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Isolation, Characterization and Selection of Bacteria that Promote Plant Growth in Grapevines (*Vitis* sp.)

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Abstract

Certain bacteria can promote and stimulate plant growth, increasing the production of biomass and reducing damage caused by phytopathogens. With that in mind, this research effort set out to select these plant growth-promoting bacteria in order to evaluate their effects on the growth of grapevines (*Vitis* sp.). The bacteria were isolated from several vineyard soil samples, and evaluated based on their production of IAA (Indole-3-Acetic Acid), siderophores and cellulase, as well as their phosphate solubilization and nitrogen fixation capabilities. *In vivo* testing included six separate treatments with the following bacterial isolates: C12, O7, B3, I3, a control group and a blended group. The tests were performed in a greenhouse with bacterial suspension inoculation placed around the roots of Paulsen 1103 rootstock cuttings. The data collected included the following: number of leaves per plant, branch lengths, chlorophyll content, fresh and dry mass, and Carbon, Hydrogen, Nitrogen and Sulfur concentrations. Forty-six separate bacteria were isolated, of which 100% produced IAA, 65.21% produced siderophores, 63.04% solubilized phosphate, 34.78% produced cellulase, and 30.43% showed nitrogen fixation. The *in vivo* testing also revealed significant increases in the length of the branch and in percentages of Carbon and Nitrogen. The C12 isolate exhibited the highest increase in branch length (76.704 cm), whereas the O7 and C12 were identified as *Bacillus amyloliquefaciens* and *Bacillus thuringiensis*, respectively.

Keywords: bacteria, bioprospection, plant growth-promotion, viticulture

1. Introduction

Viticulture is an activity of great importance for Brazil's economy, especially for its leading producers, the states of Rio Grande do Sul, Pernambuco, São Paulo, Paraná, Bahia and Santa Catarina. According to IBGE (Portuguese initials for Brazilian Institute for Geography and Statistics), in 2015, Brazil produced 1,025,536 tons of grape, representing an increase of 6.74% over the previous year. The southern region states, specifically Rio Grande do Sul, Paraná, and Santa Catarina, were responsible for 90% of that production, the bulk of which was used to produce wine and grape juice. The state of Santa Catarina has lead the cultivation of grapes for the production of wines and sparkling wines. In 2015, this state's production levels reached 69,250 tons (IBGE, 2015), representing an increase of 2.86% over 2014. Currently, production levels of 54,262 tons make the mid-west region of Santa Catarina, known as Vale do Rio do Peixe, the leading producer of grapes, with Isabella, Niagara and Bordeaux being the main varieties cultivated (Desplobins & Silva, 2005). Within its cultivation cycle, several factors, whether abiotic (Tecchio, Teixeira, Terra, Moura, & Paioli-Pires, 2011; Brunetto et al., 2008) or biotic (Garrido, Sonêgo, & Gomes, 2004), can impact or jeopardize the quality and output of the crop. Bacteria that establish symbiotic relationships with the plants play a critical role in maintaining and/or increasing plant growth rates, and can be used to promote plant growth, significantly improving crop output. As the name implies, these plant growth-promoting bacteria (PGPB) can stimulate plant growth, increasing stem and root development, as well as the production of biomass, while, at the same time, reducing damages caused by phytopathogens (Gupta, Parihar, Ahirwar, Snehi, & Singh, 2015; Ahemad & Kibret, 2014; Lugtenberg & Kamilova, 2009; Van Loon & Bakker, 2005). Direct growth-promoting mechanisms are those that affect the plant's natural balance of growth regulators, improving its nutritional proficiencies and stimulating the processes that fight systemic diseases (for example, biological nitrogen fixation, phytohormones production, synthetization

