

Antifungal and plant growth promoting activity with *Bacillus amyloliquefaciens* in *Aeonium* subs.

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Abstract

In this work, the biostimulant and biocontrol capacity of *Bacillus amyloliquefaciens* in succulent plants such as *Aeonium* was evaluated. The specific objectives were in particular: (i) Evaluate whether the use of this bacterium can increase the growth rate of *Aeonium* plants that typically have a slow growth cycle; (ii) evaluate if the use of *B. amyloliquefaciens* can lead to increased plant resistance to *Fusarium oxysporum*; (iii) considered if utilization of *B. amyloliquefaciens* allows for increased plant resistance to water and nutrient stress. The experimentation showed a significant improvement of the agronomic parameters analyzed on all *Aeonium* plants treated with *Bacillus amyloliquefaciens*. In general there was a significant increase in plant height, number of leaves, vegetative and root weight, number of new shoots. In addition, *Aeonium goochiae* and sunburst show a better protection against the fungus *Fusarium oxysporum*. The trial also highlighted the ability of *B. amyloliquefaciens* to guarantee a productive and qualitative production of the plants even under conditions of reduced water and nutritional resources. Plants, thanks to the interaction with soil microorganisms, can reach higher nutrient and water resources, resulting in greater resistance to abiotic stresses and better quality in the cultivation cycle.

Keywords: *Crassulaceae*; Microorganisms; Sustainable agriculture; Plant quality; Rhizosphere

1. Introduction

Crassulaceae are dicolythedonous plants, with succulent leaves, arranged in a rosette; they can take on the size of grasses or shrubs; only a few species are annuals or biennials. Leaves are often covered with bloom, with great variety of shapes and colors. They have a certain basic number of sepals and petals, stamens and carpels; the flowers are small, star-shaped or tubular, mostly bisexual, white, red or yellow, forming corymbs or panicles; many species are self-fertile but need pollination [1]. The fruit is a follicle that opens at maturity whose seeds, very small, are dispersed by the wind. Some species bloom in winter, others in spring or late summer. European species are mostly hardy. *Aeonium* is characterized by some forty species of perennial grasses or shrubs, with or without branches, with or without stems, bearing a rosette at the top of the stems that dies after flowering (monocarpic genus) [2]. During the dry period, the outer leaves die. Flower color ranges from white, to golden yellow, to pink. It identifies the group without stems including the species: *canariense*, *cuneatum*, *glandulosum*, *palmense*, *subplanum*, *tabuliforme*, *virgineum*. The branched, dwarf group consisting of: *haworthii*, *lindleyi*, *saundersii*, *sedifolium*, *smithii*, *spatulatum*, *viscatum* [3]. Then we have a group including large shrubs over a meter tall: *arborescens*, *balsamiferum*, *rubrolineatum*, *undulatum*, *urbicum*. These are plants from the Canary Islands, the southern Mediterranean, East Africa, Arabia [4]. They grow in general from sea level up to 2000 meters. In the wild the growing season is between October and March, coinciding with the rainy season in the Canary Islands, however the plants can be encouraged to vegetate in the summer and become more

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robust if planted in the ground during the good season and potted up again in the fall. In summer, however, they like an outdoor exposure. They are subject to rot; propagation is by apical stem cuttings or leaf cuttings resting on the soil; in late summer you can also practice the sowing [5,6].

Bacillus strains manifest their biological control capabilities by producing a broad spectrum of antibiotics, synthesizing extracellular enzymes and competing for nutrients, also resulting in processes of inducing systemic resistance in plants against pathogens [7,8]. Several studies have shown that VOCs produced by *Bacillus* spp. are able to inhibit spore germination of several fungal plant pathogens [9,10]. In addition to anti-fungal activity, VOCs can improve growth and induce resistance against plant pathogens [11,12,13]. Several studies have shown that secretion of secondary metabolites is the most important mechanism to suppress pathogens; lipopeptides are the most important secreted metabolites and have been reported to effectively antagonize plant pathogens [14]. Iturin A, a lipopeptide that is produced by *Bacillus amyloliquefaciens*, has shown excellent antifungal activity against several plant pathogens [15,16]. It may also play a positive role in biofilm formation [17,18]. The biocontrol activity and mechanism of *B. amyloliquefaciens* have been well studied in recent years. *B. amyloliquefaciens* strains also produce plant growth-promoting hormones including indole-3-acetic acid (IAA) and gibberellin A3 (GA3) and several nutrient-related enzymes [19]. In addition, phosphorus solubilization and nitrogen fixation are other characteristics that promote plant growth of *B. amyloliquefaciens* strains [20].

