Improving performance of microbial biocontrol agents against plant diseases

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Abstract

Reducing dependence on chemical pesticides is considered as an essential challenge for sustainable crop production. The use of microbial biocontrol agents (MBCAs) is a key component of sustainable pest management. Numerous antagonistic microorganisms are known to suppress plant diseases, but their practical application and commercialization are still limited in part due to poor reliability of their efficacy in the field. Although promising MBCAs achieve remarkable disease control in the laboratory or greenhouse, field control is often unsatisfactory. Thus, for MBCAs to be integrated into crop production, their field performance must be improved to provide the cost-effectiveness and efficacy required by growers. In this review, we highlight recent approaches to enhance the field performance of MBCAs.

Keywords: Microbial biocontrol agents; Genetically engineered biocontrol agents; Abiotic stress improvement; Nutrient provisioning; Microbial mixtures; Biopesticide formulation technology

Introduction

Crop pests (diseases, insects, and weeds) cause estimated losses of 40% of annual global crop yields despite the annual application of about 3 billion tons of chemical pesticides worldwide (Messing and Brodeur 2018). Chemical pesticides have certainly contributed to increased crop productivity since the mid-1900s, but overuse and dependence on pesticides has led to environmental concerns and a prevalence of pesticide-resistant pests. The discovery and commercialization of new synthetic pesticides is increasingly more difficult and costly; more than 140,000 compounds might be screened to develop one new commercially acceptable pesticide after 10 years of work and more than US$250 million (Glare et al. 2012). Therefore, the development of alternative pest control measures has become an urgent priority for sustainable crop production and reduction of pesticide use to a bare minimum.

Integrated pest management (IPM) is now accepted practice to reduce dependency on chemical control. A key component of IPM is biocontrol using beneficial microorganisms. Growing interest in the exploitation of microbial biocontrol agents (MBCAs) to control of crop pests is evidenced by the vast number of books, reviews, and articles on this topic (Ab Rahman et al. 2018; Bardin et al. 2015; Bonaterra et al. 2012; Ehlers 2011; Hyakumachi et al. 2014; Maheshwari 2013; Massart et al. 2015; Narayanasamy 2013; Nicot 2011; Parnell et al. 2016; Sharma et al. 2009). Moreover, many large global companies have demonstrated a strong interest in developing microbial biocontrol products (MBPs) by acquiring small biopesticide companies and signing licensing agreements to distribute and sell MBPs developed by smaller companies (Pelaez and Mizukawa 2017). In 2012, the global biopesticide market was growing at a 15.6% compounded annual growth rate (Glare et al. 2012); 10 MBCAs

50 were registered between 1996 and 2000 in the European Union (EU) (Droby et al. 2016),
51 and 27 microbial fungicide products were newly approved in the last 15 years
52 (European Commission 2019).

53 Although demand for MBPs is increasing, developing a practical product is not easy
54 for a variety of reasons. Field performance of the MBP must be on par with existing
55 chemical pesticides, but most of the MBCAs isolated from natural environments tend to
56 be milder-acting and less stable than chemical pesticides in the field, and consequently,
57 lack reliability. In addition, they require careful handling during preservation and
58 transportation compared with chemicals. All these drawbacks must be overcome for
59 maximal adoption of MBPs in crop production.

60 Here, we review the most relevant scientific works concerning augmentative
61 biocontrol of plant diseases from the last decade. We discuss challenges for enhancing
62 performance of the biocontrol agents and suggest possible avenues to overcome these
63 challenges and develop practical MBPs.

64

65 **Improvement of abiotic stress tolerance of MBCAs**

66 MBCAs are exposed to diverse abiotic stresses such as drought, UV radiation, ambient
67 pH, and temperature changes after they are applied to soil or plants. These stresses may
68 negatively influence the persistence and performance of MBCAs; therefore, in addition
69 to biocontrol activity, environmental stress tolerance is a necessary attribute for
70 antagonistic microorganisms used as biocontrol agents. From this aspect, the
71 improvement of MBCA abiotic stress tolerance would help assure the desired biocontrol
72 performance in harsh conditions. Generally, two strategies have been used to improve
73 MBCA stress tolerance.

The first strategy is stress preconditioning of MBCAs. Microorganisms that survive a given stress often gain tolerance to that stress or other stresses via cross-protection (Wesch et al. 2009). By using this adaptive capability, MBCAs can be preconditioned against various stresses by exposing them to a sublethal (mild) stress during mass cultivation (Cañamás et al. 2009; Cheng et al. 2016; Daranas et al. 2018; Liu et al. 2012; Puopolo et al. 2015; Sartori et al. 2010; Wang et al. 2018). For example, Puopolo et al. (2015) demonstrated that UV resistance is elevated in *Lysobacter capsici* cultivated at 15°C exhibits compared with those grown at their optimal growth temperature of 25°C. In another example, Daranas et al. (2018) reported that preconditioning of *Lactobacillus plantarum* by incubation in a hyperosmotic and acidic broth enhanced desiccation tolerance.

The second strategy to improve stress tolerance is incorporating anti-stress protectants into MBCA cells. Survival of microorganisms under a variety of abiotic stresses is correlated with the intracellular accumulation of certain protectants (Potts 1994); microbes can take up high levels of exogenously applied protectants, which accumulate in the cytoplasm and enhance tolerance to abiotic stresses (Streeter 2003). Intracellular accumulation of protectants such as trehalose, glucose, and glycine betaine by their addition to culture media help biocontrol yeasts tolerate high/low temperature and oxidative stresses (Li and Tian 2006; Sui and Liu 2014; Sui et al. 2012).

Genetic engineering of MBCAs

Although only a few genetically modified microorganisms are commercially available as plant protection products, this approach may provide a powerful alternative to the development of chemical pesticides. Identifying genes associated with biocontrol

mechanisms might enhance the expression of biocontrol traits, and/or the genes could be integrated into a single MBCA. Increased biocontrol performance by genetic engineering can be achieved by enhancing the antagonistic ability or aggressiveness of MBCAs against pathogens (e.g., by production of antimicrobial compounds) and by enhancing the colonization ability of the MBCAs.

