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Microbial bio stimulant obtained from cactus and succulent plants for rooting and growth of the castello hybrid rose (*Attilio Ragionieri*)

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Abstract

Research objective: The aim of this research was to evaluate the stimulating potential of new microbial consortia obtained from the root systems of cacti and succulents in the rooting and growth of *Attilio Ragionieri* rose.

Materials and Methods: The experiments, which began in February 2022, were conducted in the CREA-OF greenhouses in Pescia (PT), Tuscany, on cuttings obtained from a mother plant of the *Attilio Ragionieri* rose. Experimentation with the use of microbial consortia selected from cactus and succulent roots was carried out both to assess possible differences in rooting of cuttings and to highlight improvements in plant cultivation and growth. After 3 months from the start of the trial in May 2022, the following parameters were evaluated on the plants: number of cuttings rooted, average rooting speed, dead cuttings. After 5 months of cultivation from the time of transplanting, the following parameters were analysed on the plants and in the substrate in October 2022: plant height, number of leaves, leaf area, vegetative weight, roots volume and length, number of microorganisms in the substrate, number of dead plants, pH substrate value and SPAD index.

Results and Discussion: The experiment showed that the use of microorganisms introduced in the rooting medium of rose cuttings can significantly increase the percentage of rooted cuttings, reduce the rooting time and mortality of the cuttings. Furthermore, once rooted, the cuttings colonised by the microorganisms grow better, showing an increase in height, number of leaves, vegetative and root weight, increasing root length, leaf area and chlorophyll content. A very interesting aspect was also the increase in microbial biomass in the treated theses, particularly in the thesis inoculated with microorganisms obtained from cactus and succulent roots. Interestingly, there are no references in the literature on the use of these microbial selections evaluated for plant rooting, stimulation and resistance, which is why this work appears to be of particular importance. Plants living in our latitudes may be better able to adapt to climate change in the future if microorganisms from extreme environments are used.

Conclusions: Microbial biofertilisers can maintain low crop productivity and increase resistance to biotic and abiotic stresses, and in particular can improve fertiliser utilisation. The development of innovative protocols for the rooting and cultivation of old, often forgotten roses by exploiting microbial consortia that have not yet been tested seems to be a very important aspect for the recovery of important plants that might become extinct. Further research is currently underway on other ornamental species of historical and religious interest.

Keywords: Ornamental plants; Plant growth promoting rhizobacteria; Sustainable agriculture; Flowers; Ancient roses

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1. Introduction

In soil, plants influence bacterial communities by exuding root exudates that are specific to their cultivated species [1]. As a result of these substances, microorganisms are capable of multiplying and are crucial to plant biology, producing substances similar to plant hormones that stimulate cell differentiation, roots development, and changes in root hair growth [2]. Upon colonizing roots, microorganisms can initiate a symbiosis which leads to disease [3]. Plants rely on microorganisms in the rhizosphere to grow and defend themselves. Extracellular Rhizobacteria (ePGPR) reside primarily in the roots' rhizosphere, while intracellular Rhizobacteria (iPGPR) live inside the roots. In addition to *Nitrotobacter*, *Bacillus, Pseudomonas* and *Azospirillum*, there are several rhizobacteria that promote plant growth, including *Agrobacterium, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Erwinia, Flavobacterium, Chromobacterium, Caulobacter, Arthrobacter, Allorhizobium, Mesorhizobium* [4]. Besides improving soil fertility and characteristics, they can also make plant cultivation easier. In addition to forming siderophores, minerals such as potassium and phosphates are solubilized, nitrogen is fixed, and phytophores are produced during root colonization [5]. To improve crop quality today, microbial inoculants that promote plant growth, control disease, and enhance soil fertility are definitely necessary [6]. A growing number of farmers are turning to microbial inoculants that promote plant growth, disease control agents, and soil health in order to improve their agricultural production without harming the biodiversity of the agrosystem. Also, PGPRs are important for improving plant health, reducing environmental stress, and remediating soils [7].

