environmental microbiology

Environmental Microbiology (2019) **21**(3), 940–948 doi:10.1111/1462-2920.14486

Plant growth-promoting archaea trigger induced systemic resistance in *Arabidopsis thaliana* against *Pectobacterium carotovorum* and *Pseudomonas syringae*

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Summary

Archaea have inhabited the earth for a long period of time and are ubiquitously distributed in diverse environments. However, few studies have focused on the interactions of archaea with other organisms, including eukaryotes such as plants, since it is difficult to cultivate sufficient numbers of archaeal cells for analysis. In this study, we investigated the interaction between soil archaea and *Arabidopsis thaliana*. We demonstrate for the first time that soil archaea promote plant growth and trigger induced systemic resistance (ISR) against the necrotrophic bacterium *Pectobacterium carotovorum* subsp. *carotovorum* SCC1 and biotrophic bacterium *Pseudomonas syringae* pv. *tomato* DC3000. Ammonia-oxidizing archaeon *Nitrosocosmicus oleophilus* MY3 cells clearly colonized the root surface of *Arabidopsis* plants, and increased resistance against both pathogenic species via the salicylic acid-independent signalling pathway. This mechanism of bacterial resistance resembles that underlying soil bacteria- and fungi-mediated ISR signalling. Additionally, volatile emissions from *N. oleophilus* MY3 were identified as major archaeal determinants that elicit ISR. Our results lay a foundation for archaea–plant interactions as a new field of research.

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Introduction

Plants protect themselves against diverse microbial pathogens and insects using a variety of defence mechanisms (Agrios, 2005). Induced resistance represents one of these mechanisms, which is used against a broad spectrum of plant pathogens (Mysore and Ryu, 2004; Eyles *et al*., 2010). Induced resistance is classified into two major types: systemic acquired resistance (SAR), which is elicited by avirulent pathogens, and induced systemic resistance (ISR), which is induced by a certain group of root-associated bacteria, referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper *et al*., 2004). Many microbial determinants have been reported to elicit plant immunity, such as cell-wall components and secreted metabolites including bacterial volatile compounds (Ryu *et al*., 2004; Walters *et al*., 2008). Although bacteria and fungi have been shown to elicit ISR, it has not been reported in archaea.

Since the first formal recognition of the archaeal domain approximately three decades ago, our knowledge of the biology, diversity and ecology of this microbial group has grown considerably (Woese *et al*., 1990). Archaea are found in a wide variety of habitats, including hydrothermal vents (Ehrhardt *et al*., 2007), marine waters (DeLong, 1992), hypersaline sediments (Demergasso *et al*., 2004), freshwater sediments (Schleper *et al*., 1997) and soil environments (Bintrim *et al*., 1997). Advances in our understanding of archaea have largely been fuelled by the advent of molecular techniques enabling the detection of archaea in samples without cultivation (Simon *et al*., 2000). Recent progress in the field of archaeal biology includes the recognition of novel archaeal lineages, development of new functional markers and probes, cultivation of over 50 different archaeal strains (Bates *et al*., 2011) and sequencing of many archaeal genomes, including ammonia-oxidizing archaea (AOA) (Schleper *et al*., 1997; Walker *et al*., 2010). Although our knowledge of the group

is expanding, our understanding of terrestrial archaea is inadequate. To observe interactions between archaea and the environment, archaea must be cultivated and subjected to various investigations; however, until recently, cultivation of archaea has proven difficult.

Recently, a few studies have proposed potential interactions between archaea and plants because siderophore-producing archaea (Dignam *et al*., 2018), phosphorus-solubilizing haloarchaea (Yadav *et al*., 2015) and nitrogen-fixing methanogens (Leigh, 2000) behave like PGPR. In addition, an archaea phylum, *Crenarchaeota*, has been shown to colonize tomato roots (Simon *et al*., 2005). However, direct evidence supporting the role of archaea in plant growth is lacking.

Nitrosocosmicus oleophilus MY3 was first isolated from soil and characterized as AOA (Jung *et al*., 2016). Therefore, we hypothesized that *N. oleophilus* MY3 interacts with plant roots like other ammonium-oxidizing bacteria (AOB) (Briones *et al*., 2003). In the current study, we investigated whether a culturable soil archaeon *N. oleophilus* MY3 stimulate plant growth and innate immunity. Here, we demonstrate that *N. oleophilus* MY3 promote the growth of *Arabidopsis thaliana* plants, similar to PGPR as shown previously (Kloepper *et al*., 2004); we therefore refer to this archaeon as plant growth-promoting archaea (PGPA). Additionally, we show that drench application of *N. oleophilus* MY3 to the root system of *Arabidopsis* seedlings elicits ISR. Our findings serve as a foundation for further research in the field of archaea–plant interactions.

