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*Bacillus subtilis* biofilm matrix components target seed oil bodies to promote growth and anti-fungal resistance in melon

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Beneficial microorganisms are used to stimulate the germination of seeds; however, their growth-promoting mechanisms remain largely unexplored. *Bacillus subtilis* is commonly found in association with different plant organs, providing protection against pathogens or stimulating plant growth. We report that application of *B. subtilis* to melon seeds results in genetic and physiological responses in seeds that alter the metabolic and developmental status in 5-d and 1-month-old plants upon germination. We analysed mutants in different components of the extracellular matrix of *B. subtilis* biofilms in interaction with seeds and found cooperation in bacterial colonization of seed storage tissues and growth promotion. Combining confocal microscopy with fluorogenic probes, we found that two specific components of the extracellular matrix, amyloid protein TasA and fengycin, differentially increased the concentrations of reactive oxygen species inside seeds. Further, using electron and fluorescence microscopy and metabolomics, we showed that both TasA and fengycin targeted the oil bodies in the seed endosperm, resulting in specific changes in lipid metabolism and accumulation of glutathione-related molecules. In turn, this results in two different plant growth developmental programmes: TasA and fengycin stimulate the development of radicles, and fengycin alone stimulate the growth of adult plants and resistance in the phylloplane to the fungus *Botrytis cinerea*. Understanding mechanisms of bacterial growth promotion will enable the design of bespoke growth promotion strains.

Seed germination is a complex biological process that is controlled by interconnected hormone-regulated pathways. Seed inoculation with plant growth-promoting rhizobacteria is commonly used to enable bacterial colonization of plants and beneficial effects of plant growth-promoting rhizobacteria post germination. A detailed understanding of the mechanisms that underpin growth-promoting host–microbe interactions is important to enable design of innovative biotechnological probiotics.

*Bacillus subtilis* and closely related species coexist with plants, providing multiple beneficial services. Production of polyvalent secondary metabolites, sporulation or biofilm formation underpin bacterial fitness and bioactivity towards plants. Biofilms are formed by bacterial cells that are embedded in a secreted extracellular matrix (ECM) that is essential for efficient colonization of plant organs. The ECM also comprises secondary metabolites that mediate bacterial communication with the plants by triggering physiological responses associated with defence or growth.

Here we investigated the mechanism by which melon seeds respond to the stimulatory activity of *B. subtilis* and evaluated the functions of the ECM in this microbe–host interaction.

**Results**

*B. subtilis* regulates metabolism and growth of colonized plants. Beneficial bacteria can promote two different and genetically controlled stages of seed germination, namely germination itself and growth of the emergent radicle. Melon seeds treated with *B. subtilis* NCIB 3610 produced larger radicles compared with those grown from untreated seeds (Fig. 1a). However, the germination rates (initial emergence of the radicle) of treated seeds did not change compared with untreated seeds. We analysed the expression levels of *GA20ox1* and *CYP7A1*, which are two genes involved in the modulation of the determinant ratio of abscisic acid and gibberellins, and found that they are statistically similar between treated and untreated seeds (Extended Data Fig. 1a,b). Analysis of transcriptomes of three pools of seeds 16 h after treatment with a suspension of *B. subtilis* did not identify any changes in the expression levels of the genes involved in the germination-related hormone signalling pathway. However, the upregulation of plant genes involved in carbon metabolism and photosynthesis, together with repression of plant heat shock proteins and structural proteins of lipid storage vesicles (oleosin and caleosin), was detected and enabled activation of seed metabolism (Fig. 1b and Extended Data Fig. 1c,d).

Metabolomic profiles of treated and untreated seeds were compared to reveal increased abundance of energetic resources, such as glycerophospholipids and fatty acyls with analogy to triacylglycerides (TAGs) in the initial stages (0 h) after treatment with *B. subtilis* cells (Fig. 1c and Extended Data Fig. 2a). At 24 h after the treatment, metabolite levels were similar between both conditions,
although specific changes in the abundance of metabolites within these lipidic classes remained clear (Extended Data Fig. 2a).

These post-germination stimulatory effects of treatment with *B. subtilis* were long-lasting, considering that 1-month-old adult plants emerging from treated seeds developed a more vigorous radicular system and canopy than those emerging from untreated seeds (Fig. 1c). We analysed the metabolome of three adult plants that emerged from treated seeds to define putative metabolic changes that were associated with this long-lasting growth-promoting effect. Carboxylic acids, lipids and lipid-like molecules and organooxygen compounds were the main classes of metabolites differentially detected in aerial regions (leaves and stem) of plants grown from treated and untreated seeds (Extended Data Fig. 2b), while metabolomic composition of the roots was not significantly different between treated and untreated groups.

Changes in fatty acyls, carboxylic acids, organooxygen compounds and prenol lipids were the main metabolic signatures of the leaves from plants grown after bacterial treatment of the seeds (Fig. 1e). Our metabolomic analysis also revealed differential accumulation of 1-tryptophan and cinnamic acids in leaves of adult plants derived from treated seeds (Extended Data Fig. 2c). Both families of molecules are biomarkers of beneficial plant–bacteria interactions\(^\text{14,15}\).

**B. subtilis extracellular matrix and growth promotion.** We investigated the growth dynamics of the *B. subtilis* population during the first 5 d after seed treatment in the seed and emergent radicle (Fig. 2a) by colony-forming unit (c.f.u.) plating over seed and radicle extracts. In the radicles, the population remained unchanged and almost every cell sporulated upon radicle emergence (2 d after treatment). In the seeds, however, the bacterial population increased during the first 5 d post treatment and became almost entirely sporulated 4 d after bacterization. These results suggest that *B. subtilis* cells, mostly spores, may have been passively dragged by the emergent radicle or might have colonized and proliferated inside seeds. We used scanning electron microscopy analysis (SEM) of treated seeds to confirm that *B. subtilis* colonizes the inner regions of the seeds (Extended Data Fig. 3a). Confocal laser scanning microscopy (CLSM) analyses of transversely sectioned seeds previously treated with fluorescently labelled *B. subtilis* (CellTracker cm-DIH) indicated that bacterial cells accumulate in the storage tissues near the seed micropyle, which is a natural entry point for bacteria into seeds\(^\text{13}\) (Extended Data Fig. 3b). The cell densities of the wild-type (WT) strain and ∆hag (flagellum), ∆fps or ∆srf mutant strains, which are known to have altered swimming, sliding or swarming motility, respectively\(^\text{16–18}\), were unchanged in two differentiated parts inside the seeds (Fig. 2b,c). Overall, these findings reveal that growth-promoting activity might be triggered by bacterial cells that enter and colonize the seed storage tissues, which is a process that does not appear to rely specifically on any one type of bacterial motility.

Several studies have reported multifaceted contributions of bacterial biofilms to interactions with hosts\(^\text{1,19–21}\). The cell densities of single structural mutants ∆srfA, ∆tapA, ∆fps and ∆bslA, which form altered biofilms, were substantially decreased in seed extracts compared with that of the wild-type bacteria (set at 100%) 5 d after seed treatment (Fig. 2d). However, this result was not associated with the attachment of bacterial cells to the seeds a few hours after treatments (Extended Data Fig. 3c).

A similar bacterial population pattern was monitored in the emergent radicles, which, as described above for WT, may reflect dragging along of bacterial cells (Extended Data Fig. 3d). The ∆srf, ∆fps or ∆bps strains, which do not produce the secondary metabolites surfactin, bacillicene or fengycin, respectively (also present in the ECM), had unchanged population dynamics compared with the WT strain (Fig. 2d and Extended Data Fig. 3d). The decrease in the bacterial cell population size found in the structural mutants suggests that mutations led to a significant decrease in radicle growth promotion compared with treatment with the wild-type strain (Fig. 2e). In contrast to a correlation between bacterial cell density and plant growth promotion, the poorly persisting ∆srfA strain retained promoting activity, while the ∆fps and ∆bps strains failed to promote radicle growth despite considerable persistence on the seeds (Fig. 2d,e).