1.1. Growth-promoting mechanisms in plants

Phytohormone production, nitrogen fixation, and phosphorus solubilisation are examples of direct mechanisms that promote plant growth [8]. It is important to note, however, that indirect mechanisms also play a role, such as competition for space and nutrients, production of antimicrobial and antifungal substances, enzymes that break down phytopathogenic cell walls, the production of siderophores, and the development of systemic plant resistance [9]. Microorganisms can be inoculated directly through spores or indirectly through liquid cultures. When molecular nitrogen is reduced to ammonia, it can either be absorbed directly by the roots, or transformed, by bacterial nitrification, into nitrate, which plants are able to readily absorb [10]. Bio-fertilisers are microorganisms that fix atmospheric nitrogen, making it available for plants to use. One of the main macronutrients indispensable for plant growth is phosphorus. Although phosphorus is abundant in most soils, plants do not readily absorb it. There are two types of phosphates present in soil: mineral phosphates like calcium phosphate and hydroxyapatite, and organic phosphates like phytates, inositol-phosphate, and phosphoesters [11]. In rhizosphere microorganisms, phosphorus solubilisation is the most common mechanism for promoting root growth, and it happens by producing organic acids such as acetate, lactate and oxalate, which acidify the surrounding environment [12]. Acid phosphatases and phytases catalyze enzymatic reactions that mineralize organic phosphate in soil. Microorganisms from the PGPR family produce phytohormones in addition to those produced by the plant, altering the balance, influencing plant growth and development, such as overproliferation of root hairs and lateral roots, which result in an increase in ion uptake from soil solution [13,14]. Auxins, like gibberellins, are also produced by numerous microorganisms that stimulate plant growth and help fix nitrogen. Plant phytostimulants are microorganisms that produce substances with phytohormonal activity, degrade growth-inhibiting hormones, or stimulate plants to produce growth hormones [15].

1.2. The *Attilio Ragionieri* rose (Castello hybrid)

During the late nineteenth century, Dr. *Attilio Ragionieri* worked on specimens of Rosa banksiae derived from those taken by Paolo Baroni in 1868 from the Botanical Garden of Florence. Upon hybridizing the rose, *Attilio Ragionieri* named it Rosa banksiae hyb. di Castello (Gard. Chron., Ser. 3, Vol. 76, p. 73, 1924) [16]. As a sarmentosa with small, white corollas when ripe, it is associated with the Marian cult since 'Maria autem rosa fuet candida per virginitatem, rubicunda per charitatem' (Saint Bernard of Clairvaux). Rosa Banksiae hyb. di Castello is one of the earliest denominations. It descends from the cross Rosa banksiae f. lutescens Voss × Rosa 'Lamarque' 1896 (Noisette, Maréchal, 1830). Rosa 'Lamarque' is a sarmentosa that is said by some to descend from a cross of R. 'Blush Noisette' × R. Parks' Yellow Tea-scented China, two other roses that have a lot of history behind them [17]. It is an excellent rose with double, white flowers that fade in the centre to warm, creamy yellow tones that smell of lemon. The Florentine roses grown in the couches of the villa at Sesto supplied not only the Italian market but also the foreign one, until at least 1970, when both the beds that housed the roses and the large greenhouse of lemons were decommissioned, events that eventually led to the extinction of the noble breeds hybridised by the Ragionieri, which today remain paper petals [18,19]. The way of cultivating them, as Attilio expounds and illustrates in the book, is made up of complex and delicate operations, such that the attempts made even more recently were not successful, as the villa's current gardener Bruno Bruscagli confirms. Following the prize he won in Ghent, but remembering him precisely as a 'skilful and beneficent doctor, ingenious horticulturist'. in April 1923, the Unione Agraria di Sesto proclaimed Attilio as honorary president, and in

May of the same year he was awarded the title of honorary citizen by the municipality of Sesto for 'the great merits he had acquired both in the field of medical science and agriculture' [20,21].

1.3. Research Objectives

The aim of this research was to evaluate the stimulating potential of new microbial consortia obtained from the root systems of cacti and succulents in the rooting and growth of *Attilio Ragionieri* rose (Figure 1). The possible interaction between plants and substrate microorganisms was also evaluated with regard to plant mortality and the number of flowers produced.

