ACTA
AMAZONICA

http://dx.doi.org/10.1590/1809-4392201901072

ORIGINAL ARTICLE

Diversity of bacterial strains in biochar-enhanced Amazon soil and their potential for growth promotion and biological disease control in tomato

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ABSTRACT

The use of bacteria in growth promotion and biological control of plant diseases can minimize environmental contamination caused by the indiscriminate use of pesticides and chemical fertilizers. We aimed to evaluate growth promotion and biological control of *Corynespora cassiicola* in tomato seedlings mediated by beneficial bacteria isolated from a non-rhizospheric Amazon soil containing different amounts of biochar, and to identify to which groups of bacteria the strains belong. We obtained 200 strains of bacteria from experimental plots containing biochar doses of 0, 40, 80 and 120 t ha^{-1}. Of these, 53 strains were selected by root colonization tests. Based on growth promotion parameters, 25 strains were screened, identified by molecular characterization and evaluated for indoleacetic acid (IAA) production, phosphate solubilization and biological control. The best dose of biochar for colony formation was 40 t ha^{-1}, and a regression model indicated 34 t ha^{-1} as the optimal dose. The production of IAA was observed in 18 (75%) strains, and two (8%) strains were able to solubilize phosphate. The efficiency in root growth promotion was up to 125%, and the percentage of plant protection ranged from 50 to 59%. Molecular characterization showed that the bacteria used in this study belong to the genera *Bacillus* and *Lysinibacillus*.

KEYWORDS: beneficial bacteria; *Corynespora cassiicola*; *Solanum lycopersicum*; target spot

Diversidade de isolados bacterianos em solo amazônico enriquecido com biocarvão e seu potencial para a promoção de crescimento e controle biológico de doenças em tomate

RESUMO

O uso de bactérias na promoção do crescimento e no controle biológico de doenças em plantas pode minimizar a contaminação ambiental causada pela aplicação indiscriminada de pesticidas e fertilizantes químicos. Objetivamos avaliar a promoção do crescimento e o controle biológico de *Corynespora cassiicola* em mudas de tomate mediadas por bactérias benéficas isoladas de solo amazônico não-rizosférico contendo diferentes dosagens de biocarvão, e identificar a quais grupos de bactérias os isolados pertencem. Obtivemos 200 isolados de parcelas experimentais contendo doses de biocarvão de 0, 40, 80 e 120 t ha^{-1}. Destes, 53 foram selecionados por testes de colonização radicular. Com base nos parâmetros de promoção do crescimento, 25 isolados foram selecionados, identificados através de análise molecular e avaliados para produção de ácido indolacético (AIA), solubilização de fosfato e controle biológico. A melhor dose de biocarvão para a formação de colônias foi 40 t ha^{-1}, e um modelo de regressão indicou 34 t ha^{-1}como dose ótima. A produção de AIA foi observada em 18 (75%) isolados e dois (8%) isolados foram capazes de solubilizar fosfato. A eficiência na promoção do crescimento das raízes foi de até 125%, e a porcentagem de proteção das plantas variou de 50,3 a 59,0%. A caracterização molecular indicou que as bactérias utilizadas nesse estudo pertencem aos gêneros *Bacillus* e *Lysinibacillus*.

PALAVRAS-CHAVE: bactérias benéficas; *Corynespora cassiicola*; *Solanum lycopersicum*; mancha alvo

CITE AS: Caniato, M.M.; Catarino, A.M.; Sousa,T.F.; Silva, G.F.; BICHARA, K.P.A.; Cruz, J.C.; Assis, L.A.G.; Hanada, R.E. 2020. Diversity of bacterial strains in biochar-enhanced Amazon soil and their potential for growth promotion and biological disease control in tomato. Acta Amazonica 50: 278-288.

INTRODUCTION

Tomato (*Solanum lycopersicon* Linnaeus) is one of the most commonly produced vegetables worldwide (Faostat 2017). In Amazonas state, Brazil, tomato cultivation is hampered by soil acidity and low natural fertility (Cerri *et al*. 2003) and requires high doses of limestone and chemical fertilizers, which significantly increase the costs of tomato production and discourage farmers. In addition, diseases caused by fungi are a limiting factor in tomato crops. Target spot, caused by the fungus *Corynespora cassiicola* (Berk and M. A. Curtis) C.T. Wei. is among the most important diseases that affect the aerial parts of tomato seedlings (Mandal *et al*. 2017). *Corynespora cassiicola* is non-specific and occurs mainly in the tropics and subtropics (Dixon *et al*. 2009), where environmental conditions favour disease development (Teramoto *et al*. 2017). In Brazil, there are still no specific fungicides recommended by the Ministry of Agriculture for the control of target spot in tomato crops. Therefore, the disease is controlled by fungicides recommended for soybean crops, such as Carbendazim Nortox® and Comet®, which have benzimidazole and strobirulin, respectively, as active ingredients (Agrofit 2020). These agents can cause harm to the environment, plants, animals and humans (Chaturvedi *et al*. 2013).

Beneficial bacteria in soils have potential to promote both biocontrol, because some produce natural fungicides to control phytopathogens, and plant growth, because some produce phytohormones (e.g., indoleacetic acid) and siderophores, and solubilize minerals, such as silicates, phosphates and potash (Naureen *et al*. 2017). Biochar applied to the soil can enhance colonization by beneficial bacteria, including plant growth-promoting bacteria (PGPB) (Bertola *et al.* 2019). Biochar can improve soil health with or without exposure to contamination by heavy metals and/or organic pollutants (Palansooriya *et al.* 2019). Biochar improves composting processes, as well as the biochemical properties of compost, by increasing the number of plant growth-promoting rhizobacteria (PGPR), which solubilize phosphate, produce indoleacetic acid (IAA) and degrade protein and cellulose. Biochar can also synergize the use of biofertilizers for promoting sustainable agriculture (Antonius *et al*. 2015).

Through increases in bacterial abundance and changes in microbial community structure, biochar soil-enrichment can exert a significant role on disease suppression and plant growth promotion, either through direct antagonism or indirectly via induction of systemic resistance in the plant (Jaiswal *et al.* 2018). Biochar amendments in soil can reduce the severity of bacterial wilt caused by *Ralstonia solanacearum* on tomato (Lu *et al.* 2016), induce resistance to the pathogens *Botrytis cinerea* and *Leveillula taurica* in both pepper and tomato, and the pest *Polyphagotarsonemus latus* in pepper (Elad *et al*. 2010), and improve considerably the growth of tomato plants, as they become more resistant to *Fusarium oxysporum* and *Ralstonia solani* (Khalifa and Thabet 2015).

Our aim was to evaluate growth promotion and biological control of *C. cassiicola* in tomato seedlings mediated by beneficial bacteria from a non-rhizospheric Amazonian soil containing different amounts of biochar, and to identify to which groups of bacteria the strains belong.

MATERIAL AND METHODS

Influence of biochar on soil cultivable bacterial populations

The non-rhizospheric soils used were obtained at the Experimental Station for Tropical Fruit Culture of Instituto Nacional de Pesquisas da Amazônia (INPA), located at km 42 of the BR-174 highway, municipality of Manaus, Amazonas state, Brazil. The soil in the area is dystrophic yellow Oxisol with clay texture (> 60%). Soil samples were collected in 2017 from 25-m^2 plots that had been enriched with biochar (Table 1) at concentrations of 0, 40, 80 and 120 t ha^{-1} in 2006 (one plot per treatment). A maize/cowpea crop rotation was started on the plots three months after biochar application, when a chemical fertilization (66 kg ha^{-1} of urea, 177 kg ha^{-1} of triple superphosphate and 100 kg ha^{-1} of KCl) was applied. Three samples were collected from each plot at depths between 0 and 10 cm, and mixed into a composite sample. About 300 g of each sample was packed into plastic bags and transported in a polystyrene box to the Phytopathology Laboratory at INPA.

Bacterial strains were isolated by the serial dilution methodology (Silva and Romero 2004), with dilution factors varying from 10^{-1} to 10^{-4}. After dilution, strains were cultivated using solid 523 non-selective culture medium (Kado and Heskett 1970). Colonies were obtained according to the methodology of Silva and Romero (2004), using 10^{-4} dilution factor aliquots of 100 µL, which were deposited in Petri dishes containing the culture medium and maintained at 28 °C in biochemical oxygen demand (BOD) during 24-hours of light. The results were expressed in colony forming units per ml (CFU ml^{-1}) using the formula: $R = a \times 10^b$ CFU ml^{-1}, where R = result, a = average number of colonies per repetition and b = exponent of the dilution.

The experiment was conducted in a completely randomized design with four biochar treatments (0, 40, 80 and 120 t ha^{-1}) and five replications. Each replication consisted of one Petri dish. Fifty colonies were selected for each treatment, totaling

Table 1. Concentrations of chemical elements in the biochar-enriched non-rhizospheric soil collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, municipality of Manaus (Amazonas, Brazil) in 2017.

Material	C	N	Ca	Mg	K	P	Zn	Mn
			g kg^{-1}				mg kg^{-1}	
Fine coal	873.3	8.9	6.2	1.3	2.1	0.2	12.0	67.0

200 strains. Colonies were selected by appearance, for uniform color and shape. Selected strains were isolated in solid medium (Kado and Heskett 1970) and the colonies were maintained at 28 ºC during 24 h of light in BOD.

Root colonization of tomato seedlings

After colony growth of the 200 selected strains, a bacterial suspension was obtained by adding 10 ml of saline solution (0.85%) to be used in the microbiolization step. The concentrations of the suspensions were adjusted by dilution, according to the correlation between optical density and number of CFU's, to 0.2 absorbance (Abs.) (540 nm), which corresponds to approximately 10^8 CFU ml^{-1}.

Untreated *Santa Cruz Kada* tomato seeds were disinfested by immersion in ethanol (50%) for two minutes, NaCl (2%) for four minutes and washing in sterilized water. The microbiolization was performed according to Silva *et al*. (2003). After this phase, seeds were sown in tubes containing 523 Kado and Heskett culture medium for 10 days. For each bacterial strain, three tubes containing two seeds each were used. In addition to the 200 strains, we used the rhizobacterium *Bacillus cereus* Frankland & Frankland, 1887 (UFV-101 strain) as positive control, as it has proven efficiency in root colonization (Romeiro *et al*. 2010). The presence of a halo around the root was used as an indicator of colonization. Each isolate was categorized as positive or negative for root colonization capacity. A light microscope with a magnification of 100x was used to observe bacterial biofilm formation.

Growth promotion of tomato seedlings

This experiment was performed at the Von der Pahlen Experimental Station for Vegetable Crops of INPA, and was divided into two steps. First, a preliminary selection was carried out among the positive strains for root colonization. The second step consisted of the confirmation test with the most promising strains. For both steps, the bacterial strains were grown in Petri dishes containing medium 523 (Kado and Heskett 1970), for 24 to 48 h. Tomato seeds (cultivar *Santa Cruz Kada*) were immersed in the bacterial suspension of each selected strain for a period of 24 h by microbiolization, then were sown in tubes (280 g) containing Vivatto Plus® substrate (two seeds per tube), with 10 tubes per treatment. Thinning was performed seven days after germination, leaving one seedling per tube. The experimental design was completely randomized. Two controls were used, one negative, with microbiolized seeds in sterilized distilled water, and one positive, with microbiolized seeds in *B. cereus* (UFV-101), with 10 replicates each.

In the first step, the effect of the bacterial strains on tomato seedling growth was evaluated through plant height (PH), number of leaves (NL), stem diameter (SD) and dry mass of the aerial part (DMAP). In the second step, the most promising strains were reassessed through the same growth parameters (except NL), and also including the dry mass of the roots (DMR) and the total dry mass (TDM). PH was measured from the base to the apical bud of the seedling, using a millimetre ruler. NL was obtained by counting all fully expanded true leaves. SD was measured with a digital caliper (ZAAS). DMAP and DMR were obtained by weighing the aerial part and root dry mass, respectively. Samples were dried in a Digital Stove timer SSD 110L.

The growth promotion efficiency (GPE) was calculated for each variable and isolate selected in the second step using the data of both steps. GPE was calculated as $([G_T - G_C]/ G_C) \times 100$, where G_T is the growth parameter for the isolate, and G_C is the growth parameter for the negative control, as described by Almoneafy *et al*. (2014). All evaluations were performed 20 days after sowing.

Indoleacetic acid production and phosphate solubilization

The strains selected in the second step of the growth promotion assay and *B. cereus* strain (UFV-101) were grown in Petri dishes containing 523 solid medium (Kado and Heskett 1970). After 24 h, they were transferred to test tubes containing 5 ml of TS medium enhanced with hydroxytryptophan (3 g tryptone, 0.1 g soy peptone, 1.6 g NaCl, 0.2 g hydroxytryptophan, and 200 ml sterilized distilled water), and amino acid similar to L-tryptophan produced in capsules containing 50 mg of 5-HTP. After 24 h under 130 rpm stirring, the medium was transferred to 10 ml Falcon-type tubes.

The production of indoleacetic acid (IAA) was determined by the colorimetric method, following Bric *et al*. (1991), using the Salkowski reagent (0.5 M $FeCl_3.6\ H_2O$ and 35% $HClO_4$), and three replicates per strain. The tubes were centrifuged and 2 ml of the supernatant were placed in assay tubes with 1 ml (2:1) of the reagent. The strains were incubated in the absence of light for 20 min for the reaction to occur. A reddish color in the tube signaled the production of IAA by the bacteria.

The evaluation of solubilization was based on Katznelson and Bose (1959). Strains were cultivated and maintained at 28 °C for 15 days, with three replicates per strain. The colonies that formed a clear halo around them were considered calcium phosphate solubilizers. The diameters of the colonies and solubilization halos were measured to obtain the solubilization index (SI), using the formula: SI = Ø Halo (mm)/ Ø Colony (mm) (Berraquero *et al.*1976), where Ø = diameter. The bacteria were classified as low (SI < 2), medium (2 ≤ SI < 4) and high solubilizers (SI > 4). According to the starting time of solubilization, the bacteria were classified as precocious (solubilization onset before the third day) or late solubilizers (onset after the third day), and apparent non-solubilizers (that did not show visible solubilization until the 15th day of evaluation) (Hara and Oliveira 2004).

Biological control

This experiment was conducted in a greenhouse at EEH, aiming to control target spot caused by the pathogen *C. cassiicola* using the strains selected in the second step of the growth promotion assay. The INPA 2839 *C. cassiicola* strain was cultivated in potato-dextrose-agar medium (PDA). On the tenth day of growth, which coincided with the twentieth day of *Santa Cruz Kada* cultivar tomato seedling growth, the spore suspension was prepared with 1.1 x 10^5 spores ml^{-1} and applied to the seedlings using an atomizer. Subsequently, seedlings were placed in a humid chamber for 24 h. The biocontrol agents (bacterial strains) were added 20 days before the pathogen following the protocol used in the growth promotion trials. The experimental design was completely randomized, with 10 replicates for each bacterial strain and three controls, which consisted of (i) plant + *B. cereus* UFV-101+ *C. cassiicola* (positive control); (ii) plant + *C. cassiicola* (negative control); and (iii) plant + water (negative control).

The severity of the disease induced by *C. cassiicola* was evaluated on alternate days starting on the second day after inoculation with the pathogen and ending on the tenth day. Three leaflets per replicate were evaluated with the aid of an adapted Horsfall-Barratt diagrammatic scale (Oliveira *et al.* 2006) and classified according to the proportion of injured area as follows: 0 - no symptoms; 1 - < 1% injured area; 2 - 1.1 to 3%; 3 - 3.1 to 6%; 4 - 6.1 to 12%; 5 - 12.1 to 25%; 6 - 25.1 to 50%; and 7 - > 50%.

The area under the disease progression curve (AUDPC) = Σ ((Y_i +Y_{i+1}) /2) (t_{i+1}-t_i) was calculated for each strain and control from the data obtained for severity, where Y = intensity of the disease, t = time, and i = number of evaluations in time (Campbell and Greaves 1990). The protection percentage (%) was estimated by the relationship: (1-x/y), where x = AUDPC of the treated plants, and y = AUDPC of the inoculated controls (Li *et al.* 1996).

Statistical analysis

The CFU count data were compared among treatments with regression analysis using a quadratic regression, which best fitted the data. The frequencies of positive and negative strains for root colonization were compared among soil treatments with a Chi-square test, and those for growth promotion, phosphate solubilization, indole acetic acid production and resistance induction were compared with a Fischer's exact test. The growth promotion variables were compared among strains using ANOVA, except PH and SD, which were submitted to a Skott-Knott test, and NL and DMAP, which were submitted to a Dunn test by the Kruskal-Wallis non-parametric analysis. Homogeneity of variance was assessed by Cochran's Q test and normality by the Shapiro Wilk test. The AUDPC means among replicates were compared among bacterial strains and controls using the Skott-Knott test and a significance level of 5%. All analyses were performed using ASSISTAT 7.7 beta (Silva and Azevedo 2016).

