Draft genome sequence of *Bacillus amyloliquefaciens* subsp. *plantarum* strain S2784, an isolate useful for microbial control

Projecto de sequência genómica de *Bacillus amyloliquefaciens* subsp. *plantarum* strain S2784, um isolado útil para o controlo microbiano

DOI:10.34115/basrv6n3-007

Recebimento dos originais: 18/02/2022
Aceitação para publicação: 14/03/2022

Gabriela Rocha
Mestrado em Produção Vegetal pelo Programa de Pós-graduação Stricto Sensu da Universidade Estadual de Goiás (UEG)
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: gabriela_teodoro.rocha@yahoo.com.br

Priscila Grynberg
Doutorado em Bioinformática pela Universidade Federal de Minas Gerais (UFMG)
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: priscila.grynberg@embrapa.br

Paulo Queiroz
Doutorado em Biologia Animal pela Universidade de Brasília (UNB)
Institution: Centro Universitário de Brasília (CEUB)
Address: 707/907, Campus Universitário, Asa Norte, Brasília, DF, CEP: 70790-075
E-mail: prqzqueiroz@gmail.com

Roberto Togawa
Doutorado pela University of Bedfordshire
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: roberto.togawa@embrapa.br

Thifany Purcena
Graduada em Biomedicina pelo Centro Universitário de Brasília (CEUB)
Institution: Centro Universitário de Brasília (CEUB)
Address: 707/907 - Campus Universitário, Asa Norte, Brasília, DF, 70790-075
E-mail: thifanypurcena@gmail.com

Érica Martins
Doutorado em Ciências Biológicas (Biologia Molecular) pela Universidade de Brasília
Institution: Universidade de Brasília
Address: SBN Quadra 02 Bloco C, Edifício Phenícia, CEP: 70040-020
E-mail: es.martins2@gmail.com

Rose Monnerat
Doutorado em Agronomia na Ecole Nacionale Agronomique de Montpellier
Institution: Embrapa Recursos Genéticos e Biotecnologia
Address: Parque Estação Biológica, PqEB, Av. W5 Norte (final) Caixa Postal 02372, Brasília, DF, CEP: 70770-917
E-mail: rose.monnerat@embrapa.br

ABSTRACT
Bacillus amyloliquefaciens subsp. plantarum can colonize plant rhizosphere, stimulate plant growth, and suppress competing phytopathogenic microorganisms. This work describes the draft genome sequence of the B. amyloliquefaciens subsp. plantarum S2784 which contains several specific singletons including genes showing inhibitory activity against numerous plant pathogens and plant growth promotion pathways. However, selective bioassays indicated that the species has pathogenic potential for insects of the order Lepidoptera. In addition, strain S2784 was found to be resistant to the antibiotics ampicillin, penicillin, sulfamethoxazole, and polymyxin B.

Keywords: tryptophan synthase, plant pathogen, anthranilate synthase.

RESUMO
Bacillus amyloliquefaciens subsp. plantarum pode colonizar a rizosfera vegetal, estimular o crescimento das plantas, e suprimir os microrganismos fitopatogénicos concorrentes. Este trabalho descreve o projecto de sequência genómica do B. amyloliquefaciens subsp. plantarum S2784 que contém vários singletons específicos, incluindo genes que mostram actividade inibitória contra numerosos

Palavras-chave: tryptophan synthase, patogénico das plantas, anthranilate synthase.

1 INTRODUCTION

Plant-associated strains of *Bacillus amyloliquefaciens* belonging to subsp. *plantarum* (1) is distinguished from other *B. amyloliquefaciens* by its ability to colonize plant rhizosphere, stimulate plant growth, and suppress competition for phytopathogenic bacteria and fungi. Due to their biofertilizer and biocontrol properties, they are becoming increasingly important as a natural alternative to chemical pesticides and other agrochemicals (2).