In this experiment, the main objective was to:

- Evaluate whether the use of this bacterium can increase the growth rate of *Aeonium* plants that typically have a slow growth cycle;
- Evaluate if the use of *B. amyloliquefaciens* can lead to increased plant resistance to *Fusarium oxysporum*;
- Considered if utilization of *B. amyloliquefaciens* allows for increased plant resistance to water and nutrient stress.



Figure 1 - Particular of *Aeonium* subs. in greenhouse cultivation: *Aeonium goochiae* (A); *Aeonium haworthii* (B); *Aeonium decorum* (C); *Aeonium arboreum* (D); *Aeonium* cv sunbursts

2. Material and methods

The experiments, started in January 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on *Aeonium arboreum*, *Aeonium haworthii*, *Aeonium decorum*, *Aeonium goochiae*, *Aeonium* cv. *sunbursts* (Figure 1A,1B). The plants were placed in ø 10 cm pots; 30 plants per thesis, divided into 3 replicas of 10 plants each. All plants were fertilized with a controlled release fertilizer (1 kg m⁻³ Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The experimental groups were:

- Group without *B. amyloliquefaciens* (CTRL100) (peat 60% + pumice 40%), irrigated with water (100%) and substrate previously fertilized (100%);

- Group without *B. amyloliquefaciens* (CTRL50) (peat 60% + pumice 40%) irrigated with water (50%) and substrate previously fertilized (50%);
- Group with *B. amyloliquefaciens* (BAM100) (peat 60% + pumice 40%) irrigated with water (100%) and substrate previously fertilized (100%); *B. amyloliquefaciens* has been isolated by TNC Mycorr MAX (1.2×10^9 cfu/Kg)
- Group with *B. amyloliquefaciens* (BAM50) (peat 60% + pumice 40%) irrigated with water (50%) and substrate previously fertilized (50%);

The plants were watered 4 times a week in the CTRL100 and BAM100 and grown for 12 months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the fraction of leaching. On January 7, 2021, plant height, leaves number, vegetative weight, roots weight, shoots number. In addition, the number of plants that died to *Fusarium oxysporum* were evaluated.

2.1. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results and discussion

The experimentation showed a significant improvement of the agronomic parameters analyzed on all *Aeonium* plants treated with *Bacillus amyloliquefaciens*. In general there was a significant increase in plant height, number of leaves, vegetative and root weight, number of new shoots. In addition, *Aeonium goochiae* and sunburst show a better protection against the fungus *Fusarium oxysporum*. The trial also highlighted the ability of *B. amyloliquefaciens* to guarantee a productive and qualitative production of the plants even under conditions of reduced water and nutritional resources. Plants treated with *B. amyloliquefaciens* and 50% water and fertilizer showed the same agronomic results as the control with 100% water and fertilizer.

In (Table 1), in *Aeonium haworthii* there was a significant increase in plant height in (BAM100) with 13.69 cm compared to 12.64 cm in (CTRL100), 12.60 cm in (BAM50) and 11.20 cm in (CTRL50) (Figure 2). In addition, there was a significant increase in leaf number 6.70 in the (BAM100) thesis, compared to 5.71 in the control at optimal fertilization and irrigation and 4.80 in (BAM50) and (CTRL50). Regarding vegetative weight, the (BAM100) thesis was the best with 29.82 g, followed by (CTRL100) with 27.51g, (BAM50) 27.50 g and (CTRL50) with 23.99 g. The same trend for root weight where (BAM100) showed a weight of 17.86 g, (CTRL100) 15.73 g, (BAM50) 15.64 g and (CTRL50) 14.49 g (Figure 3). In terms of the number of new shoots, (BAM100) was also the best thesis with 5.40, followed by (CTRL100) and (BAM50) with 3.60 and 3.30, respectively, and finally (CTRL50) with 2.20. There were no significant differences in mortality caused by *Fusarium oxysporum* in the different experimental theses.