Molecular characterization

The genomic DNA of the bacterial strains selected in the second step of the growth promotion trials was extracted using the phenol-chloroform method adapted from Sambrook *et al.* (1989). We used the P027F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-ACGGTTACCTTGTTACGACTT-3') PCR primer pair (Weisburg *et al.* 1991) for the amplification of the 16S rRNA region and the ERIC1F (5' - ATGTAAGCTCCTGGGGATTCAG-3') and ERIC2R (5'-AAGTAAGTGACTGGGGTGAGC - 3') primer pair to evaluate strain diversity (Versalovic *et al.* 1991).

For the PCR reactions with both primers, the following concentrations were used: 100 ng total DNA, 0.2 pmol of each primer, 1X enzyme buffer (100 mM Tris-HCl (pH 8.8 at 25 °C)), 2 mM $MgCl_2$, 0.4 mM dNTPs and 1.25 units of Taq DNA Polymerase. The reaction took place in a final volume of 25 µl. Amplification conditions included an initial denaturation at 95 °C for 5 min, 35 denaturation cycles at 94 °C for 1 min, annealing at 50 °C for 1 min and extension at 65 °C for 8 min, with a final extension at 65 °C for 16 min. At the end of the cycles, the reaction was maintained at 10 °C/∞. After amplification, fragments were separated in 1.5% agarose gel with electrophoresis and visualized (L-PIX CHEMI Molecular Imaging).

PCR products generated from the P027F/1492R primer pair were treated with polyethyleneglycol (20% PEG) and sequenced using the BigDye™ Terminator v3.1 kit on the 3500 Genetic Analyzer (Applied Biosystems™) according to the manufacturer's recommendations. The consensus sequence was obtained manually based on the sequencing of the F and R strands, and new sequences generated in this study were deposited in GenBank (http://www.ncbi.nlm.nih) under accession numbers MH547253 to MH547275.

The results obtained from the ERIC1F/ERIC2R primer pair reaction were analyzed using the PAST® Program (version 2.17c; Hammer *et al.* 2000), after binary data transformation and the construction of a 0-1 matrix, where 1 indicates the presence, and 0 the absence of a band. The bands generated for each strain were compared and their similarities estimated by the Jaccard coefficient, which was obtained by the unweighted pair group method with arithmetic mean (UPGMA) algorithm, and the strains were grouped and plotted using a similarity dendrogram (Sneath and Sokal 1973).

Dataset construction was completed with 16S rRNA region sequences from the strains obtained in this study. Sequences were obtained from GenBank (http://www.ncbi.nlm.nih) using the BLASTn tool. The sequences were aligned

with the MAFFT online service (Katoh *et al*. 2017) and manually adjusted in MEGA 7.0 (Tamura *et al*. 2013).

Phylogenetic analyses were performed using the maximum likelihood (ML) and Bayesian inference (BI) methods. Partial deletion was used for the treatment gaps and missing data in the ML analysis. The 95% cut off and non-parametric bootstrap measurements were done with 1000 replicates, and the tree was generated and visualized in MEGA 7.0. Bayesian inference was based on the model selected by PAUP*4 and Mrmodeltest2 v2 (Posada 2003) through an alignment including all sites. The analysis was allowed to run for ten million generations, with the first 25% of trees discarded as burn-ins using the tool MrBayes v. 3.6, which is available on the CIPRES platform (https://www.phylo.org/). Posteriori probabilities (PP) and tree topologies were visualized with Figtree v. 1.1.2 (Rambaut 2009).

The identity analysis between the sequences was performed in the SDT v.1.2 program (Sequence Demarcation tool) by means of an array containing sequences of the strains obtained in this study and sequences obtained from GenBank (http://www.ncbi.nlm.nih) by the BLASTn tool. The alignment MAFFT algorithm was selected to calculate the identity values, and the similarity of the phylogenetic relationships was estimated with the neighbor component using two cut off values, one at 99% and the other at 78%. These cut-off values represent the species demarcation thresholds (Kim *et al*. 2014).

RESULTS

Influence of biochar on the soil cultivable bacterial population

There was a significant difference in CFU among treatments according to regression analysis. Biochar stimulated bacterial growth up to 34 t ha^{-1} and from there on a decrease was observed in the bacteria population (Figure 1). The estimated CFU for soil without biochar (dose 0) was 6.65 x 10^6 CFU ml^{-1}. The optimal dose of biochar estimated by the adjusted regression model was 34 t ha^{-1}, providing a maximum value of 7.55 x 10^6 CFU ml^{-1}. The adjusted regression model was $\hat{y} = 6.656 + 0.0532x - 0.0008x^2$, where $\hat{y}$ is the estimated CFU value and x the biochar dose. The model explained 91.4 % of the total CFU variation in response to biochar doses (Figure 1).

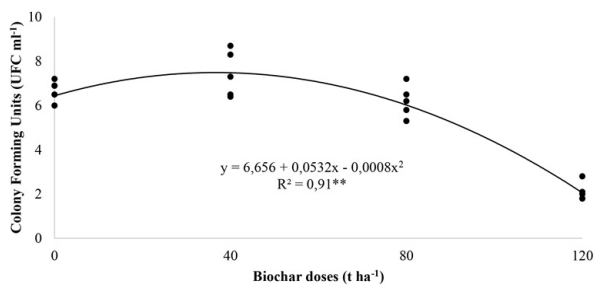

Figure 1. Relationship between biochar doses and colony forming units (CFU) of non-rhizospheric soil cultivable bacteria collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, municipality of Manaus (Amazonas, Brazil). **significant at $p \leq 0.01$.

Root colonization and growth promotion

Seventy (35%) of the initial 200 strains were positive for root colonization (Supplementary Material, Tables S1, S2), of which 53 were selected for the growth promotion test. Among the 53, eight (15%) significantly reduced the growth of tomato seedlings relative to the controls, 20 (37.7%) did not differ significantly from the control, and 25 had significantly higher DMAP (Supplementary Material, Table S3), and were considered the most promising bacterial strains. Among the 25 strains, SD differed significantly from the negative control in eight (32%), DMR differed significantly in ten (40%), and there was no significant difference in PH, DMAP and TDM (Table 2).

There was a significantly higher frequency of strains capable of colonizing the root system in the soils with 0 and 40 t ha^{-1} biochar ($\chi^2 = 28.92$; $p < 0.0001$) (Supplementary Material, Table S2). The frequency distribution of the bacterial strains positive for growth promotion did not vary significantly among soil types (Fisher's p = 0.71709) (Supplementary Material, Table S4), i.e., the strains responded in the same way to the growth promotion test, independently of the biochar dose.

Indoleacetic acid production and phosphate solubilization

Eighteen of the 25 strains were found to produce IAA, while only two showed calcium phosphate solubilization capacity: 25T4 and 12T4 (Table 2). These strains formed a solubilization halo and were categorized as precocious, with a low capacity to solubilize phosphate (SI < 2). The two strains were from the treatment with 120 t ha^{-1} biochar, resulting in a relative frequency of 66.7% positive strains for solubilization of inorganic phosphate in the form of $CaHPO_4$ (Supplementary Material, Table S5). Accordingly, the frequency distribution of strains positive for phosphate solubilization varied significantly among soil types (Fisher's p = 0.01), i.e., there was an influence of the dose of biochar on the population of phosphate solubilizing bacteria present in non-rhizospheric soil (Supplementary Material, Table S5). It is worth mentioning that only in the soil with the highest dose of biochar (120 t ha^{-1}) were found rhizobacteria belonging to the *B. megaterium* group (strain 25T4; Supplementary Material, Table S6) capable of solubilizing calcium phosphate in inorganic form.

The frequency distribution of the bacterial strains positive for production of indole acetic acid did not vary significantly

Table 2. Results of growth promotion, production of indoleacetic acid and phosphate solubilization tests with 25 bacterial strains obtained from biochar-enriched non-rhizospheric soil collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, Manaus municipality (Amazonas, Brazil), on *Santa Cruz Kada* tomato seedlings 20 days after sowing on Vivato plus® substrate. Values are the mean ± SD of 10 replicates. IAA and P indicate positive (+) or negative (–) result for the production and solubilization test, respectively. CV% = coefficient of variation.

Strain	PH[1] (cm)	GPE (%)	SD[1] (mm)	GPE (%)	DMAP[1] (g)	GPE (%)	DMR[1] (g)	GPE (%)	TDM[1] (g)	GPE (%)	IAA	P
C-*	12.5 ± 0.5^{a}	0.0	1.8 ± 0.2^{b}	0.0	0.0 ± 0.04^{a}	0.0	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	0.0	-	-
C+**	13.5 ± 0.4^{a}	8.6	2.1 ± 0.2^{a}	13.0	0.1 ± 0.06^{a}	35.7	0.0 ± 0.007^{a}	75	0.1 ± 0.06^{a}	39.1	+	-
3T4	13.3 ± 0.4^{a}	6.7	2.1 ± 0.2^{a}	12.0	0.0 ± 0.05^{a}	19.0	0.0 ± 0.005^{b}	25	0.0 ± 0.05^{a}	19.6	+	-
4T2	12.9 ± 0.3^{a}	3.6	2.1 ± 0.1^{a}	11.3	0.0 ± 0.05^{a}	16.7	0.0 ± 0.007^{a}	75	0.1 ± 0.06^{a}	23.9	-	-
6T1	12.7 ± 0.4^{a}	1.6	1.8 ± 0.3^{b}	-2.7	0.0 ± 0.04^{a}	7.1	0.0 ± 0.005^{b}	25	0.0 ± 0.05^{a}	10.8	+	-
15T2	12.4 ± 0.3^{a}	-0.8	1.7 ± 0.2^{b}	-9.2	0.0 ± 0.04^{a}	-9.5	0.0 ± 0.007^{a}	75	0.0 ± 0.04^{a}	-2.2	+	-
7T1	13.2 ± 0.3^{a}	6.3	1.9 ± 0.3^{b}	1.6	0.0 ± 0.05^{a}	28.6	0.0 ± 0.007^{a}	75	0.7 ± 0.06^{a}	34.8	+	-
25T4	13.2 ± 0.2^{a}	5.7	1.9 ± 0.3^{b}	0.5	0.0 ± 0.05^{a}	19.0	0.0 ± 0.008^{a}	100	0.1 ± 0.06^{a}	28.3	+	+
22T3	13.5 ± 0.3^{a}	8.7	2.0 ± 0.3^{a}	8.6	0.0 ± 0.05^{a}	21.4	0.0 ± 0.009^{a}	125	0.1 ± 0.06^{a}	32.6	-	-
53T1	13.3 ± 0.3^{a}	7.1	2.2 ± 0.1^{a}	17.3	0.0 ± 0.05^{a}	21.4	0.0 ± 0.007^{a}	75	0.1 ± 0.06^{a}	26.0	+	-
23T3	12.7 ± 0.2^{a}	1.9	1.8 ± 0.1^{b}	1.1	0.0 ± 0.04^{a}	0.0	0.0 ± 0.008^{a}	100	0.0 ± 0.05^{a}	10.9	+	-
52T1	12.8 ± 0.4^{a}	2.6	1.8 ± 0.3^{b}	-2.2	0.0 ± 0.04^{a}	0.0	0.0 ± 0.008^{a}	100	0.0 ± 0.05^{a}	8.7	-	-
114T1	12.8 ± 0.5^{a}	2.4	1.9 ± 0.4^{b}	0.5	0.0 ± 0.05^{a}	11.9	0.0 ± 0.006^{b}	50	0.0 ± 0.05^{a}	16.7	+	-
17T3	13.5 ± 0.2^{a}	8.1	2.1 ± 0.2^{a}	14.0	0.0 ± 0.05^{a}	21.4	0.0 ± 0.007^{a}	75	0.1 ± 0.06^{a}	28.3	-	-
113T1	12.9 ± 0.2^{a}	3.3	2.0 ± 0.2^{a}	7.0	0.0 ± 0.05^{a}	14.3	0.0 ± 0.007^{a}	75	0.0 ± 0.05^{a}	19.6	+	-
117T1	12.6 ± 0.3^{a}	1.4	1.8 ± 0.2^{b}	-0.5	0.0 ± 0.04^{a}	0.0	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	2.2	+	-
47T1	13.1 ± 0.3^{a}	5.5	1.9 ± 0.3^{b}	1.6	0.0 ± 0.04^{a}	4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	2.2	-	-
8T2	12.6 ± 0.4^{a}	1.2	1.8 ± 0.3^{b}	0.0	0.0 ± 0.04^{a}	4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	2.2	+	-
46T1	12.1 ± 0.4^{a}	-3.1	1.9 ± 0.2^{b}	0.5	0.0 ± 0.04^{a}	4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	4.3	+	-
6T3	11.7 ± 0.3^{a}	-5.8	1.8 ± 0.2^{b}	-2.2	0.0 ± 0.04^{a}	7.1	0.0 ± 0.005^{b}	25	0.0 ± 0.05^{a}	6.5	-	-
30T2	13.2 ± 0.4^{a}	5.7	2.0 ± 0.3^{a}	5.9	0.0 ± 0.04^{a}	4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	6.5	+	-
80T2	12.8 ± 0.3^{a}	2.7	2.0 ± 0.2^{a}	9.7	0.0 ± 0.05^{a}	14.3	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	13.0	+	-
28T2	12.5 ± 0.3^{a}	0.1	1.8 ± 0.3^{b}	-1.1	0.0 ± 0.04^{a}	4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	4.3	-	-
2T2	12.5 ± 0.3^{a}	0.2	1.9 ± 0.2^{b}	0.5	0.0 ± 0.04^{a}	-2.4	0.0 ± 0.004^{b}	0.0	0.0 ± 0.04^{a}	-2.2	+	-
115T1	13.0 ± 0.2^{a}	4.5	1.9 ± 0.3^{b}	2.2	0.0 ± 0.04^{a}	7.1	0.0 ± 0.004^{b}	0.0	0.0 ± 0.05^{a}	6.5	+	-
12T4	12.7 ± 0.2^{a}	2.3	1.8 ± 0.3^{b}	-3.2	0.0 ± 0.04^{a}	-4.8	0.0 ± 0.004^{b}	0.0	0.0 ± 0.04^{a}	-4.3	-	+
52T2	13.1 ± 0.3^{a}	4.8	1.9 ± 0.2^{b}	0.5	0.0 ± 0.05^{a}	14.3	0.0 ± 0.005^{b}	25	0.0 ± 0.05^{a}	13.0	+	-
CV%	14.52	-	16.55	-	17.25	-	17.82	-	16.32	-		

1 Means followed by the same letter in the column do not differ significantly from each other based on the Scott-Knott test ($p < 0.05$).
* Negative control; ** Positive control.

among soil types (Fisher's p = 0.8602) (Supplementary Material, Table S7).

Biological control

Eleven of the 25 strains did not differ statistically from the controls containing only the pathogen. Other 11 had positive results that were statistically equivalent to the positive control, and three had the most promising results, with AUDPC significantly below the positive control (Figure 2), and percentage of protection between 50 and 59% (Figure 3). There was no difference among the soil types in the frequency of bacterial strains with potential to induce resistance (Fisher's p = 0.5537) (Supplementary Material, Table S8), i.e., the strains from different soils responded in the same way to the resistance induction test, with no influence of the biochar doses on the result.

Molecular characterization

Of the 25 selected strains, 23 16S-rRNA regions were sequenced successfully. The phylogenetic analysis of 25 sequences was based on 1354 characters, including gaps, of which 30 were obtained from GenBank. The analysis was performed using the best-selected evolutionary model (HKY + G). The topology of the tree obtained was derived from the ML analysis plus the posterior probability values in the main branches containing three genera of the *Bacillaceae* family with twenty-one taxa (Figure 4). Of the 23 strains obtained, 20 were characterized as *Bacillus*, 17 belonged to the *Cereus* group, and three were characterized as *Lysinibacillus,* more closely related to *Lysinibacillus sphaericus* and *Lysinibacillus macroides*. Among the strains of the genus *Bacillus* that do not belong to the *B. cereus* group, 46T1 was more closely related to *Bacillus altitudinis*, and 25T4 and 12T4 to *Bacillus megaterium*, all with a high level of statistical support.

Based on the pairwise identity data (Figure 5), strain 114T1 showed > 99% identity with *L. sphaericus* and *L.*

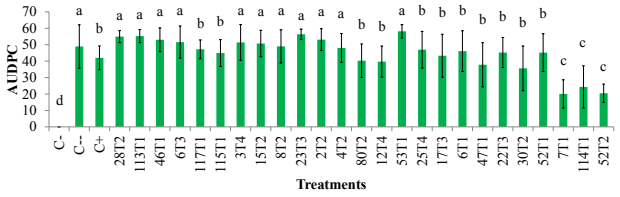

Figure 2. Mean values of the area under the target spot progression curve (AUDPC) by the diagrammatic scale in *Santa Cruz Kada* tomato seedlings, for 25 beneficial bacterial strains and three controls (C- = only water; C-- = no strain + pathogen; C+ = *Bacillus cereus* UFV 121). Columns represent the mean and bars the standard deviation. Same letters above the means indicate non-significant differences by the Skott-Knott test. This figure is in color in the electronic version.