Biocontrol efficacy has already been improved by increasing the ability of MBCAs to produce antimicrobial substances such as antibiotics, hydrolytic enzymes, and bacteriocins (Bilal et al. 2017; Jing et al. 2018; Kowsari et al. 2014; Liu et al. 2016; Sun et al. 2017; Tang et al. 2019; Yang et al. 2017; Zembek et al. 2011; Zhou et al. 2014). For example, Jing et al. (2018) constructed a *retS* mutant of *Pseudomonas protegens* Pf-5 that produced higher levels of the antifungal metabolite 2,4-diacetylphloroglucinol and were significantly superior to the parent strain in suppressing *Rhizoctonia solani*. The introduction of foreign genes for antibiotic and hydrolytic enzyme biosynthesis has also increased biocontrol performance. A recombinant strain of *Pseudomonas fluorescens* that was constructed by the introduction of a seven-gene operon from *Pseudomonas synxantha* for the biosynthesis of phenazine-1-carboxylic acid suppressed take-all disease in wheat to a greater extent than the wild-type strain, which produces an antifungal cyclic lipopeptide (Yang et al. 2017). Similarly, the introduction of foreign genes that encode antifungal chitinase and glucanase into *Streptomyces* strains strengthens their biocontrol of fungal diseases (Li et al. 2015; Wu et al. 2013a, b, 2015a).

Colonization by biocontrol bacteria can be improved by manipulation of genes associated with the signaling pathways that operate during colonization, such as those for motility, chemotaxis, and biofilm formation. Barahona et al. (2011) reported that a

hypermotile *kinB*, *sadB, wspR* mutant of *P. fluorescens* was superior to the wild-type strain in colonizing the rhizosphere and controlling *Fusarium oxysporum* and *Phytophthora cactorum*. Flagellar motility and biofilm formation in *Bacillus* species are regulated by a two-component signal transduction system, DegU-DegS, and the DegQ protein enhances phosphorylation of DegU by DegS and consequently influences flagellar motility and biofilm formation. Xu et al. (2018) constructed a recombinant *Bacillus velezensis* strain in which *degQ* was replaced with a xylose-inducible *degQ*. They then showed that biofilm formation by this recombinant strain was induced in the presence of xylose, which is a typical carbohydrate secreted by plant roots. This strain colonized cucumber and tomato roots at significantly higher levels than the wild-type strain did, and their efficacy against cucumber Fusarium wilt and tomato bacterial wilt was also higher.

Nutrient provisioning and organic amendments

The persistence of MBCAs introduced into the field is a critical factor strongly associated with biocontrol performance. Supplementation with appropriate nutrients that are preferentially utilized by MBCAs, such as chitin, chitosan, L-arabinose, D-glucose, pectin, sucrose, mannitol, nicotine, riboflavin, glycine, and Tween 80 (Cabrefiga et al. 2011; Gramisci et al. 2018; Kang 2011; Kim et al. 2008; Postma et al. 2009; Ma et al. 2018a; Wu et al. 2015b; Yandigeri et al. 2015; Zhang et al. 2017a), support the growth of MBCAs in the rhizosphere and phyllosphere and enhance biocontrol. In an interesting study by Tomada et al. (2016), pea broth supplementation enhanced the efficacy of *L. capsici* against *Plasmopara viticola* by fostering cell movement on grapevine leaves. They found that pea broth triggered cell motility associated with the

146 biogenesis of type IV pili in the bacteria, which then facilitated leaf colonization. In this
147 context, careful comparison of the nutrient preferences of both the MBCAs and the
148 pathogens is essential during the screening of candidate nutrients because provisioning
149 of inappropriate nutrients might increase the pathogen aggressiveness and disease
150 incidence. Indeed, Gramisci et al. (2018) found that provisioning with the several
151 compounds that were utilized by both biocontrol yeasts (*Vishniacozyma victoriae* or
152 *Pichia membranifaciens*) and the pathogens (*Botrytis cinerea* or *Penicillium expansum*)
153 decreased biocontrol.

154 Nutrient provisioning to strengthen the aggressiveness of MBCAs against pathogens
155 is another approach to improving biocontrol. The biocontrol activity of bacteria was
156 improved by providing nutrients that stimulated the production of antimicrobial
157 compounds and hydrolytic enzymes at the target sites (Kang 2011; Wu et al. 2015b;
158 Yandigeri et al. 2015). For instance, provisioning with pectin increased the production
159 of the cyclic lipopeptide surfactin by *Bacillus amyloliquefaciens* in the tobacco
160 rhizosphere and improved biocontrol of bacterial wilt (Wu et al. 2015b).

161 Combining MBCAs with organic amendments (OAs) as a nutrient base might also be
162 a practical way to stabilize and/or enhance the disease control by MBCAs. The use of
163 certain OAs as MBCA carriers can also provide safe niches for MBCAs (Bonanomi et
164 al. 2018). These features of OAs improve the persistence of MBCAs in hostile
165 environments. There have been many examples of the successful combination of
166 bacterial and fungal biocontrol agents with OAs such as composts, manures, and
167 organic wastes (Ding et al. 2013; Gava and Pinto 2016; Huang et al. 2011, 2012; Ling et
168 al. 2012; Ma et al. 2018b; Rao et al. 2017; Ren et al. 2012; Sotoyama et al. 2017; Zhang
169 et al. 2017b). For consistent results with these combinations, OAs should have uniform

quality, because the chemical compositions and properties of OAs vary greatly with their origin and/or maturity level. Bonanomi et al. (2018) proposed the use of ^{13}C cross-polarized magic angle spinning nuclear magnetic resonance-based nutritional profiling to aid in the preliminary identification of OA chemical properties.

