Genomic Analysis of the 1-Aminocyclopropane-1-Carboxylate Deaminase-Producing *Pseudomonas thivervalensis* SC5 Reveals Its Multifaceted Roles in Soil and in Beneficial Interactions With Plants

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Beneficial 1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing bacteria promote plant growth and stress resistance, constituting a sustainable alternative to the excessive use of chemicals in agriculture. In this work, the increased plant growth promotion activity of the ACC deaminase-producing *Pseudomonas thivervalensis* SC5, its ability to limit the growth of phytopathogens, and the genomics behind these important properties are described in detail. *P. thivervalensis* SC5 displayed several active plant growth promotion traits and significantly increased cucumber plant growth and resistance against salt stress (100 mmol/L NaCl) under greenhouse conditions. Strain SC5 also limited the *in vitro* growth of the pathogens *Botrytis cinerea* and *Pseudomonas syringae* DC3000 indicating active biological control activities. Comprehensive analysis revealed that *P. thivervalensis* SC5 genome is rich in genetic elements involved in nutrient acquisition (N, P, S, and Fe); osmotic stress tolerance (e.g., glycine-betaine, trehalose, and ectoine biosynthesis); motility, chemotaxis and attachment to plant tissues; root exudate metabolism including the modulation of plant phenolics (e.g., hydroxycinnamic acids), lignin, and flavonoids (e.g., quercetin); resistance against plant defenses (e.g., reactive oxygen species-ROS); plant hormone modulation (e.g., ethylene, auxins, cytokinins, and salicylic acid), and bacterial and fungal phytopathogen antagonistic traits (e.g., 2,4-diacetylphloroglucinol, HCN, a fragin-like non ribosomal peptide, bacteriocins, a lantipeptide, and quorum-quenching activities), bringing detailed insights into the action of this versatile plant-growth-promoting bacterium. Ultimately, the combination of both increased plant growth promotion/protection and biological control abilities makes *P. thivervalensis* SC5 a prime candidate for its development as a biofertilizer/biostimulant/biocide product. The genomic analysis of this bacterium brings new insights into the functioning of *Pseudomonas* and their role in beneficial plant-microbe interactions.

**Keywords:** *Pseudomonas*, plant-growth-promoting bacteria, 1-aminocyclopropane-1-carboxylate deaminase, genomics, plant-microbe interaction
INTRODUCTION

The increasing demand for sustainable agricultural practices creates the need for the development of novel strategies to promote plant growth and stress resistance without resorting to harmful chemical fertilizers and pesticides. One alternative to such polluting chemical compounds resides in the use of plant-growth-promoting bacteria (PGPB) that are able to efficiently promote plant growth and health (Glick, 1995; de Souza et al., 2015). These beneficial plant-associated bacteria are naturally found in soils and plant tissues, and are known to help plants overcome numerous growth limitations and stressful conditions, being of great importance for agricultural and biotechnological applications (Glick, 2012).

One of the most important mechanisms employed by PGBP involves the expression of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and the subsequent modulation of the plant hormone ethylene through the catabolism of its direct precursor, ACC (Glick et al., 1998; Glick, 2014). