Multistrain versus single-strain plant growth promoting microbial inoculants - The compatibility issue

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Summary Plant Growth Promoting Microorganisms or Plant Probiotics (PGPMs) constitute a promising solution for agricultural sustainability. The concept that inoculation of PGPM mixtures may perform better in enhancing agricultural production than single strain application dates back to the discovery of plant growth rhizobacteria (PGPR) and is gaining ground in our days. This shift is highlighted by the increasing number of research publications dealing with the positive impact of microbial mixtures in promoting plant growth, controlling plant pathogens, as well as providing abiotic stress tolerance. The continuous deposition of patents as well as commercially available formulations concerning bioprotective and/or biostimulant multistrain mixtures also underlines this shift. A major issue in engineering an effective and consistent synthetic multistrain mixture appears to be the compatibility of its components. The present review provides a thorough literature survey supporting the view that treatment of plants with compatible multistrain mixtures generally exerts a better effect in plant growth and health than single-strain inoculation. Our study focuses on multistrain mixtures based on *Pseudomonas*, *Bacillus* and beneficial fungal strains, while commercial products are also being referred.

Additional keywords: plant probiotics, biostimulants, synthetic multistrain mixtures, biological control, co-inoculation, consortia

Introduction

The plant microbiome is composed of active microorganisms that can alter plant physiology and development, perform biological control against pathogens as well as provide tolerance to various types of stress such as drought, salinity, or contaminated soils (Müller et al., 2016). These plant associated microbes can be rhizospheric, epiphytic or endophytic with overlap existing between these categories (Turner et al., 2013). However, such functions are not carried out by ‘the whole microbiome’, but by one or a few microbial species acting individually or in a cooperative manner (Hassani et al., 2018).

These microbes are defined as Plant Growth Promoting Microorganisms (PGPMs) or Plant Probiotics (PPs) (Berg, 2009; Berlec, 2012; Abhilash et al., 2016). Plant growth promotion can be direct through production of phytohormones or facilitation of nutrient bioavailability and indirect through biological control of plant pathogens by biological control agents (BCAs). Therefore, the purposeful introduction of PGPM inoculants to plants’ microbiome represents an environmentally sound option that holds a prominent position for several decades, in an effort to reduce the overuse of chemical pesticides and fertilizers (Adesemoye and Kloepper, 2009; Abhilash et al., 2016; Aloo et al., 2019).

In most cases, effective microbial inoculants consist of a single strain. However, the current research trend is shifted towards the development of synthetic bacterial and/or fungal multistrain mixtures with the rationale that they would perform better than single strains (Vorholt et al., 2018; Woo and Pepe, 2018). Although single application
could be effective, mixed inoculants could theoretically adapt to a broader range of environmental conditions and may possess a variety of modes of action (Guetsky et al., 2002; García et al., 2003; Sarma et al., 2015).

In the last two decades, hundreds of studies have been conducted evaluating synthetic mixtures of bacterial species, fungal species or both as plant growth promoting or biological control agents. The concept that combination of beneficial microbial isolates may enhance the efficacy achieved by single isolates dates back to the discovery of Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper et al., 1980). In the majority of studies, microbes used to develop microbial mixtures were selected based on their individual PGP activities and/or disease suppressive ability. Then, microbes were mixed together on the assumption that the consortium will be more effective against tested pathogens or in promoting plant growth, without taking into account that antagonistic interactions occurring among PGPMs of the mixture might reduce the expected effects (Sarma et al., 2015). Thus, the old issue of compatibility among microbial strains (Kloepper et al., 2004) regained a strong position in developing effective multistrain mixtures to use as inoculants (Sarma et al., 2015).

Human and animal multistrain probiotics have received more attention than plant probiotics in the past decade. Several multistrain probiotics are being used for human health, animal feed and aquaculture (Markowiak and Śliżewska, 2018; Sniffen et al., 2018). However, major issues remain unresolved; whether single strains or multistrain mixtures are considered more beneficial and whether strains in a mixture are compatible with each other (Korada et al., 2018; Ouwahand et al., 2018). The present study will describe the research findings on the evolution of PGPM mixtures and the compatibility issue among their components in order to provide valuable knowledge for the development of effective microbial mixtures for sustainable agricultural applications.