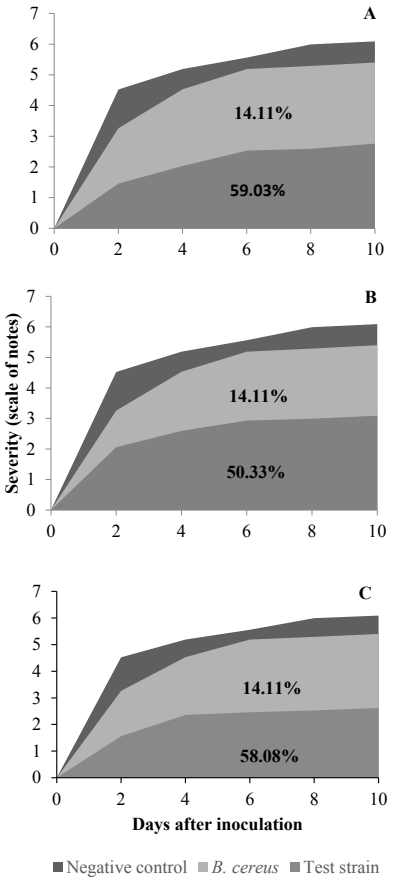

Figure 3. Progress of target spot and protection percentage (values in the boxes) in *Santa Cruz Kada* cv. tomato seedlings for three beneficial bacterial strains collected from biochar-enriched non-rhizospheric soil at the Experimental Station of Tropical Fruticulture - EEF /INPA, municipality of Manaus (Amazonas, Brazil) A – strain 7T1; B – strain 114T1; C – strain 52T2.

macroides. Strains with identity > 99% belonging to the *Bacillus* clade were grouped in the Cereus group. Strains 8T2 and 3T4 were more closely related to *Bacillus thuringiensis*, and 6T3 and 30T2 to *B. cereus*. Five strains shared a 98% identity, and thirteen shared a < 97% identity (Supplementary Material, Table S6).

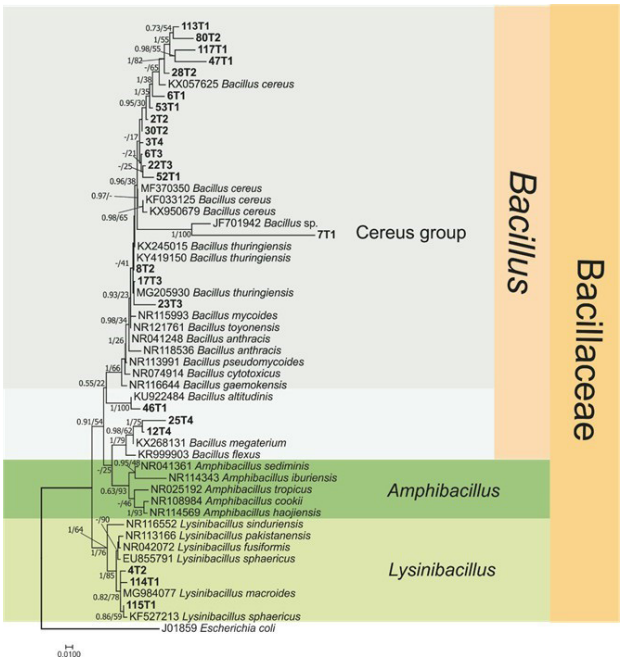

Figure 4. Phylogram resulting from a consensus of maximum likelihood and Bayesian analysis based in 16S rRNA region alignment, with the values of bootstrap and posteriori probability (ML/PP) for 25 beneficial bacterial strains obtained in this study from biochar-enriched non-rhizospheric soil collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, municipality of Manaus (Amazonas, Brazil). Test strains are marked in bold. The tree is rooted with *Escherichia coli* strain J01859. This figure is in color in the electronic version.

The dendrogram generated from ERIC-PCR had low similarity coefficients in some of the formed groups. Four main groups were formed, with their respective subgroups. Group I was subdivided into three subgroups. Strain 46T1 (subgroup Ia), which was identified as *Bacillus* sp. (*pumilus* group), did not group directly with the other strains of group I, corroborating the identification by sequencing of the 16S rRNA gene. Subgroups Ib, Ic and Id were composed exclusively of strains from group *B. cereus*. It is noteworthy that strains 17T3 and 22T3 showed the formation of only one band and two bands, respectively, which did not allow their correct grouping in the dendrogram, and provided low coefficients of similarity that do not corroborate the information obtained by sequencing the 16S rRNA gene (Figure 6).

Two strains, 3T4 and 4T2, were allocated into group II and identified as *Bacillus* sp. group *B. cereus* and *Lysinibacillus* sp., respectively. This group differed most from the others, as they grouped in the same clade and belonged to different genera, which differs from the information obtained from the 16S rRNA gene sequencing (Figure 6).

Group III was divided into two subgroups, both containing only *Bacillus* strains. In subgroup IIIa, only strains belonging to the group *B. cereus* were grouped, with two groupings (53T1-80T2 and 15T2-6T3) with 100% similarity

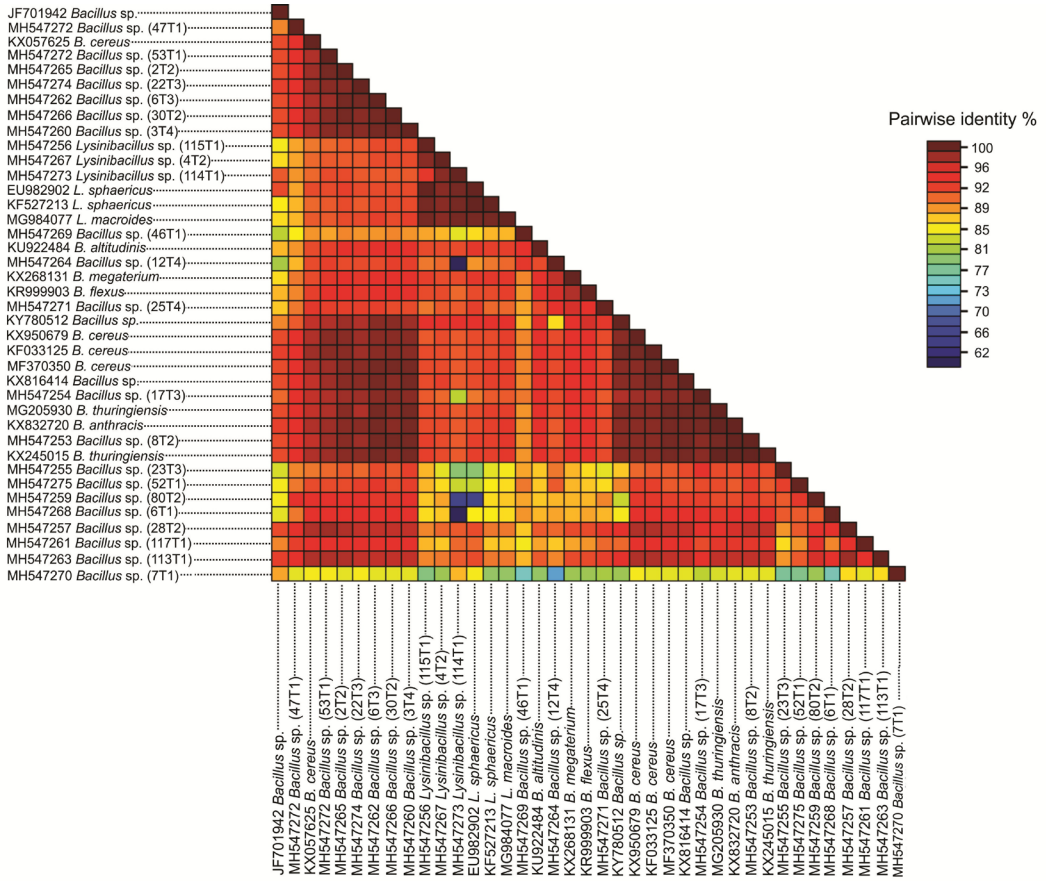

Figure 5. Colour-coded pairwise identity matrix generated from 39 sequences of the 16S rRNA region of 25 beneficial bacterial strains obtained from biochar-enriched non-rhizospheric soil collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, municipality of Manaus (Amazonas, Brazil). Each colored cell represents an identity percentage score between two sequences indicated horizontally to the left and vertically at the bottom. A colored key indicates the correspondence between pairwise identities and the colors displayed in the matrix. The strains obtained in this study are identified in parentheses. This figure is in color in the electronic version.

among the strains. Strains 25T4 and 12T4, both identified as *Bacillus* spp. (*megaterium* group), also grouped (70% similarity) into subgroup IIIb, as observed in the phylogenetic analysis (Figure 6).