However, members of the *Bacillus* genus like *B. subtilis* and *B. amyloliquefaciens* species are among the most efficient bacterial biocontrol agents isolated so far (3). Its antagonistic effect is caused by compounds, antibiotics (bacilisin, iturin, mycosubtylin), and siderophores (4). These species are also capable of inducing growth and defense responses in the host plant (5). Furthermore, *Bacillus* can produce spores resistant to UV light and heat, which allows them to withstand adverse environmental conditions and allows easy formulation for commercial purposes (6). *B. amyloliquefaciens* subspecies

plantarum has undergone two reclassifications as a later heterotypic synonym of *B. methylotrophicus* and then as *B. velezensis* (7).

2 METHODOLOGY

2.1 ISOLATION AND MORPHOLOGICAL CHARACTERIZATION OF THE STRAIN

The bacterial species were isolated from soil in the Federal District, Brasília in 2019. The strain isolation process was carried out according to the methodology established by Praça (8). For the analysis of colony morphology and bacterial strain cytomorphology, the principles of Bergey's Manual of Systematic Bacteriology were followed (9). The morphological properties were observed in a scanning electron microscope, model DSM 962-Zeiss, and transmission electron microscope, model JEM-2100-Jeol.

2.2 GENOME SEQUENCING

Purified genomic and plasmid DNA`s from strain S2784 were extracted by Masterpure Gram-positive DNA purification kit (Epicentre) and QIAGEN Plasmid Maxi Kit (QiAGEN). Afterward, these DNA`s were sequenced at Macrogen, Inc. (Seoul, Korea) using high-throughput HiSeq2000 and GS-FLX Plus platforms getting one lane of 100 bp and 1/8 region plate, respectively. The reads were assembled using Uniclycer, an assembler pipeline based on SPAdes developed for bacterial genomes (10) and annotated by prokka (11). The species identification was done with ANI – Average nucleotide Identity (12).

2.3 ANTIMICROBIAL RESISTANCE PROFILE

The antibiotic susceptibility of the S2784 strain to penicillin G, clindamycin, erythromycin, vancomycin, rifampicin, cefazolin, ceftriaxone, chloramphenicol, ampicillin, oxacillin, tetracycline, lincomycin, clarithromycin, polymyxin B, chlortetracycline, sulfamethoxazole, amikacin, clotrimazole, cloxacillin monohydrate, kanamycin, levofloxacin, and streptomycin was tested. The antimicrobial resistance profile was determined by the agar dilution test following the procedures described by the Clinical and Laboratory Standards Institute. A log-phase culture of the strain was diluted to a concentration of approximately 1×10^8 to 2×10^8 CFU/ml (McFarland standard 0.5). The concentrations determined for the assays were 30, 70, 110, 150, 200, 250, and

300 µg/ml. The solutions were individually added to 50 ml of Embrapa-agar medium in the liquefied state, at a temperature below 60 °C, to avoid antibiotic degradation. The experimental design was completely randomized, with three replications per concentration. The evaluation was performed after 24 hours of incubation at 30 °C. The evaluation consists of verifying whether there was an inhibition of bacterial growth or not, due to the action of the imposed antibiotic.

2.4 INSECT AND NEMATODE SELECTIVE BIOASSAYS

All insects used in the tests were obtained from the Plant and Insect Breeding Platform (PCPI) of Embrapa Genetic Resources and Biotechnology. In all cases, individuals were raised on an artificial diet and under controlled temperature and photoperiod conditions, following the methodologies described by (13-14).

Selective bioassays for *Spodoptera frugiperda*, *Helicoverpa armigera*, *Chrysodeixis includens*, and *Aedes aegypti* were performed as described by (15-16). Bioassays for *Anthonomus grandis* were performed according to the methodology described by (15). For *Caenorhabditis elegans*, bioassays were performed as described by (17).