The absence of the amyloid protein TasA has recently been reported to provoke important cellular and physiological changes in *B. subtilis*. To clarify whether the role of TasA in the structure of the ECM is related to TasA-dependent growth-promoting activity, we used *B. subtilis* strain JC81, which expresses a version of TasA that fails to restore biofilm formation but reverts the physiological status of ∆srfA cells to wild-type levels\(^\text{8}\). Treatment of seeds with JC81 failed to promote radicle growth, but the bacteria persisted on the seeds at a level comparable to that of the WT. ∆fps or ∆bps strains (Fig. 2d,e).

These results led us to propose three distinct and complementary contributions of ECM components to the promotion of seed radicle growth: first, EPS, BsA and TapA have a role in the persistence of *B. subtilis* cells in the seeds; second, fengycin (fps) and bacillicene (bps) have roles in the chemical dialogue of *Bacillus* with seeds; and third amyloid TasA is involved in both of these functions.

Comparison of the metabolic status of radicles 5 d after treatment of seeds with WT or various ECM mutant strains demonstrated a specific metabolic response of plants to chemicals present in the *B. subtilis* ECM (Extended Data Fig. 4a). The changes associated with bacterial treatment of seeds included a decrease in organooxygen

Fig. 1 | Interaction of *B. subtilis* with the seeds stimulates radicle development and results in growth-promoting effect on adult plants. a, Left: average ± s.d. radicle areas after seed treatments with *B. subtilis*. Statistical significance was assessed by a two-tailed t-test (n = 8; P = 0.0056). Right: representative radicles from untreated seed (left) and a *B. subtilis*-treated seed (right) 5 d after treatments. b, Volcano plot of DEGs identified by RNA-sequencing in bacterized seeds and untreated seeds 16 h after treatment. P values were calculated on the basis of the Fisher method using nominal P values provided by edgeR and DESeq2. Dashed lines represent the threshold defined for P (horizontal) and fold change (vertical) for a gene to be considered as DEG. Tags label the genes related to seed germination progress: OEE1, oxygen-evolving enhancer protein 1; OEE2-1, oxygen-evolving enhancer protein 2-1, chloroplastic; OEE2-2, oxygen-evolving enhancer 2-1, chloroplastic; Fd-like, ferredoxin-like; PSD2, photosystem I reaction centre subunit II, chloroplastic; Gdh, glutamate dehydrogenase; rbcL, ribulose bisphosphate carboxylase small chain; ppdK, pyruvate, phosphate dikinase; gckG, phosphoenolpyruvate carboxykinase; Clo, caleosin; Hisp70_2, heat shock 70 kDa protein_2; Hsp, class I heat shock protein; Hisp70, heat shock 70 kDa protein_1; and Hisp22, 22.0 kDa class IV heat shock protein. NS, not significant. c, Molecular families corresponding to fatty acyls analogous to triacylglycerides and glycerophospholipids differentially abundant in seedsOh after seed bacterization. Pie charts inside the nodes indicate the mean of the peak abundance of each metabolite in the corresponding node. Node shape indicates the level of identification for ref. \(^\text{17}\). The chemical structures of annotated features based on spectral matches to GNPS libraries are also presented for each molecular family. POPC, 1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine; PC, phosphocholine. d, Adult plants grown from seeds treated with *B. subtilis* (3610, right) or from untreated seeds (control, left). e, Circos plot showing the top 100 metabolites significantly more abundant in leaves of plants grown from bacterized seeds versus those in leaves of the control plants. Ribbon colours refer to the class of each metabolite, and thickness is proportional to the log FC values. The curved colour bar shows the contribution of each metabolite (rectangles inside the bar coloured according to their chemical class) to the total abundance (100%), ordered from highest to lowest contribution.
compound analogues of sphinganines and an increase in abundance of the prenol lipid and stilbene molecular families (Extended Data Fig. 4b). The presence of a functional ECM in the WT strain triggered the accumulation of fatty acyls belonging to two different clusters, and treatments with ECM mutants produced a clear decrease in organooxygen compounds and accumulation of specific prenol lipids (Extended Data Fig. 4b).

Lysophospholipids, glutathione and growth promotion. According to our data, TasA and fengycin are highly relevant molecules of the ECM with regards to growth promotion of \textit{B. subtilis} in seeds. TasA, in common with other amyloids, is a polymorphic protein that can adopt a variety of structural conformations that have different biochemistries and functions\textsuperscript{6,22,23}. We polymerized purified homogeneous TasA monomers to show that large aggregates...
stimulate radicle growth (Extended Data Fig. 5a). Moreover, fresh apoplast fluid extracted from melon seeds promoted the polymerization of TasA into aggregates (Extended Data Fig. 5b), indicating that this largely active polymerized form of TasA may predominate inside seeds. Evaluation of stimulatory activity showed that a 3 µM solution of the most active form of TasA or a 10 µM solution of puri-
Fig. 3 | Amyloid TasA and fengycin stimulate radicle development. a. Left: percentage of radicle growth promotion of seeds treated with purified ECM components normalized to the average radicle area of untreated seeds (100%, dotted line) 5 d after treatments. Average values ± s.d. are shown. Statistical significance was assessed by one-way ANOVA with post hoc Dunnett’s multiple comparisons test (each treatment vs control) (n = 64 in control, n = 8 and n = 27 in Fengycin 1 µM and 10 µM, respectively, n = 12 and n = 10 in TasA 1.5 µM and 3 µM, respectively, n = 4 in BslA 25 µM and n = 22 in surfactin 20 µM; *P = 0.0104 in Fen10 µM, **P = 0.0313 in TasA 3 µM). Right: representative radicles of untreated seeds or seeds treated with purified ECM 5 d after treatments. b. Molecular families corresponding to LysoPE and GSH. Pie charts represent the mean peak abundance of metabolites in each condition. Node shape indicates the level of identification according to ref. 78. c. Top: representative radicles treated with water (C), LysoPE and GSH 5 d after treatment. Bottom: left Y axis, percentage of radicle growth promotion of seeds treated with water or purified LysoPE and GSH normalized to the control radicle area (100%, dotted line) 5 d after treatment. Statistical significance was assessed by one-way ANOVA with post hoc Dunnett’s multiple comparisons test (each treatment vs control) (n = 24 in control, n = 8 in LysoPE and GSH; *P = 0.0451 in LysoPE, **P = 0.0021 in LysoPE); right Y axis, percentage of weight of adult plants grown from seeds treated with LysoPE and GSH, normalized to the weight of control plants (100%, dotted line) (n = 12 in control, n = 5 in LysoPE and n = 8 in GSH). Average values ± s.d. are shown. d. Representative CLSM images of the inner tissues of dihydrorhodamine-stained seeds immediately after seed treatments with water (control), fengycin or TasA. Graph represents the intensity of the fluorescence in each condition, measured as the mean grey value from n = 30 random sections from 3 different fields. Average values ± s.d. are shown. Statistical significance was assessed by one-way ANOVA with multiple comparisons test (P < 0.0001).
fied fengycin significantly increased the area of the radicles compared with untreated seeds (Fig. 3a). The lack of stimulatory activity of purified BsLA or surfactin confirmed our previous findings using B. subtilis mutants (Figs. 2e and 3a).

The metabolome of individual radicles (n = 3) that emerged from seeds treated with fengycin or TasA was analysed to define their contributions to the metabolic signatures associated with the promotion of radicle growth. Results of principal component analysis and heatmap analyses indicated sample clustering in three groups (Extended Data Fig. 6a,b). Statistical analysis by partial least-squares discriminant analysis indicated that glycerophospholipids, fatty acyls, organooxygen compounds or carboxylic acids were discriminating metabolites in the case of TasA or fengycin treatments, and feature-based molecular networking of these features showed integration into molecular families composed of other metabolites that generally followed the same abundance patterns (Extended Data Fig. 6c–e). Further refinement of this analysis identified two molecular families of interest: lysophosphatidylethanolamine (LysOPE), mainly associated with TasA treatment, and an analogue of reduced glutathione (GSH) that accumulated in the radicles grown from seeds treated with fengycin (Fig. 3b). The treatment of seeds with commercially available LysOPE or GSH increased radicle growth as much as treatment with fengycin or TasA, but did not produce the long-term promoting effects observed in plants 1 month after treatment of seeds with B. subtilis (Fig. 3d).

