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The plant stress hormone jasmonic acid evokes defensive responses in streptomycetes

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ABSTRACT Actinobacteria are prevalent in the rhizosphere and phyllosphere of diverse plant species where they help to enhance tolerance of plants against biotic and abiotic stresses. Here, we show that the plant hormones jasmonic acid (JA) and methyl jasmonate (MeJA) affect the growth, development, and specialized metabolism of *Streptomyces*. Exposure of *Streptomyces coelicolor* to JA or MeJA led to enhanced production of the polyketide antibiotic actinorhodin. JA also exhibited toxicity toward *Streptomyces* and *Streptacidiphilus* at higher concentrations, whereby streptomycetes were more tolerant to JA than members of the genus *Streptacidiphilus*. Tolerance to JA could be linked to its conjugation by the bacteria with glutamine. Additionally, JA conjugates with valine, tyrosine, phenylalanine, and leucine/isoleucine were identified. In contrast to JA, synthetic JA conjugates failed to activate antibiotic production and showed significantly reduced toxicity. Thus, our findings provide insights into a previously unknown defense mechanism deployed by *Streptomycetaceae* to a plant hormone. The underlying mechanism encompasses the attachment of amino acids to JA, which in turn safeguards the bacteria against the harmful impacts of the plant hormone. This study adds to the growing body of evidence that plant hormones can have a significant impact on members of the plant microbiome by affecting their growth, development, and secondary metabolism.

IMPORTANCE Microorganisms that live on or inside plants can influence plant growth and health. Among the plant-associated bacteria, streptomycetes play an important role in defense against plant diseases, but the underlying mechanisms are not well understood. Here, we demonstrate that the plant hormones jasmonic acid (JA) and methyl jasmonate directly affect the life cycle of streptomycetes by modulating antibiotic synthesis and promoting faster development. Moreover, the plant hormones specifically stimulate the synthesis of the polyketide antibiotic actinorhodin in *Streptomyces coelicolor*. JA is then modified in the cell by amino acid conjugation, thereby quenching toxicity. Collectively, these results provide new insight into the impact of a key plant hormone on diverse phenotypic responses of streptomycetes.

KEYWORDS plant hormone, antibiotic production, amino acid conjugation, *Streptomyces*, jasmonic acid conditioning

Streptomycetes are versatile bacteria that are commonly found in close association with fungi, plants, and animals, as well as free-living in soil, saline, and freshwater environments (1). Their filamentous mode of growth and reproduction via spores contribute to the successful inhabitation of ecologically diverse niches (2). *Streptomyces* species are renowned for their ability to produce a multitude of bioactive secondary metabolites, and many *Streptomyces* antibiotics have been essential in medicine to fight infections by pathogenic bacteria. However, after the golden era of drug discovery in the

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1950s and 1960s, the low-hanging fruit had been harvested, and now there is a steep decline in antibiotic discovery. The frequency of discovering a new antibiotic is estimated to be as low as one per 10^7 randomly screened actinomycetes or even less (3). Meanwhile, bacterial infections are once more a huge threat for human health, especially due to the rapid spread of multidrug resistance among the current bacterial pathogens (4, 5). One solution to boost the discovery of novel antimicrobials may lie in the activation of so-called cryptic or silent antibiotic biosynthetic pathways. Sequencing technologies have unveiled that many biosynthetic gene clusters (BGCs) for secondary metabolites are not or poorly expressed under routine laboratory conditions (6, 7). Hence, the potential diversity of microbial natural products is still large, but we need to find ways to activate their production.

Actinobacteria are prevalent in the rhizosphere and phyllosphere of diverse plant species and play a key role in protection of plants against biotic and abiotic stresses due to their ability to produce a wide variety of plant beneficial metabolites, such as antibiotics, siderophores, and plant hormones (8–11). Remarkably, plants stimulate cooperative plant-microbe interactions, and the plant's innate immune system plays an important role in sculpting microbial assemblages (12). For example, the secretion of salicylic acid by plant roots modulates colonization by specific bacterial taxa (13), and treating plants with methyl jasmonate (MeJA) alters root-associated microbial community composition (14). In this context, we showed that plant-associated *Streptomyces* had altered antibiotic activities in response to plant hormones, especially to jasmonic acid (JA), auxins, and salicylic acid (15). These findings exemplified that plant hormones may act as elicitors of antibiotic production in plant-associated *Streptomyces* species, but if and how plant hormones impact on growth and development of diverse streptomycetes are largely unknown.

Here, we show that jasmonates altered antibiotic production in *Streptomyces* strains originating from various sources, including the model organism *Streptomyces coelicolor* and *Streptomyces roseifaciens* (16). We observed enhanced antimicrobial activity and altered growth for both *Streptomyces* species in the presence of JA. Intriguingly, *S. roseifaciens* conjugated JA to jasmonoyl glutamine (JA-Gln), and molecular networking revealed conjugation of JA to various amino acids by both *Streptomyces* and *Streptacidiphilus* bacteria. Bacteria conjugate toxic molecules, such as heavy metals, antibiotics, and reactive oxygen species, with glutathione to reduce their toxicity and promote their excretion from the cell. This illustrates that conjugation can be used as an effective strategy to attenuate compound activity (17). Accordingly, tolerance toward chemically synthesized JA-aminoacyl conjugates was drastically higher than the non-conjugated version of JA, and bioactivity was abolished. Finally, we show that JA-conditioned *S. roseifaciens* has higher tolerance to the plant hormone, likely by enhanced amino acid conjugation. Together, our data show that amino acid conjugation of JA is an adaptive strategy of bacteria to survive JA toxicity. To our knowledge, this is the first report on bacterial conjugation of JA and thereby a novel example of an interkingdom hormone-mediated response.

RESULTS

Jasmonates alter antibiotic production by *Streptomyces*

The cry for help hypothesis entails that plants stimulate the production of protective molecules by its microbiome in response to pests and infections (1). We aimed to investigate whether, and if so how, plant hormones may alter the life style and specialized metabolism of streptomycetes. For this, we studied the effect of the plant stress hormones, jasmonic acid and methyl jasmonate, on growth and antibiotic production in *Streptomyces* isolates from our collection, which have predominantly been isolated from soils. Interestingly, challenging a panel of 20 isolates with either 0.5 mM JA or MeJA resulted in changes in antibiotic activity toward *Bacillus subtilis* (Fig. 1; Table S1A). Earlier trials demonstrated that when the concentration reached 5 mM, bacterial growth was significantly inhibited, prompting us to choose a concentration below that threshold. We