The correction of mortality observed in larvae of *S. frugiperda*, *H. armigera*, *C. includens*, *A. grandis*, *A. aegypti*, and in the nematode species *C. elegans* by an isolate of S2784 was performed through the equation below proposed by (18):

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} * \text{n in T after treatment}}{\text{n in Co after treatment} * \text{n in Co after treatment}}\right) * 100$$

Where: n = Insect population, T- treated, Co = control

2.5 ANTAGONISM IN *F. OXYSPORUM* F. SP. *VASINFECTUM* AND *SCLEROTINIA SCLEROTIORUM*

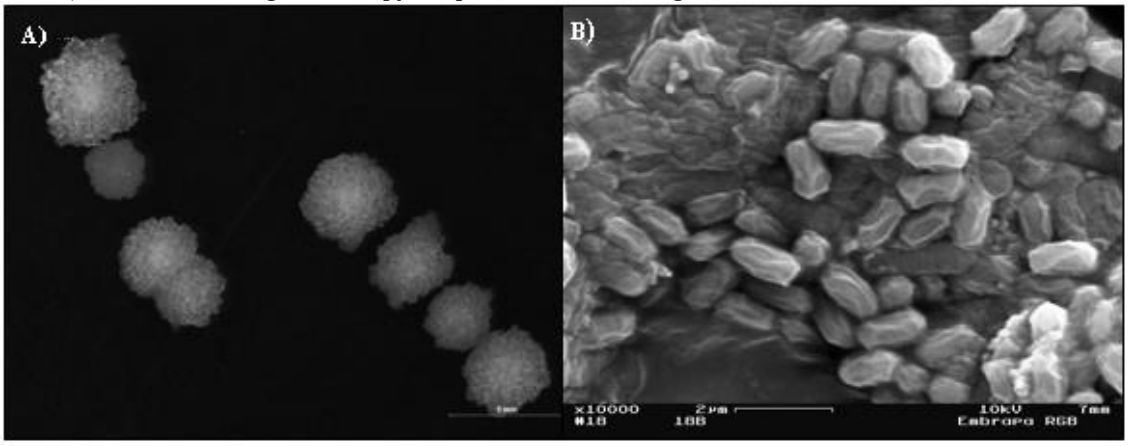
The evaluation of the antagonism of the S2784 strain to *F. oxysporum* f. sp. *vasinfectum* and *Sclerotinia sclerotiorum* was performed by confrontation, with the adoption of the method of pairing cultures in Petri dishes, according to (19-20), with adaptations, in which the selected culture medium was selected only the PDA 50% + EM 50% culture medium.

3 RESULTS

3.1 MORPHOLOGY

The visualized cells were Gram-positive, anaerobic, motile, rod-shaped, 0.7 to 0.9 µm wide, and 1 to 2 µm long. Spores were cylindrical, central in sporangia that were not swollen. Colonies were white, irregular, with lobulated margins, a dry consistency, and a rough, opaque surface (Figure 1). The ideal temperature for growth was 30 °C.

Figure 1. A) Colony morphology of the strain S2784 by electron transmission microscopy at a scale of 5 mm. B) Electron scanning microscopy of spores at 10,000x magnification