**In vitro compatibility of PGPMs in the construction of multistrain mixtures**

Based on a large number of studies, multistrain PGPM mixtures appear to have greater efficacy on improvement of plant growth and/or biological control than single strains. According to the current trend, prerequisites for successful construction of artificial microbial mixtures are: 1) use of diverse microorganisms that can promote plant growth and protect plants from biotic or abiotic stress, 2) efficacy of seed, leaf or root colonization, 3) compatibility among strains in the mixture, 4) use of microorganisms with different modes of action, 5) human and environmental safety, 6) easy application and 7) easy incorporation in an existing management system (Raupach and Kloepper, 1998; Sikora et al., 2010; Bashan et al., 2014; Großkopf and Soyer, 2014; Ahkami et al., 2017).

The issue of compatibility among microbial components of a probiotic multistrain mixture is gaining ground and is considered a basic requirement in the engineering of synthetic microbial mixtures applied to plants (Sarma et al., 2015; Friedman et al., 2017; Woo and Pepe, 2018) or humans and animals (Ouwahand et al., 2018). According to the established literature, the microbial components of a PGPM mixture are considered to be compatible when they have no growth suppressive effect on each other during their in vitro co-culture, either in contact or in proximity, or during the plant rhizosphere colonization competition assay (Jain et al., 2012; Castanheira et al., 2017; Pangesti et al., 2017; Santiago et al., 2017; Liu et al., 2018). In broader terms, compatibility between strains may be achieved when one strain produces toxic compounds and the second strain possesses a detoxifying mechanism that could lead to a certain tolerance of the compounds and vice versa (Kelsic et al., 2015; Kamou et al., 2016).

In many cases, the outcome of the in vitro co-culture compatibility tests reflects the actual nature of the interaction to some extent (Prasad and Subramanian, 2017). For example, competitive colonization assays
under controlled, greenhouse or field conditions demonstrated that *in vitro* compatible bacterial and/or fungal stains are also compatible in the rhizosphere; root population levels reached by each strain in the mixture were not significantly different from those obtained when strains were applied individually (Agusti et al., 2011; Alizadeh et al., 2013; Stefanic et al., 2015; Castanheira et al., 2017; Molina-Romero et al., 2017; Santiago et al., 2017). The same goes with *in vitro* incompatible combinations. For instance, the antagonistic strain of an *in vitro* co-culture may interfere with the root colonization capacity of the other strain (Anith et al., 2011; Stefanic et al., 2015; Pangesti et al., 2017; Santiago et al., 2017; Maroniche et al., 2018; Varkey et al., 2018). Thus, co-inoculation with *in vitro* incompatible strains may result in preventing one or both microbial agents to reaching the appropriate population threshold for plant-beneficial effects (Haas and Defago, 2005).

However, the outcome of the *in vitro* compatibility test does not always represent the actual antagonistic potential in plant conditions (Becker et al., 2012). It has been reported that variations in media used to test *in vitro* compatibility may affect the interaction (Georgakopoulos et al., 2002; Simoes et al., 2008; Deveau et al., 2016; Lyons et al., 2017). Also, microbes could colonize different ecological niches (Pliego et al., 2008), suggesting that *in vitro* incompatible microbes may not interfere with each other’s growth on the root surface. In a study of Ruano-Rosa et al. (2014) a mixture of *Pseudomonas pseu doalcaligenes* AVO110 and *Trichoderma atroviride* CH 304.1 appears as a very effective combination against *Rosellinia necatrix* to control avocado white root rot, in spite of their observed *in vitro* incompatibility. In another study, the compatible biocontrol agents *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 were added together via different modes of application, seed bacterization and fungal soil inoculation, and provided protection from *Rhizoctonia solani* (Abeyesinghe, 2009). Abeyesinghe (2009) and Ruano-Rosa et al. (2014) suggested that mixtures of bacteria and *Trichoderma* strains should be applied at different times and types of inoculation. Also, Anith et al. (2011) showed that sequential inoculation of *T. harzianum* and *Piriformospora indica* can increase the coexistence and the beneficial effects on black pepper. In some cases, the biological control agents of a microbial mixture may show *in vitro* compatibility but can be mechanistically incompatible in the sense that one strain interferes with the mechanism by which a second strain suppresses plant disease (Stockwell et al., 2011).

**Multistrain PGPM mixtures based on *Pseudomonas* or *Bacillus* strains**

A major group of PGPMs possessing many traits that make them well suited as biocontrol and plant growth promoting agents is *Pseudomonas* and *Bacillus* bacterial strains. Isolates from both taxa show a wide range of plant beneficial properties such as efficient plant colonization, plant growth promotion, biological control of phytopathogens and induction of plant tolerance to abiotic stress, through mechanisms including production of phytohormones, antibiotic compounds and enhancement of nutrient bioavailability (Hol et al., 2013; Aloo et al., 2018).