of enzymes, inorganic phosphate solubilization, and phosphate mineralization). Indirect growth regulator mechanisms, on the other hand, are the ones that reduce or inhibit the activities of pathogenic microorganisms through biocontrol, which includes the production of antibiotics and iron chelating agents (siderophores), and the synthetization of exoenzymes, such as cellulases and chitinases (Carvalhais et al., 2013; F. Ahmad, I. Ahmad, & Khan, 2008; Zahir, Asghar, Akhtar, & Arshad, 2005; Asghar, Zahir, Arshad, & Khaliq, 2002). Although several studies have reported the potential of different microorganisms to promote growth in plants such as wheat, soybeans, and potatoes, these are in short supply for the cultivation of grapevines, both in terms of the isolation of the microorganisms and when it comes to *in vitro* and *in vivo* testing (Dawwam, Elbeltagia, Emara, Abbas, & Hassan, 2013; Karagöz, Ates, Karagöz, Kotan, & Cakmakci, 2012; Smyth et al., 2011; Khalid, Arshad, & Zahir, 2004). In the last couple of decades, research efforts related to the development of biological consumables, such as inoculating agents, have mustered a lot of attention from researchers. Considering the importance of microorganisms and the attention focused on the search for alternatives that promote plant growth, the purpose of this research paper is to bioprospect plant growth-promoting bacteria by evaluating their physiological and enzymatic activities, and focusing on their application to grapevine cultivation.

2. Method

The isolation and characterization of the bacteria being studied were performed at UNOESC's (Universidade do Oeste de Santa Catarina, City of Videira Campus) Microbiology Laboratory, and the greenhouse experiment was carried out at EPAGRI (Portuguese acronym for State of Santa Catarina Agricultural, Livestock, and Rural Extension Research Company), also in the City of Videira.

2.1 Isolation of the Bacteria

The bacteria were isolated from several soil samples collected from grapevine growing properties located in the mid-west region of the state of Santa Catarina. The samples were homogenized and sieved to remove any coarser materials, and then re-suspended in 90 mL sterile peptone water. The suspended samples were then incubated for 30 minutes in a shaker unit, at room temperature. Decimal serial dilutions were performed following the homogenization. A 100 µL aliquot portion of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were seeded, using a Drigalsky agar nutrient medium. The plates were incubated for 24-72 hours, at a temperature of 30 °C. After this incubation period, the bacteria colonies were purified, preserved, and then used in the experiment.

2.2 Evaluation of Plant Growth-Promothing Agents

2.2.1 Production of Indole-3-Acetic Acid (IAA)

The isolates were cultivated in a King B medium, supplemented with L-tryptophan (5 $mM \cdot mL^{-1}$), and incubated for 48 hours, at a temperature of 30 ºC, as per methodology stipulated by Bric, Bostock, and Silverstone (1999), and adapted by Cattelan (1999). A 2 mL aliquot portion of the culture was centrifuged at 2,000 rpm, for 10 minutes. Subsequently, 1 mL of supernatant was transferred to a new test tube containing 1 mL of Salkowski solution (1.5 mL of 0.5 M of $FeCl_3 \cdot 6H_2O$ in 80 mL of 60% H_2SO_4), and left at room temperature, protected from light. After 30 minutes, a spectrophotometer reading was taken at 540 nm. The IAA concentration was determined based on an IAA standard curve (0, 1, 5, 10, 20, 40, 80 and 160 $mg \cdot mL^{-1}$).

2.2.2 Production of Siderophores

An evaluation of the bacteria's siderophore production was performed in a chrome azurol S (CAS) reagent enriched King B medium (King et al., 1954). The isolates were cultured for 24 hours in that medium, under constant agitation and at a temperature of 30 °C. A 100 µL aliquot portion of that culture was transferred to plates of the same medium, which were then incubated for five to seven days, at a temperature of 30 °C. The formation of orange colored halos around the colony was proof positive of siderophore production. The rate of siderophore production was calculated based on the relationship between the total halo diameter (THD) and the colony halo diameter (CHD) (or THD/CHD, in millimeters).

2.2.3 Solubilization of Phosphate

The isolates' ability for phosphate solubilization was qualitatively evaluated according to Nautiyal et al. (1999). Using the pin prick method, the bacteria were inoculated in a culture medium containing tricalcium phosphate (10 g of glucose; 5 g of $Ca_5(OH)(PO_4)_3$; 5 g of $MgCl_2$ $6H_2O$; 0.25 g of $MgSO_4$ $7H_2O$; 0.2 g of KCl; 0.1 g of $(NH_4)_2SO_4$; 1.5% agar and pH 7.0). The plates were then incubated for seven days, at a temperature of 30 °C. Only the isolates showing clear halos around the colonies were considered to be proof positive for the solubilization of phosphate.

2.2.4 Asymbiotic Nitrogen Fixation

For this test, the bacteria were cultured in test tubes containing a nitrogen-free medium (NFM), which were kept in an incubator for 10 days, at a temperature of 30 °C. At the end of that period, the isolates were transferred to different test tubes, containing the same type of medium, and were incubated under the exact same conditions, for the same period of time. The same procedure was repeated yet a third time. Therefore, after 30 days in a NFM, an aliquot portion of 20 µL of each culture was transferred to a nutrient agar medium so as to confirm their viability. The bacteria's ability to asymbiotically fix nitrogen was assessed in accordance with a methodology proposed by Rennie (1981), and adapted by Cattelan (1999). Isolates that actually showed growth were considered to be proof positive of nitrogen fixation.

2.2.5 Production of Cellulase

For this phase, the bacteria were cultured in a mineral medium, to which a 5% carboxymethyl cellulose solution was added. After the inoculation, using the pin prick method, the plates were kept in an incubator at a temperature of 30 ºC, for five days, at which time the plates were stained with Lugol's iodine solution. The formation of a colorless halo around the colony was proof positive of cellulase production.

2.3 Greenhouse Trial Design

Rooted and sprouted Paulsen 1103 grapevine rootstock cuttings were selected as the standard. The cuttings were washed in running water and transplanted to plastic bags containing an inert coco coir and vermiculite substrate (in a 3-to-1-proportion), and autoclaved for 20 minutes, at a temperature of 121 °C. These were kept in a climate controlled greenhouse for a period of 30 days at a maximum temperature of 25 °C, relative humidity of 60%, and a 12-hour photoperiod. The trial design consisted of repetition of six randomized blocks with five plants each. Treatments were also random within the blocks, as follows: T1 = control with no added bacteria; T2 = isolate C12; T3 = isolate B3; T4 = isolate I3; T5 = isolate O7, and T6 = isolates C12, B3, I3 and O7, together. All treatments consisted of inoculations using a 1.8×10^8 CFU/mL bacterial suspension. All selected bacteria were cultured in a brain-heart infusion (BHI) medium for 12-16 hours, under constant agitation of 125 r.p.m. and a temperature of 30 °C. A 20 mL bacterial suspension was added to each treatment. Then, for 60 days, all plants were kept in a controlled incubator with relative humidity of 80%, a maximum temperature of 25 °C, a 16-hour photoperiod, and a weekly wet down with Hoagland's nutrient solution (Hoagland & Arnon, 1950).

2.3.1 Characteristics Evaluations

The promotion of growth in grapevines (*Vitis* sp.) was evaluated using the following parameters: foliar chlorophyll content index, length of branches and number of leaves. To measure the chlorophyll content, a portable chlorophyll measuring device with diodes that emit light at 650 nm (red) and at 940 nm (infrared) was used; this because light at 650 nm is very close to the two main wavelengths associated with chlorophyll activity (645 nm and 663 nm). The length of the branches and number of leaves were measured and counted at regular intervals, (on the 1st day, 15th day, 30th day, 45th day and 60th day). On the 60th day, the plants were collected and separated into their three component parts (aerial, stem and roots). The roots were carefully manipulated in order to remove the coco coir/vermiculite substrate. After weighing them, the parts were packaged in previously dried paper bags, and stored in a plant incubator/dryer, at a temperature of 60 °C, where they were monitored on a daily basis until their weight stabilized. All samples were then macerated and sent to EPAGRI, in the City of Caçador, SC, for analysis, in order to determine their percent composition in terms of Carbon, Hydrogen, Nitrogen and Sulfur.

2.4 Identification of the Bacteria

Two of the bacteria were identified at the molecular level: the isolate, which yielded positive results in all in vitro tests; and the isolate, which showed the highest potential for promoting plant growth in the in vivo tests.

2.4.1 Molecular DNA Extraction

The bacteria were cultured in a BHI medium for 12-14 hours, at a temperature of 30 °C; their DNA was then extracted using the PureLink Quick Gel Extraction Kit, and quantified in keeping with Sambrook and Russel (2001).