GSH is an essential and polypeptide metabolite with special antioxidant functions, which maintains cellular redox homeostasis and development, growth or response of plants to a variety of stimuli. Further, an increase in the total pool of glutathione usually occurs 3 to 4 d after ROS exposure, therefore we hypothesized that one possible outcome of fengycin treatment might be ROS production. The level of ROS in seeds after treatment with fengycin was statistically higher than that in untreated seeds. An intermediate increase in ROS, which was statistically less than that elicited by fengycin, was also observed in seeds treated with TasA (Fig. 3d).

TasA and fengycin differentially target seed oil bodies. Accumulation of bacterial cells in the micropylar endosperm (Extended Data Fig. 3b), accumulation of TAGs (Fig. 1c) and changes in the lipid composition of the radicles and adult plants after treatment of seeds with bacteria or ECM components (Extended Data Figs. 2b and 6d,e) led us to hypothesize that seed oil bodies (OBs), which are nutrient reservoir organelles mainly composed of TAGs and other neutral lipids, are the target of fengycin and TasA. During germination, OBs are degraded by interaction with glyoxysomes that feed the plant embryo and varies between species; however, we propose that high affinity of fengycin for lipid membranes explains disaggregation and a reduction in the size of OBs. Fengycin has been described to efficiently interact with and disrupt artificial lipid monolayers or bilayer membranes in a concentration-dependent manner. In addition to micellar concentrations of fengycin, specific disruption of the membranes relies on the lipid composition of the target. Exact chemical composition of phospholipids of the membranes of OBs in the seeds is not known and varies between species; however, we propose that high affinity of fengycin for lipid membranes explains disaggregation and a reduction in the size of OBs.

Non-targeted metabolomic analyses provided additional information about the change in relative abundance of metabolites and potential (bio)chemical modifications of seed metabolites from 0 to 24 h after treatment with TasA or fengycin. The use of the chemical proportionality tool (ChemProp) showed putative changes in the dynamics of TAGs analogues after seed treatments (Fig. 4d). In
control seeds, many modifications in this molecular family denoted an important catalytic activity occurring over key storage metabolites in this time period. Treatments with TasA and, more notably, with fengycin led to an increase in the initial abundance of many metabolites (Fig. 4d, floating bar plots) that would reflect the faster metabolic activity of these seeds. A decrease in the Chemprop score...
indicated that the significance of the changes between 0h and 24h would be directly related with the earlier initiation of the catalytic activity over these molecules. Similar changes to TAGs were also remarkable in the dynamics of glycerophospholipids (Extended Data Fig. 8b).

**Fengycin promotes plant growth and resistance to Botrytis cinerea.** Plants that emerged from seeds treated with fengycin were larger than plants grown from untreated or TasA-treated seeds (Fig. 5a). Adult plants grown from seeds treated with LysoPE or GSH, however, did not show a significant increase in growth over time compared with plants from untreated seeds (Fig. 5c). These findings indicate that the presence of different signalling events underpin short-term radicle growth mediated by TasA, fengycin or associated LysoPE and GSH molecules, as well as long-term growth of adult plants specifically associated with fengycin. GSH associated with treatments of the seed with fengycin did not produce a sustained long-term growth-promoting effect, suggesting that a constant endogenous trigger is required that can only be achieved by fengycin treatment. Both TasA and fengycin increased the level of

**Fig. 5 | Seed treatment with fengycin stimulates the growth and immunization of adult plants.** a. Left: representative adult plants grown from seeds treated with water (control), fengycin or TasA. Right: percentage of the weight of plants grown from seeds treated with water (control), 10μM fengycin or 3μM TasA normalized to the average weight of plants grown from control seeds (100%, dotted line). Average values ± s.d. are shown. Statistical significance was assessed by one-way ANOVA with Dunnett’s multiple comparisons test (each treatment vs control treatment, n = 4, **P** = 0.0021). b. Left: necrotic symptoms in leaves of adult plants grown from seeds treated with water (control), fengycin or TasA 72h after treatment with B. cinerea spores. Right: percentage of disease calculated by measuring the lesion areas and normalizing to the average lesion area of the control leaves (100%, dotted line). Average values ± s.d. are shown. Statistical significance was assessed by one-way ANOVA with Dunnett’s multiple comparisons test (each treatment vs control treatment, n = 28 in control, n = 13 in TasA and n = 19 in Fengycin; **P** < 0.00043). c. Volcano plot representation of DEGs identified by total transcriptome analysis in leaves of plants grown from control seeds or seeds treated with fengycin 48h after treatment with B. cinerea spores. P values were calculated on the basis of the Fisher method using nominal P values provided by edgeR and DEseq2. Tags label the genes related to glutathione metabolism: GST1,2,3,4,5,6 (glutathione S-transferases 1 to 6), GSTL3 (glutathione S-transferase L3-like), GSTU81,2 (glutathione S-transferases UB-like 1 and 2) and MGST3 (microsomal glutathione S-transferase 3). Dashed lines represent the threshold defined for P (horizontal) and fold change (vertical) for a gene to be considered as DEG. d. Molecular family of oxidized glutathione and related metabolites differentially abundant in aerial regions of seedlings 120 h after seed treatment with fengycin. Pie charts indicate the mean peak abundance of metabolites in each condition. Node shape indicates the level of identification according to ref. 76.
ROS inside seeds, but the highest response was elicited by fengycin (Fig. 3d). ROS production is a normal response after the first water uptake during seed imbibition and, in a certain range of concentrations, ROS has a well-recognized role in endosperm weakening, protection against pathogens, mobilization of seed reserves and interaction with plant hormones during the process of seed germination\(^45-47\). These results suggest that the increase in ROS levels after seed treatment with fengycin, but not TasA, may act as a beneficial stimulus for further plant development mediated by GSH accumulation.

Previous studies revealed that application of fengycin elicits plant defence responses in adult plants\(^35-37\). Therefore, we investigated whether treatment of seeds with fengycin immunized adult plants against aboveground pathogens by inoculating the third leaf of adult plants with a spore suspension of Botrytis cinerea. The size of necrotic lesions induced by the fungus in plants grown from fengycin-treated seeds was significantly smaller than that of control plants or plants grown from TasA-treated seeds (Fig. 5b). Transcriptomic analyses of infected leaves revealed a high basal level of transcripts, some of them related to plant defence mechanisms such as Allene oxide cyclase/synthase, directly implied in the biosynthesis of jasmonic acid\(^39\) (Supplementary Table 5) in leaves of plants emerged from seeds treated with fengycin. Their expression was maintained or increased 48 h after inoculation with B. cinerea, while an increase was found in adult plants from non-treated seeds as a result of the response to the infection. Second, upregulation of the genes related to glutathione metabolism, specifically including glutathione S-transferases (GSTs), occurred 48 h after the challenge with B. cinerea (Fig. 5c and Extended Data Fig. 9a). Participation of GSTs in antioxidant reactions, together with the important cellular antioxidant GSH, has been proven to mitigate oxidative stress caused by necrotrophic fungus B. cinerea in infected tissues\(^48\). Metabolomic data from the radicles of fengycin-treated seeds demonstrated an increase in the levels of certain molecules of the same cluster that included GSH (Fig. 3b), and adult plants grown from bacterized seeds accumulated certain molecules belonging to the GSH cluster, especially in the aerial region (Extended Data Fig. 9b). In addition, metabolomes of aerial parts of seedlings 5 d after treatment with fengycin also showed an increase in oxidized glutathione and glutathione-related metabolites in addition to defence-related compounds such as flavonoids (that is, mangiferin and 2-O-rhamnosilvitexin) (Fig. 5d and Extended Data Fig. 9c).

Discussion

In this study, we demonstrated that in addition to a structural role of bacterial ECM in biofilm formation, it is also essential for Bacillus colonization of melon seeds and growth promotion, including induction of anti-fungal resistance in the adult plant. Our findings indicate that the beneficial effects of B. subtilis treatment on melon seeds are associated with changes in the initial ROS increase during seed imbibition, and the contents and specific pools of metabolites released from storage tissues, which were mediated by at least two ECM components, fengycin and TasA.

We propose that specific interactions of TasA or fengycin with OBs defines two different physiological responses. First, a short-term effect on radicle growth, mediated by both components and related to changes in the dynamics of TAGs after differential targeting of oil bodies and lipid catabolism optimization. On one hand, the oleosin–TasA interaction determines the OB aggregations around glyoxysomes and the subsequent accumulation of lular antioxidant GSH, has been proven to mitigate oxidative stress caused by necrotrophic fungus B. cinerea in infected tissues\(^48\). Metabolomic data from the radicles of fengycin-treated seeds demonstrated an increase in the levels of certain molecules of the same cluster that included GSH (Fig. 3b), and adult plants grown from bacterized seeds accumulated certain molecules belonging to the GSH cluster, especially in the aerial region (Extended Data Fig. 9b). In addition, metabolomes of aerial parts of seedlings 5 d after treatment with fengycin also showed an increase in oxidized glutathione and glutathione-related metabolites in addition to defence-related compounds such as flavonoids (that is, mangiferin and 2-O-rhamnosilvitexin) (Fig. 5d and Extended Data Fig. 9c).

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We propose that specific interactions of TasA or fengycin with OBs defines two different physiological responses. First, a short-term effect on radicle growth, mediated by both components and related to changes in the dynamics of TAGs after differential targeting of oil bodies and lipid catabolism optimization. On one hand, the oleosin–TasA interaction determines the OB aggregations around glyoxysomes and the subsequent accumulation of
lysophospholipids acting as signal molecules for growth. On the other hand, the OB disaggrecations caused by the action of fengycin over membranes determine a faster mobilization of their contents. Second, a long-term effect of growth promotion that persists in adult plants and is mediated by fengycin, through an initial increase in ROS that causes accumulation of GSH that is maintained over time and makes the plant more tolerant to biotic stresses (Fig. 6). Glycophospholipids have both structural and signalling roles in plants, and this bifunctionality is in part due to continuous synthesis and turnover of endogenous pools41. Accumulating evidence suggests a role for lysophospholipid derivatives in signalling processes in plant cells. LysoPE treatment has been reported to delay fruit softening when used postharvest, mitigate defoliation effects of ethephon, and delay leaf and fruit senescence in tomato and potato2,21, and is used commercially as a plant bioregulator to improve plant product quality21,41,43. On the other hand, glutathione is an essential metabolite with antioxidant properties, and is involved in cellular redox homeostasis and many other physiological processes such as the development, growth and environmental response of plants42. The accumulation of glutathione-related metabolites in radicles and aerial parts of seedlings, and the higher expression of GSTs in leaves after seed treatment with fengycin, suggest that initial treatment causing an increase in ROS might act as a beneficial stimulus for plant development, and confer enhanced antioxidant capacity to mitigate an imbalance in the redox status in adult plants imposed by infection with B. cinerea.

We propose that only those seeds containing abundant OBs, and characterized by specific morphology in their primordial tissues, respond to the beneficial interaction with B. subtilis, and that this interaction is mediated at least in part by fengycin and TasA. According to this model, wheat or maize, which are monocotyledous plants whose seeds are composed of starchy endosperm surrounded by a layer of living cells, did not react to the presence of fengycin, whereas cucumber or soybean seeds, which are anatomically different but do contain OBs, responded with a presence of fengycin, whereas cucumber or soybean seeds, which are anatomically different but do contain OBs, responded with a presence of fengycin.

**Methods**

**Strains, media and culture conditions.** The bacterial strains used in the present study are listed in Supplementary Table 1. Bacterial cultures were grown at 37°C from frozen stocks on lysogeny broth (LB: 1% tryptone (Oxoid), 0.5% yeast extract (Oxoid) and 0.5% NaCl) plates. Isolated bacteria were inoculated in appropriate media. The necrotrophic fungus B. cinerea was grown at 25°C from a frozen stock in potato dextrose agar (PDA) plates and maintained until inoculum preparation. Escherichia coli DH5α was used for cloning and plasmid replication. E. coli BL21(DE3) and BL21AI were used for protein purification. The final antibiotic concentrations for E. coli BL21AI were: MLS (1 μg ml⁻¹), spectinomycin (100 μg ml⁻¹), and 0.2 mg ml⁻¹ ampicillin, 5 μg ml⁻¹ chloramphenicol and 100 μM imidazole, and incubated at 37°C in an orbital shaker to reach an OD₆₀₀ of 0.7. Then, the expression of recombinant protein was induced by 0.2% l-arabinose. After 4 h, the cells were collected and resuspended in buffer A (20 mM sodium phosphate, 500 mM NaCl and 20 mM imidazole) containing 400 μl 0.1 M phenylmethylsulfonyl fluoride, 40 μl lysozyme and 4.45 μl cellulysin 10x (Sigma-Aldrich). After a 15 min incubation with shaking at room temperature, the cells were disrupted by sonication (3 pulses of 1 min at an amplitude of 80%). The cell lysate was centrifuged at 12,000 g for 15 min at 20°C, and the supernatant was filtered through a 0.45-μm-pore filter. The protein was purified using an AKTA Start fast protein liquid chromatography system (GE Healthcare). The solution was loaded into a 5 ml HiTrap HP column (GE Healthcare) previously equilibrated with buffer A. The protein was eluted from the column with elution buffer (20 mM sodium phosphate, 500 mM NaCl and 500 mM imidazole, pH 8). After affinity chromatography, the purified protein was loaded into a HiPrep 26/10 desalting column (GE Healthcare), and the buffer was exchanged for 20 mM Tris and 50 mM NaCl.

Oleosin protein was purified from inclusion bodies using freshly transformed E. coli Lemo21(DE3) cells expressing the pDEST17 plasmid harbouring the sequence of OLEOSIN1 from Arabidopsis thaliana, which was initially subcloned in the donor vector pENTR23 acquired from Arabidopsis Biological Resource Center using a Gateway recombination system. E. coli Lemo21(DE3) colonies were picked and resuspended in 500 ml LB containing 100 μg ml⁻¹ ampicillin, 5 μg ml⁻¹ chloramphenicol and 100 μM imidazole, and incubated at 37°C with orbital shaking to reach an OD₆₀₀ of 0.4. Then, the expression of the recombinant protein was induced by 400 μM isopropyl β-D-thiogalactopyranoside (IPTG). After 3 h, the cells were collected by centrifugation (5,000 g, 15 min, 4°C), resuspended in buffer A (50 mM Tris and 150 mM NaCl, pH 8) and then centrifuged again. The pellets were stored at −80°C until purification or processed after 15 min. After thawing, the cells were resuspended in buffer A, sonicated on ice (3× 45 s, 60% amplitude) and centrifuged (15,000 g, 60 min, 4°C). The supernatant was discarded because the protein was mainly expressed in inclusion bodies. The pellet was extensively washed with buffer A supplemented with 2% Triton X-100, incubated at 37°C with shaking for 20 min and centrifuged (15,000 g, 10 min, 4°C). The pellet was resuspended in buffer A supplemented with 2% Triton X-100, incubated at 37°C with shaking for 20 min and centrifuged (15,000 g, 10 min, 4°C). The pellet was then resuspended in denaturing buffer (50 mM Tris, 500 mM NaCl and 6 M GuHCl) and incubated at 60°C overnight for complete solubilization. The lysates were clarified by sonication on ice (3× 45 s, 60% amplitude) and centrifugation (15,000 g, 1 h, 16°C), and passed through a 0.45-μm filter before affinity chromatography. The protein was purified using a AKTA Start fast protein liquid chromatography system (GE Healthcare). Soluble inclusion bodies were loaded into a 5 ml HiTrap HP column (GE Healthcare) previously equilibrated with binding buffer (50 mM Tris, 0.5 M NaCl, 20 mM imidazole and 8 M urea, pH 8). The protein was eluted from the column with elution buffer (50 mM Tris, 0.5 M NaCl, 500 mM imidazole and 8 M urea, pH 8) and maintained under denaturing conditions.

**Apoplastic fluid extraction from the seeds.** The apoplastic fluid (AF) of the seeds was collected by a standard technique allowing the recovery of the apoplastic components present in the intercellular spaces based on vacuum infiltration and centrifugation. Brieﬂy, imbedded seeds were carefully decoted and immersed in inﬁltration buffer (50 mM Tris·HCl, pH 7.5, and 0.6% NaCl). Five vacuum pulses of 10 s (separated by 30 s intervals) were applied using a vacuum pump. Inﬁltrated seeds were recovered, dried on a ﬁlter paper, placed in plastic syringe barrels inside centrifuge tubes and centrifuged for 20 min at 400 g at 4°C. The AF was recovered.
**TasA polymerization assays.** The kinetics of polymerization of TasA were monitored using thioliavin T (TThI) and detected by fluorescence.²⁰ The assay was carried out in a 96-well microtitre plate. Briefly, purified TasA protein was diluted in buffer (20 mM Tris and 50 mM NaCl, pH 7) or AF to final concentrations of 0.4 mg mL⁻¹ or 0.2 mg mL⁻¹. TThI solution was added to the wells to a final TThI concentration of 20 μM in a final volume of 100 μL. The fluorescence signal was measured in a fluorescence microplate spectrophotometer reader (438 nm excitation and 495 nm emission, with a 475 nm cut-off) at 30 °C under shaking. Each protein concentration was assayed in triplicate.

**Infection assays in the plants.** Assays of B. cinerea infection were carried out in 5–6-week-old plants. Fungal conidia were collected from light-grown culture. For quantitative PCR with reverse transcription (RT–qPCR) assays, the RNA concentration was adjusted to 100 ng μL⁻¹. Then, 1 μg of DNA-free total RNA was reverse transcribed into complementary DNA using SuperScript III reverse transcriptase (Invitrogen) and random primers in a final reaction volume of 20 μL according to the manufacturer's instructions. The cDNA was subjected to a clean tube, dispersed in washing solution (7 M urea, 1:4 w/w) and centrifuged (10,000g, 30 min). To remove residue of washing solution, the fat pad was isolated and suspended in water (1:4 w/w) and centrifuged (10,000g, 30 min). After centrifugation, the creamy layer was collected and stored at 4 °C. Analyses were completed within 24 h.

**Pulldown assay.** To study possible TasA interactions with the proteins present in the seeds, we carried out a pulldown assay using a nickel-loaded affinity resin (Proton Ni-TED 2000, Machery-Nagel); purified TasA was used as a bait protein for protein extraction and the eluted fractions. Incubated and decoated seeds were ground to a fine powder using a mortar and a pestle in liquid nitrogen. The powder was transferred to a clean 15 ml tube and resuspended in extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM EDTA, 1% Triton X-100, 10% glycerol, 10 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF and 1% protease inhibitor cocktail). The samples were homogenized using a vortex, sonicated by 5 pulses of 20 s at 80% amplitude and centrifuged for 5 min at 9,000g at 4 °C. The supernatant was transferred to a new tube and used immediately. Purified TasA was added to the seed protein extract to a final concentration of 50 μM and incubated at 4 °C overnight. Then, the mixture was incubated with equilibrated resin at 4 °C overnight. Control resin that did not contain any protein was subjected to the seed protein extract. Flow-through, washing and elution fractions were collected and subjected to trichloroacetic acid protein precipitation. Precipitated proteins were resuspended in 1x Laemmli sample buffer (Bio-Rad) and heated at 100 °C for 5 min. The proteins were separated via SDS–PAGE through 12% polyacrylamide gels.

**Mass spectrometry analysis of protein bands.** The protein bands of interest were identified by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS). Briefly, the bands were cut out after electrophoresis, washed and destained. Subsequently, the disulfide bridges were reduced with DTT and cysteines were alkylated by iodoacetamide; in-gel tryptic digestion was performed to extract peptides from the protein samples. Mass spectrometry analysis of protein bands. The bands were cut out after electrophoresis, washed and destained. Subsequently, the disulfide bridges were reduced with DTT and cysteines were alkylated by iodoacetamide; in-gel tryptic digestion was performed to extract peptides from the protein samples. The samples were homogenized using a vortex, sonicated by 5 pulses of 20 s at 80% amplitude and centrifuged for 5 min at 9,000g at 4 °C. The supernatant was transferred to a new tube and used immediately. Purified TasA was added to the seed protein extract to a final concentration of 50 μM and incubated at 4 °C overnight. Then, the mixture was incubated with equilibrated resin at 4 °C overnight. Control resin that did not contain any protein was subjected to the seed protein extract. Flow-through, washing and elution fractions were collected and subjected to trichloroacetic acid protein precipitation. Precipitated proteins were resuspended in 1x Laemmli sample buffer (Bio-Rad) and heated at 100 °C for 5 min. The proteins were separated via SDS–PAGE through 12% polyacrylamide gels.

**Gene expression analysis by RT–qPCR.** Total RNA was extracted from the fungal tissues collected at various time points after the treatments. For quantitative PCR with reverse transcription (RT–qPCR) assays, the RNA concentration was adjusted to 100 ng μL⁻¹. Then, 1 μg of DNA-free total RNA was reverse transcribed into complementary DNA using SuperScript III reverse transcriptase (Invitrogen) and random primers in a final reaction volume of 20 μL according to the manufacturer's instructions. The cDNA was subjected to a clean tube, dispersed in washing solution (7 M urea, 1:4 w/w) and centrifuged (10,000g, 30 min). To remove residue of washing solution, the fat pad was isolated and suspended in water (1:4 w/w) and centrifuged (10,000g, 30 min). After centrifugation, the creamy layer was collected and stored at 4 °C. Analyses were completed within 24 h.

**Total transcriptome analysis.** For RNA sequencing analysis, 100 bp single-end read libraries were prepared using a TruSeq stranded total RNA kit (Illumina). The libraries were sequenced using a NextSeq550 instrument (Illumina). Raw reads were preprocessed by Nextseq System Suite v2.2.0. using specific NGS technology configuration parameters. This preprocessing removes low-quality, ambiguous and low-complexity stretches, linkers, adapters, vector fragments and contaminated sequences, and preserves the longest informative parts of the reads. SeqTrimNext also discarded sequences shorter than 25 bp. Subsequently, clean reads of the BAM files were aligned and annotated by Bowtie² using the Cucumis melo genome v.4.0.⁴ as the reference; these data were then sorted and uniquely mapped to SAMtools. Typically, locally mapped reads were used to calculate the read number values for each gene by Sam2counts (https://github.com/vsbuffalo/sam2counts). Differentially expressed genes (DEGs) in the treatment samples were analysed by DEgenes Hunter⁶³, which provides a combined P value calculated on the basis of the Fisher method⁶⁴ using nominal P values provided by edgeR⁶² and DEseq2⁶³. This combined P value was adjusted by the Benjamini-Hochberg procedure⁶⁵ (false discovery rate approach) and values were calculated using a ΔX cycle threshold (Ct) method.⁶⁶ Transcriptional data of the target genes were normalized to the act7 gene and are shown as the fold change in the expression levels of the target genes in each experimental treatment compared to those in the control treatment. The relative expression ratios were calculated as the differences between the qPCR threshold cycles (Ct) of the target gene and the act7 gene (ΔCt = Ct target gene – Ct act7). The fold-change was estimated using the 2^ΔΔCt method, assuming that a single PCR cycle represents a two-fold difference in the template abundance. RT–qPCR analyses were performed in technical triplicates using three independently isolated RNA samples (biological triplicates).

**Quantitative RT–PCR analysis of the host genes.** For quantitative PCR with reverse transcription (RT–qPCR) assays, the RNA concentration was adjusted to 100 ng μL⁻¹. Then, 1 μg of DNA-free total RNA was reverse transcribed into complementary DNA using SuperScript III reverse transcriptase (Invitrogen) and random primers in a final reaction volume of 20 μL according to the manufacturer's instructions. The cDNA was subjected to a clean tube, dispersed in washing solution (7 M urea, 1:4 w/w) and centrifuged (10,000g, 30 min). To remove residue of washing solution, the fat pad was isolated and suspended in water (1:4 w/w) and centrifuged (10,000g, 30 min). After centrifugation, the creamy layer was collected and stored at 4 °C. Analyses were completed within 24 h.