**Pseudomonas-based multistrain mixtures**

An early study by Sivasithamparam and Parker (1978) showed that co-inoculation of five *Pseudomonas fluorescens* isolates in sterile soil were highly efficient in reducing the take-all wheat disease caused by *Gaeumannomyces graminis* var. *tritici* while none of the isolates produced a similar effect when tested singly. These data raised the hypothesis that multiple *P. fluorescens* isolates may provide greater and more consistent disease suppression when applied as a mixture than the same strains used individually. This hypothesis was strengthened by the report of Weller and Cook (1983) where

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high suppression of this disease was demonstrated after seed treatment with a mixture of \textit{P. fluorescens} strains. Pierson and Weller (1994) using a large number of \textit{P. fluorescens} strains constructed different mixtures, consisting of three or five isolates and demonstrated that only a limited number of mixtures have the potential of greater biocontrol activity against \textit{G. graminis} var. \textit{tritici} compared with the same strains applied individually. However, \textit{in vitro} antagonistic studies of the effective mixtures revealed that their components were either strongly inhibitory to or strongly inhibited by other members of the mixture. A mixture of four or eight \textit{P. fluorescens} genotypes (CHAO, PF5, Q2-87, Q8R196, 1M1-96, MVP1-4, F113 and PhI1C2) producing 2,4-diacetylphorogluconol (2,4-DAPG) protected tomato plants from \textit{Ralstonia solanacearum} with greater efficacy than single application, although it consisted of strains that \textit{in vitro} inhibited the growth of one or more members of the mixture (Becker et al., 2012; Hu et al., 2016). However, in other studies, incompatible \textit{P. fluorescens} mixtures of high genotypic richness performed much worse than single strain inoculation (Jousset et al., 2014; Mehrabi et al., 2016), suggesting that antagonistic activity among the members of the mixture can lead to neutral or negative effect in the inhibition of the pathogen. Hence, the question raised is whether the antagonistic activity of the introduced strains in the rhizosphere enhances the expression of traits involved in disease control or, in contrast, leads to population reduction that consequently diminishes its synergistic effect in controlling the disease.

The development of \textit{Pseudomonas}-based microbial mixtures that was based on the beneficial properties of the individual components was sometimes successful, even without taking into account the possible lack of compatibility between the strains. For example, in a study conducted by Emami et al. (2018), a rhizospheric-endophytic mixed bacterial inoculant of two \textit{Pseudomonas} strains with multi PGP traits was constructed, without carrying out any compatibility tests. Its application clearly increased plant biomass and micronutrient assimilation into grain of wheat compared to single strain inoculation under greenhouse conditions. Emami et al. (2019) suggested that co-inoculation of eight bacterial strains from different taxa (\textit{Pseudomonas}, \textit{Bacillus}, \textit{Stenotrophomonas}, \textit{Serratia}, \textit{Nocardia} and \textit{Microbacterium}) having multiple PGP traits, increased plant growth rather than single bacterial inoculation. In another experiment, when plant growth promoting \textit{Pseudomonas} strains WCS417r and SS101 were co-inoculated as a mixture on \textit{Arabidopsis thaliana} Col-0 roots, the density of Ps. WCS417r was 44 times higher than that of Pf. SS101 (Pangesti et al., 2017). The mixed inoculation reduced shoot fresh weight compared to single inoculation of WCS417r, whereas there was no effect on root fresh weight compared to single applications. Interestingly, the two strains were also found \textit{in vitro} incompatible. Couillerot et al. (2011) reported \textit{in vitro} incompatibility between \textit{Azospirillum brasilense} Sp245 and \textit{P. fluorescens} F113 with the latter being the inhibitor. Co-inoculation of the mixture on wheat plants showed a phytostimulatory effect similar to single inoculations, but the authors concluded it may be due to the action of \textit{P. fluorescens} F113 alone since cells of \textit{A. brasilense} Sp245 were 10 times less abundant on the root. It seems that minimization of the antagonistic activity among the components in a synthetic multistrain mixture, may maximize the consistency of the beneficial effect, because the antagonistic strain tends to dominate rather quickly even in two-strain co-cultures or co-colonization competition assays (Foster and Bell, 2012; Pangesti et al., 2017). Thus, it is becoming clear that the PGP properties of the components of the microbial mixtures should be considered along with their compatibility.