2.4.2 Polymerase Chain Reaction (PCR)

In order to partially amplify the *rpoB* gene, an oligonucleotide primer pair was used, specifically AAR YTI GGM CCT GAA GAA AT and TGI ART TTR TCA TCA ACC ATG TG (Drancourt, Roux, Fournier, & Raoult, 2004). Amplification conditions matched those used by the forenamed authors. After amplification, the resulting material was purified and sent for sequencing at the Microbiology Department of the Universidade Federal do

Rio Grande do Sul (Federal University of the State of Rio Grande do Sul). The sequences were aligned, using ChromasPro 1.5, and then compared to reference species nucleotide sequences found in EMBL/GenBank's database, using NCBI's BLAST.

2.5 Statistical Analysis

Following the greenhouse trials, the collected data was submitted for analysis of variance (ANOVA), and their group means compared using the Scott Knott test ($p < 0.05$).

3. Results

From the seven soil samples that were processed, 46 bacteria were isolated, of which 28 (60.87%) were Gram-positive and 18 (39.13%) were Gram-negative. Table 1 below shows the profile for the 46 bacterial isolates based on the five enzymatic and physiological tests related to growth promotion of plants.

Table 1. Profiles of bacterial isolates based on the enzymatic and physiological *in vitro* tests related to plant growth-promotion

Isolate	Gram	IAA	Sid.	Phos.	Nit.	Cel.	Isolate	Gram	IAA	Sid.	Phos.	Nit.	Cel.
A10	-	+	-	-	+	-	I2	-	+	+	+	-	+
A11	+	+	-	-	+	+	I3*	-	+	+	+	-	-
A12	+	+	+	-	-	-	I4	+	+	+	-	-	-
A14	+	+	+	+	-	-	I5	+	+	-	-	+	-
A15	+	+	+	+	-	+	J1	+	+	+	+	+	+
A2	+	+	+	+	-	+	J2	+	+	-	-	-	-
A3	+	+	+	+	-	+	J3	+	+	+	+	-	+
A4	+	+	-	-	-	+	J4	-	+	+	+	-	-
A5	-	+	+	-	-	+	J6	+	+	+	+	+	-
A6	+	+	+	+	+	+	J7	+	+	-	+	-	-
A7	+	+	+	+	-	+	N3	-	+	+	+	-	-
A9	-	+	-	+	-	-	N4	-	+	-	+	-	-
B1	+	+	+	-	+	+	N7	-	+	-	+	+	-
B2	-	+	-	-	-	+	O11	+	+	-	+	-	-
B3*	+	+	+	+	+	-	O14	-	+	+	+	-	-
B7	-	+	+	-	+	-	O15	+	+	+	+	-	-
C11	+	+	+	-	-	+	O16	+	+	+	-	+	-
C12*	+	+	+	+	+	-	O2	+	+	-	-	-	-
C2	-	+	+	+	-	+	O3	+	+	-	+	-	-
C32	-	+	+	-	-	-	O4	+	+	-	-	-	-
C33	-	+	+	+	-	-	O7*	+	+	+	+	+	+
C35	-	+	+	+	-	-	O8	-	+	+	-	-	-
CX	+	+	-	+	+	-	O9	-	+	-	+	-	-

Note. IAA (mg/mL) = production of Indole-3-Acetic Acid; Nit = asymbiotic fixation of atmospheric nitrogen; Phos. = solubilization of phosphate; Sid. = production of siderophores; Cel. = production of cellulase; (-) NO – did not produce the enzyme/metabolite; (+) YES – did produce the enzyme/metabolite; * Isolates selected for the greenhouse *in vivo* tests.

As can be seen from this table, many of the isolates yielded positive results for more than one of the administered tests. Specifically, three of them tested positive for only one test, 14 (26.09%) were positive for two tests, 15 (32.60%) were positive for three tests, 11 (23.91%) were positive for four tests, and three of the isolates, A6, J1 and O7, were positive for all five tests. In a more overarching view, of the 46 bacterial isolates, all of them (100%) produced Indole-3-Acetic Acid, 29 (63.04%) showed solubilization of phosphate, 30 (65.21%) produced siderophores, 16 (34.78%) produced cellulase, and 14 (30.43%) fostered nitrogen fixation.

This study also revealed that levels of IAA production varied between 0.36 $mg \cdot mL^{-1}$ and 14.7 $mg \cdot mL^{-1}$, while 30 (65.21%) of the microorganisms were capable of producing siderophores, with these production rates ranging between 2.33 mm and 22 mm (See Table 2).