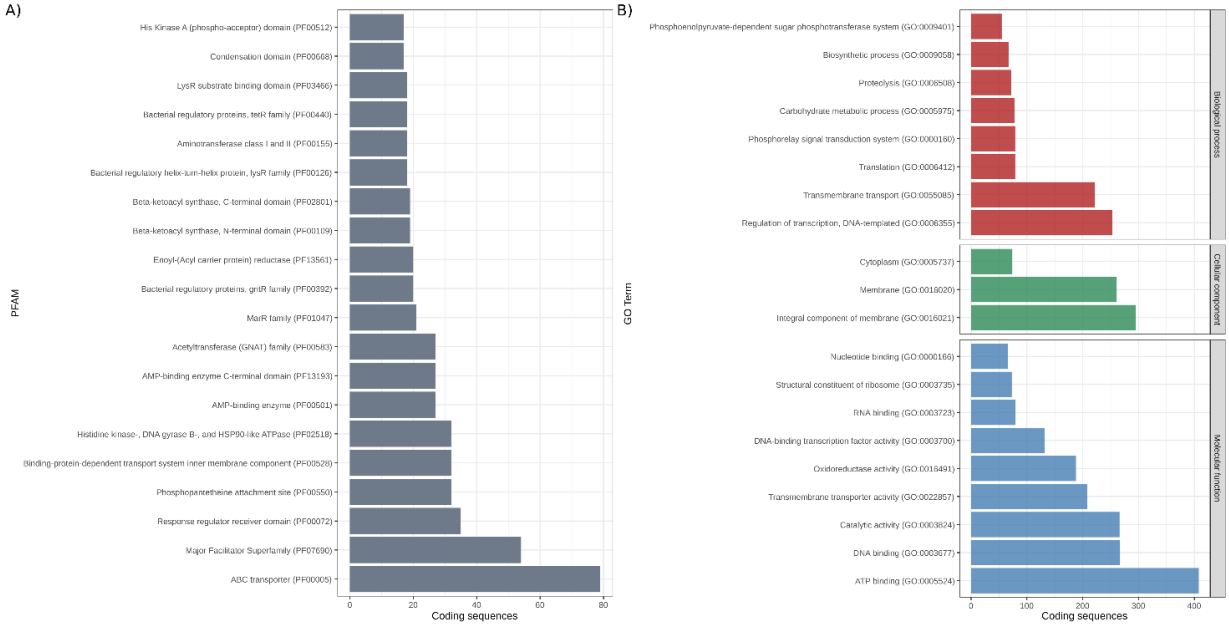
3.2 GENOME SEQUENCING

Here we present *Bacillus amyloliquefaciens* subsp. *plantarum* strain S2784 with a genome size of 3,958,137 bp assembled in 29 scaffolds, with a maximum scaffold size of 966,064 bp, an N_{50} length of 508,796 bp, 46.31% G+C content, and an average nucleotide identity (ANI) of 97,41% with *B. amyloliquefaciens* IT-45 (NC_020272.1). A total of 3,860 coding sequences, 77 tRNA, 3 rRNA operons, and 78 ncRNA were predicted.

Functional annotation was done with interproscan 5.54-87.0 (PMID: 24451626). 3,398 (88.03%) and 2,376 (61.55%) coding sequences present a PFAM or GO code. “ABC transporter” (PF00005) is the most frequent PFAM ID in S2784 coding sequences (79 CDSs), followed by “Major facilitator superfamily” (PF07690) and “Phosphopantetheine attachment site” (PF00500) with 54 and 35 CDSs respectively. The most frequent Biological Process GO terms are “Regulation of transcription, DNA-templated” (GO:0006355), “Transmembrane transport” (GO:0055085), and “Phosphorelay signal transduction system” (GO: 0000160), with 218, 178 e 68 CDSs each. “Membrane” (GO:0016020), “Integral component of membrane” (GO:0016021),

and “Ribosome” are the top three Cellular Component GO terms, with 205, 159 and 48 CDSs. Molecular Function top terms are “ATP binding” (GO:0005524), with 279 CDSs, “DNA binding” (GO:0003677) with 173, and “Oxidoreductase activity” (GO:0016491), present in 129 CDSs (Figure 2).

Figure 2. Functional annotation of the genes identified of the *B. amyloliquefaciens* subsp. *plantarum* strain S2784 by interproscan (A) and GO terms (B).



Gene ontology annotation analysis revealed that the most frequent Biological Process GO terms are “Regulation of transcription, DNA-templated” (GO:0006355), “Transmembrane transport” (GO:0055085), and “Translation” (GO: 0006412), with 253, 222, and 79 CDSs each. “Integral component of membrane” (GO:0016021), “Membrane” (GO:0016020), and “Cytoplasm” (GO:0005737) are the top three Cellular Component GO terms, with 295, 261 and 74 CDSs. Molecular Function top terms are “ATP binding” (GO:0005524), with 408 CDSs, “DNA binding” (GO:0003677) with 267 and “Catalytic activity” (GO:0003824), present in 266 CDSs.

The draft genome sequence of the strain S2784 showed the potential biocontrol activity of these bacteria is notably due to their potential to produce multiple antimicrobial compounds that have been reported for their inhibitory activity against numerous plant pathogens such as DinB family, GH25 muramidase YbfG, 8-oxo-dGTP diphosphatase, Phosphoenolpyruvate synthase, PPDK_N, Uronate isomerase (glucuronate isomerase) UxaC, Symporter, sugar (glycoside-pentoside-hexuronide) transporter, Oxidoreductase, NADB_Rossmann, Macrolactin synthesis, polyketide synthase of type I, Macrolactin

synthesis, polyketide synthase of type I, Macrolactin synthesis, putative penicillin binding protein, 2-keto-3-deoxygluconokinase KdgK, 2-keto-3-deoxygluconate-6-phosphate aldolase KdgA, H+/gluconate symporter and related permeases, Isochorismatase, cystein hydrolase, Difficidin synthesis; modular polyketide synthase of type I, sochorismatase hydrolase, cysteine hydrolase and Putative acetoacetate decarboxylase.