Group IV presented two subdivisions (IVa and IVb). Subgroup IVa only included strains of group *B. cereus*. Subgroup IVb formed two sub-subgroups (IVb.1 and IVb.2). Only strain 7T1 (group *B. cereus*) was allocated to sub-subgroup IVb.1, and strains 115T1 and 114T1, both identified as *Lysinibacillus* sp.with 85% similarity coefficient, were allocated to sub-subgroup IVb.2, corroborating the phylogeny based on 16S rRNA sequencing (Figure 6).

DISCUSSION

Our results suggest that biochar enhancement in higher doses reduces the population of cultivable bacteria in non-rhizospheric soil and intere negatively with the survival of bacterial groups capable of actively colonizing the rhizosphere of tomato seedlings, while having beneficial effects on bacteria of the *B. megaterium* group capable of solubilizing phosphate. However, no selective effect of biochar dose was observed

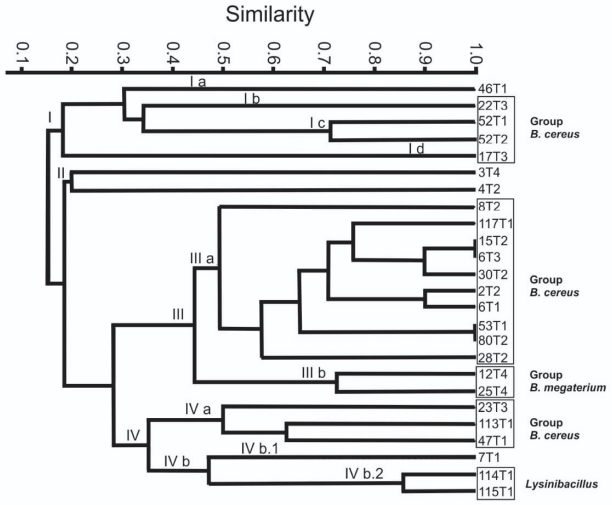

Figure 6. Dendrogram generated by the UPGMA cluster, based on the Jaccard coefficient of similarity, from the analysis of polymorphic bands generated by Eric-PCR of 25 beneficial bacterial strains obtained from biochar-enriched non-rhizospheric soil collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, municipality of Manaus (Amazonas, Brazil).

on rhizobacteria capable of producing indole acetic acid or promoting growth or inducing resistance against *C. cassiicola* in tomato seedlings.

Despite the negative effects of higher doses of biochar on bacterial populations in general, and on bacteria capable of root colonization in particular, observed in this study, there are a few studies reporting the influence of biochar on specific groups of rhizobacteria that are capable of forming effective plant-rhizobacterial associations that provide plant growth (e.g. Egamberdieva *et al*. 2016; Nadeem *et al*. 2017; Ren *et al*. 2020). Therefore, more studies are necessary, testing a wider range of strains and experimental designs with more independent replicates for each treatment level, in order to further evaluate the effects of biochar enhancement on growth-promoting bacteria for tomato seedlings.

Biochar amendments increase the population densities of soil bacteria and actinomycetes, modify soil fungi/bacteria and fungi/actinomycetes ratios and increase soil microbial activity (Lu *et al.* 2016). However, higher rates may not be beneficial and can even become detrimental (Zwart and Kim 2012), which seems to have occurred in the soil analyzed here, as our results pointed to an optimal dosage for bacterial development below that of the lowest experimental biochar concentration. The dosage and type of biochar added to the soil can influence the morphology and topography of biofilms, as the binding force, or even biochar colonization, depends on the type of dominant molecule readily available on the surface of the biochar (e.g., phenolic components, silica and metal oxides). These act as chemical signals that induce cell lysis and biofilm formation, and promote interaction pathways that condition the adaptation and survival of bacterial species (Bueno *et al*. 2018). For example, the growth rate of *Bacillus subtilis* SL-13 in NB medium increased with the addition of biochar, because it contains nutrients for the growth of these bacteria and the special porous structure of the biochar has a positive effect on the adsorption of bacteria, being a potentially suitable carrier of PGPR for agriculture (Tao *et al*. 2018).

Molecular analysis showed that the three most promising strains for biological control are species of *Bacillus* (Cereus group) and *Lysinibacillus*, and those that showed the best results for growth promotion belong to *Bacillus*. A study in greenhouse conditions demonstrated that maize plants inoculated with *B. subtilis* and *Lysinibacillus fusiformis* in biochar-enhanced substrate presented better growth and nutrient concentration than biochar and bacterial treatments alone (Rafique *et al.* 2017). These improvements in plant growth were mainly attributed by the authors to phosphate-solubilization by the bacterial strains in the soil, phosphate from the biochar and IAA, cytokinin and gerbilline production.

Bacillus thuringiensis inhibited the growth of the *C. cassiicola* in-vitro and *in-planta* (Giau and Quoc 2017). The production of antimicrobial lipopeptides synthesized in a nonribosomal mode is one of the possible means for *Bacillus* strains to use their antimicrobial action (Almoneafy *et al.* 2014). The fresh and dry mass of tomato plants were also enhanced by *Bacillus* strains (Almoneafy*et al*. 2014). *Bacillus* sp., *B. amiloliquefaciens, B. pumilus* and *B. subtilis* significantly increased the length, pseudostem diameter, fresh mass and dry mass in *Prata Anã* banana seedlings (Souza *et al.* 2017). Plant growth was also increased when a *L. sphaericus* strain was inoculated on *Trigonella feonum-graecum* (methi) and *Vigna radiata* (mung beans) seeds (Sharma and Saharan 2015). Genes coding for secondary metabolites, such as bacillibactin, bacilysin, microcin, bacillaene, difficidin, fengycin, macrolactin and surfactin, were found in bacterial strains that promoted plant growth and controlled multiple diseases (Liu *et al*. 2017), showing that mechanisms of growth promotion and plant protection may be genetically mediated.

The distribution based on pairwise identity corroborated the data obtained by phylogenetic analyses (ML and BI) for strain identification. However, for the *Lysinibacillus* clade, the relationship between *L. sphaericus* and *L. macroides*, which showed identity > 99%, was not well defined, making identification difficult through the 16S region due to the high homology between these taxa. For the genus *Bacillus*, the 16S region also had a low phylogenetic resolution (Janda *et al*. 2007). The 16S sequencing method has limitations, as in the closely related *Bacillus anthracis*, *B. cereus* and *B. thuringiensis*, which have identical 16S rDNA sequences, making their differentiation difficult using only this barcode (Han 2006) .

Strains that showed the highest identity with *B. cereus* and *B. thuringiensis* responded to the clustering tendency observed both in the pairwise identity analysis by the neighbor component and in the phylogenetic inferences, despite the low support of PP and bootstrap. According to Petti (2007), an identity of < 97% may indicate the existence of a new species.

CONCLUSIONS

The Cereus group responded more than other rhyzobacteria to the dosages of biochar used in this study. Three strains showed potential for promoting growth: 7T1, with the highest GPE for DMAP; 22T3, with the highest GPE for PH, DMR and TDM; and 53T1, with the highest GPE for SD. The 7T1, 114T1 and 52T2 strains showed potential for biological control, presenting the lowest AUCPD. 7T1 stood out for being beneficial for both growth promotion and biological control against *C. cassiicola* and is recommended for future studies. Furthermore, some strains were capable of solubilizing phosphate and producing IAA. Strains that presented high growth promotion efficiency and reduced the severity of target spot in tomato seedlings have the potential for use as additional tools for the integrated management of this disease.

ACKNOWLEDGMENTS

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for the financial assistance.

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RECEIVED: 11/03/2019
ACCEPTED: 25/09/2020
ASSOCIATE EDITOR: Antonio R. Fernandes

SUPPLEMENTARY MATERIAL (only available in the electronic version)

Caniato *et al*. Diversity of bacterial strains in biochar-enhanced Amazon soil and their potential for growth promotion and biological disease control in tomato.

Table S1. Presence (+) or absence (–) of *in vitro* root colonization capacity of 200 bacterial strains from biochar-enriched Amazon soil in tomato seedlings, *Santa Cruz Kada* cultivar.