Table 2. Indole-3-Acetic Acid (in mg/mL) and siderophores (in mm) produced by post-cultured bacterial isolates

Bacteria	IAA ($mg \cdot mL^{-1}$)	*Siderophores	Bacteria	IAA ($mg \cdot mL^{-1}$)	*Siderophores
A10	0.91	-	I2	1.84	15
A11	2.82	-	I3	14.77	10.33
A12	0.42	22.00	I4	1.51	3.67
A14	2.76	10.00	I5	0.91	-
A15	5.49	4.33	J1	0.45	6.0
A2	4.62	9.67	J2	10.62	-
A3	0.85	9.67	J3	5.49	20.0
A4	4.07	-	J4	3.64	4.33
A5	6.69	2.33	J6	0.69	7.67
A6	0.53	8.00	J7	9.09	-
A7	0.69	3.33	N3	1.89	13.33
A9	1.56	-	N4	1.18	-
B1	1.51	19.67	N7	0.69	-
B2	0.47	-	O11	0.69	-
B3	3.47	6.33	O14	4.51	10.33
B7	1.18	9.67	O15	0.63	16.33
C11	3.15	10.00	O16	0.47	3.33
C12	5.22	10.33	O2	0.74	-
C2	0.42	20.67	O3	3.47	-
C32	0.36	6.33	O4	0.91	-
C33	0.63	20.0	O7	2.44	8.0
C35	1.02	12.33	O8	0.53	20.33
CX	3.25	-	O9	0.63	-

Note. * Relationship between total halo diameter and the colony halo diameter.

Of the 46 isolates, 20 (43.48%) produced IAA levels below 1 $mg \cdot mL^{-1}$, 19 (41.30%) produced levels between 1 $mg \cdot mL^{-1}$ and 5 $mg \cdot mL^{-1}$, five (10.87%) produced between 5 $mg \cdot mL^{-1}$ and 10 $mg \cdot mL^{-1}$, and isolates J2 and I3 produced 10.62 $mg \cdot mL^{-1}$ and 14.77 $mg \cdot mL^{-1}$ of IAA, respectively. Results also show that, among the bacteria isolates, A2, C2, O8, C33 and J3 produced siderophores, yielding rates of 22.00 mm, 20.67 mm, 20.33 mm, 20.00 mm and 20.00 mm, respectively**.**

Table 3 shows the average values for branch lengths, foliar chlorophyll content index, and number of leaves, measured and counted after each treatment.

Table 3. Average branch length (in cm), foliar chlorophyll content index, and number of leaves after treatment of the *Vitis* sp. soil with the various bacterial isolates

	Length (cm)	Chlorophyll	# of Leaves
Treat. 1	66.880^{b}	24.9736^{a}	28.840^{a}
Treat. 2	76.704^{a}	22.9924^{b}	30.824^{a}
Treat. 3	69.200^{b}	24.1208^{b}	29.624^{a}
Treat. 4	58.288^{c}	24.7944^{a}	28.736^{a}
Treat. 5	63.688^{c}	24.3028^{b}	27.112^{a}
Treat. 6	68.328^{b}	24.0056^{b}	28.232^{a}
CV	42.24%	14.9%	33.61%

Note. CV = Coefficient of Variation. Within each column, the averages with the same superscripted letter do not differ statistically from the Scott Knott test ($p < 0.05$). Treatment 1 = control group; Treatment 2 = bacteria C12; Treatment 3 = bacteria O7; Treatment 4 = bacteria B3; Treatment 5 = bacteria I3; and Treatment 6 = blend of bacteria C12, O7, B3 and I3.

In Treatment 2, the soil was inoculated with bacteria C12; results for this treatment tested positive for nitrogen fixation and phosphate solubilization, had a siderophore total halo/colony halo relationship of 10.33 mm, and produced 5.22 $mg \cdot mL^{-1}$ of IAA. The C12 treatment also showed an increase of 14.7% in branch length when compared to a control group, which shows good potential for plant growth-promotion and corroborates results obtained during the *in vitro* tests. It did not, however, show significant increase in the number of leaves and foliar chlorophyll content.

In Figure 1 below, one can see the variation in branch lengths between the different treatments, over time. The chart depicts a quadratic curve behavior, which shows that the biggest difference in branch lengths occurred in the first month.