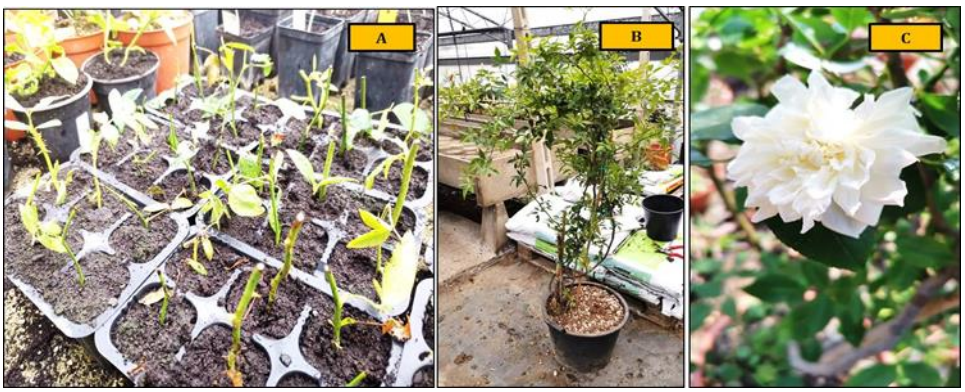

Figure 1 Detail of the rooting phase (A), pot cultivation (B) and flowering (C) of the *Attilio Ragionieri* rose

2. Materials and methods

The experiments, which began in February 2022, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on cuttings obtained from a mother plant of the *Attilio Ragionieri* rose. The cuttings were placed in 6-hole trays, 6 trays of 6 cuttings per thesis, for a total of 36 cuttings each.The experimental groups were:

- Control group (CTRL) (peat 80% + pumice 20%), (CLONEX GEL, 0.33% indolibutric acid) irrigated with water;
- Group with Symbac® (SYB) micro-organisms obtained from the root systems of cacti and succulents in (peat 80% + pumice 20%), irrigated with water (*Lactobacillus spp.*, *Streptomyces spp.*, *Trichoderma spp.*, *Bacillus spp.*, *Pseudomonas spp.*, *Aspergillus spp.*) (2.5 x 10^9 cfu/kg);
- Group with beneficial bacteria (BAC1) (peat 80% + pumice 20%) irrigated with water, (TNC Bactorrs13: *Bacillus amyloliquefaciens*, *B. Brevis*, *B. Cirulans*, *B. Coagulans*, *B. Firmus*, *B. Halodenitrificans*, *B. Laterosporus*, *B. Licheniformis*, *B. Megaterium*, *B. Mycoides*, *B. Pasteuri*, *B. Polymyxa*, *B. Subtilis* (1.3×10^{11} cfu/kg); Mix 1.5 g (approx. 1/2 tsp) per litre of soil;
- Group with beneficial bacteria (BAC2) (peat 80% + pumice 20%) irrigated with water and previously fertilised substrate, Tarantula powder Advanced nutrients: *A. Globiformis* 25,000 cfu/ml, *B. Brevis* 2,000,000 cfu/ml, *B. Coagulans* 500,000 cfu/ml, *B. Licheniformis* 5,000,000 cfu/ml, *B. Megaterium* 500,000 cfu/ml, *B. Polymyxa* 50,000 cfu/ml, *B. Pumilis* 50,000 cfu/ml, *B. Subtilis* 1,000,000 cfu/ml, *B. Thuringiensis* 100,000 cfu/ml, *B. Thuringiensis Canadiensis* 50,000 cfu/ml, *P. Polymyxa* 300,000 cfu/ml. Mix 2gr per litre of water.

The plants during the rooting process were sprayed twice a day for 1 minute. Irrigation was activated by a timer, the programme of which was adjusted weekly according to the weather conditions and the leaching fraction. At this stage, the percentage of rooted cuttings and the rooting rate was evaluated. After 3 months from the start of the trial in May 2022, the following parameters were evaluated on the plants: number of cuttings rooted, average rooting speed, dead cuttings. Each rooted cutting was subsequently placed in a 10-diameter pot and the evaluation of the growth phase started from that point. All experimental theses were managed with a substrate (60% peat + 40% pumice) and suitably fertilised with a slow-release fertiliser (3 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 theses remained the same as in the rooting phase. In this experimental part, the plants were irrigated once a day using a timer, the schedule of which was adjusted according to the weather conditions. After 5 months of cultivation from the time of transplanting, the following parameters were analysed on the plants and in the substrate in October 2022: plant height, number of leaves, leaf area, vegetative weight, root volume and length, number of microorganisms in the substrate, number of dead plants and pH

substrate value. In addition, the SPAD index was measured on three pinched leaves from the base to the apex of the crown of each plant (a total of 90 measurements per treatment).