Strain	Colonization	Strain	Colonization	Strain	Colonization	Strain	Colonization
1T1	-	1T2	+	1T3	-	1T4	-
2T1	-	2T2	+	2T3	-	2T4	-
3T1	+	3T2	+	3T3	-	3T4	+
4T1	+	4T2	+	4T3	-	4T4	+
5T1	-	5T2	-	5T3	-	5T4	-
6T1	+	6T2	+	6T3	+	6T4	-
7T1	+	7T2	-	7T3	-	7T4	-
8T1	+	8T2	+	8T3	+	8T4	-
9T1	-	9T2	+	9T3	-	9T4	-
10T1	+	10T2	-	10T3	-	10T4	-
11T1	-	11T2	-	11T3	-	11T4	-
12T1	+	12T2	-	12T3	-	12T4	+
13T1	-	13T2	-	13T3	+	13T4	-
14T1	+	14T2	+	14T3	-	14T4	+
15T1	-	15T2	+	15T3	+	15T4	+
16T1	-	16T2	-	16T3	-	16T4	-
17T1	-	17T2	+	17T3	+	17T4	-
18T1	-	18T2	+	18T3	-	18T4	-
19T1	-	19T2	-	19T3	-	19T4	-
20T1	-	20T2	-	20T3	-	20T4	-
21T1	-	21T2	-	21T3	-	21T4	-
22T1	+	22T2	-	22T3	+	22T4	-
23T1	-	23T2	-	23T3	+	23T4	+
24T1	-	24T2	-	24T3	-	24T4	-
25T1	-	25T2	-	25T3	-	25T4	+
26T1	-	26T2	-	26T3	-	26T4	-
27T1	+	27T2	+	27T3	-	27T4	-
28T1	+	28T2	+	28T3	-	28T4	-
29T1	-	29T2	+	29T3	-	29T4	-
30T1	-	30T2	+	30T3	-	30T4	-
31T1	-	31T2	+	31T3	-	31T4	-
32T1	-	32T2	-	32T3	-	32T4	-
33T1	-	33T2	-	33T3	-	33T4	-
34T1	+	34T2	+	34T3	+	34T4	-
35T1	-	35T2	-	35T3	-	35T4	+
36T1	-	36T2	-	36T3	-	36T4	-
37T1	-	37T2	-	37T3	-	37T4	-
41T1	+	38T2	+	38T3	-	38T4	-
46T1	+	39T2	+	39T3	-	39T4	-
47T1	+	40T2	+	40T3	-	40T4	-
51T1	+	41T2	+	41T3	-	41T4	-
52T1	+	42T2	-	42T3	-	42T4	-
53T1	+	43T2	-	43T3	-	43T4	-
102T1	+	45T2	+	44T3	-	44T4	-
108T1	+	52T2	+	45T3	-	45T4	-
109T1	+	63T2	+	46T3	-	46T4	-
113T1	+	72T2	+	47T3	-	47T4	-
114T1	+	79T2	+	48T3	-	48T4	-
115T1	+	80T2	+	49T3	-	49T4	-
117T1	+	85T2	+	62T3	+	50T4	-

Table S2. Absolute frequency (N) and relative frequency (%) of positive and negative results of the root colonization test of 200 bacterial strains obtained from non-rhizospheric soil enriched with different doses of biochar collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, in Manaus, Amazonas state (Brazil).

Biochar dose (t ha^{-1})	Growth promotion				Total
	Positive		Negative		
	N	%	N	%	
0	25	50	25	50	50 (25%)
40	28	56	22	44	50 (25%)
80	9	18	41	82	50 (25%)
120	8	16	42	84	50 (25%)
Total	70	35	130	65	200 (100%)

Table S3. Average plant height (PH), stem diameter (SD), number of leaves (NL) and dry mass of the aerial part (DMAP) of tomato seedlings 20 days after sowing on Vivatto Plus® substrate and inoculated with each of 53 bacterial strains from Amazon biochar-enriched soil. Values are the mean ± SD of five replicates. Ctrl- = negative control. Different upper-case letters in the same columns indicate statistically significant differences at $p < 0.05$. [1]Scott-Knott test; [2]Dunn test. CV% = coefficient of variance.

Strain	PH[1] (cm)	NL[2]	DMAP (g)[1]	SD (mm)[1]
Ctrl-*	11.6 ± 0.4^{b}	2.0 ± 0.7^{d}	0.0 ± 0.0^{b}	2.0 ± 0.2^{b}
6T3	14.9 ± 0.3^{a}	3.0 ± 0.6^{abcd}	0.1 ± 0.0^{a}	2.5 ± 0.2^{a}
6T2	10.4 ± 0.6^{c}	2.7 ± 0.6^{cd}	0.0 ± 0.0^{b}	1.9 ± 0.3^{b}
62T3	11.8 ± 0.3^{b}	2.7 ± 0.6^{bcd}	0.1 ± 0.0^{b}	2.1 ± 0.3^{b}
72T2	11.5 ± 0.4^{b}	2.3 ± 0.7^{cd}	0.1 ± 0.0^{b}	2.0 ± 0.3^{b}
34T1	11.4 ± 0.7^{b}	3.3 ± 0.4^{abc}	0.1 ± 0.0^{b}	1.9 ± 0.2^{b}
109T1	10.8 ± 0.3^{b}	2.3 ± 0.4^{cd}	0.0 ± 0.0^{b}	1.9 ± 0.2^{b}
117T1	13.1 ± 0.3^{a}	2.7 ± 0.5^{cd}	0.1 ± 0.0^{a}	2.0 ± 0.2^{b}
3T4	13.8 ± 0.2^{a}	3.7 ± 0.5^{abc}	0.1 ± 0.0^{b}	2.1 ± 0.2^{b}
28T1	11.7 ± 0.2^{b}	3.3 ± 0.4^{abcd}	0.1 ± 0.0^{b}	2.1 ± 0.2^{b}
30T2	14.5 ± 0.5^{a}	4.7 ± 0.5^{a}	0.1 ± 0.0^{a}	2.5 ± 0.2^{a}
47T1	14.0 ± 0.5^{a}	3.0 ± 0.4^{abcd}	0.1 ± 0.0^{a}	2.4 ± 0.3^{a}
80T2	14.0 ± 0.5^{a}	3.7 ± 0.4^{abc}	0.1 ± 0.0^{a}	2.5 ± 0.3^{a}
10T1	11.8 ± 0.4^{b}	2.7 ± 0.5^{bcd}	0.1 ± 0.0^{b}	1.9 ± 0.3^{b}
28T2	14.1 ± 0.3^{a}	3.0 ± 0.4^{abcd}	0.1 ± 0.0^{a}	2.4 ± 0.3^{a}
46T1	14.3 ± 0.6^{a}	3.3 ± 0.4^{abc}	0.1 ± 0.0^{a}	2.3 ± 0.2^{a}
1T2	10.3 ± 0.2^{c}	2.0 ± 0.5^{d}	0.0 ± 0.0^{b}	1.6 ± 0.3^{b}
4T2	13.1 ± 0.3^{a}	2.0 ± 0.6^{d}	0.1 ± 0.0^{a}	2.2 ± 0.3^{b}
4T4	10.2 ± 0.3^{c}	2.0 ± 0.5^{d}	0.1 ± 0.0^{b}	1.7 ± 0.2^{b}
8T1	10.1 ± 0.3^{c}	3.0 ± 0.4^{abcd}	0.0 ± 0.0^{b}	1.9 ± 0.2^{b}
85T2	11.6 ± 0.2^{b}	3.0 ± 0.5^{abcd}	0.1 ± 0.0^{b}	2.1 ± 0.2^{b}
2T2	13.6 ± 0.4^{a}	3.0 ± 0.4^{abcd}	0.1 ± 0.0^{a}	2.6 ± 0.2^{a}
41T1	11.6 ± 0.5^{b}	2.3 ± 0.4^{cd}	0.1 ± 0.0^{b}	1.9 ± 0.3^{b}
79T2	11.4 ± 0.2^{b}	2.3 ± 0.7^{cd}	0.1 ± 0.0^{b}	1.9 ± 0.3^{b}
8T2	13.6 ± 0.3^{a}	3.7 ± 0.4^{abc}	0.1 ± 0.0^{a}	2.2 ± 0.2^{a}
115T1	14.6 ± 0.3^{a}	3.7 ± 0.5^{abc}	0.1 ± 0.0^{a}	2.3 ± 0.2^{a}
12T4	14.7 ± 0.4^{a}	3.3 ± 0.4^{abc}	0.1 ± 0.0^{a}	2.6 ± 0.2^{a}
8T3	12.1 ± 0.4^{b}	3.0 ± 0.5^{abcd}	0.1 ± 0.0^{b}	1.9 ± 0.2^{b}
12T1	11.3 ± 0.4^{b}	2.3 ± 0.7^{cd}	0.0 ± 0.0^{b}	1.9 ± 0.2^{b}
6T1	12.9 ± 0.4^{a}	3.0 ± 0.4^{abcd}	0.1 ± 0.0^{a}	2.1 ± 0.2^{b}
15T4	10.3 ± 0.5^{c}	2.0 ± 0.6^{d}	0.0 ± 0.0^{b}	1.7 ± 0.3^{b}
15T2	13.0 ± 0.3^{a}	3.0 ± 0.4^{abcd}	0.1 ± 0.0^{a}	1.6 ± 0.2^{b}
15T3	7.7 ± 0.4^{d}	2.3 ± 0.6^{cd}	0.0 ± 0.0^{b}	1.1 ± 0.3^{b}
13T3	11.5 ± 0.2^{b}	2.0 ± 0.5^{d}	0.1 ± 0.0^{b}	2.0 ± 0.2^{b}
7T1	12.9 ± 0.4^{a}	3.3 ± 0.5^{abc}	0.1 ± 0.0^{a}	2.4 ± 0.3^{a}
52T2	13.2 ± 0.3^{a}	2.7 ± 0.7^{bcd}	0.1 ± 0.0^{a}	2.5 ± 0.2^{a}
35T4	13.0 ± 0.4^{a}	2.0 ± 0.5^{d}	0.1 ± 0.0^{b}	1.7 ± 0.2^{b}
25T4	13.2 ± 0.3^{a}	2.3 ± 0.7^{cd}	0.1 ± 0.0^{a}	1.9 ± 0.3^{b}
22T3	15.7 ± 0.2^{a}	3.7 ± 0.5^{abc}	0.1 ± 0.0^{a}	2.7 ± 0.2^{a}
53T1	15.1 ± 0.4^{a}	2.0 ± 0.7^{d}	0.1 ± 0.0^{a}	2.1 ± 0.2^{b}
23T3	13.9 ± 0.3^{a}	2.3 ± 0.4^{cd}	0.1 ± 0.0^{a}	2.4 ± 0.2a
52T1	13.6 ± 0.3^{a}	4.3 ± 0.5^{ab}	0.1 ± 0.0^{a}	2.4 ± 0.3a
22T1	8.6 ± 0.4^{d}	2.3 ± 0.5^{cd}	0.0 ± 0.0^{b}	1.5 ± 0.3^{b}
102T1	11.5 ± 0.6^{b}	2.7 ± 0.4^{cd}	0.1 ± 0.0^{b}	2.1 ± 0.3b
14T1	10.9 ± 0.3^{b}	2.0 ± 0.5^{d}	0.1 ± 0.0^{b}	1.7 ± 0.2b
27T1	11.8 ± 0.5^{b}	2.0 ± 0.4^{d}	0.1 ± 0.0^{b}	2.1 ± 0.3b
108T1	11.0 ± 0.3^{b}	3.0 ± 0.5^{abcd}	0.1 ± 0.0^{b}	2.4 ± 0.3a
114T1	13.9 ± 0.4^{a}	3.0 ± 0.6^{abcd}	0.1 ± 0.0^{a}	2.4 ± 0.2a
17T3	14.5 ± 0.3^{a}	2.3 ± 0.4^{cd}	0.1 ± 0.0^{a}	2.7 ± 0.2a
17T2	11.0 ± 0.3^{b}	2.0 ± 0.6^{d}	0.1 ± 0.0^{b}	1.9 ± 0.2b
51T1	11.4 ± 0.3^{b}	3.3 ± 0.5^{abc}	0.1 ± 0.0^{b}	2.0 ± 0.3b
39T2	10.1 ± 0.4^{c}	2.3 ± 0.7^{cd}	0.0 ± 0.0^{b}	1.9 ± 0.2b
63T2	11.5 ± 0.4^{b}	2.7 ± 0.7^{cd}	0.1 ± 0.0^{b}	2.1 ± 0.2b
113T1	12.6 ± 0.4^{a}	3.0 ± 0.5^{abcd}	0.1 ± 0.0^{a}	2.4 ± 0.2a
CV%	**8.90**	**-**	**14.54**	**16.17**