**Data analysis.** The Ct values of the target genes were normalized to the act7 gene and are shown as the fold change in the expression levels of the target genes in each experimental treatment compared to those in the control treatment. The relative expression ratios were calculated as the differences between the qPCR threshold cycles (Ct) of the target gene and the act7 gene (ΔCt = Ct target gene – Ct act7). The fold-change was estimated using the 2^ΔΔCt method, assuming that a single PCR cycle represents a two-fold difference in the template abundance. RT–qPCR analyses were performed in technical triplicates using three independently isolated RNA samples (biological triplicates).
CLSM. Bacteria, ROS and OBs inside the seeds were visualized by CLSM. Seeds were collected at the specified hours after treatment and were transversely cut with a scalpel. A drop of glycerol was applied to the sections, which were placed into 1.5 ml microfuge tubes and stored at −80°C. The mass spectrum of bacteria was performed using in situ prep-mixing inoculum preparation by CellTracker CM-Dil dye or in situ by Hoechst solution. Oil bodies and ROS were stained with Nile red (1:1,000 v/v) or dihydrodichromide 123 (1:5000 v/v), respectively. The images were acquired using a Leica SP5 confocal microscope with a ×63HCX IARO FLUO 1.25×0.95 WATER objective. To image the purified OB suspension, a drop of Nile red and Fast Green FCF-stained preparation was applied onto a patch of polymerized 1% agarose on a glass slide, which was covered with a coverslip. The images were acquired using a Leica SP5 confocal microscope with HCX PL APO lambda blue 63.0 × 0.95 W ATER objective. To image the OBs, seeds were embedded in capsule moulds containing pure resin for 72 h at 70 °C. To enrich the bacteria, the samples were incubated in-lab in 2% paraformaldehyde, 2.5% glutaraldehyde and 0.2 M sucrose mixture in 0.1 M TEM.

TEM. For TEM analysis, 16 h after treatment, seeds were fixed directly using 2% paraformaldehyde, 2.5% glutaraldehyde and 0.2 M sucrose mixture in 0.1 M phosphate buffer (PB) overnight at 4 °C. After 3 washes in PB, the sections were excised from the microtip endosperm region and postfixed in 1% osmium tetroxide solution in PB for 90 min at room temperature, followed by PB washes and 15 min of stepwise dehydration in an ethanol series (30%, 50%, 70%, 90% and 100% twice). Between the 50% and 70% steps, the samples were incubated in-bluc in 2% uranyl acetate in 50% ethanol for overnight. At the 70%–100% stages, the samples were gradually embedded in low-viscosity Spurr’s resin (resinethanol, 1:1, 4h; resinethanol, 3:1, 4h; and pure resin overnight). The sample blocks were embedded in capsule moulds containing pure resin for 72h at 70°C. To image purified OBs in suspension or TassA protein under various polymerization conditions, carbon-coated copper grids were deposited over the sample drops and incubated at 4°C overnight for 2 h. After 2 h, the grids were washed in phosphate-buffered saline for 5 min and blocked with Pierce protein-free (TBS) blocking buffer (Thermo Fisher) for 30 min. An anti-TassA primary antibody was used at a 1:150 dilution in blocking buffer, and grids were deposited over the drops of antibody solution and incubated for 1 h at room temperature. The samples were washed three times with TBS-T (30 mM NaCl, 150 mM NaCl, pH 7.5, and 0.1% Tween 20) for 5 min and then exposed to a 10 nm–diameter immunogold–conjugated secondary antibody (20 nm gold anti-rabbit conjugate, BRL solutions) for 1 h at a 1:50 dilution. The samples were then washed twice with TBS-T and once with water for 5 min each. Finally, the grids were treated with 2% glutaraldehyde for 10 min, washed in water for 5 min, negatively stained with 1% uranyl acetate for 20 s and washed once with water for 30 s. The samples were left to dry and imaged under an FEI Tecnai G2 20 TWIN transmission electron microscope at an accelerating voltage of 80 kV. The images were acquired using TIA FEI Imaging Software v4.14.

Metabolite extraction from plant tissues. Metabolites from the samples of adult melon plants or radicles (MSV000084674, MSV000084278 and MSV000086360) were extracted by adding 1 ml methanol and vigorous vortexing. At least 2 or 3 replicates were done for each experiment, except for adult melon plants where only 1 replicate was done due to the high number of sections analysed for each plant. Due to the large sample number, weighting and normalization by biomass was not feasible. However, the size of plant sections was kept constant. To enable relative quantitative comparisons between samples, we normalized peak areas to total ion count, which is a robust and widely applied strategy in non-targeted metabolomics. In addition, sulfamethazine (1 μM) was added to all samples as an internal standard and was used for normalization. After incubation for 2h, the extracts were centrifuged at 14,000g for 30 min. The acquired supernatants were stored at −80°C until use in liquid chromatography–mass spectrometry (LC–MS/MS).

Metabolites from seeds and seedlings (MSV000088139) were extracted after grinding the tissue in liquid nitrogen and freeze-drying the samples. Three replicates were done for each condition. The samples were transferred to weighted vials and the methanol was evaporated at room temperature. Extracts were then resuspended in 1 ml methanol and centrifuged at 14,000g for 20 min at 4°C. Vials were weighed after the extractions to adjust the final concentrations to 1 mg ml−1, thus no internal standard was used.

In both cases, extraction controls were performed by adding 3 samples with pure methanol used for the extraction as a blank. Differences in abundance of metabolites were estimated on the basis of relative comparisons between equally treated samples due to the absence of authentic standards in the non-targeted metabolomics approach.

LC–MS/MS. Non-targeted LC–MS/MS analysis was performed using a Q-Exactive Quadrupole–Orbitrap mass spectrometer coupled to a Vanquish ultra-high-performance liquid chromatography (UHPLC) system (Thermo Fisher) (MSV000083630, MSV000084674 and MSV000084278) or a Shimadzu Nexera X2 UHPLC system, with attached photodiode array detector coupled to a Shimadzu 9030 HPLC system. The automatic workflow for the analysis of the radicles from seeds bacterized with VWallas substris subspecies subterraneus was performed using the GNPS environment77 using Ion Identity Network1.

Preprocessing for data analysis and MS/MS network analysis. Raw spectra were converted to mzXML files using MConvert (ProteoWizard). Feature identification was performed by MZmine open source software version 2.37 using the settings listed in Supplementary Table 3; this generated the .mgf files and quantification tab files. Statistical analysis of Metaboanalyst69 and feature-based molecular network in the Global Natural Product Social Molecular Networking (GNPS) environment using Ion Identity Network1.

Feature-based molecular networking and spectral library search. Molecular networking was performed using the GNPS environment with the settings listed in Supplementary Table 4 and visualized using Cytoscape software. Putative annotation of detected features was performed using automatic library search through the GNPS environment1, network annotation propagation1, chemical classification using ClassyFire1 and MolNetEnhancer workflows1. Mirror plots were done using GNPS and https://metabolomics-usi.ucsd.edu/77, comparing mszspec of the selected features and the metabolites recorded in MS/MS databases (Extended Data Fig. 10). Annotations were done according to guidelines in ref. 1 (Supplementary Table 8), and levels are indicated in each figure.

The automatic workflow for the analysis of the radicles from seeds bacterized with VWallas substris subspecies subterraneus (MSV000086360) and sulfamethazine and sulfamethizole (MSV000086360 and MSV000083630) can be accessed at: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=b2d36333ddc42da72ce93be86f3; feature-based molecular networking: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=81bd911de4f 48b1d59510215559a2f; network annotation propagation: https://proteomics2.ucsd.edu/ProteoSAFe/status.jsp?task=5d229b7ea5784c9807e297960a3; and
MoNetEnhancer: https://proteomics2.ucsd.edu/ProteoSAFe/status.jsp?task=ka45823e576954e7b6b2e8c8e037342.