2.1. Analysis methods

- pH: For pH measurement, 1 kg of the substrate was taken from each plant, and 50 g of the mixture was placed in a beaker containing 100 ml of distilled water. After 2 hours, the water was filtered and analyzed [22].
- Microbial count: direct determination of total microbial count by microscopy of cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [23].
- Analytical instruments: IP67 PHmeter HI99 series - Hanna instruments; Combined test kit for soil analysis - HI3896 - Hanna instruments; Microbial diversity of culturable cells [24].

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed 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 tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of microorganisms introduced into the rooting substrate of *Attilio Ragionieri* rose cuttings can significantly increase the percentage of rooted cuttings (Figure 2 and Figure 3), reduce rooting time and cuttings mortality. Furthermore, once rooted, the cuttings colonised by microorganisms grow better, showing an increase in height, number of leaves, vegetative and root weight, increasing root length, leaf area and chlorophyll content. A very interesting aspect was also the increase in microbial biomass in the treated theses, particularly in the (SYB) thesis, an inoculum of microorganisms obtained from the roots of cacti and succulents.

The thesis (SYB), proved to be the best for all agronomic parameters analysed, followed by the other two treatments with microbial consortia of various kinds, the untreated control thesis was the worst for both rooting of cuttings and subsequent plant growth and mortality.

Table 1 Evaluation of the use of selected microbial consortia from cacti and succulents on rooting and mortality of cuttings rose *Attilio Ragionieri*

Groups	Cuttings rooted (n°)	Average rooting speed (days)	Dead cuttings (n°)
CTRL	7,60 c	27,62 a	6,00 a
SYB	30,60 a	17,26 d	0,42 b
BAC1	13,40 b	19,84 c	1,40 b
BAC2	13,80 b	22,21 b	1,60 b
ANOVA	***	***	***

One-way ANOVA; n.s. – non-significant; *,**,*** – significant at P ≤ 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: (CTRL) control; (SYB) Symbac® ;(BAC1) TNC Bactorrs13;(BAC2) Tarantula powder Advanced nutrients

In Table 1, in the (SYB) thesis 85% of the rose cuttings rooted, compared to (BAC1) and (BAC2) with 37% and the control with 21%. The rooting speed in (SYB) was also significantly lower than in the other theses, 17 days, compared to 19 and 22 days in (BAC1) and (BAC2), and 27 days in (CTRL). The number of dead cuttings was also significantly higher in the control than in the other theses. In Table 2, the (SYB) thesis was the best for all agronomic parameters analysed in terms of height (Figure 4), vegetative and root growth, followed by the (BAC1) and (BAC2) theses, the control again proving to be the worst treatment.

Table 3, on the other hand, shows that the treatment with selected microorganisms from cacti and succulents (SYB), colonised the substrate better than the other experimental theses, a slight increase in pH in the thesis (BAC1) and a significant increase in plant mortality in the control thesis. With regard to SPAD, the thesis (SYB), showed a significantly higher chlorophyll content than the other theses.

Table 2 Evaluation of the use of selected microbial consortia from cacti and succulents on vegetative growth and roots biomass of rose plants *Attilio Ragionieri*

Groups	Plant height (n°)	Leaves number (n°)	Leaves surface area (cm^2)	Vegetative weight (g)	Roots volume (cm^3)	Roots length (cm)
CTRL	83,14 c	36,43 c	18,53 d	35,97 c	24,85 d	11,19 d
SYB	119,46 a	52,60 a	26,57 a	42,95 a	32,52 a	19,87 a
BAC1	109,39 b	44,20 b	20,18 c	40,24 b	28,59 b	13,62 c
BAC2	106,51 b	42,21 b	21,61 b	39,41 b	27,41 c	15,38 b
ANOVA	***	***	***	***	***	***

One-way ANOVA; n.s. – non-significant; *,**,*** – significant at P ≤ 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: (CTRL) control; (SYB) Symbac® ;(BAC1) TNC Bactorrs13;(BAC2) Tarantula powder Advanced nutrients

Table 3 Evaluation of the use of selected microbial consortia from cacti and succulents on the microbial biomass of the growing medium and physiological analysis of rose plants *Attilio Ragionieri*