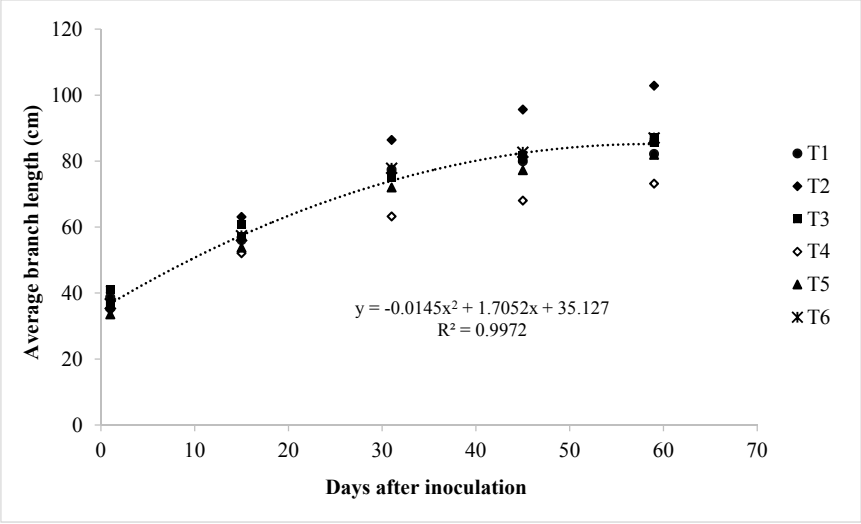

Figure 1. Average branch lengths of the *Vitis* sp. after the different treatments, during 60 days of incubation

Note that, basically, all treatments exhibited the same behavior, with Treatment 2 (T2) being the one that, statistically, most differed from the rest, showing the highest branch length increase, over time.

At the end of the experiment, fresh and dry masses (branches leaves and roots) were also determined, and those results are organized in Table 4.

Table 4. Average dry mass (in mg) and average fresh mass (in mg) for the roots, leaves and branches corresponding to each of the treatments, after 60 days

	DMR	DML	DMB	FMR	FML	FMB	TFM	TDM
Treat. 1	5.482	4.532	3.383	19.386	15.652	7.999	43.037	13.398
Treat. 2	5.312	5.495	3.987	19.195	18.400	8.888	46.484	14.795
Treat. 3	6.524	5.335	3.931	22.477	16.614	8.434	47.527	15.791
Treat. 4	5.093	4.708	2.858	18.159	14.852	6.521	39.532	12.660
Treat. 5	5.258	5.112	3.632	19.887	16.744	8.088	44.720	14.004
Treat. 6	6.285	4.874	3.500	22.348	15.158	7.766	45.273	14.660

Note. DMR = dry mass – roots; DML = dry mass – leaves; DMB = dry mass – branches; FMR = fresh mass – roots; FML = fresh mass – leaves; FMB = fresh mass – branches; TFM = total fresh mass; TDM = total dry mass; Treatment 1 = control group; Treatment 2 = bacteria C12; Treatment 3 = bacteria O7; Treatment 4 = bacteria B3; Treatment 5 = bacteria I3; and Treatment 6 = blend of bacteria C12, O7, B3 and I3.

The average total fresh mass (TFM) ranged from 39.532 mg to 47.527 mg, and the average total dry mass (TDM) from 12.660 mg to 15.791 mg. Both the TFM and the TDM were highest for Treatment 2 (46.484 mg and 14.795 mg) and Treatment 3 (47.527 mg and 15.791 mg). When comparing these against the control group, one will note an increase of 9.45% for the TFM and 15.15% for the TDM. Conversely, with results for TFM and TDM of

39.532 mg and 12.660 mg, respectively, Treatment 4 showed the lowest biomass production, yielding even lower levels than the control group. Though dry and fresh root biomass levels showed no significant difference between the control group and the other treatments, it is interesting to note that Treatments 3 and 6 yielded increases in DMR of 19% and 14.64%, respectively, with both showing increases in FMR above 15%.

On the 60th day, all samples were tested to determine content levels of Carbon, Hydrogen, Nitrogen and Sulfur, and results for each treatment are organized in Table 5.