Results and discussion

A plant growth-promoting archaeon Nitrosocosmicus oleophilus *MY3*

To investigate whether archaea promote plant growth, *Arabidopsis* plants were treated with *N. oleophilus* MY3 culture in a gnotobiotic hydroponic system (Song *et al*., 2015). The average shoot weight per plant of plants grown in the presence of *N. oleophilus* MY3 cells was 85.67 $\pm$ 7.88 mg, which was 36.67 mg higher than that of the control (-MY3; 49 $\pm$ 2.65 mg) ($p < 0.05$) (Fig. 1A). The soil plant analysis development (SPAD) values, an indicator of photosynthesis, were twofold higher in plants treated with *N. oleophilus* MY3 than in the control (Fig. 1B). Plants absorb nitrogen from the soil in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Nitrification occurs in aerobic soils, and NO_3^- is the predominant form of nitrogen available for absorption by plant roots (Nadelhoffer *et al*., 1984). The AOA oxidize ammonia to nitrite (NO_2^-), which improves the ability of plants to absorb nitrogenous compounds (Stahl and de la Torre, 2012), which may explain the growth-promoting effects of *N. oleophilus* MY3. Thus, *N. oleophilus* MY3 is the first PGPA observed experimentally.

Multiplication of N. oleophilus *MY3 on* Arabidopsis *roots*

Next, we examined the interaction of *N. oleophilus* MY3 with plants. To visualize the interaction between *N. oleophilus* MY3 cells and *Arabidopsis* plants, we conducted fluorescence *in situ* hybridization (FISH) assay using a Cy3-labelled archaea-specific probe. Initially (day 0), no archaeal cells were visible on *Arabidopsis* roots. However, Cy3-labelled *N. oleophilus* MY3 cells became visible at day 7, and the number of cells increased at day 14 (Fig. 2B). Next, quantitative real-time PCR (qRT-PCR) analysis was used for the quantification of *N. oleophilus* MY3 cells in the medium used to inoculate *N. oleophilus* MY3 and on plant roots inoculated with the MY3 culture. Although 16S ribosomal RNA (rRNA) of *N. oleophilus* MY3 was detected in the plant growth medium at day 0, the 16S rRNA copy number declined at days 7 and 14 (Fig. 2C). This suggests that *N. oleophilus* MY3 cells may not survive before plants actively growth since the plant's medium is not optimal for *N. oleophilus* MY3. By contrast, the copy number of *N. oleophilus* MY3 16S rRNA was higher at day 7 than at day 0, and increased further at day 14 (Fig. 2D), consistent with the results of FISH assay (Fig. 2D). These results indicate that *N. oleophilus* MY3 cells interact with plants by colonizing plant roots.

Induced systemic resistance against Pseudomonas syringae *and* Pectobacterium carotovorum *by* N. oleophilus *MY3*

Since PGPR promote the resistance of plants against leaf pathogens (Kloepper *et al*., 2004; van Loon, 2007), we tested whether PGPA could also elicit ISR against foliar pathogens. Since benzothiadiazole (BTH) is a chemical inducer of induced resistance such as SAR, we used BTH as a positive control before pathogen inoculation. At 5 days post-inoculation (dpi) with *Pseudomonas syringae* pv. *tomato* DC3000, plants treated with BTH or *N. oleophilus* MY3 (before pathogen inoculation) showed significantly weaker symptoms of infection than did plants treated with the growth medium (control; $p < 0.05$; Fig. 3A). This shows that *N. oleophilus* MY3 elicited induced resistance against the hemibiotrophic bacterium, similar to PGPR. To determine the effects of *N. oleophilus* MY3 on plants grown in soil, 3 week-old soil-grown *Arabidopsis* seedlings were treated with drench application of 2 week-old *N. oleophilus* MY3 liquid culture (10^8 cells per ml) (Jung *et al*., 2016). Leaves of *Arabidopsis* plants were infiltrated with the necrotrophic and biotrophic pathogens *P. carotovorum* subsp. *carotovorum* SCC1

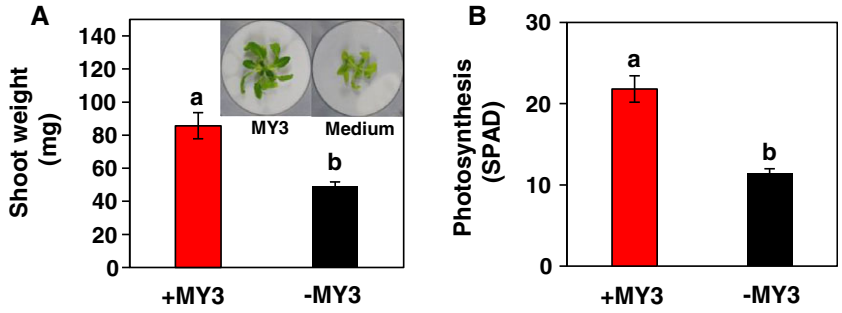

Fig. 1. Effect of plant growth-promoting archaeon *Nitrososcosmicus oleophilus* MY3 on plant growth.
Arabidopsis seedlings grown in a hydroponic system were inoculated with *N. oleophilus* MY3 cells, and plant growth parameters were measured 14 days post-inoculation (dpi).A. Representative photographs and shoot weight.
B. SPAD values. Data represent mean ± standard error (S.E.; n = 6). Different letters indicate significant differences between treatments (p < 0.05) according to Fisher's least significant difference (LSD) test. The experiment was repeated four times with similar results.