Automatic workflow for the analysis of the radicles from the seeds treated with fengycin and TasA (MSV000084279) can be accessed at: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=f0c5e1989647e40735d6e99a044f9a26; feature-based molecular networking: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ka46bcbde9a94946b98a9ed46e83c5cbdd7; network annotation propagation: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=kb767b2c85f838282514e5d36c85e1; and MoNetEnhancer: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ka45316008d47f49a3ae0f0ed44c951314.

Automatic workflow for the analysis of adult plants grown from control seeds or seeds bacterized with Bacillus subtilis subsp. subtilis NCBI 3610 (MSV000088360) can be accessed at the following addresses: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=ka109903289a94e6ba9be10a58a5d50f, feature-based molecular networking: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ka636d0c95b3e4d846e4d36e9194b55e4; feature-based molecular networking: https://proteomics2.ucsd.edu/ProteoSAFe/status.jsp?task=ka7194293e23d44329b6403d7c49b26; and MoNetEnhancer: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ka72bb16205a47e7878006efc92235a8.

Automatic workflow for the analysis of the seeds and seedlings after seed treatment with bacteria, fengycin or TasA (MSV000088139) at 0, 24 and 120 h after treatment can be accessed at: https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=ka272b67e5b8d85d3a5c36c9050a425d; feature-based molecular networking: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ka5cc6c717ec2b9f0eefebc8b68b56e96d55; network annotation propagation: https://proteomics2.ucsd.edu/ProteoSAFe/status.jsp?task=ka6b6665e8e50f0d88c581b34636e7c2.

Proportionality score. The proportionality score was calculated between two directly connected nodes across the entire molecular network using the following equation:

Proportionality = \log \left( \frac{N_{\text{Si}} \times M_{\text{Si}}}{N_{\text{Msi}} \times M_{\text{Msi}}} \right),

where $N_{\text{Si}}$ and $M_{\text{Si}}$ correspond to the peak area of the detected features $N$ and $M$ in sample $S_1$, while $N_{\text{Msi}}$ and $M_{\text{Msi}}$ correspond to the peak area of the seeds or seeds bacterized with Bacillus subtilis (MSV000084674) from radicles from seeds bacterized with Bacillus subtilis). Source data are provided with this paper.

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Author contributions
D.R. conceived and designed the work, drafted and edited the text; M.V.B.-C. collected most of the experimental data, and drafted the manuscript; C.M.-S. designed, collected and analysed MS data, and edited the manuscript; A.M.C.-R. collected and analysed MS data, and edited the text; D.P. collected MS data and edited the text; L.D.-M. informatically analysed data and drafted figures; V.J.C. supervised MS analyses and edited the text; A.d.V. substantially revised and edited the text; A.P.-G. substantially revised and edited the text; P.C.D. substantially revised and edited the text.

Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Seed treatment with *B. subtilis* cells does not induce an increase in the germination rate but accelerates post germination progression. 

**a** Germination rates of untreated and bacterized seeds represented as the percentage of germinated seeds versus time. 

**b** Relative expression levels of the GA20ox1 and CYP707A1 genes in bacterized seeds 0 and 16 hours after the treatment compared with those in the untreated seeds. The average values of two biological replicates with three technical replicates are shown with error bars representing SD. 

**c** Heatmap of fold changes of selected DEGs identified by total transcriptomic analysis of bacterized and control seeds (untreated) 16 hours after the treatment. Colour scale indicates the Log2(FC) of each DEG. 

**d** KEGG pathway enrichment analysis of all DEGs identified by RNA-seq of bacterized and control seeds 16 hours after the treatment.
Extended Data Fig. 2  | See next page for caption.
Extended Data Fig. 2 | Carboxylic acids and lipids represent the major metabolic changes in bacterized seeds. **a** Top 20 features with high median weighted sum of absolute regression coefficient scores determined by PLS-DA and calculated using MetaboAnalyst, selected as features discriminating bacterized seeds from control seeds 0 hours after the treatment (left) or 24 hours after the treatment (right). Feature IDs are accompanied by chemical class according to Classyfire classification. Features in bold are selected as features discriminating any growth-promoting treatment from the control treatment. **b** Volcano plots showing the metabolites significantly increased or decreased in leaves (left) and stem (right) of plants emerged from bacterized seeds respecting control plants. Colours indicate the chemical class of each metabolite according to Classyfire. **c** Distribution of features identified by spectral match as L-tryptophan and cinnamic acid in 3D models representing the plants grown from the control or *B. subtilis*-treated seeds.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | B. subtilis cells enter the seeds and colonize the inner regions. a Scanning electron microscopy micrographs of bacterized seeds 5 days after the treatment shows B. subtilis cells colonizing the inner side of the seed coat. Scale bars: 100 = µm (left image) and 30 µm (right image); r: radicle, i: inner seed coat, o: outer seed coat. b Confocal microscopy images of transversally cut bacterized seeds 16 hours after the treatment with fluorescently labeled B. subtilis cells. Scale bars: 100 µm (upper panels) or 10 µm (lower panels); e: embryo. c CFU counts of the surface extracts of the seeds bacterized with the WT (3610) strain and ECM mutants 1 hour after the treatment. Average values are shown with error bars representing SD (n = 6). d Radicle colonization of the ECM mutants relative to that of the WT assumed as 100% (discontinued line) in radicle extracts five days after seed treatment. Average values are shown. Error bars represent SEM. Statistical significance was assessed by one-way ANOVA with post hoc Dunnett’s multiple comparisons test (each treatment vs. WT treatment except for Δsrf and Δpks) (n = 3 in WT, n = 4 in all ECM mutants except for Δsrf and Δpks, with n = 2; Δeps \( P = 0.0391 \), ΔbslA \( P = 0.0007 \), ΔtasA \( P = 0.0014 \), JC81 \( P = 0.0091 \), ΔtapA \( P = 0.0009 \).)
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Components of the extracellular matrix of *B. subtilis* trigger metabolic reprogramming of the seed radicles related to plant growth stimulation. **a** Heatmap of the hierarchical clustering of the top 50 features of impacted molecular families in the radicles from bacterized seeds. The color code inside the heatmap depicts the relative fold change of each metabolite between groups. Color code accompanying feature names indicates their chemical class according to Classyfire. **b** Network analysis of representative features related to the presence of bacteria or ECM. Normalized abundances in the radicles in seeds subjected to various treatments are represented in features of all groups. Average values are shown with error bars representing SD. Statistical significance was assessed by one-way ANOVA with post hoc Dunnett’s multiple comparisons test (*n* = 6 except in control, where *n* = 12; *p* < 0.0001). Created with BioRender.com.
Extended Data Fig. 5 | Aggregated forms of TasA are preferred under the apoplast conditions and have the highest radicle growth-promoting activity after seed treatments. 

**a** Left: Polymerization dynamics of TasA in the fluorescence emission assays using ThT. Discontinued lines indicate time points when fractions were used for seed treatments. Right: Percentage of radicle growth promotion from the seeds treated with TasA at various polymerization states normalized to the radicle area of the control samples (100%, discontinued line) five days after seed treatment. Average values are shown. Error bars represent SD. Statistical significance was assessed by after one-way ANOVA with post hoc Dunnett’s multiple comparisons test (each treatment vs. control treatment) \((n = 11, 12, 10, 11 and 9 for control, n = 9, 10, 9, 12 and 9 for TasA 1.5 \mu M and n = 11, 10, 9, 10 and 10 for TasA 3 \mu M, referring to 0, 4, 24, 48 and 120 hours respectively). **b** Left: Fluorescence emission of ThT over time in the TasA polymerization assay in polymerization buffer (buffer) or in apoplastic fluid (AF). Right: Representative transmission electron microscopy micrographs of TasA after 16 hours of polymerization in buffer or AF.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Treatments of the seeds with TasA and fengycin influence the final metabolic patterns of emerged radicles. a PCA 3D score plot of the metabolome of the radicles showing clustering of the samples based on seed treatment. The percentage of variation explained by each principal component is indicated on the axes. b Heatmap of hierarchical clustering results of the top 50 features impacted in the radicles grown from TasA- and fengycin-treated seeds. The color code inside the heatmap depicts the relative fold change of each metabolite between groups. c Top 20 features with high median weighted sum of absolute regression coefficient scores determined by PLS-DA and calculated using MetaboAnalyst. Feature IDs are accompanied by chemical class according to Classyfire classification. Features in bold are selected as features discriminating any growth-promoting treatment from the control treatment. d, e Molecular family of features discriminating fengycin-treated seeds (D) or TasA-treated seeds (E) from the control seeds according to PLS-DA. The chemical structures of annotated features and their average mass based on spectral matches to GNPS libraries are also represented for the corresponding molecular families. Pie charts indicate the peak abundance of each metabolite in the corresponding condition. Node shape indicates the level of identification according to Sumner et al., 78.
Extended Data Fig. 7 | The size of OBs in the seeds is altered after seed treatment with *B. subtilis* cells or with purified TasA and fengycin.