The custom genes database showed the plant growth promotion pathway composed of the genes Tryptophan synthase subunits A and B, plant growth promotion (trpBA), Anthranilate synthase, transferase, plant growth promotion (trpED) and Putative IAA acetyl transferase, plant growth promotion.

3.3 ANTIMICROBIAL RESISTANCE PROFILE

Strain S2784 was resistant to penicillin at a minimum inhibitory concentration (MIC) of 300 µg/ml, following ampicillin of 70 µg/ml, sulfamethoxazole of 70 µg/ml, and polymyxin B of 30 µg/ml. In relation to the other antibiotics, the strain 2784 was susceptible to all (Table 1).

Curiously, the genomic analysis revealed the ampC gene related to the cefazolin resistance. The same finding was observed to the ImrA/ImrB genes and tetD gene related to the lincomycin and chlortetracycline resistance, respectively. Otherwise, the strain 2784 was susceptible to the three antibiotics. This finding suggests an absence of expression of these genes.

Table 1. Antimicrobial resistance profile of the S2784 strain.

Antimicrobial agents	**Strain S2784**	
	Resistant	**Susceptible**
Amikacin	-	+
Ampicillin	70 µg/ml	-
Azithromycin	-	+
Cefazolin	-	+
Ceftriaxone	-	+
Chloramphenicol	-	+
Chlortetracycline	-	+
Clarithromycin	-	+
Clindamycin	-	+
Clotrimazole	-	+
Cloxacillin Monohydrate	-	+
Erythromycin	-	+
Kanamycin	-	+
Levofloxacin	-	+
Lincomycin	-	+
Oxacillin	-	+
Penicillin	300 µg/ml	-
Polymyxin B	30 µg/ml	-
Rifampicin	-	+

Streptomycin	-	+
Sulfamethoxazole	70 µg/ml	-
Tetracycline	-	+
Vancomycin	-	+

+ positive growth in medium supplemented with the antibiotic; - negative growth.

3.4 SELECTIVE BIOASSAY

Through selective bioassays, it was possible to verify that the S2784 strain was potentially toxic, with a mortality percentage upper than 50%, only for insect species of the Lepidoptera order. It is not possible to determine the pathogenic potential of the strain on other species of pests and phytopathogens.

Table 2. Mortality percentage of S2784 strains against *H. armigera* (*H.a*), *S. frugiperda* (*S.f*), *C. includes* (*C.i*), *A. grandis* (*A.gr*), *A. aegypt* (*A.gy*), *C. elegans* (*C.e*), *F. oxysporum* f. sp. *vasinfectum* (*F.ox*), and *Sclerotinia sclerotiorum* (*S.scl*).

% Mortality in selective bioassays of the S2784 strain							
Lepidoptera			**Coleoptera**	**Diptera**	**Nematode**	**Fungi**	
H.a	***S.f***	***C.i***	***A.gr***	***A.gy***	***C.e***	***F.ox***	***S.scl***
85%	50%	70%	20%	0%	5%	0%	25%

4 DISCUSSION

For a better understanding, little is known about antibiotic susceptibility patterns for *B. amyloliquefaciens* species. Studies on this type of subject are focused on antimicrobial activity, which the species has over other organisms. However, in the work reported by (21), *B. amyloliquefaciens* CPA-8 was susceptible to gentamicin, ampicillin and nalidixic acid at all concentrations evaluated, but resistant to hygromycin. In another study conducted by (22), it was found that *B. amyloliquefaciens* VJ-1, with except of antibiotics gentamicin (10 µg) and kanamycin (30 µg), all tested antimicrobials inhibited the growth of the strain. In addition, the antibiotics of the β-lactamase inhibitor group, ampicillin, and imipenem, presented the largest zone of inhibition, differently from what was observed in this study.

