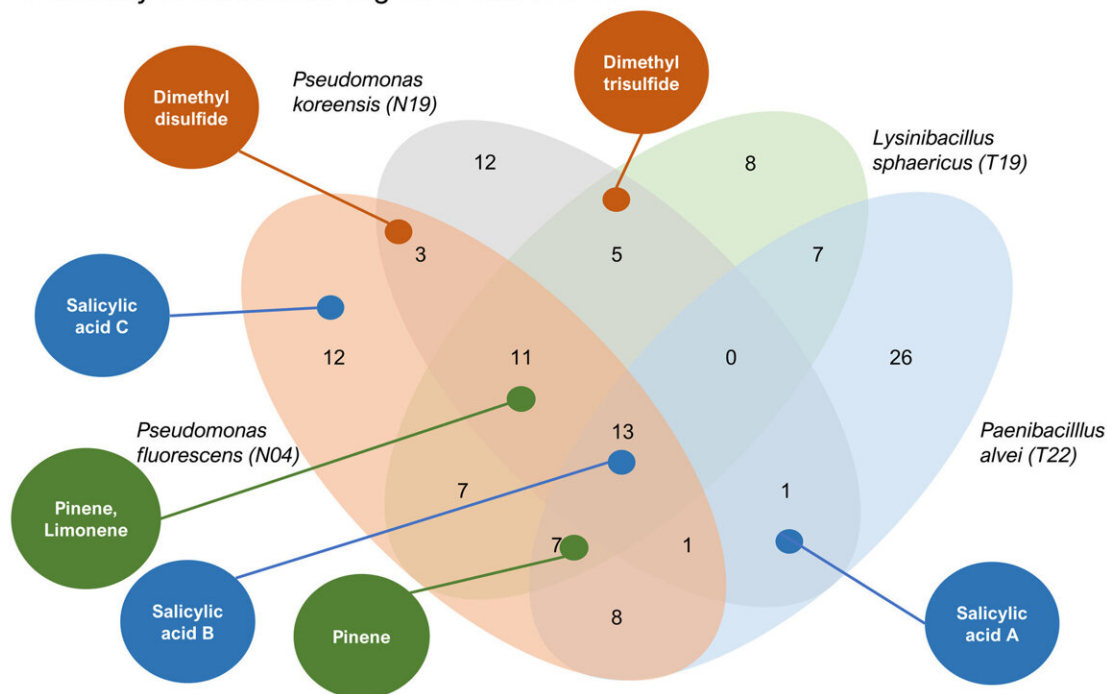


Faster screen of biologicals for growth stimulants, disease protection in wheat and corn

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Surprising haul of volatile compounds identified

Just four strains of rhizobacteria in the root zone of row crops can produce dozens of different Volatile Organic Compounds, researchers from the University of Johannesburg have discovered.



Credit: Therese van Wyk. Adapted from graphic in by Dubery, Mhlongo et al in *Metabolites*. (<https://doi.org/10.3390/metabo12080763>) <https://creativecommons.org/licenses/by/4.0/>

In an open research study in published in *Metabolites*, researchers from the University of Johannesburg show the discovery that just four strains of rhizobacteria can produce at least 121 different Volatile Organic Compounds (VOCs).

Rhizobacteria in the root zone “partner” with plants, and use the VOCs to signal to the plant’s roots or leaves, to signal to other plants, and even to signal to insects, to help protect the host plant.

The graphic shows how four strains of bacteria produced 121 different Volatile Organic Compounds, and how some of the compounds are produced by more than one strain.

Bacteria in the root zone of crops like wheat, tomatoes and corn produce volatile organic compounds that can trigger enhanced plant growth and Induced Systemic Resistance (ISR). ISR helps the plant resist stresses like heat, pests and lack of water. Salicycyclic acid is known to trigger ISR in host plants. Credit: Therese van Wyk. Adapted from graphic by Dubery, Mhlongo et al. in *Metabolites*.

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Researchers found a much faster way to screen soil bacteria as potential biostimulants and bio-pesticides. UJ scientists identified ten times more volatile signal compounds from the bacteria, compared to most recent studies.

Rhizobacteria can protect crops from abiotic and biotic stresses by boosting plant growth and plant self-defense. Farmers apply the bacteria as seed coatings or inoculants. "Biologicals" are friendly to the environment and can reduce the need for chemical fertilizers and pesticides.

For crops such as wheat, corn and tomatoes, plant-beneficial bacteria living in their [root zone](#) can significantly protect plants and boost their growth. Bacterial protection can be effective also when plants are stressed.

In a study published in *Metabolites*, researchers from the University of Johannesburg (UJ) show a much faster approach for screening bacteria for potential use as natural fertilizers and pesticides.

They grew four strains of promising rhizobacteria in the laboratory. Then they analyzed the [signaling molecules](#) the bacteria produced with metabolomic techniques.

Using a new approach, they identified a surprising haul of 121 different [volatile organic compounds](#) (VOCs) produced by just the four strains. This is between 10 and 20 times what most current published research studies identify.

Natural growth boost and protection

Among the 121 VOCs, they found three forms of [salicylic acid](#). These VOCs were produced by three of the four strains of bacteria.

"We found three derivatives of salicylic acid: methyl salicylate, isoamyl salicylate and n-hexyl salicylate.

"Salicylic acid is a plant hormone that plays a big role in [plant growth](#) and induction of resistance against disease," says Dr. Msizi Mhlongo. Mhlongo is the lead author of the study and senior lecturer at the UJ Department of Biochemistry.

Methyl salicylate is better known outside research circles for being the chemical behind the smell of "wintergreen," which is added to body lotions, sports injury patches and food.

"Methyl salicylate is volatile and can travel right around the plant in the air. But it is also a mobile signal within the plant—it can travel from the roots to the leaves of a plant. The 'roots can tell the leaves to wake up and trigger resistance'—using [methyl salicylate](#). This form of resistance is Systemic Acquired Resistance (SAR)," says Prof. Ian Dubery, also from the UJ Department of Biochemistry and supervisor of the study.

Telling signals apart

The researchers used [high-resolution mass spectrometry](#) to differentiate between the 121 VOC molecules.

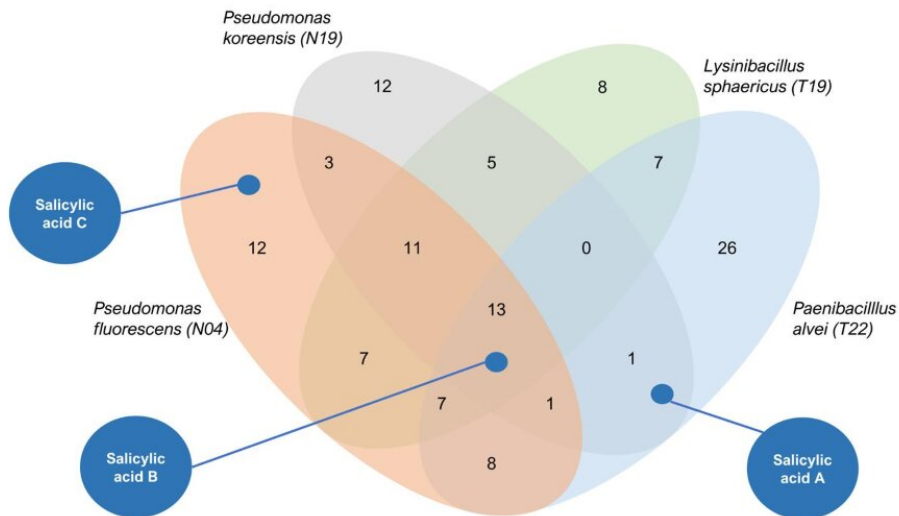
"It is the instrument's sensitivity, high scanning rate, and its ability to distinguish molecular masses that are very close to each other, that made this possible. Also, the software's ability to separate out molecular

peaks," adds Mhlongo.

All three forms of salicylic acid they found, are valuable for their ability to trigger Induced Systemic Resistance (ISR) in some crops. ISR is the root-driven "induced immune system" that helps plants protect themselves when they are stressed or under attack. Farmers can boost ISR in their crops with rhizobacteria seed coatings or inoculants.

Bacteria volatiles trigger ISR

Bacteria in the root zone of crops like wheat, tomatoes and corn produce volatile organic compounds that can trigger enhanced plant growth and Induced Systemic Resistance (ISR). ISR helps the plant resist stresses like heat, pests and lack of water. Salicylic acid is known to trigger ISR in host plants.