In (Table 2), in *Aeonium goochiae* there was a significant increase in plant height in (BAM100) with 9.93 cm compared to 8.84 cm in (BAM50), 8.51 cm in (CTRL100) and 7.60 cm in (CTRL50). In the treated thesis (BAM100) there was also a significant increase in the number of leaves 8.30, compared to 7.20 (BAM50), 6.40 (CTRL100) and 5.30 (CTRL50). In terms of vegetative weight, the thesis (BAM100) was the best with 42.42 g, followed by (BAM50) with 40.43 g, (CTRL100) 39.45 g and (CTRL50) with 35.95 g (Figure 4A). The same trend for root weight where (BAM100) showed a weight of 30.85 g, (CTRL100) 27.69 g, (BAM50) 27.42 g and (CTRL50) 24.89 g. For number of new shoots, (BAM100) was also the best thesis with 3.40, followed by (CTRL100) with 2.20 and (BAM50) and (CTRL50) with 1.80. Regarding the biocontrol of *F.oxysporum*, theses with *B. amyloliquefaciens* were the one with significantly lower mortality.

In (Table 3), in *Aeonium decorum* there was a significant increment in plant height in (BAM100) with 6.27 cm compared to 5.25 cm in (BAM50), 4.99 cm in (CTRL100) and 3.95 cm in (CTRL50). In the treated thesis (BAM100) there was also a significant increase in leaf number 5.80, compared to 4.70 (BAM50), 4.60 (CTRL100), and 4.20 (CTRL50). In relation to vegetative weight, the (BAM100) thesis was the best with 27.61 g, compared to (CTRL100) with 25.98 g, (BAM50) 25.62 g and (CTRL50) with 24.23 g (Figure 4B). The same tendency for root weight where (BAM100) showed a weight of 19.24 g, (BAM50) 18.43 g, (CTRL100) 17.57 g and (CTRL50) 15.18 g. As for number of new shoots, (BAM100) was also the best thesis with 3.60, followed by (CTRL100) and (BAM50) with 2.20 and (CTRL50) with 1.10. Concerning the biocontrol of *F.oxysporum*, there were no significant differences among the experimental theses.

In (Table 4), in *Aeonium arboreum* there was a significant increase in plant height in (BAM100) with 18.05 cm compared to 14.97 cm in (BAM50), 14.91 cm in (CTRL100) and 13.41 cm in (CTRL50) (Figure 5). In the treated thesis (BAM100) there was also a significant increase in leaf number 6.40, compared to 5.00 in (BAM50) and (CTRL100) and 3.80 (CTRL50). Regarding vegetative weight, the (BAM100) thesis was the best with 43.48 g, succeeded by (CTRL100) with 42.66 g and (BAM50) 42.25 g and finally (CTRL50) with 38.22 g. Same performance for root weight where (BAM100) showed a weight of 30.58 g, (CTRL100) 26.68 g, (CTRL50) 22.77 g and (BAM50) 21.79 g. For number of new shoots, (BAM100) was also the best thesis with 2.80, followed by (CTRL100) with 1.61 and (BAM50) with 1.42 and (CTRL50) with 1.00. Concerning the biocontrol of *F.oxysporum* also on this succulent species, there were no significant differences among the various experimental theses.

In (Table 5), in *Aeonium cv sunburst* there was a significant increase in plant height in (BAM100) with 8.61 cm compared to 7.56 cm in (CTRL100), 7.18 cm in (BAM50) and 6.43 cm in (CTRL50). In the treated thesis (BAM100) there was also a significant increase in leaf number 6.40, compared to 5.20 in (BAM50) and (CTRL100) and 4.10 (CTRL50). In terms of vegetative weight, the (BAM100) thesis was the best with 26.84 g, followed by (CTRL100) with 24.68 g and (BAM50) 24.61 g and finally (CTRL50) with 22.97 g. Same trend for root weight where (BAM100) showed a weight of 15.94 g, (CTRL100) 13.35 g, (BAM50) 13.29 g and (CTRL50) 12.91 g. For the number of new shoots, (BAM100) was also the best thesis with 5.60, followed by (CTRL100) with 4.20 and (CTRL50) with 3.40 and (BAM50) with 3.20 (Figure 6). Regarding the biocontrol of *F.oxysporum*, the treated experimental theses (BAM100) and (BAM50) were the best.

Table 1- Evaluation of *B. amyloliquefaciens* on the agronomic characters of *Aeonium haworthii*

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Roots weight (g)	Shoots number (n°)	<i>F.oxysporum</i> plants affected (n°)
CTRL100	12,64 b	5,71 b	27,51 b	15,73 b	3,60 b	0,61 a
CTRL50	11,20 c	4,80 c	23,99 c	14,49 c	2,20 c	0,44 ab
BAM100	13,69 a	6,70 a	29,82 a	17,86 a	5,40 a	0,00 b
BAM50	12,60 b	4,80 c	27,50 b	15,64 b	3,30 b	0,00 b
ANOVA	***	***	***	***	***	ns

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (BAM100): *B. amyloliquefaciens* with 100% water and fertilization; (BAM50): *B. amyloliquefaciens* with 50% water and fertilization.