Based on a large number of studies, \textit{Pseudomonas}-based multistrain mixtures appear to have a consistently greater efficacy on improvement of plant growth and/or biological control than the single strains. A microbial mixture consisted of \textit{in vitro} compatible
strains *P. fluorescens* PF1 and *A. brasilense* TNAU enhanced groundnut plant growth more efficiently than each single inoculation, depending on the type of application (Prasad and Subramanian, 2017). The interaction between *Pseudomonas* and *Azospirillum* taxa may be influenced by the species or even strains. Indeed, growth of *A. brasilense* strains is differentially inhibited or enhanced by distinct *P. fluorescens* strains (Maroniche et al., 2018), confirming this hypothesis. In vitro compatible PGPR *Pseudomonas fluorescens* FAP2 and *Bacillus licheniformis* B642, successfully colonized rhizosphere and rhizoplane of wheat seedlings individually and by co-inoculation, increasing plant growth parameters compared to control (Ansari and Ahmad, 2019). Co-inoculation with the combination of *P. fluorescens* compatible strains RE8 and RS111 gave significant disease suppression of *Fusarium* wilt of radish in comparison with combination of incompatible strains RE8 and RS111a in a potting soil bioassay (de Boer et al., 1999). Similarly, the introduction of three compatible *P. fluorescens* isolates PF1, TDK1, and PY15 was very effective in controlling population of the root-feeding nematode *Meloidogyne graminicola* in a field trial (Seenivasan et al., 2012), as well as in controlling sheath rot *Sarocladium oryzae* in rice (Saravankumar et al., 2009). Co-inoculation of salt-sensitive pepper plants with *Pseudomonas* strains that were compatible in the rhizosphere improved the plant physiological properties under salinity stress compared to single inoculation (Samaddar et al., 2019).

Combining strains with different modes of action may increase the likelihood of building a consistently effective mixture against plant pathogens (Ruano-Rosa et al., 2014). Agusti et al. (2011) selected two compatible *P. fluorescens* strains which differed in secondary metabolite production and found that dual inoculations lead to better control of *Phytophthora cactorum* in strawberry compared to single introductions, suggesting that the different mechanisms of action between strains may act complementary or synergistically. Co-inoculation of detached potato leaves with two compatible *Pseudomonas* strains, weakly interfering with each other's growth, which had complementary modes of action against *Phytophthora infestans* was particularly efficient as compared to single-strain inoculation (De Vrieze et al., 2018). Also, in vitro compatibility tests showed antagonism between certain strains of *Pseudomonas* spp. and plant beneficial fungal strains of *Trichoderma* spp., but also permitted the selection of compatible strains for the construction of mixtures that promoted plant health and growth compared to each strain alone (Mishra et al., 2013).

A literature survey revealed an increasing number of examples where plant inoculation with compatible strains’ mixtures of *P. fluorescens* and plant mutualistic bacteria (Sundaramoorthy and Balabaskar, 2012; Sundaramoorthy et al., 2012; Sundaramoorthy and Balabaskar, 2013; Rathi et al., 2015; Kumar et al., 2016; Sharma et al., 2018) or beneficial fungi including species of *Trichoderma* (Thilagavathi et al., 2007, Jain et al., 2012, 2013, 2014, 2015; Singh et al., 2013a, 2013b, 2014; Ruano-Rosa et al., 2014; Thakkar and Saraf, 2015; Chemelrotit et al., 2017; Patel et al., 2017; Yadav et al., 2017; Jambhulkar et al., 2018), *Beauveria* (Karthiba et al., 2010; Senthilraja et al., 2013), *Pochonia* (Siddiqui et al., 2003) and *Clonostachys* (Karlsson et al., 2015) showed better results than inoculation with individual strains or control treatment, under controlled and field conditions. Furthermore, co-inoculation of specific *Pseudomonas* strains that function as mycorrhiza helper bacteria (MHB) in combination with various arbuscular mucorrhiza fungi (AMF) promoted the growth of maize plants in field conditions better than single AM inoculation (Berta et al., 2014). Prior testing of compatibility among strains is more likely to lead to the construction of a successful mixture.