Combined application of multiple MBCAs

Combining two or more MBCAs can have possible synergistic biocontrol effects. Our co-inoculation of two antagonistic rhizobacteria, namely *Mitsuaria* sp. TWR114 and *Ralstonia* sp. TCR112, protected tomato plants from bacterial wilt for at least 4 weeks, whereas protection by the individual strains ended within 2 weeks (Marian et al. 2019). Similarly, synergistic action against mostly soil-borne pathogens has been obtained with various combinations of bacterial–bacterial, bacterial–fungal and fungal–fungal combinations of BCAs such as *Pseudomonas* + *Bacillus*, *Pseudomonas* + *Trichoderma*, *Serratia* + *Trichoderma* and *Glomus* + *Trichoderma* (Chemeltorit et al. 2017; Grosch et al. 2012; Jambhulkar et al. 2018; Kavino and Manoranjitha 2018; Manjukarunambika et al. 2013; Sennoi et al. 2013). As evidenced by these reports, certain combinations of MBCAs have the potential to generate a substantial synergistic effect. However, according to the literature review by Xu et al. (2011), 98% of past biocontrol studies using MBCA mixtures found only slight or no improvement in biocontrol efficacy. This lack may mainly be due to competition for spatial and nutritional niches and/or mutual antagonism among the selected microorganisms. Therefore, careful investigation of colonization site and nutrient utilization patterns of each MBCA and any antagonistic interactions among MBCAs are important to identify compatible combinations that produce the desired effectiveness. Additionally, unfavorable natural incompatibility

194 among MBCAs can be overcome by adjusting the inoculum ratio in mixed biocontrol
195 preparations. Singh et al. (1999) reported that the suppressive effect of a combination of
196 *Paenibacillus* and *Streptomyces* isolates against cucumber Fusarium wilt varied with
197 inoculum ratio: i.e., ratios of 1:1, 3:2, and 4:1 produced significantly higher efficacy
198 than individual isolates, whereas the suppressive effects of 2:3 and 1:4 ratios were
199 similar to that of the *Paenibacillus* isolate alone. We also reported that the combined
200 application of *Mitsuaria* and nonpathogenic *Ralstonia* isolates at a 2:1 ratio produced
201 the best suppression of tomato bacterial wilt among all the ratios tested (Marian et al.
202 2019). The reason for these effects of inoculum mixture ratios is not fully understood,
203 but does highlight the need for an in-depth understanding of the various interactions
204 between MBCAs, plants, and pathogens to develop a product with more reliable,
205 effective mixtures of MBCAs.
206

207 **Formulation procedures**

208 Product formulation has been recognized as the key to the commercial success of
209 MBCAs because they can affect many aspects of MBCA shelf life and field
210 performance (Fravel 2005). Although the details of the formulation process are often
211 company secrets and thus not generally accessible, many reports have addressed
212 formulation optimization (Aeron et al. 2011; Angeli et al. 2017; Bejarano et al. 2017;
213 Crozier et al. 2015; Segarra et al. 2015; Wei et al. 2015; Wiyono et al. 2008; Yang et al.
214 2011). Most MBCAs have been commercialized as wettable powders, liquids, or
215 granular formulations. Wettable powder formulations are the main form of biocontrol
216 pesticides because of their easy handling, lower storage and transportation costs, and
217 lower risk of contamination with undesirable microorganisms. Therefore, a great deal of

effort has been devoted to the technological improvement of commonly used drying methods, such as air-drying (Schisler et al. 2016), spray-drying (Meng et al. 2015), fluidized bed-drying (Carbó et al. 2017), freeze-drying (Zhan et al. 2012), and vacuum-drying (Melin et al. 2011). Low-temperature low-humidity drying (LTLHD) and fluid-bed spray-drying (FBSD) have also recently been investigated as alternative drying methods (Gotor-Vila et al. 2017a; Umashankar et al. 2018). Both methods use lower air temperatures for drying (50°C for LTLHD and 65°C for FBSD) compared with spray drying (100−200°C), thus facilitating the drying of heat-sensitive microorganisms such as Gram-negative bacteria and yeasts. Moreover, these drying methods enable a reduction of the drying time compared with conventional drying methods and thus cost less. Gotor-Vila et al. (2017a, b) examined the shelf life and biocontrol efficacy of *B. amyloliquefaciens*, subjected to liquid formulation, freeze-drying, and FBSD, and demonstrated the superiority of FBSD over the other methods. Generally, desiccation stress in the dry formulation process often causes serious damage to microbial cells, and thus decreases the viability of microorganisms, particularly non-sporulating bacteria (Berninger et al. 2018; Nocker et al. 2012). In this context, stress adaptation of microbial cells and the external addition of protectants during cultivation or before drying are feasible approaches to overcome this drawback of dry formulations. For example, osmoadaptation using NaCl and glycine betaine supplementation of the growth medium increased the survival of *Pantoea agglomerans* during freeze-drying and storage (Pusey and Wend 2012). The addition of fructose and trehalose before air-drying also improves the viability of several *P. fluorescens* strains (Schisler et al. 2016).

Encapsulation of MBCAs as beads or capsules is another promising formulation approach to improve stability and stress resistance (John et al. 2011; Locatelli et al.

2018; Ma et al. 2015). Encapsulation within a polymer matrix improves the resistance of microbial cells to abiotic stress factors such as dryness and temperature and extends the shelf life of the bead/capsule formulation without reducing the metabolic activity of active microbial ingredients (Vemmer and Patel 2013). Alginate is the preferred material for most encapsulations because it is nontoxic, biodegradable, and slowly releases the MBCAs into the soil. Although its high cost has markedly limited its commercial application, it is now relatively cheap (US$2/kg for a Chinese product), making encapsulation more feasible (Bashan 2016). Furthermore, blending alginate with other low-cost materials such as gelatin was demonstrated to be a feasible way to prepare uniform, rounded shape, and well-dispersed micron microcapsules of *Bacillus subtilis* via emulsification/internal gelation (Tu et al. 2015). Ma et al. (2015) reported that maltodextrin could be used for microencapsulation of biocontrol *Bacillus* strain as an alternative to alginate.

Three basic methods are used to formulate microbial cells in beads or capsules: physical processes such as spray-drying, spray-chilling/cooling, extrusion, or fluid bed spray coating; chemical processes such as co-crystallization, molecular inclusion, or interfacial polymerization; and also physiochemical processes such as coacervation, and gelation/inverse gelation (Schoebitz et al. 2013). Most encapsulation methods for MBCAs are based on the ionic gelation method due to its biocompatibility (Vemmer and Patel 2013). However, one of the biggest disadvantages of this method is that the beads are often porous to cells (Schoebitz et al. 2013). The addition of filler materials such as starch, kaolin, chitin, bentonite, or perlite to the formulations can produce more stable beads containing a high concentration of bacterial cells by improving bead mechanical strength (Li et al. 2016; Liffourrena and Lucchesi 2018; Schoebitz et al.

2013; Zohar-Perez et al. 2003). Many encapsulation devices are designed to produce beads in the laboratory and at a very small scale, so innovative encapsulation equipment that can produce large amounts of inoculum must also be designed (Schoebitz et al. 2013). Very recently, Strobel et al. (2018) successfully developed a novel and highly scalable single-step process that encapsulates Gram-negative bacteria in a cross-linked alginate matrix by spray-drying a mixture of bacterial suspension, alginate, insoluble $CaHPO_4$, and succinic acid that is atomized at the nozzle. As the droplets dry into microcapsules, vaporization of the volatile base reduces the pH, which dissolves $CaHPO_4$ and releases calcium ions, which cross-link the alginate. Another useful commercially available high-performance device for bead generation is based on a laminar jet break-up extrusion technique such as the jet-cutting technique developed by GeniaLab Biotechnologie (http://www.genialab.com/).

Multiple microorganisms have also been encapsulated together to achieve synergistic effects (De Jaeger et al. 2011; Loján et al. 2017) or with nutrients to preserve their viability and promote their proliferation (Kim et al. 2012). Encapsulation may, therefore, represent an innovative technology that can perhaps be fine-tuned to develop more efficient MBCA formulations.

Conclusion and future prospects

Reducing the dependency on chemical pesticides is a key issue for the sustainability of global crop production. Toward this goal, various countries, particularly in Europe, are promoting the use of MBCAs against crop diseases and insect pests as an alternative or supplement to chemical pesticides. Thus, the market for MBCAs in these countries has been rapidly growing. However, many other countries are lagging in the implementation of MBCAs. For example, in Japan, the proportion of biofungicide sales to total fungicide sales has remained low (ca. 0.6%–0.7%) over the last 17 years (Japan Plant Protection Association 2005, 2017). This lack of growth in the Japanese biopesticide market may be because the efficacy of MBCAs often does not meet expectations, and thus farmers and pesticide companies do not place much confidence in MBPs. However, biocontrol using beneficial microorganisms will undoubtedly become a more important tool for sustainable pest management worldwide. To develop stable, augmentative biocontrol measures and accelerate the commercialization of MBCAs as practical MBPs, further improving MBCA field performance, usability and cost are significant challenges that must be met. As noted in this review, these challenges can certainly be overcome by contriving methods of mass cultivation, formulation, and application of MBCAs based on the insights gained through current research into the physiology, metabolism, and genomics of these microorganisms and into the plant–microbe and microbe–microbe interactions. Although we did not discuss screening strategies to identify MBCAs, it is very important to select candidate strains from microbial assemblages that have the potential to survive in competitive microbial communities at the target sites. In this regard, studying plant microbiomes using advanced omic technologies will help in selecting the most suitable microbial assemblages among the

307 complex microflora of the rhizosphere, phyllosphere, or endosphere. Because plant-
308 associated bacteria play an important role in the disease resistance of resistant cultivars
309 (Kwak et al. 2018), combining MBCAs with plant cultivars that are genetically
310 compatible with the MBCAs may be a new approach for sustainable disease
311 management. As we discussed here, field performance and usability also need to be
312 improved and addressed from various perspectives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Ab Rahman SFS, Singh E, Pieterse CMJ, Schenk PM (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267:102–111

Aeron A, Dubey RC, Maheshwari DK, Pandey P, Bajpai VK, Kang SC (2011) Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L.). Eur J Plant Pathol 131:81–93

Angeli D, Saharan K, Segarra G, Sicher C, Pertot I (2017) Production of *Ampelomyces quisqualis* conidia in submerged fermentation and improvements in the formulation for increased shelf-life. Crop Protect 97:135–144

Barahona E, Navazo A, Martínez-Granero F, Zea-Bonilla T, Pérez-Jiménez RM, Martín M, Rivilla R (2011) *Pseudomonas fluorescens* F113 mutant with enhanced competitive colonization ability and improved biocontrol activity against fungal root pathogens. Appl Environ Microbiol 77:5412–5419

Bardin M, Ajouz S, Comby M, Lopez-Ferber M, Graillot B, Siegwart M, Nicot PC (2015) Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front Plant Sci 6:566

Bashan N (2016) Inoculant formulations are essential for successful inoculation with plant growth-promoting bacteria and business opportunities. Indian Phytopathol 69:739–743

Bejarano A, Sauer U, Preininger C (2017) Design and development of a workflow for microbial spray formulations including decision criteria. Appl Microbiol Biotechnol 101:7335–7346

347 Berninger T, González López Ó, Bejarano A, Preininger C, Sessitsch A (2018)
348 Maintenance and assessment of cell viability in formulation of non-sporulating
349 bacterial inoculants. Microb Biotechnol 11:277–301
350 Bilal M, Guo S, Iqbal HMN, Hu H, Wang W, Zhang X (2017) Engineering
351 *Pseudomonas* for phenazine biosynthesis, regulation, and biotechnological
352 applications: a review. World J Microbiol Biotechnol 33:191
353 Bonanomi G, Lorito M, Vinale F, Woo SL (2018) Organic amendments, beneficial
354 microbes, and soil microbiota: toward a unified framework for disease
355 suppression. Annu Rev Phytopathol 56:1–20
356 Bonaterra A, Badosa E, Cabrefiga J, Francés J, Montesinos E (2012) Prospects and
357 limitations of microbial pesticides for control of bacterial and fungal pomefruit tree
358 diseases. Trees 26:215–226
359 Cabrefiga J, Francés J, Montesinos E, Bonaterra A (2011) Improvement of fitness and
360 efficacy of a fire blight biocontrol agent via nutritional enhancement combined with
361 osmoadaptation. Appl Environ Microbiol 77:3174–3181
362 Cañamás TP, Viñas I, Abadias M, Usall J, Torres R, Teixidó N (2009) Acid tolerance
363 response induced in the biocontrol agent *Pantoea agglomerans* CPA-2 and effect on
364 its survival ability in acidic environments. Microbiol Res 164:438–450
365 Carbó A, Torres R, Usall J, Fons E, Teixidó N (2017) Dry formulations of the
366 biocontrol agent *Candida sake* CPA-1 using fluidised bed drying to control the main
367 postharvest diseases on fruits. J Sci Food Agric 97:3691–3698
368 Chemeltorit PP, Mutaqin KH, Widodo W (2017) Combining *Trichoderma hamatum*
369 THSW13 and *Pseudomonas aeruginosa* BJ10–86: a synergistic chili pepper seed
370 treatment for *Phytophthora capsici* infested soil. Eur J Plant Pathol 147:157–166

371 Cheng Z, Chi M, Li G, Chen H, Sui Y, Sun H, Wisniewski M, Liu Y, Liu J (2016) Heat
372 shock improves stress tolerance and biocontrol performance of *Rhodotorula*
373 *mucilaginosa*. Biol Control 95:49–56
374 Crozier J, Arroyo C, Morales H, Melnick RL, Strem MD, Vinyard BT, Bailey BA
375 (2015) The influence of formulation on *Trichoderma* biological activity and frosty
376 pod rot management in *Theobroma cacao*. Plant Pathol 64:1385–1395
377 Daranas N, Badosa E, Francés J, Montesinos E, Bonaterra A (2018) Enhancing water
378 stress tolerance improves fitness in biological control strains of *Lactobacillus*
379 *plantarum* in plant environments. PLoS ONE 13:e0190931
380 De Jaeger N, de la Providencia IE, Rouhier H, Declerck S (2011) Co-entrapment of
381 *Trichoderma harzianum* and *Glomus* sp. within alginate beads: impact on the
382 arbuscular mycorrhizal fungi life cycle. J Appl Microbiol 111:125–135
383 Ding C, Shen Q, Zhang R, Chen W (2013) Evaluation of rhizosphere bacteria and
384 derived bio-organic fertilizers as potential biocontrol agents against bacterial wilt
385 (*Ralstonia solanacearum*) of potato. Plant Soil 366:453–466
386 Droby S, Wisniewski M, Teixidó N, Spadaro D, Jijakli MH (2016) The science,
387 development, and commercialization of postharvest biocontrol products. Postharvest
388 Biol Technol 122:22–29
389 Ehlers RU (ed) (2011) Regulation of biological control agents. Springer, Berlin,
390 Germany
391 European Commission (2019) EU Pesticides database. European Commission, Brussels,
392 Belgium Available via http:// ec.europa.eu/food/plant/pesticides/eu-
393 pesticidesdatabase/public/?event=homepage&language=EN. Cited 10 March 2019

394 Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev
395 Phytopathol 43:337–359
396 Gava CAT, Pinto JM (2016) Biocontrol of melon wilt caused by *Fusarium oxysporum*
397 Schlect f. sp. *melonis* using seed treatment with *Trichoderma* spp. and liquid
398 compost. Biol Control 97:13–20
399 Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L,
400 Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30: 250–258
401 Gotor-Vila A, Usall J, Torres R, Abadias M, Teixidó N (2017a) Formulation of the
402 biocontrol agent *Bacillus amyloliquefaciens* CPA-8 using different approaches:
403 liquid, freeze-drying and fluid-bed spray-drying. BioControl 62:545–555
404 Gotor-Vila A, Usall J, Torres R, Solsona C, Teixidó N (2017b) Biocontrol products
405 based on *Bacillus amyloliquefaciens* CPA-8 using fluid-bed spray-drying process to
406 control postharvest brown rot in stone fruit. LWT-Food Sci Technol 82:274–282
407 Gramisci BR, Lutz MC, Lopes CA, Sangorrín MP (2018) Enhancing the efficacy of
408 yeast biocontrol agents against postharvest pathogens through nutrient profiling and
409 the use of other additives. Biol Control 121:151–158
410 Grosch R, Dealtry S, Schreiter S, Berg G, Mendonça-Hagler, L, Smalla K (2012)
411 Biocontrol of *Rhizoctonia solani*: complex interaction of biocontrol strains, pathogen
412 and indigenous microbial community in the rhizosphere of lettuce shown by
413 molecular methods. Plant Soil 361:343–357
414 Huang X, Chen L, Ran W, Shen Q, Yang X (2011) *Trichoderma harzianum* strain
415 SQR-T37 and its bio-organic fertilizer could control *Rhizoctonia solani* damping-off
416 disease in cucumber seedlings mainly by the mycoparasitism. Appl Microbiol
417 Biotechnol 91:741–755

418 Huang X, Zhang N, Yong X, Yang X, Shen Q (2012) Biocontrol of *Rhizoctonia solani*
419 damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. Microbiol
420 Res 167:135–143
421 Hyakumachi M, Takahashi H, Matsubara Y, Someya N, Shimizu M, Kobayashi K,
422 Nishiguchi M (2014) Recent studies on biological control of plant diseases in
423 Japan. J Gen Plant Pathol 80:287–302
424 Jambhulkar PP, Sharma P, Manokaran R, Lakshman DK, Rokadia P, Jambhulkar N
425 (2018) Assessing synergism of combined applications of *Trichoderma harzianum*
426 and *Pseudomonas fluorescens* to control blast and bacterial leaf blight of rice. Eur J
427 Plant Pathol 152:747–757
428 Japan Plant Protection Association (2005) The shipments of agricultural chemicals. In:
429 Japan Plant Protection Association (eds) Agricultural Chemicals Handbook. Tokyo,
430 Japan (in Japanese), pp 3–83
431 Japan Plant Protection Association (2017) The shipments of agricultural chemicals. In:
432 Japan Plant Protection Association (ed) Agricultural Chemicals Handbook. Tokyo,
433 Japan (in Japanese), pp 3–89
434 Jing X, Cui Q, Li X, Yin J, Ravichandran V, Pan D, Fu J, Tu Q, Wang H, Bian X,
435 Zhang Y (2018) Engineering *Pseudomonas protegens* Pf-5 to improve its antifungal
436 activity and nitrogen fixation. Microb Biotechnol. doi:10.1111/1751-7915.13335
437 John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D (2011) Bio-encapsulation of
438 microbial cells for targeted agricultural delivery. Crit Rev Biotechnol 31:211–226
439 Kang BR (2011) Mannitol amendment as a carbon source in a bean-based formulation
440 enhances biocontrol efficacy of a 2, 4-diacetylphloroglucinol-producing
441 *Pseudomonas* sp. nj134 against tomato Fusarium wilt. Plant Pathol J 27:390–395

442 Kavino M, Manoranjitham SK (2018) *In vitro* bacterization of banana (*Musa* spp.) with
443 native endophytic and rhizospheric bacterial isolates: Novel ways to combat
444 Fusarium wilt. Eur J Plant Pathol 151:371–387
445 Kim IY, Pusey PL, Zhao Y, Korban SS, Choi H, Kim KK (2012) Controlled release of
446 *Pantoea agglomerans* E325 for biocontrol of fire blight disease of apple. J Control
447 Release 161:109–115
448 Kim YS, Jang BR, Chung IM, Sang MK, Ku HM, Kim KD, Chun SC (2008)
449 Enhancement of biocontrol activity of antagonistic *Chryseobacterium* strain KJ1R5
450 by adding carbon sources against *Phytophthora capsici*. Plant Pathol J 24:164–170
451 Kowsari M, Zamani MR, Motallebi M (2014) Enhancement of *Trichoderma harzianum*
452 activity against *Sclerotinia sclerotiorum* by overexpression of Chit42. Iran J
453 Biotechnol 12:26–31
454 Kwak MJ, Kong HG, Choi K, Kwon SK, Song JY, Lee J, Lee PA, Choi SY, Seo M, Lee
455 HJ, Jung EJ (2018) Rhizosphere microbiome structure alters to enable wilt resistance
456 in tomato. Nat Biotechnol 36:1100–1109
457 Li BQ, Tian SP (2006) Effects of trehalose on stress tolerance and biocontrol efficacy of
458 *Cryptococcus laurentii*. J Appl Microbiol 100:854–861
459 Li J, Liu W, Luo L, Dong D, Liu T, Zhang T, Lu C, Liu D, Zhang D, Wu H (2015)
460 Expression of *Paenibacillus polymyxa* β-1, 3-1, 4-glucanase in *Streptomyces lydicus*
461 A01 improves its biocontrol effect against *Botrytis cinerea*. Biol Control 90:141–147
462 Li M, Tshabalala, MA, Buschle-Diller G (2016) Formulation and characterization of
463 polysaccharide beads for controlled release of plant growth regulators. J Mater
464 Sci 51:4609–4617

Liffourrena AS, Lucchesi GI (2018) Alginate-perlite encapsulated *Pseudomonas putida* A (ATCC 12633) cells: Preparation, characterization and potential use as plant inoculants. J Biotechnol 278:28–33

Ling N, Zhang W, Tan S, Huang Q, Shen Q (2012) Effect of the nursery application of bioorganic fertilizer on spatial distribution of *Fusarium oxysporum* f. sp. *niveum* and its antagonistic bacterium in the rhizosphere of watermelon. Appl Soil Ecol 59:13–19

Liu J, Wisniewski M, Droby S, Norelli J, Hershkovitz V, Tian S, Farrell R (2012) Increase in antioxidant gene transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* following sublethal oxidative stress exposure. FEMS Microbiol Ecol 80:578–590

Liu K, Hu H, Wang W, Zhang X (2016) Genetic engineering of *Pseudomonas chlororaphis* GP72 for the enhanced production of 2-hydroxyphenazine. Microb cell Fact 15:131

Locatelli GO, dos Santos GF, Botelho PS, Finkler CLL, Bueno LA (2018) Development of *Trichoderma* sp. formulations in encapsulated granules (CG) and evaluation of conidia shelf-life. Biol Control 117:21–29

Loján P, Demortier M, Velivelli SLS, Pfeiffer S, Suárez JP, De Vos P, Prestwich BD, Sessitsch A, Declerck S (2017) Impact of plant growth-promoting rhizobacteria on root colonization potential and life cycle of *Rhizophagus irregularis* following co-entrapment into alginate beads. J Appl Microbiol 122:429–440

Ma L, Zheng SC, Zhang TK, Liu ZY, Wang XJ, Zhou XK, Yang CG, Duo JL, Mo MH (2018a) Effect of nicotine from tobacco root exudates on chemotaxis, growth, biocontrol efficiency, and colonization by *Pseudomonas aeruginosa* NXHG29. Antonie van Leeuwenhoek 111:1237–1257

Ma L, Zhang HY, Zhou XK, Yang CG, Zheng SC, Duo JL, Mo MH (2018b) Biological control tobacco bacterial wilt and black shank and root colonization by bio-organic fertilizer containing bacterium *Pseudomonas aeruginosa* NXHG29. Appl Soil Ecol 129:136–144

Ma X, Wang X, Cheng J, Nie X, Yu X, Zhao Y, Wang W (2015) Microencapsulation of *Bacillus subtilis* B99-2 and its biocontrol efficiency against *Rhizoctonia solani* in tomato. Biol Control 90:34–41

Maheshwari DK (ed) (2013). Bacteria in agrobiology: disease management. Springer Berlin, Germany

Manjukarunambika K, Ponmurugan P, Marimuthu S (2013) Efficacy of various fungicides and indigenous biocontrol agents against red root rot disease of tea plants. Eur J Plant Pathol 137:67–78

Marian M, Morita A, Koyama H, Suga H, Shimizu M (2019) Enhanced biocontrol of tomato bacterial wilt using the combined application of *Mitsuaria* sp. TWR114 and nonpathogenic *Ralstonia* sp. TCR112. J Gen Plant Pathol 85:142–154

Massart S, Martinez-Medina M, Jijakli MH (2015) Biological control in the microbiome era: challenges and opportunities. Biol Control 89:98–108

Melin P, Schnürer J, Håkansson S (2011) Formulation and stabilisation of the biocontrol yeast *Pichia anomala*. Antonie van Leeuwenhoek 99:107–112

Meng X, Yu J, Yu M, Yin X, Liu Y (2015) Dry flowable formulations of antagonistic *Bacillus subtilis* strain T429 by spray drying to control rice blast disease. Biol Control 85:46–51

Messing R, Brodeur J (2018) Current challenges to the implementation of classical biological control. BioControl 63:1–9