Table 5. Average percentages of Carbon, Hydrogen, Nitrogen and Sulfur found in the biomass for all treatments, on the 60th day of the experiment

Treatment	C (%)	H (%)	N (%)	S (%)
Treat. 1	35.16^{b}	3.56^{a}	0.58^{c}	0.42^{a}
Treat. 2	38.88^{a}	4.38^{a}	0.94^{b}	0.70^{a}
Treat. 3	40.08^{a}	4.50^{a}	1.24^{a}	0.70^{a}
Treat. 4	39.20^{a}	4.38^{a}	1.28^{a}	0.48^{a}
Treat. 5	38.94^{a}	4.82^{a}	1.40^{a}	0.82^{a}
Treat. 6	30.34^{c}	4.16^{a}	1.28^{a}	0.60^{a}

Note. Within each column, the averages with the same superscripted letter do not differ statistically from the Scott Knott test ($p < 0.05$). Treatment 1 = control group; Treatment 2 = bacteria C12; Treatment 3 = bacteria O7; Treatment 4 = bacteria B3; Treatment 5 = bacteria I3; and Treatment 6 = blend of bacteria C12, O7, B3 and I3.

For the Carbon and Nitrogen contents, a significant difference was observed between treatments. When assessing the percentage content of Carbon, specifically, one can see that the control (Treat. 1) and blended (Treat. 6) groups have the lowest levels of Carbon (35.16% and 30.34%, respectively) when compared to Treatments 2 thru 5, for which the samples were inoculated with the individual bacteria C12, O7, B3 and I3, respectively, yielding an increase of Carbon content of 10%, 14%, 11.5% and 10.8%, also respectively. For Nitrogen, on the other hand, only the control group had a lower content percentage than the rest of the treatments, with the percentage increase in Nitrogen content ranging from 61.7% (Treat. 2) to 140% (Treat. 5).

Of the 46 bacteria, isolates O7 and C12 were identified based on their *rpoB* gene partial nucleotide sequence. Isolate O7 stood out for having yielded positive results in all tests, and isolate C12 for obtaining the best *in vivo* results, yielding longer branch lengths, and higher Carbon and Nitrogen contents, when compared to non-inoculated plants. The *rpoB* gene sequence amplification for isolate O7 produced 627 base-pairs, which was a 99% match to the *Bacillus amyloliquefaciens*. By the same token, isolate C12 produced a sequence amplification of 669 base-pairs, which was a 98% match to the *Bacillus thuringiensis.*

4. Discussion

This research showed that the majority of the bacteria isolated from vineyard soil samples have multiple capabilities. These results are similar to the ones achieved by Marasco et al. (2013) in an experiment that isolated 769 bacteria from soil and root samples of grapevines originally from Italy, Tunisia and Egypt. In that experiment, 95% of the isolates yielded positive results for more then one of the tests. On that occasion, the authors found that 82% of the isolates produced IAA, 61% solubilized phosphate, and 47% produced siderophores. Another group of authors, specifically Ahamad et al. (2008), reported isolating 72 bacteria, for which 80% of the *Azotobacter*, *Pseudomonas* and *Mesorhizobium* isolates produced IAA, while 56.68% showed solubilization of phosphate. In our study, all isolates tested positive for the production of IAA, results that are similar to those obtained by Kuss, Kuss, Lovato, and Flores (2007). According to Dobbelaere, Vanderleyden, and Okon (2003) the ability to synthesize phytohormones is widespread among bacteria associated with plants, and these hormones stimulate plant growth and promote an increase in root area, allowing for better nutrient absorption from the soil. In yet another study, Dawwam et al. (2013) verified that all seven bacteria isolated from the *Ipomoea batatas L*. rhizosphere produced IAA with concentrations ranging from 0.6 $\mu g \cdot mL^{-1}$ to 10.73 $\mu g \cdot mL^{-1}$; in this study, for which IAA concentrations ranged from 0.36 $mg \cdot mL^{-1}$ a 14.77 $mg \cdot mL^{-1}$.