Bacillus amyloliquefaciens species are used as biocontrol agents for the suppression of many soil-plant pathogens. In fact, due to the sketch of the genomic sequence of the S2784 strain, it was found that the pathogenic potential of this isolate is mainly due to the strain's ability to produce various antimicrobial and antifungal compounds. One of these compounds found through sequencing was macrolactin, a molecule with antiseptic capacity used in agricultural and medical applications (23). In a study conducted by Yuan (23) it was found that macrolactin was able to exhibit significant antagonistic effects against *Fusarium oxysporum* and *Ralstonia solanacearum*. However,

strain S2784 was not equally pathogenic against *F. oxysporum* f. sp. *vasinfectum*, and *S. sclerotiorum*, in this study.

So, the genomic analysis of promising beneficial microbes will help to obtain improved bioformulations to save considerable amounts of agrochemicals, especially chemical fertilizers, and chemical pesticides (24).

Nucleotide sequence accession numbers. The sequence of the *B. amyloliquefaciens* strain S2784 has been deposited in GenBank with the accession number SAMN12230145.

Acknowledgment

We would like to thank Dr. Lilian Praça for the technical assistance.

REFERENCES

1 Borriss R, Chen XH, Rueckert C, Blom J, Becker A, Baumgarth B, Fan B, Pukall R, Schumann P, Sproer C, Junge H, Vater J, Pühler A, Klenk HP (2011). Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM 7T and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on their discriminating complete genome sequences. Int J Syst Evol Microbiol 61:1786–1801. doi: 10.1099/ijs.0.023267-0.

2 Krebs B, Hoding B, Kubart S, Workie MA, Junge H, Schmiedeknecht G, Grosch R, Bochow H, Hevesi M (1998) Use of *Bacillus subtilis* as biocontrol agent I. Activities and characterization of *Bacillus subtilis* strains. Z Pflanzenk Pflanzen 105:181–197. https://www.jstor.org/stable/43215233.

3 Borriss R (2011) Use of plant-associated *Bacillus* strains as biofertilizers and biocontrol agents. In: Maheshwari DK (ed) Bacteria in agrobiology: plant growth responses. Springer, Heidelberg, pp 41–76. doi: 10.1007/978-3-642-20332-9_3.

4 Shoda M (2000) Bacterial control of plant diseases. J of Bios and Bioeng 89: 515-521. doi: 10.1016/S1389-1723(00)80049-3.

5 Raupach GS, Kloepper JW (1998). Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88:1158-64.11. doi: 10.1094/PHYTO.1998.88.11.1158.

6 Raaijmakers JM, Vlami M, de Souza JT (2022) Antibiotic productionby bacterial biocontrol agents. Antonie Van Leeuwenhoek 81:537-47. doi: 10.1023/A:1020501420831.

7 Dunlap CA, Kim SJ, Kwon SW, Rooney AP (2016) *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus velezensis*, *Bacillus amyloliquefaciens* subsp. *Plantarum* and *Bacillus oryzicola* are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. Int J Syst Evol Microbiol 66: 1212–1217. doi:10.1099/ijsem.0.000858.

8 Praça LB, Morinaga C, Medeiros PT, Melatti VM, Martins ÉS, Dumas VF, Monnerat RG (2009) Isolamento e caracterização de estirpes de *Bacillus thuringiensis* coletadas em solos do oeste baiano. Universitas: Ciências da Saúde: 7: 1-18. https://www.cienciasaude.uniceub.br/cienciasaude/article/view/999.

9 De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer KH, Whitman W (Eds.) (2009) Bergey's Manual of Systematic Bacteriology. Vol. 3: The Firmicutes. Springer Science; Business Media, p. 1450.

10 Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13(6): e1005595. https://doi.org/10.1371/journal.pcbi.1005595.

11 Seemann T. Prokka: rapid prokaryotic genome annotation. Vol. 30 no. 14 2014, pages 2068–2069. doi:10.1093/bioinformatics/btu153.