and *P. syringae* pv. *tomato* DC3000, respectively, using a needleless syringe, and evaluated for disease severity at 0, 5 and 7 days after drench application of *N. oleophilus* MY3. Soil drench application of *N. oleophilus* MY3 reduced symptom development on leaves infiltrated with *P. syringae* pv. *tomato* DC3000 (p < 0.05; Fig. 3B). Soft-rot symptoms caused by *P. carotovorum* subsp. *carotovorum* SCC1 were attenuated at 1 day after pathogen

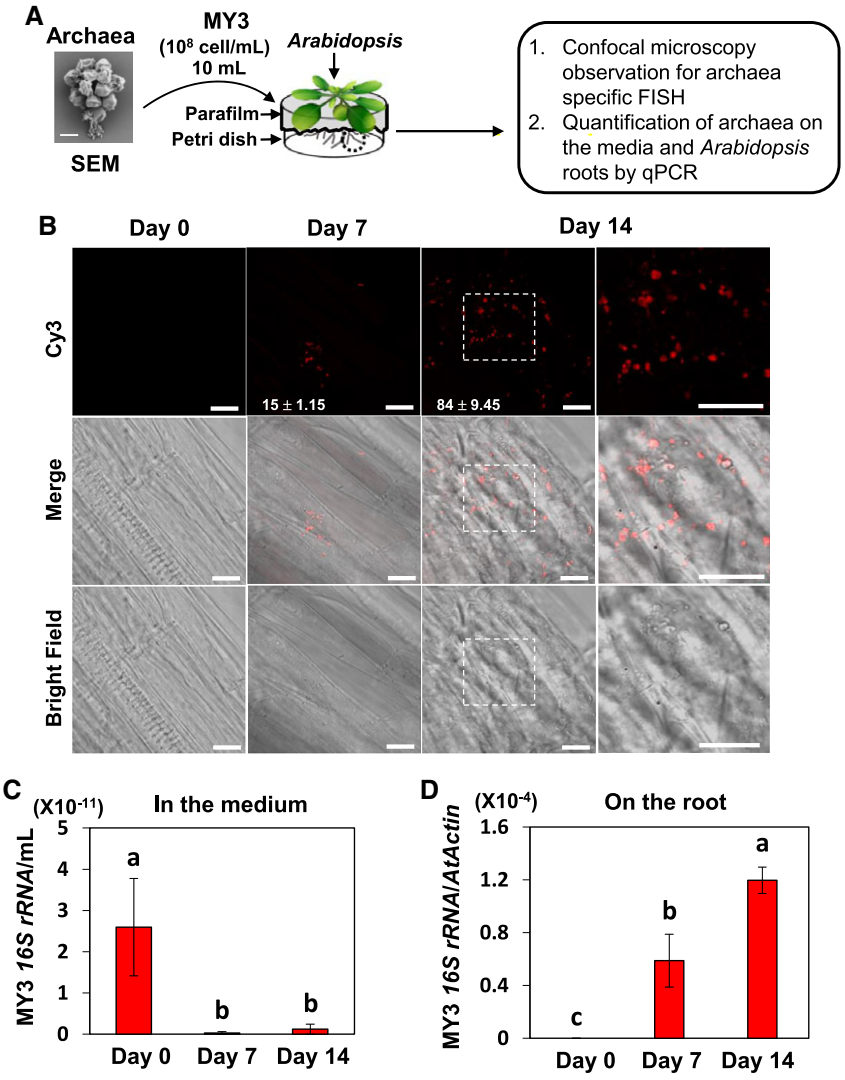

Fig. 2. Colonization of *Arabidopsis* roots by *N. oleophilus* MY3.
A. Schematic representation of the experimental set up and analysis of *Arabidopsis* seedlings in the hydroponic system. Scanning electron microscope image of *N. oleophilus* MY3 is shown on the left.
B. Confocal laser scanning microscopy images of Cy3-stained archaeal cells (red) taken at 0, 7 and 14 days after *N. oleophilus* MY3 inoculation (scale bar: 10 μm). The area outlined with a white dashed line in the left panel in the 14 day image is magnified in the right panel. The Cy3-labelled fluorescent cells were counted at 7 and 14 dpi (n = 3, mean ± S.E.).
C and D. qRT-PCR analysis of archaea in hydroponic plant media (C) and on the surface of *Arabidopsis* roots (D) at 0, 7 and 14 dpi. Data represent mean ± S.E. (n = 3). Different letters indicate significant differences among treatments (p < 0.05; Fisher's LSD test).