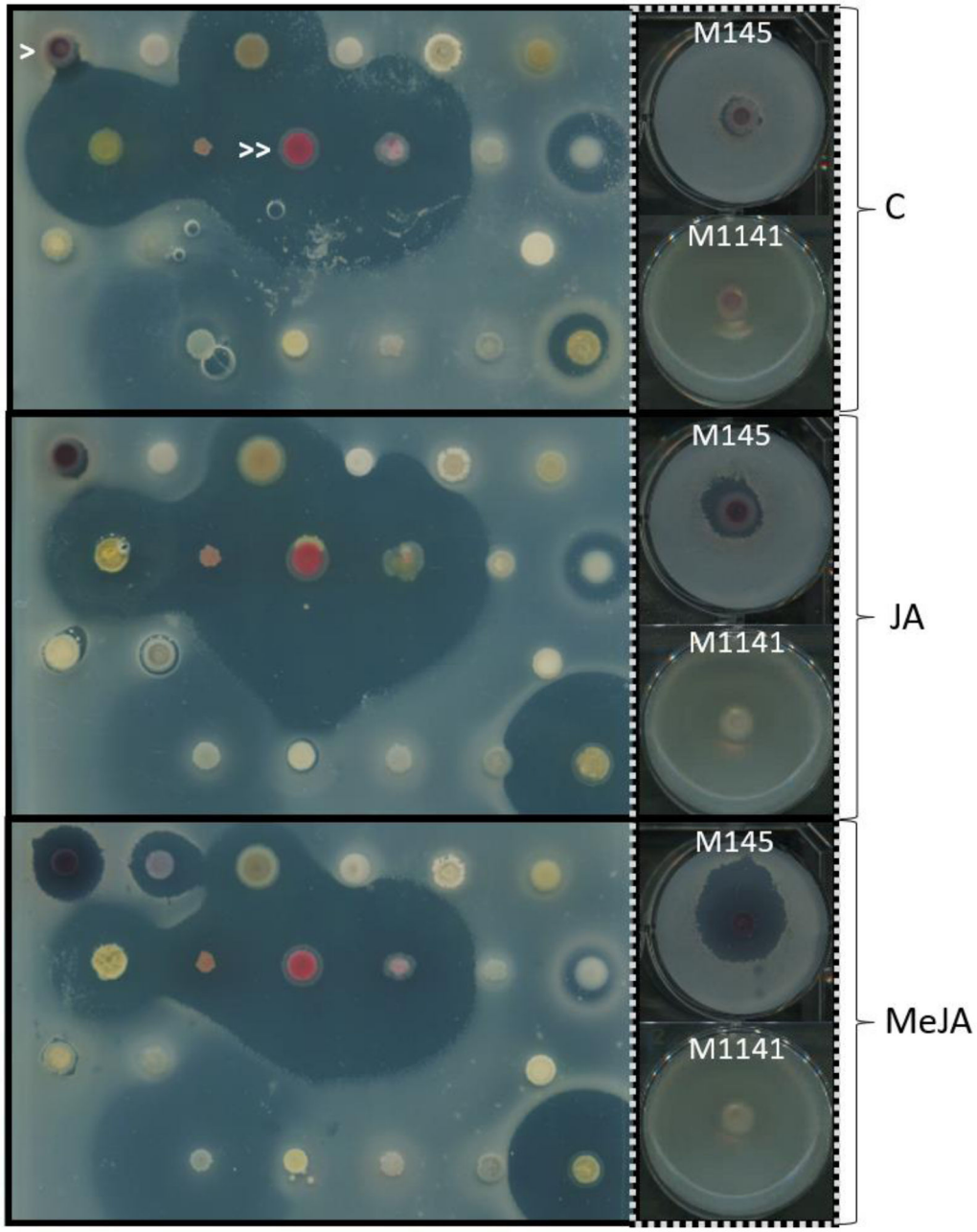

FIG 1 JA and MeJA alter antibiotic production by streptomycetes. Left panels: *Streptomyces* species were grown as spots on minimal medium (MM) (C), which served as a control, or MM supplemented with either 0.5 mM JA (JA) or 0.5 mM MeJA (MeJA). Growth inhibition of indicator strain *B. subtilis* is apparent from the zones of clearance. The single arrowhead indicates the position of the *S. coelicolor* spots, and the double arrowheads indicate the position of the *S. roseifaciens* spots. A complete overview of strains and their positions is provided in Table S1A. Right panels: *S. coelicolor* M145 and its *act* null mutant M1141 on MM (C), MM supplemented with 0.5 mM JA (JA) or 0.5 mM MeJA (MeJA). The lack of bioactivity of the *act* null mutant M1141 strongly suggests that actinorhodin is the causative agent of the plant hormone-enhanced bioactivity.

observed both increased and reduced antimicrobial activity against *B. subtilis* based on a qualitative soft agar overlay assays (Fig. 1, left panel). To better understand the responses to JA and MeJA, we studied their effects on two JA-responsive *Streptomyces* strains in more detail. These were *S. coelicolor,* a well-studied model organism, and *Streptomyces roseifaciens* MBT76^T, a gifted producer of secondary metabolites isolated from Qinling mountain soil (7, 18).

Jasmonates have distinct effects on growth and antimicrobial production

When *S. coelicolor* was exposed to JA or MeJA at 0.5 mM concentration, enhanced antimicrobial activity was observed against *B. subtilis*, whereby MeJA had a stronger eliciting effect than JA (Fig. 1, right panel). Jasmonates themselves do not sensitize *B. subtilis* (15), and hence, the observed enhanced antibiosis is therefore most likely due to elicitation of antimicrobial activity by the two strains. *S. coelicolor* produces several antibiotics, including the blue-pigmented antibiotic actinorhodin (Act), which has bioactivity against Gram-positive bacteria (19). Therefore, we tested the effect of JA and MeJA on the Act non-producing strain *S. coelicolor* M1141, which lacks the *act* gene cluster (20), while all other BGCs are still intact. In contrast to the parent *S. coelicolor* M145, its *act* null mutant *S. coelicolor* M1141 failed to inhibit the growth of *B. subtilis* when exposed to JA or MeJA (Fig. 1, right panel). This strongly suggests that under the chosen conditions both the JA- and MeJA-elicited antibiotic activity corresponds to Act and its derivatives, with MeJA being the most effective elicitor. Enhanced Act production was also observed in liquid-grown cultures supplemented with MeJA. After 24 h of growth on MeJA, a vast increase in blue pigmentation was observed (Fig. 2G and H). Mycelial biomass of liquid-grown cultures supplemented with JA also showed enhanced production of a red pigments (Fig. S1), which are most likely the result of the accelerated production of red-pigmented prodiginines (21, 22). On solid media, the addition of JA accelerated development, as shown by the appearance of gray-pigmented

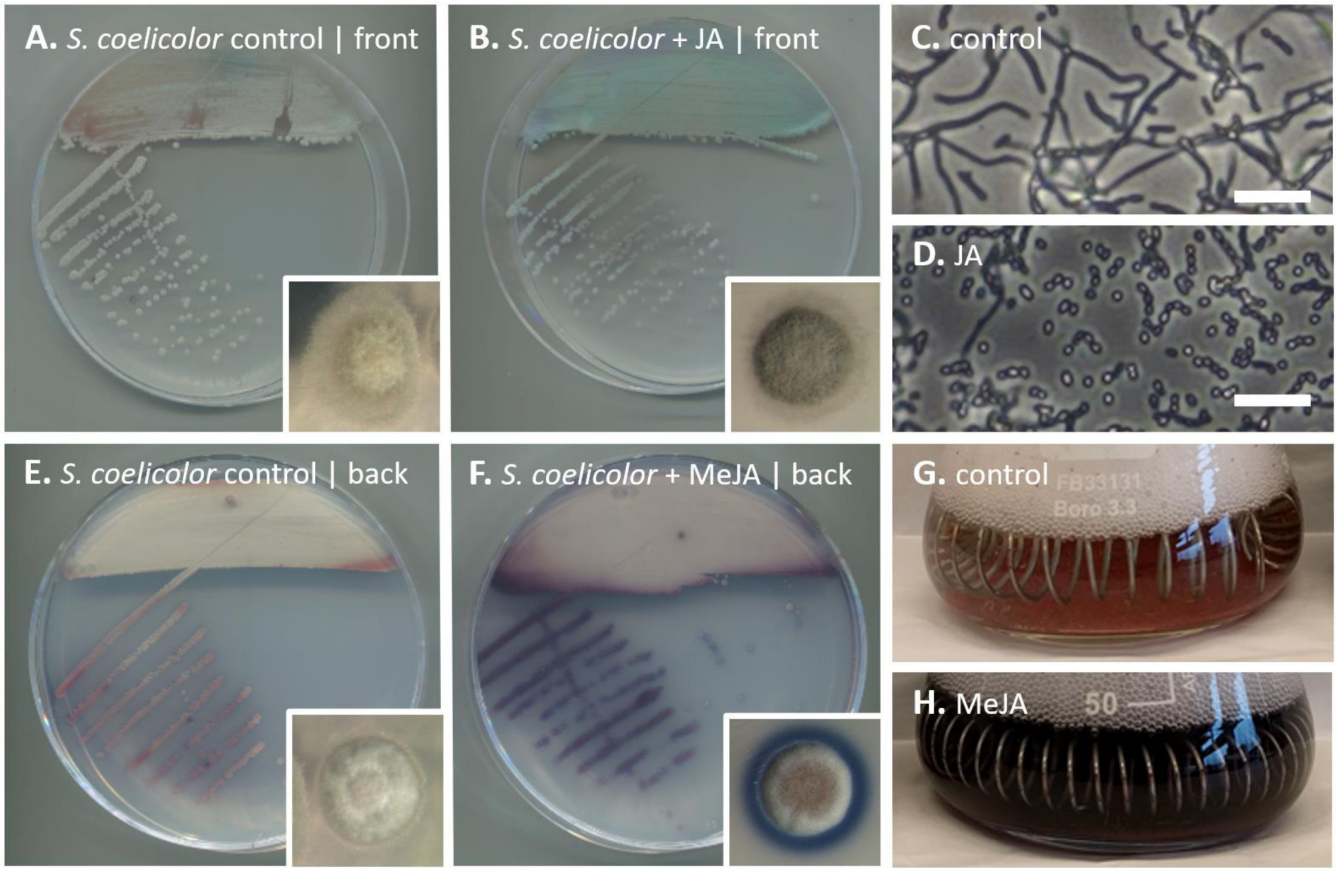

FIG 2 Altered development and actinorhodin production of *S. coelicolor* M145 in response to jasmonates. Aerial hyphae were observed 2 days after inoculation of minimal medium (MM) agar plates with spores of *S. coelicolor* M145 (A). On MM supplemented with 0.5 mM JA (B), the bacterium had a gray appearance indicating accelerated sporulation. Light micrographs confirm the production of aerial hyphae on MM (C) and spores on MM + JA (D). Supplementation with 0.5 mM MeJA resulted in enhanced production of the blue-pigmented Act surrounding the *S. coelicolor* colonies (F) as compared to growth on MM without MeJA (E). Two days old liquid-grown cultures supplemented with MeJA (H) also produced more Act as compared to under control conditions (G). Scalebars: 10 µm. Inserts in the down right corner of the petri dishes show single colonies grown under the same conditions.

colonies due to the production of the gray spore pigment WhiE as well as the presence of spores already after 2 days of growth (Fig. 2A through D). Interestingly, spores of *S. coelicolor* grown on JA germinated prematurely in the developing spore chains (Fig. S2). The hyphae emerging from the germinating spores were much thinner as compared to the surrounding aerial hyphae, consistent with young vegetative hyphae. It is important to note that while 0.5 mM JA accelerates development, at higher concentrations (5 mM) JA inhibits normal growth (data not shown).

Like *S. coelicolor*, *S. roseifaciens* also showed increased antimicrobial activity on media supplemented with 0.5 mM JA, but this elicitation was not observed for MeJA (Fig. S3). This shows that different streptomycetes may respond differently to JA and MeJA. In addition to increased antibiotic activity, *S. roseifaciens* showed reduced red pigmentation during the first 3 days of growth on JA as compared to control conditions, which was another indicator of altered specialized metabolism (Fig. S4). Again, we detected altered growth when 0.5 mM JA was added to cultures of *S. roseifaciens*. Growth of the bacteria on minimal medium (MM) with JA accelerated red pigmentation typical of *S. roseifaciens* by about 6 h as compared to media without JA (Fig. S5), illustrating that JA also affects growth of *S. roseifaciens*. As was also seen for *S. coelicolor*, at high concentration (5 mM) JA inhibited growth of *S. roseifaciens* (see below).

Amino acid conjugation of jasmonic acid in *Streptomyces roseifaciens*

S. roseifaciens produces a plethora of bioactive compounds, including a variety of isocoumarins (18). To analyze the effect of JA on the metabolome of *S. roseifaciens*, crude extracts of JA-grown *S. roseifaciens* MM agar-grown cultures were analyzed with liquid chromatography-mass spectrometry (LC-MS). Statistically significant changes in the metabolite patterns of *S. roseifaciens* were caused by the addition of JA, including changes in isocoumarins production (Fig. S6). However, we could not observe a specific pattern in the isocoumarin profile in response to JA, with some mass features upregulated, while others were downregulated. One mass feature stood out that was exclusively present in JA-treated cultures and had a monoisotopic mass of 339.1915 m/z, eluting at a retention time of 4.53 min (Fig. 3). Database searches (in Antibase 2012, Reaxys, and ChemSpider) using the exact mass and molecular formula did not return any hits for a known bacterial natural product. The same was true when the MS/MS spectrum was searched against the mass spectral libraries available on the global natural product social molecular networking (GNPS) platform (23). To identify the compound, metabolites were isolated from 5 L of liquid-grown tryptic soy broth with 10% sucrose (TSBS) cultures because higher titers could be obtained than from MM agar-grown cultures. Following several chromatographic separation steps, compound 1 was purified as a mixture of two stereoisomers (Fig. 4). The molecular formula of 1 was established as $C_{17}H_{26}N_2O_5$, with six degrees of unsaturation, based on high resolution electrospray ionisation mass spectroscopy (HRESIMS) analysis (Fig. S7). 1D and 2D Nuclear Magnetic Resonance (NMR) analysis (Fig. S8 to S13; Table S2) showed that part of the compound contained a jasmonoyl moiety based on the observed NMR features consistent with jasmonic acid, like the ^{1}H triplet signal for the methyl group at C-12 (δ_H 0.90), the two olefinic protons at C-9 and C-10 (δ_H 5.36 and 5.23), and the ketocarbonyl group at C-6 (δ_C 219.1), which forms part of the 2,3-disubstituted cyclopentanone ring identified through the 2D correlations (Fig. 4A). Generally, the ^{1}H and ^{13}C chemical shifts assigned to the jasmonoyl moiety were within around 1 ppm difference from those previously reported to jasmonic acid (24) apart from the ^{13}C chemical shift of the acid carbonyl group at C-1 (δ_C 169.7 versus 177.4 for jasmonic acid), indicating a substitution at C-1. The other part of the structure was identified as glutamine, based on the homonuclear correlation spectroscopy (COSY) spin system connecting the 1′-NH (δ_H 7.33) to the α-proton of 1′-CH (δ_H 3.82), which was further connected to two CH_2 groups positioned at 2′ (δ_H 1.84 and 1.70) and 3′ (δ_H 2.01) (Fig. 4A). Combined with COSY, the Heteronuclear Multiple Bond Correlation (HMBC) spectrum confirmed the observed spin system and established its connection to the carboxylic acid group and the terminal carboxamide group through

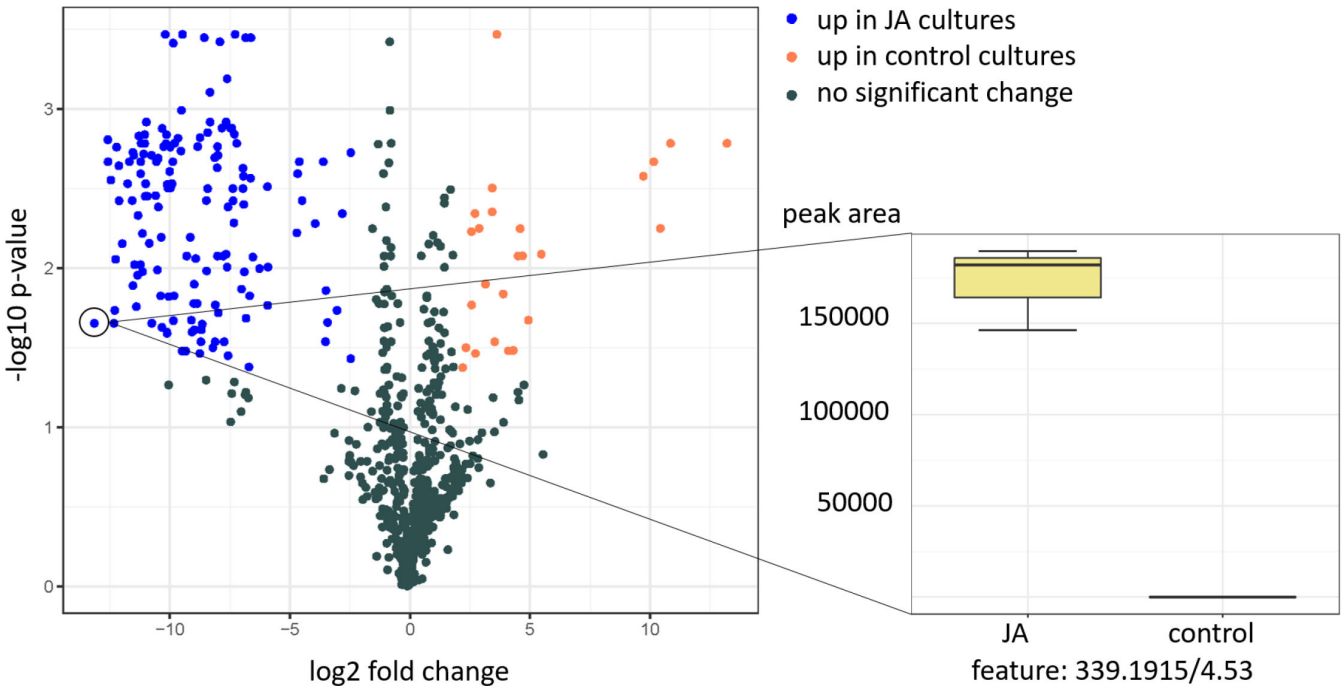

FIG 3 Changes in the metabolite profiles of *S. roseifaciens* in response to JA. Volcano plot of mass features upregulated by JA challenge (blue features) and those that are downregulated by JA (orange features). Dark gray-colored features show non-significant changes. The threshold settings were >fourfold changes in peak intensity (log2 fold change ≥2 or ≤−2) at a *P*-value ≤0.05 (−log10 *P*-value ≥1.3). The boxplot of the peak area of mass feature 339.1915 (RT 4.53, highlighted in the circle in the volcano plot) represents the most upregulated mass feature in the JA-treated *Streptomyces* culture (JA) compared to the control (control).

the HMBC correlations observed from the protons of 1′-NH (δ_H 7.33) and 2′-CH_2 (δ_H 1.84 and 1.70) to the carboxylic acid carbonyl carbon positioned at 5′ (δ_C 173.4), and from the protons of 2′-CH_2 (δ_H 1.84 and 1.70) and the terminal amino group 4′-NH_2 (δ_H 7.44 and 6.60) to the amide carbonyl carbon positioned at 4′ (δ_C 174.7). The glutamine residue was connected to the jasmonoyl moiety through an amide bond between 1′-NH of the glutamine residue and 1-CO of the jasmonoyl moiety, based on the HMBC correlations observed from the protons of 1′-NH (δ_H 7.33), 1′-CH (δ_H 3.82), and 2-CH_2 (δ_H 2.41 and 2.15) to the carbonyl carbon of the jasmonoyl group 1-CO (δ_C 169.7). Thus, we could positively identify compound 1 as jasmonoyl-glutamine (Fig. 4B). Two diastereomers of JA-Gln could be isolated, which is likely due to the addition of a racemic mixture of JA to the cultures of *S. roseifaciens*. While conjugation of JA to amino acids has been reported in plants and fungi, this is the first report of amino acylation of JA in bacteria.

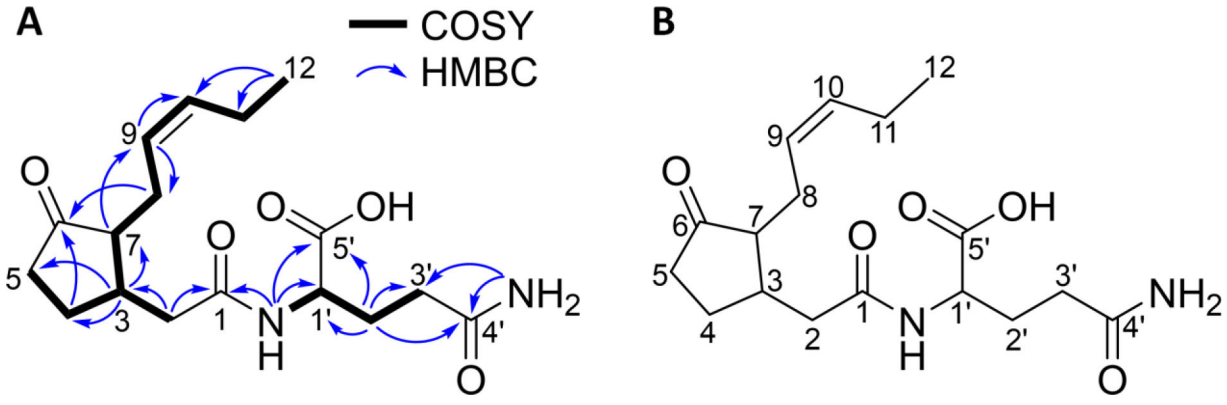

FIG 4 NMR data for jasmonoyl glutamine (1). (A) Key COSY and HMBC correlations of 1, (B) structure of jasmonoyl glutamine (1).

JA conjugation is a common feature of *Streptomycetaceae*

Since JA-Gln had only been purified and identified from *S. roseifaciens*, we wondered how widespread aminoacylation of JA is among the family *Streptomycetaceae*. We, therefore, tested a selection of 15 *Streptomyces* and 13 *Streptacidiphilus* strains, a sister genus of *Streptomyces*. They were grown in liquid media supplemented with 0.5 mM JA, followed by isolation of the specialized metabolites. The strains are listed in Table S1B.

Surprisingly, while all streptomycetes grew well on 0.5 mM JA, only three *Streptacidiphilus* isolates (*Streptacidiphilus* spp. P03-D6a, P15-A2a, and P18-A5a) produced biomass when grown under these conditions, indicating that members of the genus *Streptacidiphilus* are more sensitive to JA than streptomycetes. Importantly, in JA-deficient cultures, biomass could be obtained for all 13 *Streptacidiphilus* strains. The remaining 18 bacterial cultures were then extracted and analyzed using LC-MS/MS. Since we identified JA-Gln in *S. roseifaciens*, molecular networking was chosen to identify JA-Gln and possibly also other structurally related metabolites in the 18 remaining bacterial extracts. For this, the LC-MS/MS data were uploaded to the GNPS platform (23). Cytoscape was then used to visualize the obtained network, which consisted of 1,881 nodes representing all the ions with associated MS2 spectra that are detected in all the bacterial extracts analyzed (Fig. 5). Each node represents a cluster of MS^2 fragmentation spectra for a certain precursor ion that is detected in any or all of the samples. The nodes were connected by an edge if there is some similarity in their MS^2 spectra, which accordingly indicate similarity in the chemical structure of the respective molecules. Based on this, 1,382 nodes were grouped into 210 spectral families.

Of the nodes, 129 were attributed to ions detected only in JA-treated cultures, 8 of which were additionally detected in the control media with JA and no bacteria. The node associated with JA-Gln could be readily detected in the network. Looking at its MS^2 spectrum, it was possible to identify the characteristic fragments a–c, which are consistent with the loss of the Gln moiety, and further fragmentation of the jasmonoyl moiety (Fig. S14 and S15; Table S4). Fragment d was identified based on the $[M + H]^+$ ion of glutamine. The JA-Gln node formed a network with two other nodes, whose precursor masses were 354.186 and 353.207, and they were both exclusively detected in JA-treated cultures (Fig. 5). The molecule with a precursor mass of 353.207 did show an MS^2 spectrum consistent with an amino acid conjugate of JA. The mass difference between the 353.207 node and that of JA-Gln indicated a molecule with an extra methyl group. MS^2 spectrum of the 353.207 node still retained the same fragments a–c due to the jasmonoyl moiety. There was also a fragment due to a loss of HCOOH group as in JA-Gln, but there was no fragment due to a loss in NH_3. Thus, the observed node was annotated as a JA conjugate of *N*-methyl-glutamine (JA-*N*-MeGln). The node with a precursor mass of 354.186 turned out to be an artifact.

Another spectral family in the molecular network consisted of four nodes all exclusively detected in JA-treated bacterial cultures (Fig. 5). We analyzed a node with a precursor mass of 374.199, corresponding to an $[M + H]^+$ ion for a molecule with molecular formula $C_{21}H_{27}NO_5$. The MS^2 spectrum of this ion showed that fragments a–c due to the jasmonoyl moiety were 16 Da higher (Fig. S16 and S17; Table S5), which corresponds to an additional oxygen based on the accurate mass measured in the orbitrap. Accordingly, the observed ion was deduced as an amino acid conjugate of hydroxyjasmonic acid (OHJA). The amino acid was identified as phenylalanine (Phe), based on the molecular formula of the precursor mass together with the observed fragment d due to the amino acid. OHJA and its amino acid conjugates have been previously reported in plants as one of the inactivation pathways for the hormone, with hydroxylation at C-12 (25, 26). A similar position is highly likely for the observed molecule, especially considering the intense fragment ion e which is likely due to the loss of the hydroxylated side chain comprising C-9 to C-12 in the jasmonoyl moiety (Fig. S16). Further scrutiny of the molecular network revealed that all the nodes connected to OHJA-Phe had a fragmentation pattern and molecular formula consistent with amino acid conjugates of OHJA. The three additional nodes were thus annotated as OHJA

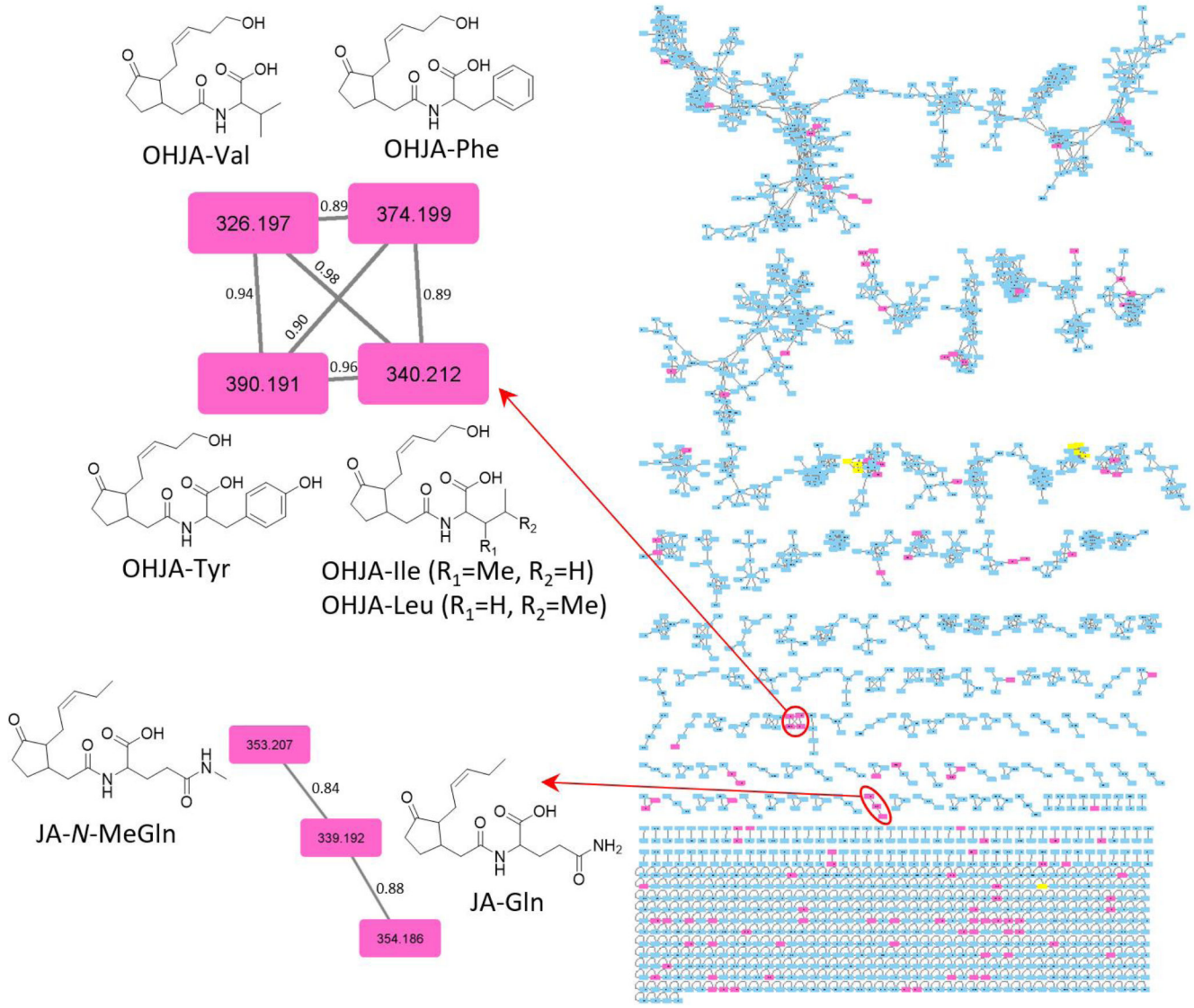

FIG 5 Molecular network of extracts of Actinobacteria grown on medium with the plant hormone jasmonic acid. Pink nodes represent ions detected only in jasmonic acid-treated actinobacterial cultures, while yellow nodes represent the ions detected in jasmonic acid-treated cultures together with blank media. Two networks are enlarged, which include amino acid conjugates of both JA and hydroxyjasmonic acid (OHJA). The nodes are labeled by their precursor masses and the edges by their cosine scores.

conjugates of valine (Val), tyrosine (Tyr), and leucine/isoleucine (Leu/Ile) (Fig. 5). The network also showed nodes which could be due to conjugates of the same amino acids to JA rather than OHJA, but their intensity was very low, and consequently, we failed to obtain enough fragments to cluster closely with JA-Gln or with each other.