**Bacillus-based multistrain mixtures**

Among PGPMs, strains of *Bacillus* are the most widely used as biopesticides and biofertilizers (Aloo et al., 2018). As discussed
above, it is reasonable to assume that multistrain mixtures based on them may function in synergistic and additive manner compared to single-strain inoculants. Researchers have successfully engineered effective *Bacillus*-based multistrain mixtures without taking into account the compatibility of their components. A multistrain mixture consisted of *B. subtilis* AR12, *B. subtilis* SM21, and *Chryseobacterium* sp. R89, was shown to be a promising biocontrol agent against various diseases including *Ralstonia* wilt, *Phytophthora* blight and *Meloidogyne* root-knot of pepper under greenhouse and field conditions (Liu et al., 2014). Zhang et al. (2010) evaluated the efficacy of several *Bacillus*-based mixtures constructed using a pool of 12 bacilli strains known for their capacity to suppress *Phytophthora* blight on squash. Certain combinations of PGPR strains applied further increased the efficacy of disease control against *Phytophthora capsici* relative to their individual application but the authors concluded that the effect of mixtures cannot be predicted just by the performance of individual strains.

Brewer and Larkin (2005) screened various combinations of field and commercial bacterial and fungal strains and indicated that co-inoculation of *B. subtilis* GB03 (Kodiak, Gustafson) and *Trichoderma virens* GL-21 (SoilGard, Certis) provided a somewhat better control of stem canker caused by *Rhizoctonia solani* on potatoes than each organism alone, thus suggesting that certain bacterial and fungal mixtures may provide some synergistic effect in biocontrol efficacy. The other combinations did not show the desirable effect. Furthermore, several studies have demonstrated that mixtures of *Bacillus* spp. and *Trichoderma* spp. increased plant growth or the biocontrol efficiency against fungal phytopathogens more than each organism alone (Jisha and Alagawadi, 1996; Yobo et al., 2011; Ali et al., 2018; Alamri et al., 2019). They demonstrated that only a small fraction of the engineered mixtures exerted a better effect in controlling blight than the individual strains. Treatment with commercial formulation Trisan (*T. harzianum* AP-01) and Larminar (*B. subtilis* AP-01), applied alone or in combination, suppressed bacterial wilt (*R. solanacearum*), damping-off (*Pythium aphanidermatum*) and frogeye leaf spot (*Cercospora nicotiana*) of tobacco and protected the plant more effectively compared to the individual products (Maketon et al., 2008). Treatment of tomato with a mixture of commercial product BioYield (*Bacillus* spp. GBO3 and IN937a) and *B. licheniformis* CECT5106 showed a far better effect on tomato growth parameters and protection against *R. solani* than BioYield alone or the individual strains suggesting that increasing the diversity of microbial mixture may enhance the efficacy of the *Bacillus*-based mixture (Domenech et al., 2006). The effect of four different PGPB strains, *B. subtilis* GB03 and FZB24, *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* SE34, applied individually and in different combinations of dual mixtures revealed that only the combination of IN937a and GB03 strains provided a higher control efficacy against *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato than the individual strains (Myresiotis et al., 2012). In this study, data concerning the compatibility of the microbial strains used are not presented, suggesting that construction of effective *Bacillus*-based multistrain mixture can be possible, but only when appropriate combinations are used. The issue of compatibility among the components of a *Bacillus*-based multistrain mixture was early realized by researchers, thoroughly discussed and gradually implemented in their studies (Jetiyanon et al., 2003; Kloepper et al., 2004). A combination of *Bacillus* spp. strains BB11 and FH17, showing compatibility in the rhizosphere, enhanced yield and increased biocontrol efficiency against *Phytophthora* blight of bell pepper better than single strain inoculations (Jiang et al., 2006). Seed treatments with a mixture of *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a, showing rhizosphere compatibility, exhibited a greater plant growth promotion and protection against pathogens than any of the individual components (Kokalis-Burelle et al., 2006; Ryu et
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al., 2007). The two-strain combination of Bacillus spp. GBO3 and IN937a was selected for the development of the product BioYield by Gustafson (Dallas, TX).