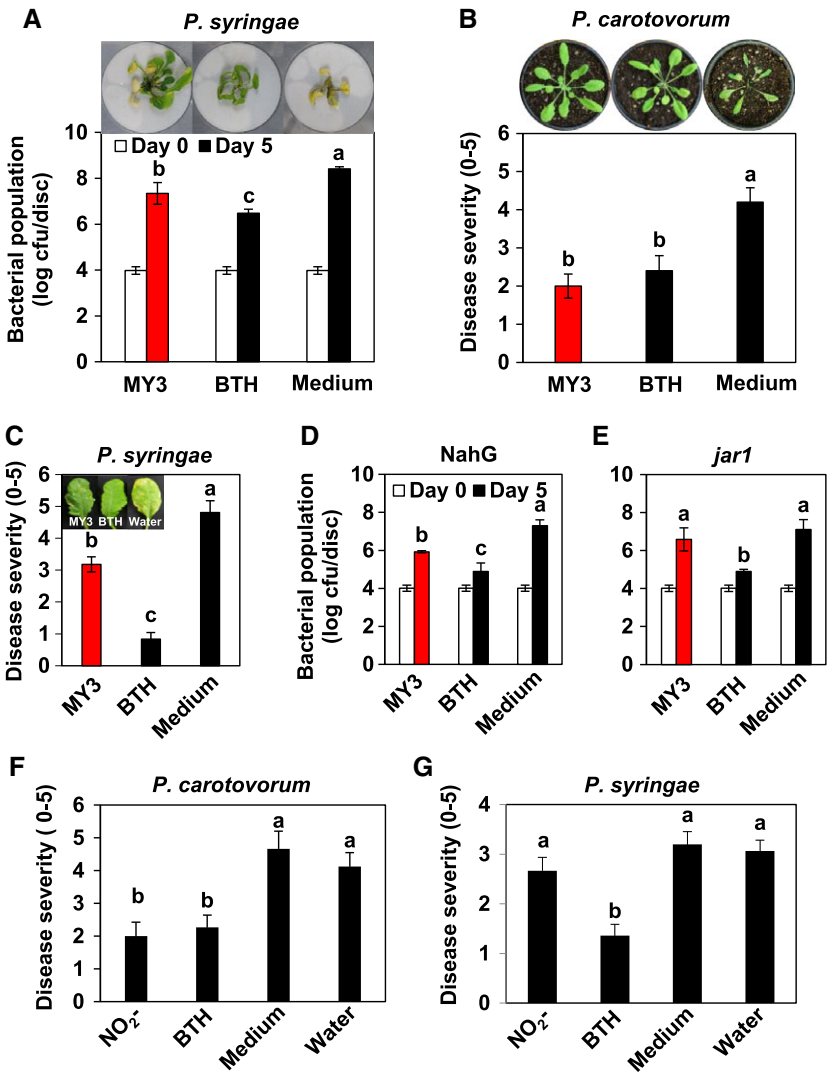

Fig. 3. *N. oleophilus* MY3 trigger induced systemic resistance in *Arabidopsis*.
A. Representative photographs and bacterial cell count of *N. oleophilus* MY3-treated *Arabidopsis* plants inoculated with *Pseudomonas syringae* pv. *tomato* DC3000 (10^8 cfu ml^{-1}). Images were taken at 7 dpi, and bacterial cell count was measured at 0 and 5 dpi.
B. Representative photographs of *N. oleophilus* MY3-treated *Arabidopsis* plants inoculated with *Pectobacterium carotovorum* subsp. *carotovorum* SCC1 taken at 1 dpi. Symptoms of disease severity were recorded 24 h after pathogen challenge (0, no symptoms; 1, mild yellowing of the inoculated leaf; 2, partial softening or collapse of the leaf at the inoculation site; 3, almost complete softening or collapse of the leaf at the inoculation site; 4, intensification of leaf soft rot on other leaves; 5, complete plant collapse).
C. Symptoms of disease severity on plants inoculated with *P. syringae* pv. *tomato* DC3000 were scored on a scale of 0–5 at 7 dpi as follows: 0, no symptoms; 1, yellowish colour; 2, chlorosis only; 3, partial necrosis and chlorosis; 4, necrosis of the inoculated area and expanded chlorosis; 5, complete necrosis of the inoculated area.
D and E. Bacterial cell count of *P. syringae* pv. *tomato* DC3000 in NahG (D) and *jar1* (E) plants measured at 0 and 5 dpi.
F and G. Symptoms of disease severity on *Arabidopsis* plants inoculated with *P. syringae* pv. *tomato* DC3000 (F) and *P. carotovorum* subsp. *carotovorum* (G) in the presence of *N. oleophilus* MY3 secondary metabolites. Plants in (A) were grown in the hydroponic system, and those in (B–G) were grown in soil. Data represent mean ± S.E. (n = 6 replications per treatment). Different letters indicate significant differences between treatments ($p < 0.05$; Fisher's LSD test). The experiment was repeated three times with similar results.

infiltration to levels similar to those triggered by BTH treatment ($p < 0.05$; Fig. 3B). *N. oleophilus* MY3 also elicited ISR against *P. syringae* pv. *tomato* DC3000 at 5 days after pathogen infiltration ($p < 0.05$; Fig. 3C).