**a** Representative CLSM images of transversally cut seeds 16 hours after the treatment with *B. subtilis*. Oil bodies were stained with Nile red, and bacteria were stained with Hoechst. Scale bar: 20 µm.

**b** Transmission electron microscopy images of thin sections of the seeds (top) or purified OB suspensions (bottom) 16 hours after the treatment with water (control), fengycin, or TasA. Arrows indicate oil bodies.

**c** Representative transmission electron microscopy images of thin sections of the seeds 16 hours after the treatment with water (control), fengycin or TasA. G: glyoxysomes; arrows: oil bodies.

**d** Transmission electron microscopy images of purified OB suspension 16 hours after the treatment with TasA. Samples were immunolabeled with a TasA antibody (1:150) and secondary anti-rabbit antibodies conjugated to 10 nm gold particles (1:50).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | TasA and fengycin induce changes in OBs morphology and glycerophospholipids dynamics in seeds. a  Representative CLSM images of transversally cut seeds 16 hours after the treatment with 10 µM fengycin or 3 µM TasA. Oil bodies were stained with Nile red (scale bar: 20 µm). b  Chemical proportionality analysis of a glycerophospholipids molecular family in non-treated seeds (control) and seeds treated with fengycin or TasA from 0 h to 24 h after treatment. The size of the nodes is directly related with the abundance of the features at time 0 h. Arrows indicate chemical directionality of the modifications found and the color scale of the arrow represents the ChemProp score, the value obtained after the measurement of the peak area changes of connected nodes in a molecular network across a sequential data frame by comparing their proportions.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Treatment of the seeds with fengycin increase the expression and accumulation of metabolites related to glutathione metabolism and flavonoids. a. KEGG pathway enrichment analysis of all DEGs identified by total transcriptomic analysis of the leaves of adult plants grown from control or fengycin-treated seeds 48 hours after inoculation with *B. cinerea*. b 3D plant models of adult plants grown from control (left) or *B. subtilis*-treated seeds (right) showing global distribution of the feature with m/z identical to discriminant feature 1785 of the GSH molecular family identified in the metabolome of the radicles grown from the seeds treated with purified TasA or fengycin. c Molecular family with features differentially abundant in the aerial regions of seedlings from fengycin-treated seeds vs. control seedlings 5 days after seed treatment, annotated as flavonoids according to Classyfire. The chemical structures of annotated features and their average mass based on spectral matches to GNPS libraries are also represented for the corresponding molecular families. Pie charts indicate the peak abundance of each metabolite in the corresponding condition. Node shape indicates the level of identification according to Sumner et al., 78.
Extended Data Fig. 10 | See next page for caption.
Extended Data Fig. 10 | Mirror plots comparing spectra from named features along the manuscript to standard spectra deposited in GNPS. In the upper part of the plot (black lines) is represented the MS spectra of the candidate feature and in the lower part (green lines); the MS spectra of the standard compound. Mirror plots have been generated using https://metabolomics-usi.ucsd.edu/.
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### Software and code

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#### Data collection

RNA-seq data were collected using NextSeq System Suite v2.2.0. Confocal microscopy images were taken using Leica Application Suite Advance Fluorescence v2.7.3.9723. Electron microscopy images were taken using TIA FEI Imaging Software v4.14. Metabolomics data were obtained with the commercial software of Thermo Scientific for UPLC systems. HPLC-ESI-MS/MS data corresponding to the identification of proteins in gel cut bands was acquired using ProteinScape 3 software from Bruker coupled to Mascot v3.1 (Matrix Science).

#### Data analysis

For statistics and representation of all experimental data, analysis were done using GraphPad Prism v.6.01. Raw reads were preprocessed by NextSeq System Suite v2.2.0. using specific NGS technology configuration parameters. Clean reads of the BAM files were aligned and annotated using the Cucumis melo genome (v4.0) as the reference by Bowtie2; these data were then sorted and indexed using SAMtools v1.48411074. Uniquely localized reads were used to calculate the read number values for each gene by Sam2counts (https://github.com/vsbuffalo/sam2counts). Differentially expressed genes (DEGs) in the treatment samples were analyzed by DEgenes Hunter, which provides a combined p value calculated based on the Fisher method using nominal p values provided by edgeRand DEseq2. This combined p value was adjusted by the Benjamini-Hochberg (BH) procedure (false discovery rate approach) and used to rank all obtained differentially expressed genes.

A heatmap and DEG clustering were generated using ComplexHeatmap in R Studio and Kobas 2.0. DEGs annotated using the Cucumis melo genome were processed to identify the Gene Ontology functional categories using sma3s and TopGo software.

KEGG pathways and enrichment were estimated using Bioconductor packages (Bioconductor.org) GGplot2, ClusterProfiler, DOSE, and EnrichPlot in R Studio.

For LC-MS/MS data analysis, raw spectra were converted to .mzXML files using MSconvert (ProteoWizard). MS1 and MS/MS feature extraction was performed with Mzmine2.30. For molecular networking and spectrum library matching the .mgf file was uploaded to GNPS.
Molecular networks were visualized with Cytoscape v. 3.4. For CLSM data analysis, image processing was performed using Leica LAS AF (LCS Lite, Leica Microsystems) and ImageJ 1.51s.

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The RNA-seq data were deposited in the GEO database under the reference GSE175611.

Metabolomics data are deposited at https://massive.ucsd.edu/ with the identifiers MSV000084674 (data from radicles from seeds bacterized with Bacillus subtilis subsp subtilis NCBI 3610 and mutant strains; https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=b2e3636335d24c2da72634b7c5c8b63f), MSV000084278 (data from radicles from the seeds treated with fengycin and TasA; https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=fc05e1b986cb4073b56d9f9a044f0a2f), MSV000086360 (data from adult plants grown from control seeds or seeds bacterized with Bacillus subtilis subsp subtilis NCBI 3610; https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=1090c82889a04e4ba9be1058a8a5d6db) and MSV000088139 (data from seeds and seedlings treated with fengycin, TasA or bacteria; https://massive.ucsd.edu/ProteoSAFe/dataset.jsp?task=72bb762b7b8d45da8d3c5c9005a425d8).

Cucurbit genome databases used for mass spectrometry analysis of protein bands were UniProtKB/TrEMBL TrEMBL Cucumis melo, v 2017.10.25.

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No data were excluded from the study.

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At least three replicates were taken in all the experiments performed. In all the cases the experiments were successfully replicated.

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Antibodies

The anti-TasA antibody used for immunodetection studies in this work was kindly provided by professor Adam Driks (Stover and Driks, 1999) and it consists in a polyclonal antiserum obtained from blood 23 days after injecting the protein into rabbit. The secondary antibody used in western blot was commercially available at Bio-Rad (Goat Anti-Rabbit IgG (H + L)-HRP Conjugate cat. no. 1706515). The immunogold-conjugated secondary antibody used was commercially available at BBI solutions (EM Grade 20nm Goat anti-Rabbit Conjugate cat. no. EM.GAR20/1).

Validation

The anti-TasA primary antibody was validated elsewhere in the literature (Stover and Driks, 1999). The Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (cat. no. 1706515) secondary antibody from BioRad was validated by the supplier as follows: “Specific for rabbit IgG, heavy and light chain. The cross-reactivities of anti-rabbit IgG antibody are tested in an ELISA. Minimum cross-reactivity to human IgG. 1:2,000-1:5,000 dilution can be used (Coligan, J., 1997)".