Liu et al. (2016a, 2016b, 2017, 2018) engineered synthetic Bacillus-based mixtures taking into account the biological control and plant growth promoting activities of individual strains as well as their in vitro compatibility. As a result, all the synthetic mixtures consistently showed a better efficacy in exerting the desirable effect in an additive or synergistic manner. In another study, the mixture of compatible B. amyloliquefaciens strain BLB369, B. subtilis strain BLB277 and Paenibacillus polymyxa strain 267 has been shown to stimulate wheat seed germination and exhibit better efficacy in controlling head blight caused by Fusarium graminearum than treatments with the individual strains or mixtures of two-strain combination (Zalilla-Kolsi et al., 2016). The combined application of three compatible (colonization levels of cotton stems were similar for each strain) biocontrol strains on (colonization levels of cotton stems were combined application of three compatible combination (Zalila-Kolsi et al., 2016). Application of a compatible combination of B. subtilis MF352017 and T. harzianum controlled chickpea wilt caused by Fusarium oxysporum f. sp. ciceris and enhanced plant growth as compared to individual application (Zaim et al., 2018). Treatment with a combination of compatible B. subtilis ATCC 11774, T. harzianum and Trichoderma koningii suppressed the development of potato stem canker as well as promoted growth and yield (Ali et al., 2018). Combinations of compatible B. subtilis and Beauveria bassiana have been successfully used for the control of wilt disease and fruit borer in tomato plants, broadening the range of the beneficial fungi that can be used for preparing Bacillus-based compatible mixtures (Prabhukarthikeyan et al., 2013). In another study, B. pumilus INR7 and Rhizosphaghus sp. were found to be compatible with each other. Combined application of INR7 and mycorrhiza not only suppressed plant disease caused by R. solani but also improved common bean dry weight either in simultaneous or delayed pathogen inoculation (Hussein et al., 2018).

On the contrary, application of commercial formulations of Serenade (B. subtilis) and Trianum (T. harzianum T22) or Sentinel (T. atroviride LC52) applied simultaneously or sequentially did not improve disease control compared to single application (Xu et al., 2010). The BCAs B. amyloliquefaciens CPA28 and Penicillium frequentans strain 909 (PF909) in a mixture were less effective in controlling stone fruit brown rot caused by Monilinia spp. compared to their individual application. P. frequentans and B. amyloliquefaciens could not be combined because bacteria inhibited the germination and growth of P. frequentans. Furthermore, B. amyloliquefaciens outcompetes P. frequentans once applied on fruit surface (Guijarro et al., 2018). In the study of Thilagavathi et
al. (2017) mixture of incompatible B. subtilis Bs16 with Trichoderma viride strains Tvl and/or Tvl3, had the same or less effect on inhibition of Macrophomina phaseolina and produced greengram plants with a lower vigour index and germination percentage compared to their individual application. Bacillus species show strong antagonistic activity against other beneficial bacteria (Simoes et al., 2007) and fungi (Kim et al., 2008; Fuga et al., 2016), thus making the prior examination of compatibility a necessary step for the construction of an effective Bacillus-based mixture.

**Fungal mixtures**

Several studies have demonstrated that treatment of plants with mixtures of endophytic fungi have improved plant growth and health (Lugtenberg et al., 2016; Kashyap et al., 2017). Abundant endophytic fungi isolates applied to their own host or different hosts as a mixture significantly reduced disease symptoms by fungal pathogens, suggesting that endophytes suppress growth of invading pathogens either directly or indirectly (Arnold et al., 2003). A mixture of endophytic fungi isolated from wild barley effectively suppressed the seed-borne infections in a barley cultivar (Murphy et al., 2015). A fungal endophyte consortium consistently improved barley grain yield over several seasons under a variety of chemical fertilizer inputs and low seasonal rainfall (Murphy et al., 2017). Intra- or interspecies fungal consortia consisting of Clonostachys, Beauveria, Metarhizium or Trichoderma spp. are known to contribute to plant growth and health as biopesticides, biofertilizers, biostimulants and inducers of natural resistance to biotic and abiotic stress (Krauss and Soberanis, 2001; García et al., 2003; Hidalgo et al., 2003; Cota et al., 2008; Kapongo et al., 2008; Keyser et al., 2015; Chirino-Valle et al., 2016; Ren et al., 2016). However, the construction of the microbial mixtures was based on the effectiveness of each single isolate and the issue of compatibility among the isolates was not considered.

Inter- and intraspecies incompatibility among beneficial fungal isolates is quite often found (Reaves and Crawford, 1994; Krauss et al., 2004; Ruano-Rosa and López-Herrera, 2009; ten Hoopen et al., 2010; Krauss et al., 2013). Thus, antagonistic interactions between beneficial fungal strains could occur and decrease the efficacy of the treatment. Evaluation of in vitro interactions between Clonostachys and Trichoderma isolates revealed the dominant antagonistic activity of Clonostachys over Trichoderma strains suggesting that these two mycoparasites may be incompatible (Krauss et al., 2013). Co-inoculation of a mixture (1:10) of Clonostachys rosea and Trichoderma spp. on cocoa pods, temporarily suppressed C. rosea, whereas two weeks after application, C. rosea was the dominant and persistent pod colonizer (Krauss et al., 2013). However, these interactions may not be always antagonistic.