Salicylic acid (SA) and jasmonic acid (JA) are major plant defence hormones. To elucidate whether *N. oleophilus* MY3-induced pathogen resistance is mediated via SA or JA signalling, we investigated the activation of ISR in NahG transgenic and *jar1* mutant *Arabidopsis* plants. Application of *N. oleophilus* MY3 elicited ISR against *P. syringae* pv. *tomato* DC3000 in NahG transgenic plants, which catabolize SA to the inactive molecule catechol, but not in JA-insensitive *jar1* mutant plants (Fig. 3D and E). These results indicate that *N. oleophilus* MY3 elicits ISR in a not SA-dependent but JA-dependent manner.

Next, we attempted to identify ISR determinants from AOA. Several microbial determinants have been shown to elicit plant immunity (Walters *et al*., 2008), including microbial (fungal and bacterial) cell-wall components and secreted metabolites. Among these secreted metabolites, NO_2^- is a major product released from the AOA (phylum *Thaumarchaeota*) (Jung *et al*., 2016). Therefore, we investigated whether the intermediate and final compounds obtained from the association of AOA and AOB with plants are sufficient to elicit ISR against the two pathogens. The product of AOA (NO_2^-) in soil clearly triggered ISR against the necrotrophic pathogen *P. carotovorum* subsp. *carotovorum* SCC1 but failed to elicit ISR against the biotrophic pathogen *P. syringae* pv. *tomato* DC3000 (Fig. 3F and G). Since *N. oleophilus* MY3 induced resistance against both pathogens, products of AOA (NO_2^-) may not be the main factor responsible for *N. oleophilus* MY3-induced resistance. Taken together, these findings suggest that metabolites secreted by AOA partially trigger JA-dependent signalling, resulting in ISR against *P. carotovorum* subsp. *carotovorum* SCC1 (Fig. 3E).