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Just four strains of rhizobacteria can produce three forms of salicylic acid, researchers from the University of Johannesburg found, in an open study published in *Metabolites*. These are A: methyl salicylate; B: isoamyl salicylate; and C: n-hexyl salicylate. Soil bacteria produce salicylic acid to trigger ISR in their partner plants.

The graphic shows how four strains of bacteria produced 121 different Volatile Organic Compounds, and how some of the compounds are produced by more than one strain.

Just four strains of rhizobacteria in the root zone of row crops can produce dozens of different Volatile Organic Compounds, researchers from the University of Johannesburg have discovered. Credit: Therese van Wyk. Adapted from graphic by Dubery, Mhlongo et al. in *Metabolites*.

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Screening for biostimulants

"We were looking for the important signal molecules that these bacteria secrete to trigger a state of resistance in plants," says Dubery.

"Not all bacteria in the rhizosphere are active inducers of SAR or ISR. The purpose of this study was to find out which strains are better at inducing ISR," he adds.

They used Southern African strains of the rhizobacteria *Pseudomonas koreensis*, *Pseudomonas fluorescens*, *Lysinibacillus sphaericus* and *Paenibacillus alvei*. Prof Nico Labuschagne from the University of Pretoria supplied the strains from his collection of rhizobacteria isolates.

Previous glass house trials by Labuschagne in South Africa had identified the four strains for their biostimulant and bio-pesticide potential for wheat, corn and tomatoes.

"In general, the process of screening bacteria starts with isolating them from an environment such as a type of soil or wetland. You can screen them for the presence of a single molecule or a group of molecules. Then you would screen for safety, followed by glass house trials, then field trials, before releasing them commercially," says Dubery.

The plant 'gut biome'

During good conditions without much stress, rhizobacteria partner with plants and their roots, much like gut bacteria partner with their human hosts, says Dubery. The rhizobacteria enable plants to absorb nutrients such as nitrogen and phosphorus, among many other activities.

During times of abiotic stresses, the rhizobacteria help defend their partner plants. This could be lack of water, high temperatures, or high

salinity in the soil. Also, during biotic stresses such as being infected by fungi, viruses or pathogenic bacteria; or threatened by weeds or other plants; rhizobacteria step up to boost the chemical defenses of their partner plants.

The rhizobacteria accomplish all of this by producing volatile organic compounds (VOCs), such as those identified by the UJ researchers.

The VOCs act as communication signals such as "calling for help," biochemical defenses and biofertilizers in a variety of ways.

Rhizobacteria can signal to other microbes using VOCs, says Mhlongo. Microbes can signal to plants. It is even possible for plants to signal to microbes, he adds. They have previously authored a [comprehensive review of research](#) on this topic that the current study draws upon.

Screening before field trials

The results of the study show the high number of signaling molecules produced by the four strains of bacteria. Follow-up work would need to investigate the volume, concentration, and consistency of production.

"As more research is done, biostimulant approaches become more reliable," says Mhlongo.

"Plant growth promoting rhizobacteria (PGPR) are crop specific. What works well for tomatoes, wheat, and maize (corn) might not work for spinach. This kind of research helps us know which plant-specific bacteria will promote growth and induced resistance," he adds.

VOC analyses like this study can create "biomarkers" for future studies, adds Mhlongo. As an example, one could define the three forms of salicylic acid they found in this study as a biomarker for induced plant

resistance SAR and ISR.

Then, when screening other rhizobacteria for inducing plant resistance, it's not necessary to go do glass house trials or field trials to rule out non-starters. Instead, one can do a similar analysis of the VOCs from those [bacteria](#), using high-resolution mass spectrometry. This makes it possible to rule out strains or combinations of strains early on, which do not produce a desired group of molecules such as the different forms of salicylic acid.

More information: Msizi I. Mhlongo et al, Profiling of Volatile Organic Compounds from Four Plant Growth-Promoting Rhizobacteria by SPME–GC–MS: A Metabolomics Study, *Metabolites* (2022). [DOI: 10.3390/metabo12080763](#)

Provided by University of Johannesburg

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