Table S4. Absolute frequency (N) and relative frequency (%) of positive and negative results of the growth promotion test of 25 bacterial strains obtained from non-rhizospheric soil enriched with different doses of biochar collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, in Manaus, Amazonas state (Brazil).

Biochar dose (t ha^{-1})	Growth promotion				Total
	Positive		Negative		
	N	%	N	%	
0	4	40.0	6	60.0	10 (40%)
40	4	50.0	4	50.0	8 (32%)
80	3	62.5	1	37.5	4 (16%)
120	2	66.7	1	33.3	3 (12%)
Total	13	52.0	12	48.0	25 (100%)

Table S5. Absolute frequency (N) and relative frequency (%) of positive and negative results of the phosphate solubilization test of 25 bacterial strains obtained from non-rhizospheric soil enriched with different doses of biochar collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, in Manaus, Amazonas state (Brazil).

Biochar dose (t ha^{-1})	Phosphate solubilization				Total
	Positive		Negative		
	N	%	N	%	
0	0	0	10	100	10 (40%)
40	0	0	8	100	8 (32%)
80	0	0	4	100	4 (16%)
120	2	66.7	1	33.3	3 (12%)
Total	2	8.0	23	92.0	25 (100%)

Table S6. Identity between the bacterial-strain sequences obtained from a central Amazon soil sample enriched with biochar and sequences deposited in GenBank.

Strain	GenBank code	Size (bp)	ID Blast %	SDT ID score%*	GenBank code/Related species
8T2	MH547253	1354	99	99.9	KX245015/*Bacillus thuringiensis*
17T3	MH547254	1256	99	91.9	MG205930/*Bacillus thuringiensis*
23T3	MH547255	1293	99	95.9	MG205930/*Bacillus thuringiensis*
115T1	MH547256	1265	99	92.9	KF527213/*Lysinibacillus sphaericus*
28T2	MH547257	1354	98	98.5	KX057625/*Bacillus cereus*
53T1	MH547258	1354	98	98.6	KX950679/ *Bacillus cereus*
80T2	MH547259	908	95	64.0	KX057625/ *Bacillus cereus*
117T1	MH547261	1354	94	94.3	KX057625/ *Bacillus cereus*
3T4	MH547260	1355	99	99.3	KX245015 /*Bacillus thuringiensis*
6T3	MH547262	1356	99	99.4	MF370350/ *Bacillus cereus*
113T1	MH547263	1311	98	96.9	KX057625/ *Bacillus cereus*
2T2	MH547265	1354	99	98.8	KF033125/ *Bacillus cereus*
30T2	MH547266	1353	99	99.1	MF370350/ *Bacillus cereus*
12T4	MH547264	815	98	58.3	KR999903/ *Bacillus flexus*
4T2	MH547267	1358	99	91.7	MG984077/*Lysinibacillus macroides*
6T1	MH547268	815	94	91.4	KY780512/*Bacillus cereus*
7T1	MH547270	1354	88	87.7	JF701942/*Bacillus* sp.
46T1	MH547269	1315	96	97.4	KU922484/*Bacillus altitudinis*
25T4	MH547271	1355	96	96.1	KX268131/*Bacillus megaterium*
47T1	MH547272	1363	95	95.3	KX057625/*Bacillus cereus*
114T1	MH547273	768	99	99.5	EU982902/*Lysinibacillus sphaericus*
22 T3	MH547274	1358	98	98.8	KX816414/*Bacillus cereus*
52T1	MH547275	1307	95	96.7	KF033125/*Bacillus cereus*

* Based on the Sequence Demarcation Tool (SDT v.1.2)

Table S7. Absolute frequency (N) and relative frequency (%) of positive and negative results of the indole acetic acid production test of 25 bacterial strains obtained from non-rhizospheric soil enriched with different doses of biochar collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, in Manaus, Amazonas state (Brazil).

Biochar dose (t ha^{-1})	IAA production				Total
	Positive		Negative		
	N	%	N	%	
0	7	70.0	3	30.0	10 (40%)
40	6	75.0	2	25.0	8 (32%)
80	2	50.0	2	50.0	4 (16%)
120	2	66.7	1	33.3	3 (12%)
Total	17	68.0	8	32.0	25 (100%)

Table S8. Absolute frequency (N) and relative frequency (%) of positive and negative results of the resistance induction test of 25 bacterial strains obtained from non-rhizospheric soil enriched with different doses of biochar collected at the Experimental Station of Tropical Fruticulture - EEFT/INPA, in Manaus, Amazonas state (Brazil).

Biochar dose (t ha^{-1})	Resistance induction				Total
	Positive		Negative		
	N	%	N	%	
0	7	70.0	3	30.0	10 (40%)
40	3	37.5	5	62.5	8 (32%)
80	2	50.0	2	50.0	4 (16%)
120	2	0	1	100	3 (12%)
Total	14	56.0	11	44.0	25 (100%)