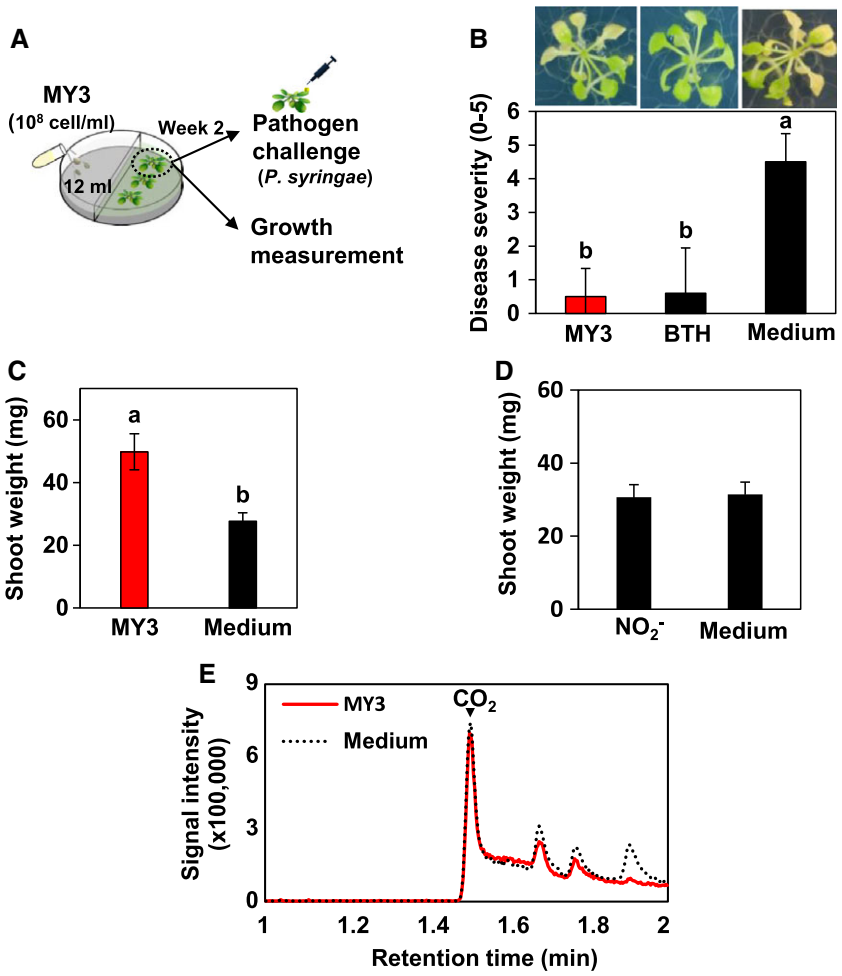

Fig. 4. PGPA-emitted volatile-mediated ISR and plant growth promotion.
A. The I-plate system used to test *N. oleophilus* MY3 volatiles.
B and C. The capacity of *N. oleophilus* MY3 volatiles to induce resistance against *P. syringae* pv. *tomato* DC3000 (B) and promote growth of *Arabidopsis* plants (C).
D. Volatile effect of *N. oleophilus* MY3 secondary metabolites, NO_2^-, on the growth of *Arabidopsis* plants.
E. Analysis of volatile compounds of *N. oleophilus* MY3 and *N. oleophilus* MY3 culture medium using gas chromatography followed by tandem mass spectrometry (GC–MS/MS). Data represent mean ± S.E. (n = 15 replications per treatment). Carbon dioxide (CO_2) concentrations were determined after adsorption for 12 h using SPME DVB/CAR/PDMS fibres in an I-plate containing *N. oleophilus* MY3 medium. Different letters indicate significant differences between treatments ($p < 0.05$; Fisher's LSD test). The experiment was repeated three times with similar results.

PGPA-emitted volatile-mediated induced systemic resistance and plant growth promotion

We further investigated potential determinant(s) of *N. oleophilus* MY3 capable of eliciting ISR against *P. syringae* pv. *tomato* DC3000. We previously showed that volatile compounds emitted by PGPR elicit ISR against *P. syringae* pv. *tomato* DC3000 when applied to plants (Lee *et al*., 2012). Therefore, we investigated the possibility that volatile compounds are responsible for the resistance induced by the PGPA *N. oleophilus* MY3. Using compartmented Petri dishes, referred to as 'I-plates' (Fig. 4A), we detected ISR in *Arabidopsis* seedlings against *P. carotovorum* subsp. *carotovorum* SCC1 (data not shown) and *P. syringae* pv. *tomato* DC3000 (Fig. 4B), even when the *N. oleophilus* MY3 culture was spatially separated from the seedlings. This result strongly suggests that volatile emissions from *N. oleophilus* MY3 are major bacterial determinants that elicit ISR against both pathogens. In addition, volatile compounds from *N. oleophilus* MY3 promoted plant growth (Fig. 4C and D).

PGPR-emitted volatiles such as acetoin and 2,3-butanediol have been shown to trigger ISR (Ryu *et al*., 2004). We therefore examined the genome sequence of *N. oleophilus* MY3 and searched for genes involved in volatile biosynthesis pathways such as the acetoin metabolic pathway. Numerous studies have identified other bacterial volatiles and determined their effects on plant responses to pathogen infection. Signalling pathways involved in major plant defence mechanisms, such as those that mediate the effects of SA, JA and ethylene, have been intensively studied using only 2,3-butanediol, acetoin and tridecane volatiles within the *A. thaliana–P. syringae* pathosystem (Kwon *et al*., 2010; Lee *et al*., 2012). Of these, the most well characterized microbial volatiles include 2,3-butanediol and its precursor acetoin, the last intermediate in the 2,3-butanediol biosynthesis pathway. Metabolic conversion of acetoin to 2,3-butanediol is reversible in most bacteria but irreversible in fungi such as yeast (Syu, 2001). A search for key enzymes in the acetoin biosynthesis pathway encoded by the *N. oleophilus* MY3 genome revealed genes encoding acetolactate synthase (*alsS*) and acetolactate decarboxylase (*alsD*). Acetoin is converted to 2,3-butanediol by acetoin reductase, encoded by the *budC* gene. The *N. oleophilus* MY3

genome harbours only the *alsS* gene (NMY3_03009), which encodes an enzyme that converts pyruvate to α-acetoin. However, gas chromatography–mass spectrometry (GC–MS) analysis failed to detect acetoin or 2,3-butanediol in *N. oleophilus* MY3 cultures (data not shown). It is possible that α-acetoin was converted to a molecule similar to acetoin that induces disease resistance. Therefore, we searched for archaea-specific acetoin derivatives or other volatile compounds that promote plant growth. Gas chromatography followed by tandem mass spectrometry (GC–MS/MS) revealed similar volatile compounds in *N. oleophilus* MY3-treated and control samples. Although some studies suggest carbon dioxide (CO_2) as a factor affecting plant growth (Nautiyal *et al*., 2010; Gamage *et al*., 2018), no significant differences were observed in the amount of CO_2 in *N. oleophilus* MY3-treated and control samples (Fig. 4E). Therefore, we concluded that plant growth promotion by *N. oleophilus* MY3 is not induced by CO_2 but by unknown volatile compounds. Future investigations should focus on identifying key determinants of archaea that affect plant growth.