Of the strains tested, *S. roseifaciens* showed the highest level of JA-Gln, with a peak that was more intense than any other JA or OHJA conjugate. In addition to *S. roseifaciens*, JA-Gln was also detected in *S. scabies* and *S. lividans* and in the *Streptacidiphilus* sp. P03-D6a and P18-A5a (Table S3). However, JA-Gln production was not detected in *S. coelicolor*. JA-*N*-MeGln was mainly detected in *S. roseifaciens* and as a minor peak in the plant-associated strain *Streptomyces* sp. Atmos39. The other amino acid conjugates were mainly detected in the extracts of the *Streptacidiphilus* bacteria, particularly in *Streptacidiphilus* sp. P15-A2a, together with *Streptomyces* sp. Atmos31.

Conjugation of JA reduces bioactivity

Since JA inhibited growth of the vast majority of *Streptacidiphilus* bacteria at 0.5 mM and at higher concentrations for *S. coelicolor* and *S. roseifaciens*, we hypothesized that amino acid conjugation may be a way for the bacteria to protect themselves against the plant hormone. To further probe this hypothesis, we prepared two of the previously identified JA-amino acid conjugates, JA-Gln and JA-Phe, via organic synthesis (for details see the Materials and Methods section). *S. roseifaciens* bacteria were spotted onto MM agar with the growth-inhibiting concentration (5 mM) of JA or JA-Gln, the dominant JA conjugate identified in this strain. While growth on JA was inhibited as expected, *S. roseifaciens* grew equally well on control media or those supplemented with JA-Gln (Fig. 6—top panel). This shows that glutamine conjugation circumvents the toxicity of JA. To confirm this, we tested whether conjugation of JA would abolish its toxicity against *Streptacidiphilus* P18-A5a. Indeed, while concentrations above 0.5 mM JA inhibited growth of the *Streptacidiphilus* strain, chemically synthesized JA-Gln did not affect growth under the conditions tested (Fig. S18).

We then wondered if conjugation of JA abolishes the bioactivity of the plant hormone. To test this, *S. roseifaciens* was exposed to JA and JA-Gln at concentrations

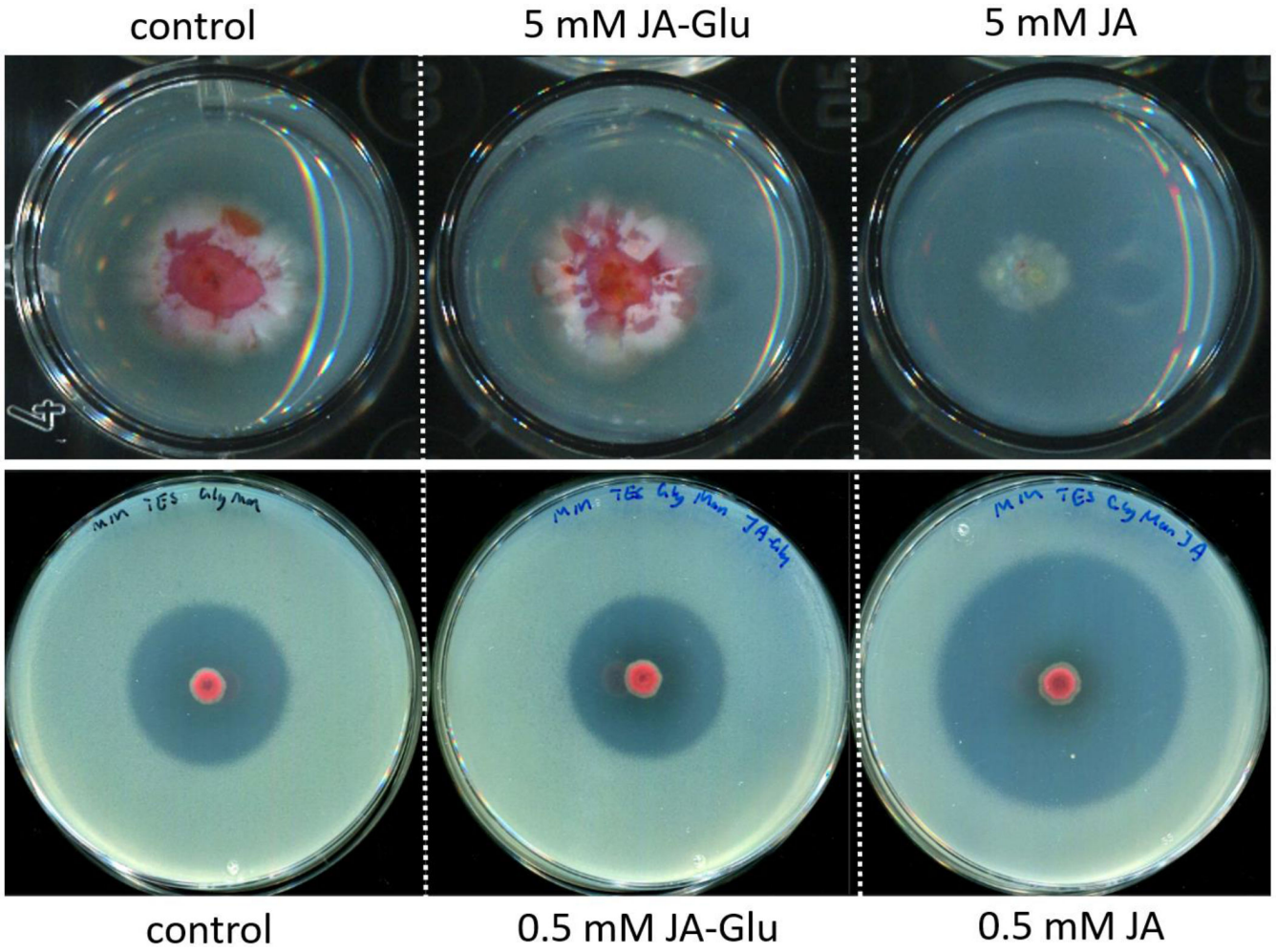

FIG 6 Eliciting bioactivity of JA is abolished by amino acid conjugation to JA-Gln. Top panel: top view of *S. roseifaciens* spores spotted on a 24-well plate. When spores of *S. roseifaciens* were spotted onto MM agar or in the presence of 5 mM JA-Gln, normal growth was observed, whereas growth was strongly inhibited when the media contained 5 mM JA. Bottom panel: back view of *S. roseifaciens* spores spotted on a petri-dish. Antimicrobial activity by *S. roseifaciens* against *B. subtilis* is increased when exposed to 0.5 mM JA. When the medium is supplemented with 0.5 mM JA-Gln, the observed antimicrobial activity approximates the antimicrobial activity observed for the control condition.

where JA stimulates antibiotic production in *S. roseifaciens* (0.5 mM). JA-Gln failed to enhance antibiotic production by *S. roseifaciens* against *B. subtilis* (Fig. 6, bottom panel). Taken together, these experiments show that JA affects growth and secondary metabolism of *Streptomycetaceae*. Furthermore, bacterial conjugation of the plant hormone reduces its toxicity suggesting this may represent a survival strategy.

Adaptation of *S. roseifaciens* to jasmonic acid through sequential exposure and enhanced amino acid conjugation

We reasoned that if bacteria conjugate JA as a means to reduce its toxicity, the bacteria might be "conditioned" by successive rounds of growth on JA. Therefore, *S. roseifaciens* was sequentially streaked on media supplemented with 0.5 mM JA. Interestingly, the red pigmentation typical of this strain gradually decreased after each transfer, whereby after eight rounds the strain hardly produced any red pigments. Instead, it now produced a bright yellow pigment (Fig. S19C). Light microscopy of the yellow JA-conditioned derivative showed hyphae forming bundles, while branching was reduced as compared to the parent (Fig. S19A and C). To image the mycelium at higher resolution, single colonies were subjected to scanning electron microscopy (SEM). The wild-type strain showed a peculiar hyphal morphology in the center of the colony, demonstrated by relatively thick hyphal "cables" of up to 1.2 µM in diameter. These bundles likely consist of multiple hyphae (Fig. S19B, insert) contained within a yet unidentified extracellular matrix. Neither the extracellular matrix nor the bundles were observed for the yellow JA-conditioned derivative. Here, merely the aggregation of multiple hyphae was observed with clusters of up to eight hyphae (Fig. S19D). Importantly, JA-conditioned *S. roseifaciens* was desensitized to JA. Exposure to the plant hormone did not alter antimicrobial activity nor did otherwise toxic concentrations of the plant hormone inhibit growth (Fig. S20). Metabolomic analysis of extracts of JA-adapted *S. roseifaciens* grown on MM agar with 0.5 mM JA revealed strongly increased peak intensity of JA-Gln as compared to extracts of the parental strain (Fig. 7). Conversely, the conditioned strain had only a minor peak corresponding to JA itself. These data show that *S. roseifaciens* adapts to JA, likely by inducing the enzyme(s) responsible for its amino acid conjugation. In turn, this Gln conjugation of JA abolishes the impact of the plant hormone on growth and antibiotic production by *Streptomyces*.

DISCUSSION

Jasmonates play an important role in biotic stress responses like insect herbivory and necrotrophic pathogen attack *in planta* (27, 28). Metabolic profiling of *A. thaliana* root exudates revealed that JA is present in those as well (29). Whereas the role of jasmonates in plant stress responses is relatively well understood, their role in root exudates is yet to be explained. Here, we show that the effects of jasmonates go beyond the plant kingdom and may have an effect on members of the plant microbiome. Jasmonates affected antibiotic production by the streptomycetes *S. coelicolor* and *S. roseifaciens*. Exposure to JA also resulted in accelerated development, premature germination, and enhanced antibiotic induction in *S. coelicolor*, while MeJA primarily stimulated antimicrobial activity and strongly enhanced production of the blue-pigmented antibiotic actinorhodin. While these responses do indicate stress, they also imply that plant hormones potentially override a developmental checkpoint that regulates the precise timing of chemical and morphological differentiation, processes that are usually interconnected throughout the life cycle of *Streptomyces*. As a result, the increased production of antibiotics resulting from exposure to JA could be attributed to the earlier initiation of development. While this may indeed explain the effect of JA on antibiotic production, this is not likely the case for the response to MeJA in *S. coelicolor*. First, MeJA does not have a strong effect on development. Second, after 3–4 days of growth on MM, dark blue rings of Act accumulated around single colonies when MeJA is present, which is not observed for *S. coelicolor* during regular growth on this medium. Thus,

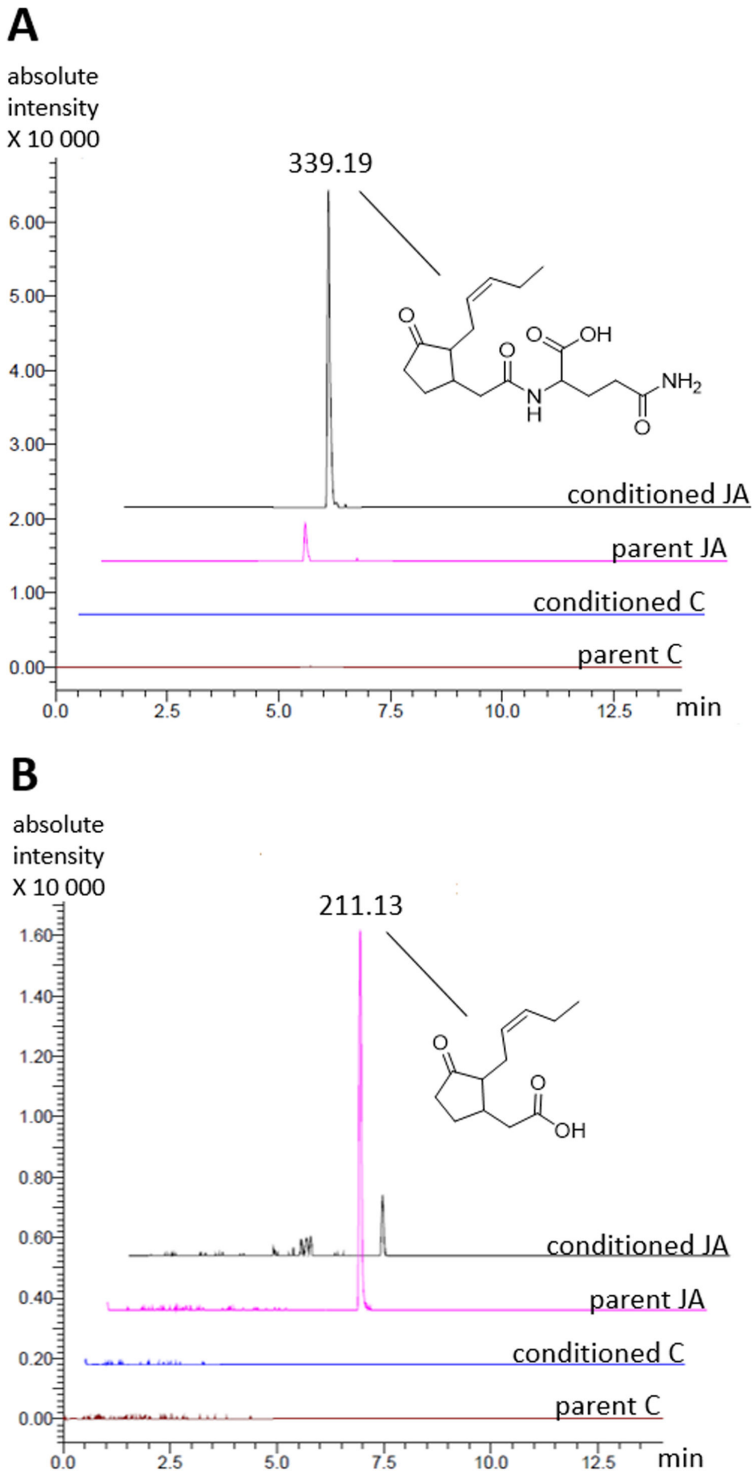

FIG 7 Extracted ion chromatograms of JA-Gln and JA. (A) The intensity of the peak representing JA-Gln (339.19 m/z) is higher in JA-conditioned *S. roseifaciens* as compared to the parent. The peak was not observed when parent and JA-conditioned *S. roseifaciens* were grown in the absence of JA. (B) The intensity of the peak representing JA was higher in extracts of parent *S. roseifaciens*. The absence of the two JA-related peaks under control conditions further validates that these compounds originate from externally added JA.

MeJA specifically acts as an elicitor of Act production. This reinforces the potential for hormones as elicitors of cryptic gene clusters in *Streptomyces* (15, 30).

Our work shows that JA exerts selective pressure on streptomycetes. Indeed, despite phenotypic and physiological effects of JA, it also imposes a toxic effect at high concentrations. Importantly, the biological significance of the JA concentrations that were tested remains uncertain. For example, it was previously shown that the amount of JA that can be isolated from a gram of fresh plant material is in the nanomolar range, but amounts highly depend on the growth conditions (31, 32). Furthermore, local JA concentrations on a micro scale *in planta* are still difficult to deduce from those experiments (33). We, therefore, argue that higher concentrations of JA may exist locally.

We isolated JA-conditioned *S. roseifaciens* derivatives that are morphologically different from the parent strain as exemplified by altered pigmentation, hyphal aggregation, and reduced branching. Importantly, our results indicated that the JA-conditioned phenotype withstands the JA selective pressure through conjugation of JA to JA-Gln, thereby neutralizing the growth-inhibiting effects of the plant hormone. LC-MS analysis of *Streptomycetaceae* exposed to JA revealed conjugation of JA to a range of aminoacyl variants, primarily (OH)JA-Val, (OH)JA-Phe, (OH)JA-Ile, (OH)JA-Leu, (OH)JA-Tyr, and JA-Gln. To our knowledge, this is the first time such diversity of JA conjugation is shown within the family of *Streptomycetaceae*. Most conjugates were found in *Streptomyces* extracts, with JA-Gln being one of the most abundantly detected conjugates among the family of *Streptomycetaceae*. The nodes observed for amino acid conjugates of hydroxylated JA (OHJA) were mainly observed in the extracts of the *Streptacidiphilus* isolates, together with some plant-associated *Streptomyces* species. Neither the lab strains nor the soil isolates tested were able to produce detectable amounts of Phe, Tyr, Val, or Leu/Ile conjugates of OHJA, suggesting this feature might be attributed to specific groups of microbes within the family of *Streptomycetaceae*.

JA was also conjugated to *N*-methyl-Gln by *S. roseifaciens* and the plant-associated strain *Streptomyces* sp. Atmos39. Only the amino acid part *N*-methyl-Gln was previously described and detected as a metabolic intermediate in bacteria utilizing monomethylamine as a carbon and/or nitrogen source (34). The amino acid was also found as one of the constituents of green tea extract (35). The effect(s) of *N*-methyl-Gln are largely unknown, and this compound will be an interesting target for future plant physiology and plant-microbe studies.

Conjugation of JA to either Gln or Phe lowers JA toxicity toward *Streptacidiphilus*, while JA-Gln does not have a major effect on growth and antibiotic production, illustrating again that amino acid conjugation of JA abolishes bioactivity. We hypothesize that JA conjugation with amino acids alters the hormone's biochemical properties like water solubility and/or target affinity. Follow-up work, including transcriptome profiling and chemical probes, based on JA mimics is now planned to elucidate the signal transduction pathway and regulatory network that respond to JA in bacteria. Also, a large number of mutants deleted for regulatory genes are available for *S. coelicolor*, and testing these mutants for their ability to respond to JA may be a very useful first step in the study of the JA-responsive regulatory network.

JA conjugation in plants gives function and specificity to the phytohormone, and hydroxylation is believed to deactivate JA and its conjugates (28). Conjugation of JA with amino acids, such as Ile, Leu, Val, Ala, Tyr, and Phe, is believed to play a role in the signal transduction pathway of jasmonate-responsive events, like the emission of volatile organic compounds that activate the defense systems in neighboring plants (36). However, to our knowledge, the JA-Gln conjugate was only reported once in a study that investigated the leaves of *Nicotiana attenuata* plants after treatment with oral secretions of the herbivorous tobacco hornworm, but the function and enzymes responsible for the conjugation to JA-Gln remained unclear (37). It is tempting to speculate that the responsible enzymes were not found because the conjugation of JA with Gln was done by members of the plant's microbiome. However, the enzymes responsible for JA conjugation in *Streptomyces* and *Streptacidiphilus* have not yet been

elucidated, which is a first step to validate that microbes may be (in part) responsible for the accumulation of conjugated JA variants in plants. The increased hormone levels in response to stress are beneficial but, for extended times, toxic to their producing organism, and deactivation mechanisms are always tightly connected to their biosynthesis. As for JA, plants hydroxylate JA-Ile to deactivate the hormone, as a means to attenuate unwanted negative effects (28). What then is the ecological significance of the uptake and subsequent modification of JA by *Streptomycetaceae*? A benefit may be the accelerated development in response to JA and, for the plant, the removal of access hormone.

In summary, our work shows that jasmonates alter growth and specialized metabolism of various *Streptomyces* species. The strains detoxify JA through conjugation with various amino acids. Together, these results are the first to underline the impact of the plant stress hormone JA on development, secondary metabolism, and fitness of bacteria and its potential role in interkingdom communication.

MATERIALS AND METHODS

Bacterial strains and growth conditions

Streptomyces roseifaciens MBT76^T (DSM 106196T, NCCB 100637T) (16) was obtained from the Leiden University strain collection. The strain had previously been isolated from the Qinling Mountains, Shanxi province, China (38). *Bacillus subtilis* 168 or *Escherichia coli* ASD19 (39) were used as indicator strains. The *Streptomycetaceae* used for the antimicrobial assays and GNPS networking are listed in Table S1A and B, respectively. *Streptomyces* spp. were grown on MM agar plates supplemented with 1% glycerol and 0.5% mannitol and 50 mM TES buffer for 5 days at 30°C, unless indicated differently. *Streptacidiphilus* strains were grown on ½ PDA (2 mg/L potato extract, 10 g/L dextrose, 20 g/L Iberian agar) supplemented with 25 mM MES, pH 5.5. To test plant hormone tolerance, approximately 2 µL of bacterial stock solutions was added on ½ PDA by using a replicator. The agar plates were supplemented with (±)-jasmonic acid (Cayman chemical company, CAS No.: 77026–92-7) or in-house synthesized jasmonic acid-L-amino acid conjugates (see below).

Antimicrobial assays

Antimicrobial assays were conducted using the double-layer agar method. In short, *S. roseifaciens* was inoculated on minimal medium agar plates containing both mannitol (0.5% wt/vol) and glycerol (1% wt/vol) as non-repressing carbon sources and 50 mM TES buffer. The agar plates were supplemented with either (±)-jasmonic acid (Cayman chemical company, cas: 77026-92-7) or 0.01% (±)-jasmonic acid methyl ester (MeJA) (Cayman chemical company, cas: 39924-52-2). Typically, 2 µL spots of *S. roseifaciens* were incubated for 5 days at 30°C, following which they were overlaid with LB soft agar (0.6% wt/vol agar) containing 300 µL of one of the indicator strains (OD_{600} 0.4–0.6) and then incubated overnight at 37°C. The following day, antibacterial activity was determined by measuring the inhibition zones (mm) of the indicator strain surrounding the colonies.

Time-lapse growth analysis

A volume of 2 µL of mycelium stock was spotted on MM agar supplemented with mannitol. The plates were then placed upside down in Perfection V370 scanner (Epson, Nagano, Japan) located inside a 30°C incubator. A scanning picture was taken every hour, and images were processed using a custom python script to get the brightness value of the plate (40). Specifically, the pictures were first converted to grayscale. Seventy percent of the diameter of the colony from the center was selected as the region of interest (ROI). The average gray value of all the pixels within the ROI was used as the brightness value. After measurement, all values were blanked using the first three data points.

Microscopy

Light microscopy

Stereo microscopy was done using a Zeiss Lumar V12 microscope equipped with an AxioCam MRc. Bright-field images were taken with the Zeiss Axio Lab A1 upright microscope, equipped with an Axiocam MRc.

Electron microscopy

Morphological studies on *S. roseifaciens* by SEM were performed using a JEOL JSM6700F scanning electron microscope. For *S. roseifaciens*, pieces of agar with biomass from single colonies grown on MM or MM with 0.5 mM jasmonic acid were cut and fixed with 1.5% glutaraldehyde (1 h). Subsequently, samples were dehydrated (70% acetone 15 min, 80% acetone 15 min, 90% acetone 15 min, and 100% acetone 15 min), and critical point dried (Baltec CPD-030). Hereafter, the samples were coated with palladium using a sputter coater and directly imaged using a JEOL JSM6700F.

Extraction of secondary metabolites and LC-MS analysis

Agar plates with 5 days old *S. roseifaciens* on MM or MM supplemented with 0.5 mM jasmonic acid were extracted with 50 mL of ethyl acetate. Blank MM agar plates with or without JA were also extracted as a negative control. The crude extracts were dried under vacuum. LC-MS/MS acquisition was performed using Shimadzu Nexera X2 UHPLC system, with attached PDA, coupled to Shimadzu 9030 QTOF mass spectrometer, equipped with a standard Electrospray Ionization (ESI) source unit, in which a calibrant delivery system (CDS) is installed. The dry extracts were dissolved in methanol (MeOH) to a final concentration of 1 mg/mL, and 2 µL was injected into a Waters Acquity HSS C_{18} column (1.8 µm, 100 Å, 2.1 × 100 mm). The column was maintained at 30°C, and run at a flow rate of 0.5 mL/min, using 0.1% formic acid in H_2O as solvent A and 0.1% formic acid in acetonitrile (ACN) as solvent B. A gradient was employed for chromatographic separation starting at 5% B for 1 min, then 5%–85% B for 9 min, 85%–100% B for 1 min, and finally held at 100% B for 4 min. The column was re-equilibrated to 5% B for 3 min before the next run was started. The LC flow was switched to the waste for the first 0.5 min, then to the MS for 13.5 min, then back to the waste to the end of the run. The PDA acquisition was performed in the range of 200–600 nm, at 4.2 Hz, with 1.2-nm slit width. The flow cell was maintained at 40°C.

The MS system was tuned using standard NaI solution (Shimadzu). The same solution was used to calibrate the system before starting. Additionally, a calibrant solution made from Agilent API-TOF reference mass solution kit was introduced through the CDS system, the first 0.5 min of each run, and the masses detected were used for post-run mass correction of the file, ensuring stable accurate mass measurements. System suitability was checked by including a standard sample made of 5 µg/mL paracetamol, reserpine, and sodium dodecyl sulfate; which was analyzed regularly in between the batch of samples.

All the samples were analyzed in positive polarity, using data-dependent acquisition mode. In this regard, full-scan MS spectra (m/z 100–2,000, scan rate 20 Hz) were followed by three data-dependent MS/MS spectra (m/z 100–2,000, scan rate 20 Hz) for the three most intense ions per scan. The ions were selected when they reach an intensity threshold of 1,000, isolated at the tuning file Q1 resolution, fragmented using collision induced dissociation (CID) with collision energy ramp (CE 20–50 eV), and excluded for 0.05 s (one MS scan) before being re-selected for fragmentation. The parameters used for the ESI source were interface voltage 4 kV, interface temperature 300°C, nebulizing gas flow 3 L/min, and drying gas flow 10 L/min. The parameters used for the CDS probe were interface voltage 4.5 kV and nebulizing gas flow 1 L/min.

Statistical analysis

Prior to statistical analysis, raw data were converted to mzXML centroid files using Shimadzu LabSolutions Postrun Analysis. The converted files were imported into MZmine 2.40.1 (41) for data processing. Mass ion peaks were detected using the centroid algorithm with a noise level set to 2×10^2. Afterward, chromatograms were built for the detected masses using ADAP Chromatogram Builder with the minimum group size in number of scans set to 10, group intensity threshold set to 2×10^2, minimum highest intensity of 5.0×10^2, and mass tolerance of 0.002 m/z or 10 ppm. Chromatograms were smoothed with a filter width of 9 before being deconvoluted using local minimum search algorithm (search minimum in RT range 0.05 min, chromatographic threshold 90%, minimum relative height 1%, minimum absolute height 5.0×10^2, minimum ratio of peak top/edge 2, and peak duration range 0.03–3 min). In the generated peak lists, isotopes were identified using isotopic peaks grouper (m/z tolerance 0.002 or 10 ppm and retention time tolerance 0.1 min). All the peak lists were subsequently aligned using join aligner (m/z tolerance 0.002 or 10 ppm, m/z weight 20, retention time tolerance 0.1 min, and retention time weight 20), and missing peaks were detected through gap filling using peak finder (intensity threshold 1%, m/z tolerance 0.002 or 10 ppm, and retention time tolerance 0.1 min). Fragments, adducts, and complexes were identified, and duplicate peaks and peaks due to detector ringing were filtered from the generated peak list. Finally, the peak list was exported as a CSV file for further cleaning in Excel.

In Excel, each feature was given a name made of its m/z and retention time, and the intensity of the feature across different samples was expressed by the area of the detected peak. Then features that were not consistently present or had an intensity less than 3,000, in all the replicates, were removed from the file. Additionally, features that originate from background peaks or the culture medium were removed by retaining only those features whose average intensity was at least 50 times greater in the bacterial extracts than in the culture medium extracts. Finally, missing values or zero intensities were replaced by half of the minimum non-zero value. In order to identify discriminatory features between the different groups, volcano plots were constructed in R (x64 3.6.0) using Rstudio. Features which were shown to be interesting were checked back in the chromatograms. The R package ggplot2 was used to generate the volcano plots and boxplots.

LC-MS-based dereplication

Dereplication of the discriminatory feature of interest was done by searching its accurate mass and likely molecular formulae for matching natural products in the databases Antibase 2012, Reaxys, and ChemSpider. Additionally, its MS/MS spectrum was searched for hits in the MS/MS libraries available on the GNPS platform (23). In order to do this, the raw LC-MS data file of an extract of a JA-treated *S. roseifaciens* culture was converted to a 32-bit uncompressed mzML file using MSConvert tool in Proteowizard (42). The converted file was submitted for MS/MS library search through GNPS using default search and filtering options. The file was searched against the two spectral libraries, speclibs and CCMS_Spectral Libraries.

Purification and structure elucidation of jasmonoyl glutamine

In order to identify the discriminatory peak observed in the extracts of JA-treated *S. roseifaciens* cultures, the strain was grown in a total of 5 L TSBS medium to which 0.5 mM jasmonic acid was added (tryptic soy broth 30 g/L, sucrose 100 g/L, jasmonic acid solution 1 mL/L, and pH 7). The culture was incubated at 30°C and shaken at 200 rpm. After 3 days, (5% wt/vol) Diaion HP20 (Resindion) was added to the culture and shaken for 3 h. HP20 and the mycelia were filtered off the liquid media, washed with distilled water, and then extracted with MeOH three times by overnight soaking. The MeOH extract was then evaporated under reduced pressure to yield 30 g of crude extract.

The crude extract was dry loaded on a Vacuum Liquid Chromatography (VLC) column (12.5 × 5.5 cm) packed with silica gel 60 (Sigma Aldrich) and eluted with a gradient of EtOAc–MeOH (100:0–0:100, vol/vol). The column elution was monitored by both TLC and LC-MS to yield seven fractions Q1–Q7. Fraction Q5 (4 g) was subjected to a VLC column (6.5 × 10 cm) eluted with a gradient of DCM–MeOH (100:0–0:100) to obtain eight sub-fractions, Q51–Q58. The target peak turned out to be two isomers, which were purified from sub-fraction Q57 (784 mg) using BUCHI Reveleris prep purification system, by first injecting it on Vydac 150HC C_{18} column (10 µm, 150 Å, 22 × 150 mm) eluted with an aqueous MeOH gradient (5%–30% over 16 min at 15 mL/min), followed by purification on SunFire C_{18} column (5 µm, 100 Å, 10 × 250 mm) eluted with an aqueous ACN gradient (10%–20% over 20 min at 3 mL/min), to yield the two isomers of 1 (31 and 3 mg). NMR data for the purified compounds were recorded on Bruker AV 600 MHz NMR spectrometer equipped with 5 mm TCI cryoprobe (Bruker BioSpin GmbH), using DMSO-d6 as solvent.

Jasmonoyl glutamine (1): colorless solid; UV (MeOH, LC-MS) λ_{max} 217, 290 nm; HRESIMS m/z 339.1919 $[M + H]^+$ (calcd for $C_{17}H_{27}N_2O_5^+$, 339.1914), 361.1736 $[M + Na]^+$ (calcd for $C_{17}H_{26}N_2O_5Na^+$, 361.1734); ^1H and ^{13}C NMR data (Fig. S7 to S13; Table S2).

GNPS molecular networking

LC-MS spectra for molecular networking were obtained using a Waters Acquity UPLC system coupled to a Thermo Instruments MS system (LTQ Orbitrap XL). The UPLC system was run using SunFire C18 column (3.5 µm, 100 Å, 4.6 × 150 mm). Solvent A was 0.1% formic acid, 95% H_2O, and 5% ACN. Solvent B was 0.1% formic acid and 100% ACN. The gradient used was 2% B for 1 min, 2%–85% for 15 min, 85%–100% for 3 min, and 100% for 3 min. The flow rate used was 0.9 mL/min. A fixed injection volume of 5 µL was used for all the analyzed extracts. The MS conditions used were as followed: spray voltage 3.5 kV, capillary voltage 5 V, capillary temperature 300°C, auxiliary gas flow rate 10 arbitrary units, and sheath gas flow rate 50 arbitrary units. The mass analyzer was calibrated using Agilent low concentration tuning mix before each run. The MS method used for molecular networking comprised full-scan MS in the orbitrap in the positive mode at a mass range of 100–2,000 m/z and FT resolution of 30,000, followed by data-dependent MS2 in the ion trap for the three most intense ions using CID with normalized collision energy of 35 and isolation width of 1.5 m/z. Dynamic exclusion was enabled after three counts with an exclusion duration set to 10 s. A run was also performed with data-dependent MS2 being scanned in the orbitrap at an FT resolution of 7,500 to deduce the molecular formulae of fragment ions of interest. Raw MS/MS data were centroided and converted to 32-bit uncompressed mzXML files using MSConvert (ProteoWizard) (41). The data were then uploaded to the GNPS platform (https://gnps.ucsd.edu) for molecular networking (23). The parameters used for molecular networking were a parent and fragment ions tolerance of 0.5 Da, a minimum cosine score of 0.5, minimum matched peaks of 4, maximum node size of 200, and MSCluster enabled with a minimum cluster size of 3 spectra. The spectra were also searched against GNPS spectral libraries using default settings. Group and Attributes files were generated according to the GNPS documentation, and the generated molecular networks were visualized in Cytoscape (43) . The LC-MS/MS data and their associated metadata were submitted to MassIVE repository (ID MSV000092688), and the generated molecular network on GNPS can be accessed at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=1a8d97d0861944a8990454f6a04aa581.

Synthesis of jasmonic acid and jasmonic acid amino acid conjugates

Jasmonic acid and JA amino acid conjugates were prepared according to reference (44).

Racemic jasmonic acid

To a solution of racemic methyl jasmonate (4.85 mL, 22.3 mmol) in methanol (50 mL), a 5-M KOH aqueous solution (7.5 mL) was added while stirring at room temperature for 5 h. The reaction mixture was neutralized with 6 M aqueous HCl and concentrated *in vacuo*. The residue was dissolved in H_2O (50 mL), and the solution was adjusted to pH 2–3 with 6 M aqueous HCl, before being extracted with ethyl acetate (50 mL, three times). The combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified with a column of silica gel using a mixture of n-hexane:ethyl acetate:acetic acid (14:6:1, vol/vol/vol) as an eluent, to give racemic-JA (3.8 g, 18 mmol, 75% yield). NMR (Fig. S21) was in accordance with reference (44).

Jasmonic acid-N-hydroxysuccinimide ester

To a mixture of racemic jasmonic acid (1 g, 4.8 mmol) in ACN (10 mL) and *N*-hydroxysuccinimide (1.5 g, 13 mmol) in *N,N*-dimethylformamide (7.5 mL), dicyclohexylcarbodiimide (DCC; 1.25 g, 6.1 mmol) in ACN (5 mL) was added while stirring at room temperature for 48 h. Water (20 mL) was added to decompose the excess DCC, and the reaction mixture was filtered to remove the dicyclohexylurea. The filtrate was concentrated *in vacuo* and purified with a column of silica gel using a mixture of n-hexane:ethyl acetate (1:3, vol/vol) as an eluent, to give the jasmonic acid-*N*-hydroxysuccinimide ester (1.2 g, 3.9 mmol, 81% yield). NMR (Fig. S22) was in accordance with reference (44).

General procedure for preparing the jasmonic acid-l-glutamine and L-phenylalanine conjugates

Jasmonic acid-*N*-hydroxysuccinimide ester (200 mg, 0.65 mmol) in can (10 mL) was mixed with a solution of 1 mmol L-glutamine or 1 mmol L-phenylalanine in H_2O (10 mL). To this mixture, triethylamine (1 mL, 8.5 mmol) was added while stirring at room temperature for 16 h. The resulting reaction mixture was concentrated *in vacuo*. The concentrate was dissolved in 0.1 M aqueous HCl (50 mL) and extracted with ethyl acetate (50 mL, three times). The combined ethyl acetate layers were dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was purified by a column of silica, using a mixture of ethyl acetate:acetic acid (99:1, vol/vol) as an eluent, to give racemic jasmonoyl phenylalanine (180 mg, 0.50 mmol, 78% yield) and jasmonoyl glutamine (400 mg, 1.18 mmol, quantitative yield). NMR and HRESIMS data of jasmonoyl phenylalanine (Fig. S23 and S24) were in accordance with those previously reported (45) . As for jasmonoyl glutamine, the NMR and HRESIMS (Fig. S25 to S27 for JA-Gln) data were in accordance with those obtained for compound 1 purified during this work.

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ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental Information (AEM01239-23-s0001.pdf). All supplemental tables and figures

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