12 Figueras MJ, Beaz-Hidalgo R, Hossain MJ, Liles MR. Taxonomic affiliation of new genomes should be verified using average nucleotide identity and multilocus phylogenetic analysis. Genome Announc. 2014;2(6): e00927-14. Published 2014 Dec 4. doi:10.1128/genomeA.00927-14.

13 Monnerat RG, Dias SC, Oliveira-Neto OB, Nobre SD, Silva-Werneck JO, Sá MFG de (2002) Criação massal do bicudo do algodoeiro *Anthonomus grandis* em laboratório. Comunicado Técnico 46**.** 4p. Brasília: Embrapa-Cenargen. 4p. https://www.infoteca.cnptia.embrapa.br/bitstream/doc/184630/1/bp029.pdf.

14 Shmidt FGV, Monnerat RG, Borges M, Carvalho R da S (2001) Metodologia de criação de insetos para avaliação de agentes entomopatogênicos. Circular Técnica 11, 20 p. Brasília: Embrapa Recursos Genéticos e Biotecnologia. http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=AGB.xis&method=post&formato=2&cantidad=1&expresion=mfn=208922.

15 Monnerat RG, Silva SF, Silva-Werneck JO (2001) Catálogo do banco de germoplasma de bactérias do gênero *Bacillus*. Documentos 60, 65p. Brasília: Embrapa-Cenargen. https://livimagens.sct.embrapa.br/amostras/00050530.pdf.

16 Silva-Werneck JO, Monnerat R (2002). Metodologias para caracterização de isolados de *Bacillus thuringiensis*. Circular Técnica 10, 5p. Brasília: Embrapa Recursos Genéticos e Biotecnologia. https://ainfo.cnptia.embrapa.br/digital/bitstream/CENARGEN/23206/1/cot070.pdf.

17 Montalvão SCL, Castro MTD, Soares CMS, Blum LEB, Monnerat RG (2018) *Caenorhabditis elegans* as an indicator of toxicity of *Bacillus thuringiensis* strains to *Meloidogyne incognita* race 3. Ciên. Rur. 48.

18 Henderson CF, Tilton EW (1955) Tests with Acaricides against the Brown Wheat Mite12. J of Econ Ent 48: 157–161. doi: 10.1093/jee/48.2.157.

19 Dennis C, Webster J (1971) Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society 57: 25-IN3. doi: 10.1016/S0007-1536(71)80077-3.

20 Montalvão SC, de Castro MT, Blum LE, Monnerat RG (2021) Biocontrol of *Fusarium oxysporum* f. sp. *vasinfectum* with *Bacillus spp* strains. J of Agric Science 13: 9. doi: 10.5539/jas.v13n9p1.

21 Gotor-Vila A, Teixidó N, Sisquella M, Torres R, Usall J (2017) Biological Characterization of the Biocontrol Agent *Bacillus amyloliquefaciens* CPA-8: The Effect of Temperature, pH and Water Activity on Growth, Susceptibility to Antibiotics and Detection of Enterotoxic Genes. Curr Microbiol 74: 1089–1099. doi: 10.1007/s00284-017-1289-8.

22 Kadaikunnan S, Rejiniemon TS, Khaled JM, Alharbi NS, Mothana R (2015) *In-vitro* antibacterial, antifungal, antioxidant and functional properties of *Bacillus*

amyloliquefaciens. Ann Clin Microbiol Antimicrob 14:9. doi: 10.1186/s12941-015-0069-1.

23 Yuan J, Li B, Zhang N, Waseem R, Shen Q, Huang Q (2012) Production of bacillomycin-and macrolactin-type antibiotics by *Bacillus amyloliquefaciens* NJN-6 for suppressing soilborne plant pathogens. J of Agric and Food Chem 60: 2976-2981. doi: 10.1021/jf204868z.

24 Qiao et al.: Stimulation of plant growth and biocontrol by *Bacillus amyloliquefaciens* subsp. *plantarum* FZB42 engineered for improved action. (2014) Chemical and Biological Technologies in Agriculture 2014 1:12. doi:10.1186/s40538-014-0012-2