Conclusion

To the best of our knowledge, this is the first study demonstrating that soil archaea promote growth and trigger ISR in plants via a SA-independent signalling pathway. This result is consistent with previously described soil bacteria- and fungi-mediated ISR signalling. Furthermore, we showed that volatile compounds of soil archaea are the major bacterial determinants of ISR capacity. Our findings lay a strong foundation for future research in the new field of archaea–plant interactions. However, the mechanism underlying downstream ISR signalling in response to archaea as well as additional determinants of the AOA *N. oleophilus* MY3 remain unknown and need further investigation.

Experimental procedures

Plant material and treatments

Wild-type plants of *Arabidopsis thaliana* ecotype Columbia (Col-0), SAR transgenic line NahG (Wu *et al*., 2012) and JA mutant *jar1* (Staswick *et al*., 2002) were used in this study. Seeds were surface sterilized with 3% sodium hypochlorite, washed four times with sterilized distilled water and incubated on half-strength Murashige and Skoog (1/2 MS) salt medium (0.6% agar and 1.5% sucrose [pH 5.8]) at 23 °C under a 16 h light/8 h dark cycle for 4 days until germination. To perform bioassays in the hydroponic system, 4 day-old seedlings were transferred to Petri plates (60 × 15 mm; SPL) containing 26 ml of 1/2× MS liquid medium and incubated in a Phytohealth plant culturing container (103 × 78.6 mm; SPL) (Song *et al*., 2015). Plants were treated with 10 ml of *N. oleophilus* MY3 culture, BTH or media (control; Fig. 2). To evaluate the effect of various treatments, shoot weight and SPAD values (SPAD-502 plus; Konica Minolta, Tokyo, Japan) were measured at day 14. The SPAD value is an index of leaf chlorophyll content, which indicates the photosynthetic ability of leaves. To perform bioassay in soil, 7 day-old *Arabidopsis* seedlings were transferred to a soil (Sangto 2; Punong, Gyeongju, South Korea) containing 300 p.p.m. of total nitrogen and incubated in a growth chamber maintained at 23 °C under a 16 h light/8 h dark cycle for 14 days. Subsequently, plants were treated with a drench application of *N. oleophilus* MY3, BTH or media (control) dissolved in 10 ml of 0.5 mM NO2- solution. For volatile experiment, 10 μl of 0.5 mM NO_2^- solution was drop-inoculated on MS media in one compartment of the I-plate, while *Arabidopsis* seedlings were transferred the other compartment of the plate. To evaluate plant growth, shoot weight was measured at 2 weeks post-inoculation.

Cultivation of N. oleophilus *MY3*

N. oleophilus MY3 was cultured in artificial freshwater medium (AFM), as described previously (Jung *et al*., 2011), and the cultures were grown aerobically in the dark without shaking at 25 °C. After autoclaving AFM, 1 ml of non-chelated trace element solution (Widdel and Bak, 1992), 1 ml of NaFeEDTA solution (7.5 mM) and 3 ml of $NaHCO_3$ (1 M) were added to the medium. The pH of the final medium was adjusted to 7 using 1 N NaOH or HCl. Unless otherwise stated, each starting batch culture was supplemented with 1 mM ammonium chloride as the sole energy source. The medium containing ammonia was used as a control. Plants were treated with culture medium after incubation at 30 °C for 2 weeks.

Assessment of disease severity and bacterial growth

P. carotovorum subsp. *carotovorum* SCC1 and *P. syringae* pv. *tomato* DC3000 were grown on solid LB and King's B medium, respectively, containing 100 μg ml^{-1} rifampicin at 30 °C for 2 days. Subsequently, bacteria were scraped off the plates and re-suspended in 10 mM $MgCl_2$ (Song *et al*., 2013). To inoculate plants in the hydroponic system, five leaves from each of four 4 week-old plants were treated with 2 μl drops containing *P. carotovorum* subsp. *carotovorum* SCC1 and *P. syringae* pv. *tomato* DC3000 suspension (1×10^8 colony forming units [cfu] ml^{-1}). To inoculate plants grown in pots, five leaves from each of four 3 week-old plants were infiltrated with 1×10^4 cfu ml^{-1} suspension of *P. carotovorum*

subsp. *carotovorum* SCC1 and *P. syringae* pv. *tomato* DC3000. The total number of bacterial colonies in leaves was counted using serial dilution method of leaf disc (1 cm^2) at 0 and 5 days after drop inoculation and infiltration of pathogen respectively. The severity of infection was scored as described previously (Song *et al*., 2013).