A mixture of C. rosea and B. bassiana (1:20) applied to flowers and leaves of tomato vectored by bees reduced significantly both grey mold and the insect pest (whitefly) suggesting that some kind of compatibility between these fungal species may occur under natural conditions (Kapongo et al., 2008). Application of a mixture of two compatible C. rosea isolates (Cr1 and Cr2) reduced the infection of cowpea seedlings by Macrophomina phaseolina in a pot experiment more efficiently, as well as resulted in higher yields compared to single-strain application (Ndiaye et al., 2010). Combinations of compatible Trichoderma isolates revealed that most of the mixtures performed more efficiently in controlling avocado white root rot than the single application of BCAs (Ruano-Rosa and López-Herrera, 2009). Also, the majority of the combinations of four compatible Trichoderma isolates were more effective in controlling postharvest crown rot of banana than a single isolate (Sangeetha et al., 2009). Mendoza and Sikora (2009) demonstrated that the combination of two compatible beneficial fungi, a nematode-antagonistic endophyte (Fusarium oxysporum strain 162) and an egg pathogen-
ic fungus (*Paecilomyces lilacinus* strain 251) were more effective in controlling *Radopholus similis* on banana than any antagonist applied alone.

**Are the commercial multistrain mixtures consisted of compatible strains?**

Currently, the majority of the PGPMs marketed as biopesticides, biofertilizers and biostimulants are comprised of a single strain, according to the label. However, bacterial and/or fungal multistrain mixtures are gradually becoming popular (Woo *et al.*, 2014; Woo and Pepe, 2018), indicating a general shift in replacing the single strain inoculants. This shift is reflected in the increasing number of research publications, as discussed above, the boosting of patent files depositions and the interest of several companies in developing and launching multistrain microbial mixtures.

A number of companies are ready to launch multistrain mixtures into the market. An example is biofungicidal seed treatment Velondis Extra (BASF) containing *B. subtilis* strain BU1814 and *B. amyloliquefaciens* strain MBI 600 as a mixture. Another example is the combination of the rhizobia inoculant *Bradyrhizobium japonicum* with the biofungicide Velondis Flex (*B. subtilis* strain BU1814) under the name Nodulator Duo ([https://agrow.agribusinessintelligence.informa.com/-/media/agri/agrow/ag-market-reviews_pdfs/supplements/agrow_biologicals_2017_online.pdf](https://agrow.agribusinessintelligence.informa.com/-/media/agri/agrow/ag-market-reviews_pdfs/supplements/agrow_biologicals_2017_online.pdf)).

Microbial multistrain mixtures developed by BioConsortia are in second or third year field trials for drought tolerance, nutrient use efficiency and yield improvement in stressed and standard agronomic conditions, while some new consortia for biofungicide activity are moving into their first year of field trials ([https://agrow.agribusinessintelligence.informa.com/-/media/agri/agrow/ag-market-reviews-pdfs/supplements/agrow_biologicals_2017_online.pdf](https://agrow.agribusinessintelligence.informa.com/-/media/agri/agrow/ag-market-reviews-pdfs/supplements/agrow_biologicals_2017_online.pdf)).

Recently, the Canadian authorities granted registration to Rootwin Plus-S, a combination of *Bradyrhizobium* spp. and *Trichoderma* spp., specifically to aid the soybean crop with rhizobium nodulation and to stimulate a healthy root system ([https://www.andermattbiocontrol.com/](https://www.andermattbiocontrol.com/)).

Syngenta Agrochemical Company has launched the biofungicide Tellus (*Trichoderma asperellum* and *T. gamsii*) licensed from Italian company Isagro ([https://agrow.agribusinessintelligence.informa.com/AG002647/Syngenta-presents-Tellus-biofungicide-in-Spain](https://agrow.agribusinessintelligence.informa.com/AG002647/Syngenta-presents-Tellus-biofungicide-in-Spain)).

Monsanto BioAg in a new product, TagTeam, combines a rhizobial inoculant with the phosphorus solubilising fungus *Penicillium bilaiae* (O’Callaghan, 2016).

Adaptive Symbiotic Technologies have developed several fungal mixtures conferring tolerance to abiotic stresses ([http://www.adaptivesymbiotictechnologies.com/products.html](http://www.adaptivesymbiotictechnologies.com/products.html)).