Groups	Substrate total bacteria (Log CFU/g soil)	pH substrate	Plants dead number (n°)	Spad
CTRL	2,30 c	6,83 b	2,20 a	22,61 c
SYB	4,57 a	6,84 b	0,40 b	32,40 a
BAC1	3,41 b	6,90 a	0,60 b	26,00 b
BAC2	3,30 b	6,82 b	0,80 b	25,40 b
ANOVA	***	***	**	***

One-way ANOVA; n.s. – non-significant; *,**,*** – significant at P ≤ 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: (CTRL) control; (SYB) Symbac® ;(BAC1) TNC Bactorrs13;(BAC2) Tarantula powder Advanced nutrients

Figure 2 Comparison of the Symbac® (SYM) and beneficial bacteria (BAC1) thesis in the rooting process of *Attilio Ragionieri* rose cuttings

Figure 3 Effect of Symbac® (SYM) treatment on root development of *Attilio Ragionieri* rose cuttings compared to rooting hormone treatment (CTRL)

Figure 4 Comparison of the Symbac® (SYM) and beneficial bacteria (BAC2) thesis in the stimulation of plant growth of *Attilio Ragionieri* rose

4. Discussion

The term microbial inoculant refers to inoculants that contain microorganisms from plant roots and root zones. In addition to promoting seed germination and plant growth, they improve plant growth by up to 40% by colonizing the rhizospheres or roots of plants. Microorganisms have been shown to improve soil fertility and plant productivity [25, 26] by improving nutrient solubilization and root accessibility. Furthermore, Rhizobacteria have biocontrol capabilities, so they can control pests and diseases and promote plant growth [27, 28]. It has been shown that plant growth-promoting rhizobacteria (PGPR) improve root development, prolong plant and flower life, degrade harmful substances, and make young plants more resistant to biotic and abiotic stress [29,30,31]. Additionally, the use of microbial inoculants can usually be reduced over time, because they colonize surfaces slowly and can multiply independently over time. It has been shown that some microorganisms commonly used as biofertilizers are capable of fixing nitrogen and solubilizing phosphate [32, 33]. The stimulation of bacteria on plants produces many phytohormones, many of which are used as biofertilisers. Plants can benefit from their growth-promoting components, such as indole-acetic acid (IAA), amino acids, and vitamins [34]. The primary function of PGPRs is to supply nutrients to plants (nitrogen, phosphorous, potassium and essential minerals) or to produce plant hormones directly. As biocontrol agents, environmental protectors, and root colonizers, PGPRs can also indirectly increase plant growth by reducing the inhibitory effects of a variety of pathogens on growth and development [35, 36, 37]. Through PGPRs, we are indirectly achieving sustainable soil fertility and plant growth through a sustainable and ecological approach. PGPRs can be exploited in a variety of ways to reduce the need for agrochemicals, such as fertilizers and pesticides, improve soil fertility through a variety of mechanisms, including the production of antibiotics, HCNs, siderophores, and hydrolytic enzymes [38, 39, 40]. The use of microorganisms from the rhizosphere in this experiment significantly influenced the rooting of rose cuttings, survival under biotic and abiotic stresses and improved growth during the nursery phase [41, 42]. Interestingly, there are no

references in the literature on the use of microorganisms selected from cactus and succulent roots and evaluated for plant stimulation and rutting, which is why this work appears to be of particular importance. Plants living in our latitudes may be better able to adapt to climate change in the future if microorganisms from extreme environments are used. The *Attilio Ragionieri* rose is a plant that roots with difficulty, so developing sustainable and environmentally friendly multiplication protocols, based in particular on the use of innovative microbial consortia, appears to be of particular interest [21].

5. Conclusion

In addition to soil and growing media properties, organic matter and phosphorous content certainly contribute to bacterial growth. In order to achieve sustainable agricultural goals, plant growth must be improved through bacterial activity. Biofertiliser composition is essential to exploit the potential of biofertilisers. Microbes play a key role in nutrient cycling in the ecosystem. nergistic action of the various microbes. Microbes are often strain-specific, so it is necessary to assess whether they are actually functional on the plant to be cultivated. Evaluating new microbial selections from plants such as cacti and succulents that live in extreme environments is also of particular interest in view of possible climate change. Microbial biofertilisers can maintain crop productivity with low environmental impact and increase resistance to biotic and abiotic stresses, and in particular can improve fertiliser utilisation. Developing innovative protocols for the rooting and cultivation of old, often forgotten roses appears to be a very important aspect for the recovery of important plants that might become extinct.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

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