Results for this study showed that 63.04% of the bacteria solubilized phosphate and 65.21% produced siderophores, which is comparable to results attained in other research efforts (Marasco et al., 2013). One of the main contributors to the solubilization of phosphate in the soil is its reduced pH value caused by the bacteria's production of organic acids (Karagöz et al., 2012). In terms of siderophores, according to Benite, Machado, and

Machado (2002), in the last three decades, over one hundred naturally occurring siderophores have been isolated and characterized, including those of bacterial (*Streptomyces*, *E. coli*, *Pseudomonas*, *Bacillus*, *Micobacterium*) and fungal origin (*Aspergillus*, *Penicillium*). Production of these iron chelating agents can be very diverse and have many benefits, the most important ones being that they not only act as biocontrol, biosensor and bioremediation agents, but also promote plant growth (Ahmed & Holmström, 2014). Of all isolated bacteria, 34.8% produced cellulase and, according to Asghar et al. (2002) and Glick (2012), plant growth can be indirectly promoted by reducing or inhibiting the activities of pathogenic microorganisms through the production of enzymes (such as cellulase and chitinases), antibiotics and siderophores by growth-promoting bacteria.

Fourteen of the 46 isolates tested positive for nitrogen fixation. According to Beneduzi et al. (2010), microorganisms present in the rhizosphere show great capacity for assimilating nitrogen. Chagas, Oliveira, and Oliveira (2009) go a step further and claim that they are also capable of producing phytohormones. An analysis of our *in vitro* test results revealed that the isolates had the potential to promote plant growth, especially C12, O7, B3 and I3, which were, therefore, selected for the *in vivo* tests.

The *in vivo* tests, in turn, showed a significant difference in branch lengths between the different treatments, with the C12 isolate promoting the most growth (706.704 cm). In general, all growth was more accentuated in the first 30 days. As claimed by Dias et al. (2009), the initial growth can be attributed to the metabolic substances produced after the bacterial inoculation. Under incubator conditions, the microorganisms *Bacillus* spp and *Sphingopyxis* sp. showed the potential to enhance root development, and increase branch length, dry weight, number of leaves, petiole length and aerial dry weight.

Although differences were observed between fresh mass and dry mass averages, they weren't statistically significant. Such results were comparable to those obtained by Passos et al. (2014) who also did not achieve any statistically significant results in terms of the root's dry mass when they inoculated apple seedlings with five bacterial isolates from the rhizosphere. Dawwam et al. (2013) observed an increase in nitrogen (50.5%) and phosphorus content (48.3%) in the *Ipomoea batatas* L.'s dry mass when compared to the control group; increases that were also observed in this study with respect to carbon (10% to 14%) and nitrogen (61.7% to 140%) contents. Passos et al. (2014) analyzed apple seedlings in terms of absorption of nitrogen, phosphorus and potassium, and determined that only plants inoculated with *Burkholderia sp.* showed high levels of phosphorus absorption. The element that showed significant differences in this study was nitrogen, with all treatments yielding a higher content percentage of it than the control group.

The O7 and C12 isolates, which produced positive results in all *in vitro* tests, were identified as *Bacillus amyloliquefaciens* and *Bacillus thuringiensis*, respectively. Studies about plant growth-promoting bacteria have demonstrated that rhizobacteria of the *Bacillus* genus are frequently found in soil; among these the *B. amyloliquefaciens* has stood out for its ability to promote plant growth and control phytopathogens (Fan et al., 2015; Cavalhais et al., 2013). With strawberry plants, Dias et al. (2009) established that bacteria of the *Bacillus spp.* genus have the potential to enhance root development, and increase branch length, dry weight, number of leaves, petiole length and aerial dry weight. Bobrowski, Fiuza, Pasqualis, and Bodanese-Zanettini (2003), on the other hand, assert that the *Bacillus thuringiensis* is a biotechnological alternative to ward off crop diseases and pesty insects.

5. Conclusions

The *in vitro* tests performed during this study determined that the majority of the bacterial isolates have multiple capabilities, many of which reveal their potential as promoters of plant growth. According to the results herein exposed, which include parameters such as branch length, chlorophyll content index, and carbon and nitrogen contents, one can establish that inoculation of *Vitis* sp. plants with specific bacteria showed an auspicious potential for plant development. Under the specific experimental conditions in which they were evaluated, the bacterial isolates C12, O7, B3 and I3 stood out for significantly increasing Carbon and Nitrogen contents in the plants. Additionally, the C12 isolate, for which 98% of its sequence matched the *Bacillus thuringiensis*, produced the highest branch length growth (76.704 cm). New studies are needed to evaluate the effects of *in vivo* inoculations and to optimize soil and tissue colonization, which, consequently, would promote higher plant growth activities in grapevines.

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