Table 2 - Evaluation of *B. amyloliquefaciens* on the agronomic characters of *Aeonium goochiae*

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Roots weight (g)	Shoots number (n°)	<i>F.oxysporum</i> plants affected (n°)
CTRL100	8,51 b	6,40 c	39,45 b	27,69 b	2,20 b	1,81 a
CTRL50	7,60 c	5,30 d	35,95 c	24,89 c	1,80 b	1,64 a
BAM100	9,93 a	8,30 a	42,42 a	30,85 a	3,40 a	0,45 b
BAM50	8,84 b	7,20 b	40,43 b	27,42 b	1,80 b	0,62 b
ANOVA	***	***	***	***	***	**

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (BAM100): *B. amyloliquefaciens* with 100% water and fertilization; (BAM50): *B. amyloliquefaciens* with 50% water and fertilization

Table 3 - Evaluation of *B. amyloliquefaciens* on the agronomic characters of *Aeonium decorum*

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Roots weight (g)	Shoots number (n°)	<i>F.oxysporum</i> plants affected (n°)
CTRL100	4,99 c	4,60 b	25,98 b	17,57 b	2,20 b	0,60 a
CTRL50	3,95 d	4,20 b	24,23 c	15,18 c	1,10 c	0,41 ab
BAM100	6,27 a	5,80 a	27,61 a	19,24 a	3,60 a	0,00 b
BAM50	5,25 b	4,70 b	25,62 b	18,43 ab	2,20 b	0,21 ab
ANOVA	***	***	***	***	***	ns

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (BAM100): *B. amyloliquefaciens* with 100% water and fertilization; (BAM50): *B. amyloliquefaciens* with 50% water and fertilization.

Table 4 - Evaluation of *B. amyloliquefaciens* on the agronomic characters of *Aeonium arboreum*

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Roots weight (g)	Shoots number (n°)	<i>F.oxysporum</i> plants affected (n°)
CTRL100	14,91 b	5,00 b	42,66 b	26,68 b	1,61 b	0,40 ab
CTRL50	13,41 c	3,80 c	38,22 c	22,77 c	1,00 c	0,80 a
BAM100	18,05 a	6,40 a	43,48 a	30,58 a	2,80 a	0,00 b
BAM50	14,97 b	5,00 b	42,25 b	21,79 c	1,42 bc	0,20 ab
ANOVA	***	***	***	***	***	ns

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (BAM100): *B. amyloliquefaciens* with 100% water and fertilization; (BAM50): *B. amyloliquefaciens* with 50% water and fertilization.

Table 5 - Evaluation of *B. amyloliquefaciens* on the agronomic characters of *Aeonium cv sunburst*

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Root weight (g)	Shoots number (n°)	<i>F.oxysporum</i> plants affected (n°)
CTRL100	7,56 b	5,20 b	24,68 b	13,35 b	4,20 b	1,21 a
CTRL50	6,43 c	4,10 c	22,97 c	12,91 b	3,40 bc	0,85 a
BAM100	8,61 a	6,40 a	26,84 a	15,94 a	5,60 a	0,21 b
BAM50	7,18 b	5,20 b	24,61 b	13,29 b	3,20 c	0,22 b
ANOVA	***	***	***	***	***	**

One-way ANOVA; n.s. – non significant; *, **, *** – significant at $P \leq 0.05$, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ($P = 0.05$). Legend: (CTRL100): control with 100% water and fertilization; (CTRL50): control with 50% water and fertilization; (BAM100): *B. amyloliquefaciens* with 100% water and fertilization; (BAM50): *B. amyloliquefaciens* with 50% water and fertilization.



Figure 2 - Comparison between *Bacillus amyloliquefaciens* (BAM100) and control with 100% water and fertilization (CTRL100) on growth of *Aeonium haworthii*



Figure 3 - Effect of *Bacillus amyloliquefaciens* with 50% water and fertilization (BAM50) on growth roots of *Aeonium haworthii*

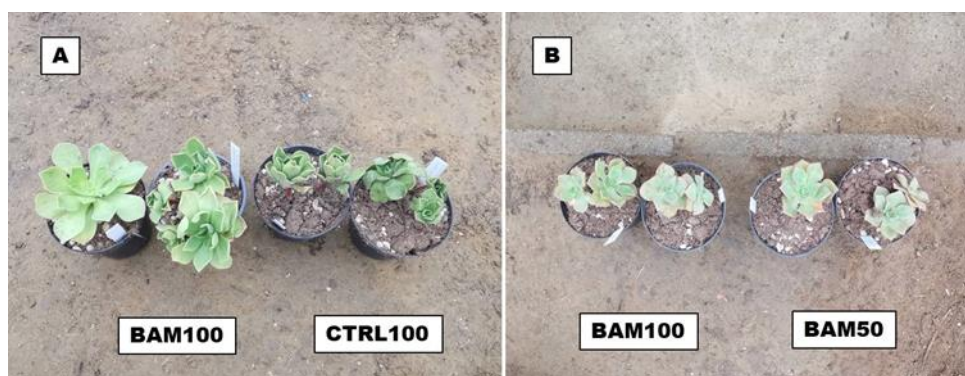


Figure 4 - Effect of *Bacillus amyloliquefaciens* with 100% water and fertilization (BAM100) on vegetative growth of *Aeonium goochiae* (A); comparison of *Bacillus amyloliquefaciens* with 100% water and fertilization (BAM100) and *Bacillus amyloliquefaciens* with 50% water and fertilization (BAM50) on *Aeonium decorum* (B)



Figure 5 - Effect of *Bacillus amyloliquefaciens* with 100% water and fertilization (BAM100) on vegetative and roots growth of *Aeonium arboreum*



Figure 6 - Effect of *Bacillus amyloliquefaciens* with 50% water and fertilization (BAM50) on vegetative and roots growth of *Aeonium cv sunbursts*

4. Discussion

The presence of diverse microbial species in the soil ensures a high number of interactions between plant roots and bacteria and fungi in the soil. This can ensure an increase in crop production as a result of direct growth mechanisms mediated by the presence of PGPMs (Plant Growth Promoting Microorganisms), which can result in an increase in the production of growth regulators such as auxins, cytokinins and gibberellins, reduction in the production of stress hormones such as ethylene, increased uptake of water and nutrients. Indirect mechanisms such as the production of antibiotics, cell wall lytic enzymes or the induction of plant defense processes can inhibit the growth of phytopathogens [21].

Microorganisms play important roles in the biogeochemical cycles of organic and inorganic soil nutrients and ensure plant health and soil fertility. The application of microbial consortia during cultivation cycles can therefore create benefits during plant cultivation and promote environmental sustainability leading to increases in agricultural production [22,23].

In this study, theses treated with *B. amyloliquefaciens* showed improvement in all agronomic parameters analyzed on *Aeonium* plants and also increased protection against *Fusarium oxysporum*. This improvement was definitely driven by the abilities of *B. amyloliquefaciens* to increase root development and consequently ensure greater water and nutrient uptake. This bacterium is also known to have the ability to stimulate plants on a hormonal and metabolic level.

The rhizosphere is a complex habitat where the plant through the exudates of its roots can promote microbial growth and metabolism [24]. Soil microbiology greatly influences plant physiology and growth, but also protection from harmful pathogenic microorganisms. Beneficial microorganisms include plant growth-promoting rhizobacteria (PGPR), which generally live near roots or within specialized cells and are able to stimulate plant growth through a variety of mechanisms [20]. There are few studies involving the application of microorganisms and biostimulants in succulent and cactus cultivation that have demonstrated improvements in plant vegetative and root development, flower production, and especially with regard to increased protection from abiotic stresses and phytopathogens [25,26]. The main objective of this work is to enrich the literature with new studies concerning the application of microbial biostimulants on succulent plants, in this case *Aeonium*, providing more information to the farmer on new cultivation methods that are more environmentally friendly.

5. Conclusion

The trial on *Aeonium* plants showed how the application of *B. amyloliquefaciens* can significantly improve plant growth and defense. In particular, the use of microorganisms from the rhizosphere showed not only an increase in the agronomic characteristics of plants and roots, but also an increased defense against *Fusarium oxysporum*. Plants, thanks to the interaction with soil microorganisms, can reach higher nutrient and water resources, resulting in greater resistance to abiotic stresses and better quality in the cultivation cycle. In plants that have a fairly slow growth cycle, the use of *B. amyloliquefaciens* can certainly ensure an increase in the speed of development, with a reduction in water and nutrient inputs.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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