FISH assay

FISH was conducted on root samples of *Arabidopsis* seedlings grown in the axenic liquid system. To visualize *N. oleophilus* MY3 colonization on the root surface and interior, 2 week-old *Arabidopsis* seedlings grown in the hydroponic system were inoculated with 2 ml of MY3 liquid culture (10^8 cells ml^{-1}; Song *et al*., 2015; Fig. 2A), and roots were sampled at 0, 7 and 14 days post-inoculation. Since plants were free of contamination, the application of an archaea-specific 16S rRNA probe targeting Arch-915 (5′-GTGCTCCCCCGCCAATTCCT-3′) was sufficient. FISH was conducted as described previously (Jung *et al*., 2016), with minor modifications. After dehydration in an ethanol series (50%, 80% and 100% ethanol for 3 min each), 100 μl of hybridization buffer (0.7 ml of Formamide, 360 μl of 5 M NaCl, 40 μl of 1 M Tris/HCl [pH 8.0], 2 μl of 10% SDS [w/v] and 0.9 ml of deionized sterile water) as well as 10 μl of probe (20 ng μl^{-1}) solution was added to the fixed sample. Hybridization was performed at 46 °C for 12 h. This was followed by a stringent (35%) washing step for 15 min at 48 °C in washing buffer (700 μl of 5 M NaCl, 500 μl of 0.5 M EDTA [pH 8.0], 1 ml of 1 M Tris/HCl [pH 8.0], 50 μl of 10% SDS [m/v] and 50 ml of sterile deionized water). Samples were quickly rinsed with ice-cold sterile deionized water and dried at room temperature in the dark. Subsequently, samples were placed on a microscope slide, covered with a cover slip and examined under a confocal laser scanning microscope (LSM 800; Carl Zeiss International, Oberkochen, Germany). Images were analysed using the ZEN 2 (blue edition) software.

qRT-PCR analysis

Two-week-old *Arabidopsis* seedlings grown in the hydroponic system were inoculated with 2 ml of *N. oleophilus* MY3 cells (10^8 cells ml^{-1}), and root samples were collected at 0, 7 and 14 days post-inoculation (Fig. 2A). Samples were ground in liquid nitrogen and homogenized with 1.5 mm glass beads at 6500 × *g* for 20 s to lyse archaeal cells. Subsequently, gDNA was extracted from root samples using the PureLink gDNA mini kit (Invitrogen, USA), according to the manufacturer's instructions. Copy number of root-colonizing MY3 gDNA was quantified relative to that of *Arabidopsis* root gDNA by qRT-PCR using *N. oleophilus* MY3-specific 16S rRNA primers (forward: 5′-gcaataggcgaaagcttgac-3′, reverse: 5′-ccgagtctgcatcgtttaca-3′), and *Arabidopsis actin* primers (forward: 5′-gaagaactatgaattacccgatgg-3′, reverse: 5′-tacagatccttcctgatatccaca-3′). The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 42 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s.

Volatile collection and analysis

Two commercially available solid phase microextraction (SPME) fibres suitable for volatile analysis (Supelco, Bellefonte, PA, USA) were utilized in this study. Vials containing samples were placed in a heating block (Gerstel MultiPurpose Sampler MPS 2, Baltimore, MD, USA) at 50 °C, with the SPME fibre inserted into the headspace above the *N. oleophilus* MY3 sample. Adsorption was performed for 30 min. The SPME fibres were desorbed at 210 °C for 1 min in the injection port of an HP 5890A GC/MS (Hewlett–Packard, Palo Alto, CA, USA) instrument equipped with a DB5 column (length = 60 m, internal diameter = 0.25 mm, film thickness = 0.25 μm; J&W Scientific, Olsom, CA, USA). Samples were subjected to GC–MS analysis for 25 min per run, and the fibre was retained in the injection port for 10 min after each run. The injection port was operated in splitless mode with a constant He flow rate of 1.0 ml min^{-1}. The initial oven temperature was held at 33 °C for 3 min, and then increased at a rate of 10 °C min^{-1} to 180 °C, followed by 40 °C min^{-1} to 220 °C, which was held for 5 min. The HP quadrupole mass spectrometer was operated in electron ionization mode at 70 eV with source and quadrupole temperature of 200 °C and 150 °C, respectively, and a continuous scan from 40 to 500 m/z.

Statistical analysis

Analysis of variance (ANOVA) of the experimental data sets was performed using the JMP software version 5.0 (SAS Institute, Cary, NC, USA; www.sas.com). Significant effects of treatments were determined based on the *F*-value ($p < 0.05$), and separation of means was performed using the Fisher's protected least square difference (LSD) test.

Acknowledgements

This research was supported by grants from the Woo Jang-Choon Project (PJ01093904) of the Rural Development Administration (RDA), Strategic Initiative for Microbiomes in Agriculture and Food, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (as part of the [multi-ministerial] Genome Technology to Business

Translation Program 918017-4) and KRIBB Initiative Program (KGM2111844), South Korea. S.-K.R. was supported by a grant from National Research Foundation of Korea (NRF-2015R1A4A1041869). The authors declare no conflict of interest.

Author contributions

G.C.S., H.I. and C.-M.R. planned and designed the research. G.C.S., J.J., S.L. and H.I. performed the experiments and analysed data. M.-Y.J. and S.-K.R. developed archaea cultures. G.C.S. and C.-M.R. wrote the manuscript.

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