional regulator | Biofilm formation                   |
| abrB     | 45645–45929   | Transition state regulator Abh | Biofilm formation                   |
| resE     | 2260204–2261985 | Sensor histidine kinase | Biofilm formation                   |
| ytcE     | 2757501–2759282 | Sensor histidine kinase | Biofilm formation                   |
| ycbA     | 248181–249473 | Sensor histidine kinase | Biofilm formation                   |
| Efp      | 2452165–2452722 | Elongation factor P  | Essential for swarming motility     |
| flID     | 3414679–3416199 | Flagellar capping protein | Elicitation of plant basal defence  |
| flgK     | 3419847–3421364 | Flagellar hook-associated protein FlgK | Elicitation of plant basal defence  |
| xynB     | 1846654–1848069 | Glycoside hydrolase | Carbohydrate metabolism            |
| lacR     | 1216343–1217104 | Lactose phosphotransferase system repressor | Lactose metabolism               |
| lacG     | 121490–1216090 | Beta-galactosidase     | Hydrolyzation of phospholactose     |
| lacE     | 1212603–1214300 | Phosphotransferase system | Cellulose degradation            |
| lacF     | 1214312–1214626 | Phosphotransferase system | Cellulose degradation            |
| Pta      | 3639358–3640329 | Phosphate acetyltransferase | Strongly upregulated by root exudate |
| moaA     | 3549409–3550434 | Molybdenum cofactor biosynthesis protein A | Cofactor for nitrogen assimilation |
| narT     | 3598984–3599565 | Nitrate reductase | Nitrate reduction reduction        |
| narJ     | 3599562–3600119 | Nitrate reductase molybdenum cofactor assembly | Nitrate reduction               |
| narH     | 3600145–3601608 | Nitrate reductase | Nitrate reduction                   |
| narG     | 3601598–3605284 | Nitrate reductase | Nitrate reduction                   |
| ilvH     | 2697631–2698149 | Acetolactate synthase | Promote plant growth               |
| ilvB     | 2698146–2699963 | Acetolactate synthase | Promote plant growth               |
| alsD     | 3488161–3488928 | Acetolactate decarboxylase | Promote plant growth         |
| butB     | 629733–630773  | Butanediol dehydrogenase | Promote plant growth               |
Secondary metabolites related to biocontrol

AntiSMASH version 6.0.1 analysis identified 14 clusters of secondary metabolites (Fig. 1). Six clusters of secondary metabolites were related to the synthesis of plantazolicin, macrolactin H, bacillaein, fengycin, difficidin and bacilysin, which can directly inhibit the growth of fungi and bacteria (Chowdhury et al. 2015). In addition, fengycin was found to induce resistance to plant diseases and suppress Sclerotinia sclerotiorum (Farzand et al. 2019).

In addition, based on digital DNA–DNA hybridizations (dDDH), B. velezensis YYC was most closely related with B. velezensis FZB42 (Table 1). And it shared 96.80% identity with the strain that was used as biofertilizer and biocontrol agent (B. velezensis FZB42) (Borriss 2011). Compared with FZB42, clusters 2, 3, 6, 7, 8, 11, 13 and 14 showed 89, 94, 93, 94, 96, 93, 96 and 98% amino acid sequence homologies with known gene clusters that synthesize surfactin, plantazolicin, macrolactin H, bacillaein, fengycin, difficidin, bacillibactin and bacilysin, respectively. Clusters 1, 4, 5, 9, 10 and 12 were not reported previously to be present in the genome of strain FZB42 and only existed in the genome of B. velezensis YYC. Comparative analysis of secondary metabolite clusters of the two strains were summarized for comparisons (Supplementary Table S1).

Genes involved in promoting plant growth and triggering plant immunity

Some strains could colonize the rhizosphere of the plant, promote plant growth and elicit plant defenses (Wu et al. 2015). In addition to producing secondary metabolites with antifungal or antibacterial activity, B. velezensis YYC contains various of genes involved in root colonization and biofilm formation, such as sacB and spo0A (Bezzate et al. 2000; Branda et al. 2001) (Table 2). In addition, the moaA (encoding molybdenum cofactor biosynthesis protein A) was found in the YYC genome, which may be related to nitrogen assimilation (Bird et al. 2003). It also contains the genes encoding acetolactate synthase (ilvH, ilvB), acetolactate decarboxylase (alsD), and butanediol dehydrogenase (butB), which have plant growth-promoting effects including stimulating root formation and increasing systemic disease resistance (He et al. 2013; Jayakumar et al. 2021). B. velezensis YYC also has the genes required for synthesis of 2, 3-butanediol (alsD), the compound reported to trigger systemic resistance (He et al. 2013).

Nucleotide sequence accession number

The complete genome sequence of B. velezensis YYC was deposited in GenBank under accession number CP075055. (BioProject: PRJNA728388, BioSample: SAMN19079027). The strain was stored in China Center for Type Culture Collection (CCTCC) in Wuhan with the accession number of CCTCC M 20,211,227.

Conclusions

The complete genome of the strain YYC was sequenced. It was identified as B. velezensis. The genome sequencing suggests that B. velezensis YYC has a good potential for plant growth promotion and biocontrol. In addition, genome information of B. velezensis YYC will be helpful to reveal the growth-promoting mechanism and biocontrol mechanism.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00203-021-02709-5.

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Declarations

Conflict of interest

The author(s) declare no conflict of interest.

References

Ashburner M, Ball CA, Blake JA et al (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29. https://doi.org/10.1038/75556
Besemer J, Borodovsky M (2005) GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res 33:W451–W454. https://doi.org/10.1093/nar/gki487
Bezzate S, Aymerich S, Chambert R et al (2000) Disruption of the Pae- nibacillus polymyxa levansucrase gene impairs its ability to aggre- gate soil in the wheat rhizosphere. Environ Microbiol 2:333–342. https://doi.org/10.1046/j.1462-2920.2000.00114.x
Bird C, Wyman M (2003) Nitrate/nitrite assimilation system of the marine picoplanktonic cyanobacterium Synechococcus sp. strain WH 8103: effect of nitrogen source and availability on gene expression. Appl Environ Microbiol 69:7009–7018. https://doi.org/10.1128/AEM.69.12.7009-7018.2003
Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, Weber T (2021) antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res 49:W29–W35. https://doi.org/10.1093/nar/gkaab335
Borriss R (2011) Use of plant-Associated Bacillus strains as biofertilizers and biocontrol agents in agriculture. In: Maheshwari DK (ed) Bacteria in agrobiology: plant growth responses. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 41–76
Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R, Kolter R (2001) Fruiting body formation by Bacillus subtilis. PNAS 98:11621–11626. https://doi.org/10.1073/pnas.191384198
Canellas LP, Balmori DM, Medièc LO et al (2013) A combination of humic substances and Herbaspirillum seropedicae inoculation enhances the growth of maize (Zea mays L.). Plant Soil 366:119–132. https://doi.org/10.1007/s11104-012-1382-5
Chan PP, Lowe TM (2019) tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol 1962:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1

Chen M, Wang J, Liu B et al (2020) Biocontrol of tomato bacterial wilt by the new strain Bacillus velezensis FJAT-46737 and its lipopeptides. BMC Microbiol 20:160. https://doi.org/10.1186/s12866-020-01851-2

Chowdhury SP, Hartmann A, Gao X, Borriss R (2015) Biocontrol mechanism by root-associated Bacillus amyloliquefaciens FZB42-a review. Front Microbiol 6:780. https://doi.org/10.3389/fmicb.2015.00780

Farzand A, Moosa A, Zubair M, Khan AR, Massawe VC, Tahir HAS, Sheikh TMM, Ayaz M, Gao X (2019) Suppression of sclerotinia sclerotiorum by the induction of systemic resistance and regulation of antioxidant pathways in tomato using fengycin produced by Bacillus amyloliquefaciens FZB42. Biomolecules 9:613. https://doi.org/10.3390/biom9100613

Feng H, Fu R, Hou X et al (2021) Chemotaxis of beneficial rhizobacteria to root exudates: the first step towards root-microbe rhizosphere interactions. Int J Mol Sci 22:6655. https://doi.org/10.3390/ijms22136655

Galperin MY, Makarova KS, Wolf YI, Koonin EV (2015) Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res 43:D261–D269. https://doi.org/10.1093/nar/gku1223

Guo S, Li X, He P, Ho H, Wu Y, He Y (2015) Whole-genome sequencing of Bacillus subtilis XF-1 reveals mechanisms for biological control and multiple beneficial properties in plants. J Microbiol Biotechnol 42:925–937. https://doi.org/10.1007/s10399-015-1612-y

He PF, Hao K, Blom J et al (2013) Genome sequence of the plant growth promoting strain Bacillus amyloliquefaciens subsp. plantarum B9601–Y2 and expression of mersacidin and other secondary metabolites. J Biotechnol 164:281–291. https://doi.org/10.1016/j.jbiotec.2012.12.014

Jayakumar A, Nair IC, Krishnankutty RE (2021) Environmental adaptations of an extremely plant beneficial Bacillus subtilis Dc1 identified through the genomic and metabolomic analysis. Microb Ecol 81:687–702. https://doi.org/10.1007/s00248-020-01605-7

Jiang C, Liao M, Wang H, Zheng M, Xu J, Guo J (2018) Bacillus velezensis, a potential and efficient biocontrol agent in control of pepper gray mold caused by Botrytis cinerea. Biol Control 126:147–157. https://doi.org/10.1016/j.biocontrol.2018.07.017

Kan J, Fang R, Jia Y (2017) Interkingdom signaling in plant-microbe interactions. Sci China Life Sci 60:785–796. https://doi.org/10.1007/s11427-017-9092-3

Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M (2016) KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res 44:D457–D462. https://doi.org/10.1093/nar/gkv1070

Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom 3:e000132. https://doi.org/10.1101/160614

Wu L, Wu H, Qiao J, Gao X, Borriss R (2015) Novel routes for improving biocontrol activity of Bacillus based bioinoculants. Front Microbiol 6:1395. https://doi.org/10.3389/fmicb.2015.01395

Ye M, Tang X, Yang R, Zhang H, Li F, Tao F, Li F, Wang Z (2018) Characteristic and application of a novel species of Bacillus: Bacillus velezensis. ACS Chem Biol 13:500–505. https://doi.org/10.1021/acschembio.7b00874

Zeng T, Rodriguez-Moreno L, Mansurkhodzaev A et al (2020) A lysoin motif effector subverts chitin-triggered immunity to facilitate arbuscular mycorrhizal symbiosis. New Phytol 225:448–460. https://doi.org/10.1111/nph.16245

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