513 Narayanasamy P (ed) (2013) Biological management of diseases of crops. Springer,
514 Dordrecht, Netherlands
515 Nicot P (ed) (2011) Classical and augmentative biological control against diseases and
516 pests: critical status analysis and review of factors influencing their success.
517 International Organisation for Biological and Integrated Control of Noxious Animals
518 and Plants, West Palaearctic Regional Section (IOBC-WPRS)Nocker A, Fernández
519 PS, Montijn R, Schuren F (2012) Effect of air drying on bacterial viability: a
520 multiparameter viability assessment. J Microbiol Methods 90:86–95
521 Parnell JJ, Berka R, Young HA, Sturino JM, Kang Y, Barnhart DM, DiLeo MV (2016)
522 From the lab to the farm: an industrial perspective of plant beneficial
523 microorganisms. Front Plant Sci 7:1110
524 Pelaez V, Mizukawa G (2017) Diversification strategies in the pesticide industry: from
525 seeds to biopesticides. Ciênc Rural 47.doi: 10.1590/0103-8478cr20160007
526 Postma J, Stevens LH, Wiegers GL, Davelaar E, Nijhuis EH (2009) Biological control
527 of *Pythium aphanidermatum* in cucumber with a combined application of *Lysobacter*
528 *enzymogenes* strain 3.1 T8 and chitosan. Biol Control 48:301–309
529 Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58: 755–805
530 Puopolo G, Palmieri MC, Giovannini O, Pertot I (2015) Impact of temperature on the
531 survival and the biocontrol efficacy of *Lysobacter capsici* AZ78 against
532 *Phytophthora infestans*. BioControl 60:681–689
533 Pusey PL, Wend C (2012) Potential of osmoadaptation for improving *Pantoea*
534 *agglomerans* E325 as biocontrol agent for fire blight of apple and pear. Biol
535 Control 62:29–37

536 Rao MS, Kamalnath M, Umamaheswari R, Rajinikanth R, Prabu P, Priti K, Grace GN,
537 Chaya MK, Gopalakrishnan C (2017) *Bacillus subtilis* IIHR BS-2 enriched
538 vermicompost controls root knot nematode and soft rot disease complex in carrot. Sci
539 Hort 218:56–62
540 Ren X, Zhang N, Cao M, Wu K, Shen Q, Huang Q (2012) Biological control of tobacco
541 black shank and colonization of tobacco roots by a *Paenibacillus polymyxa* strain
542 C5. Biol Fertil Soils 48:613–620
543 Sartori M, Nesci A, Etcheverry M (2010) Impact of osmotic/matric stress and heat
544 shock on environmental tolerance induction of bacterial biocontrol agents against
545 *Fusarium verticillioides*. Res Microbiol 161:681–686
546 Schisler DA, Slininger PJ, Olsen NL (2016) Appraisal of selected osmoprotectants and
547 carriers for formulating Gram-negative biocontrol agents active against Fusarium dry
548 rot on potatoes in storage. Biol Control 98:1–10
549 Schoebitz M, López MD, Roldán A (2013) Bioencapsulation of microbial inoculants for
550 better soil–plant fertilization. A review. Agron Sustain Dev 33:751–765
551 Segarra G, Puopolo G, Giovannini O, Pertot I (2015) Stepwise flow diagram for the
552 development of formulations of non spore-forming bacteria against foliar pathogens:
553 the case of *Lysobacter capsici* AZ78. J Biotechnol 216:56–64
554 Sennoi R, Singkham N, Jogloy S, Boonlue S, Saksirirat W, Kesmala T, Patanothai A
555 (2013) Biological control of southern stem rot caused by *Sclerotium rolfsii* using
556 *Trichoderma harzianum* and arbuscular mycorrhizal fungi on Jerusalem artichoke
557 (*Helianthus tuberosus* L.). Crop Protect 54:148–153
558 Sharma RR, Singh D, Singh R (2009) Biological control of postharvest diseases of
559 fruits and vegetables by microbial antagonists: A review. Biol Control 50:205–221

560 Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of Fusarium wilt of
561 cucumber by chitinolytic bacteria. Phytopathology 89:92–99
562 Sotoyama K, Akutsu K, Nakajima M (2017) Suppression of bacterial wilt of tomato by
563 soil amendment with mushroom compost containing *Bacillus amyloliquefaciens*
564 IUMC7. J Gen Plant Pathol 83:51-55
565 Streeter JG (2003) Effect of trehalose on survival of *Bradyrhizobium japonicum* during
566 desiccation. J Appl Microbiol 95:484–491
567 Strobel SA, Allen K, Roberts C, Jimenez D, Scher HB, Jeoh T (2018) Industrially-
568 scalable microencapsulation of plant beneficial bacteria in dry cross-linked alginate
569 matrix. Ind Biotechnol 14:138–147
570 Sui Y, Liu J (2014) Effect of glucose on thermotolerance and biocontrol efficacy of the
571 antagonistic yeast *Pichia guilliermondii*. Biol Control 74:59–64
572 Sui Y, Liu J, Wisniewski M, Droby S, Norelli J, Hershkovitz V (2012) Pretreatment of
573 the yeast antagonist, *Candida oleophila*, with glycine betaine increases oxidative
574 stress tolerance in the microenvironment of apple wounds. Int
575 J Food Microbiol 157:45–51
576 Sun ZB, Sun MH, Zhou M, Li SD (2017) Transformation of the endochitinase gene
577 Chi67-1 in *Clonostachys rosea* 67-1 increases its biocontrol activity against
578 *Sclerotinia sclerotiorum*. AMB Express 7:1
579 Tang B, Laborda P, Sun C, Xu G, Zhao Y, Liu F (2019) Improving the production of a
580 novel antifungal alteramide B in *Lysobacter enzymogenes* OH11 by strengthening
581 metabolic flux and precursor supply. Bioresour Technol 273:196–202