Bio Innovation AB filed a patent for the combination of antagonists *T. virens* isolate ATCC58678 and *B. subtilis var. amyloliquefaciens* strain FZB24 ([https://patents.google.com/patent/CA2485796C/en](https://patents.google.com/patent/CA2485796C/en)). Another product, marketed under the trade name QuickRoots, contains a patented combination of the bacterium *B. amyloliquefaciens* and the fungus *T. virens*. The combination enhances the bioavailability of nitrogen, phosphorus and potassium in the soil resulting in expanded root volume and subsequent potential of enhanced yield (Parnell *et al.*, 2016).

The Brazilian Ministry of Agriculture, Livestock and Supply has already issued the registration for the new multistrain mixture Shocker, recommended for the control of diseases, such as rhizoctoniosis and white mold, which mainly attack soy, coffee, cotton and minor crops. Shocker is composed of the bacteria *B. amyloliquefaciens* strain CPQBA 040-11DRM 01 and *B. amyloliquefaciens* strain CPQBA 040-11RRM 04 ([http://news.agropages.com/News/NewsDetail---29634.htm](http://news.agropages.com/News/NewsDetail---29634.htm)).
Conclusion

The application of Plant Growth Promoting Microorganisms (PGPMs) or Plant Probiotics (PPs) as plant inoculants represents an environmentally friendly option for the reduction of chemical fertilizers and pesticides overuse. In general, synthetic microbial multistrain mixtures show better effect in promoting plant growth and suppressing plant disease compared to individual strains. Selection of the components is usually based on their individual plant growth promoting traits, not taking into account their possible antagonistic interaction. It seems, however, that the major issue of compatibility among the strains should be considered in the process of designing a mixture. Minimizing their antagonism may lead to a more consistent mixture, since they will not interfere with each other’s growth and colonization capacity. Construction of even a dual strain successful mixture consisting of compatible components is not an easy task; nevertheless, it is an achievable one. Well-designed synthetic consortia of microbes can greatly increase the plant yield or control of plant pathogens in an environmentally sustainable way.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Σύγκριση μικροβιακών εμβολίων που προάγουν την ανάπτυξη των φυτών αποτελούμενων από μονά ή/και πολλαπλά στελέχη μικροοργανισμών – Το ζήτημα της συμβατότητας

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Περίληψη Οι μικροοργανισμοί που προάγουν την ανάπτυξη των φυτών (Plant Growth Promoting Microbes) ή οι φυτικοί προβιοτικοί μικροοργανισμοί, αποτελούν μια ιδιαίτερα υποσχόμενη λύση για την αειφόρο γεωργία. Η άποψη ότι ο εμβολιασμός φυτών με μίγματα που περιέχουν τους εν λόγω μικροοργανισμούς είναι αποτελεσματικότερος, σε σχέση με την εφαρμογή μεμονωμένων στελεχών τους, χρονολογείται από την ανακάλυψη των πιθανότερων που επάγουν την ανάπτυξη των φυτών και ανακτά έδαφος στις μέρες μας. Ο αυξανόμενος αριθμός επιστημονικών δημοσιεύσεων για τη θετική επίδραση των μικροβιακών μιγμάτων στην προαγωγή της ανάπτυξης των φυτών, στον έλεγχο των παθογόνων των φυτών καθώς και στην επιμόρφωση της αντοχής υπό αβιοτική καταπόνηση, επιβεβαιώνει την παγκόσμια τάση εφαρμογής μικροβιακών εμβολίων. Η συνεχής κατάθεση ευρεσιτεχνιών καθώς και η διαθεσιμότητα εμπορικών σκευασμάτων που αφορούν σε βιοπροστατικά ή/και βιοδιεγερτικά μίγματα πολλαπλών στελεχών, επίσης ενισχύουν την τάση αυτή. Ένα σημαντικό ζήτημα για το σχεδιασμό ενός πιο αποτελεσματικού και σταθερού συνθετικού μίγματος πολλαπλών στελεχών, αποτελεί η συμβατότητα μεταξύ των μικροβιακών. Το παρόν άρθρο ανασκόπησης παρέχει μια διεξοδική βιβλιογραφική έρευνα που υποστηρίζει την άποψη ότι η μεταχείριση των φυτών με μίγματα πολλαπλών στελεχών, συμβατά μεταξύ τους, συμβάλει στην αποδοτικότητα ανάπτυξης και υγείας των φυτών υπό εφαρμογή μικροβιακών μιγμάτων. Η μελέτη μας επικεντρώνεται σε μίγματα πολλαπλών στελεχών που έχουν ως βάση στελέχη του γένους Pseudomonas και Bacillus καθώς και στελέχη μυκητών, ενώ γίνεται αναφορά σε διαθέσιμα εμπορικά σκευάσματα.

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