582 Tomada S, Puopolo G, Perazzolli M, Musetti R, Loi N, Pertot I (2016) Pea broth
583 enhances the biocontrol efficacy of *Lysobacter capsici* AZ78 by triggering cell
584 motility associated with biogenesis of type IV pilus. Front Microbiol 7:1136
585 Tu L, He Y, Yang H, Wu Z, Yi L (2015) Preparation and characterization of alginate–
586 gelatin microencapsulated *Bacillus subtilis* SL-13 by emulsification/internal gelation.
587 J Biomater Sci Polym Ed 26:735–749
588 Umashankar K, Chandralekha A, Dandavate T, Tavanandi HA, Raghavarao KSMS
589 (2018) A nonconventional method for drying of *Pseudomonas aeruginosa* and its
590 comparison with conventional methods. Dry Technol 1–15
591 Vemmer M, Patel AV (2013) Review of encapsulation methods suitable for microbial
592 biological control agents. Biol Control 67:380–389
593 Wang Y, Luo Y, Sui Y, Xie Z, Liu Y, Jiang M, Liu J (2018) Exposure of *Candida*
594 *oleophila* to sublethal salt stress induces an antioxidant response and improves
595 biocontrol efficacy. Biol Control 127:109–115
596 Wei Z, Huang J, Yang C, Xu Y, Shen Q, Chen W (2015) Screening of suitable carriers
597 for *Bacillus amyloliquefaciens* strain QL-18 to enhance the biocontrol of tomato
598 bacterial wilt. Crop Protect 75:96–103
599 Wesche AM, Gurtler JB, Marks BP, Ryser ET (2009) Stress, sublethal injury,
600 resuscitation, and virulence of bacterial foodborne pathogens. J Food Prot 72:1121–
601 1138
602 Wiyono S, Schulz DF, Wolf GA (2008) Improvement of the formulation and
603 antagonistic activity of *Pseudomonas fluorescens* B5 through selective additives in
604 the pelleting process. Biol Control 46:348–357

605 Wu H, Li J, Dong D, Liu T, Zhang T, Zhang D, Liu W (2015a) Heterologous
606 coexpression of *Vitreoscilla* hemoglobin and *Bacillus megaterium* glucanase in
607 *Streptomyces lydicus* A02 enhanced its production of antifungal metabolites. Enzyme
608 Microb Technol 81:80–87
609 Wu K, Fang Z, Guo R, Pan B, Shi W, Yuan S, Guan H, Gong M, Shen B, Shen Q
610 (2015b) Pectin enhances bio-control efficacy by inducing colonization and secretion
611 of secondary metabolites by *Bacillus amyloliquefaciens* SQY 162 in the rhizosphere
612 of tobacco. PLoS ONE 10:e0127418
613 Wu Q, Bai L, Liu W, Li Y, Lu C, Li Y, Fu K, Yu C, Chen J (2013a) Construction of a
614 *Streptomyces lydicus* A01 transformant with a *chit42* gene from *Trichoderma*
615 *harzianum* P1 and evaluation of its biocontrol activity against *Botrytis cinerea*. J
616 Microbiol 51:166–173
617 Wu Q, Bai L, Liu W, Li Y, Lu C, Li Y, Lin Z, Wang M, Xue C, Chen J (2013b)
618 Construction of *Streptomyces lydicus* A01 transformant with the *chit33* gene from
619 *Trichoderma harzianum* CECT2413 and its biocontrol effect on Fusaria. Chi Sci
620 Bull 58:3266–3273
621 Xu XM, Jeffries P, Pautasso M, Jeger MJ (2011) Combined use of biocontrol agents to
622 manage plant diseases in theory and practice. Phytopathology 101:1024–1031
623 Xu Z, Xie J, Zhang H, Wang D, Shen Q, Zhang R (2018) Enhanced control of plant wilt
624 disease by a xylose-inducible *degQ* gene engineered into *Bacillus velezensis* strain
625 SQR9XYQ. Phytopathology 109:36–43
626 Yandigeri MS, Malviya N, Solanki MK, Shrivastava P, Sivakumar G (2015)
627 Chitinolytic *Streptomyces vinaceusdrappus* S5MW2 isolated from Chilika lake, India

enhances plant growth and biocontrol efficacy through chitin supplementation against *Rhizoctonia solani*. World J Microbiol Biotechnol 31:1217–1225

Yang M, Mavrodi DV, Mavrodi OV, Thomashow LS, Weller DM (2017) Construction of a recombinant strain of *Pseudomonas fluorescens* producing both phenazine-1-carboxylic acid and cyclic lipopeptide for the biocontrol of take-all disease of wheat. Eur J Plant Pathol 149:683–694

Yang X, Chen L, Yong X, Shen Q (2011) Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against Fusarium wilt of cucumbers. Biol Fertil Soils 47:239–248

Zembek P, Perlińska-Lenart U, Brunner K, Reithner B, Palamarczyk G, Mach RL, Kruszewska, JS (2011) Elevated activity of dolichyl phosphate mannose synthase enhances biocontrol abilities of *Trichoderma atroviride*. Mol Plant–Microbe Interact 24:1522–1529

Zhan Y, Xu Q, Yang MM, Yang HT, Liu HX, Wang YP, Guo JH (2012) Screening of freeze-dried protective agents for the formulation of biocontrol strains, *Bacillus cereus* AR156, *Burkholderia vietnamiensis* B418 and *Pantoea agglomerans* 2Re40. Lett Appl Microbiol 54:10–17

Zhang C, Gao J, Han T, Tian X, Wang F (2017a) Integrated control of tobacco black shank by combined use of riboflavin and *Bacillus subtilis* strain Tpb55. BioControl 62:835–845

Zhang N, Pan R, Shen Y, Yuan J, Wang L, Luo X, Raza W, Ling N, Huang Q, Shen Q (2017b) Development of a novel bio-organic fertilizer for plant growth promotion and suppression of rhizome rot in ginger. Biol Control 114:97–105

651 Zhou TT, Li CY, Chen D, Wu K, Shen QR, Shen B (2014) *phlF*⁻ mutant of
652 *Pseudomonas fluorescens* J2 improved 2,4-DAPG biosynthesis and biocontrol
653 efficacy against tomato bacterial wilt. Biol Control 78:1–8
654 Zohar-Perez C, Chernin L, Chet I, Nussinovitch A (2003) Structure of dried cellular
655 alginate matrix containing fillers provides extra protection for microorganisms
656 against UVC radiation. Radiat Res 160:198–204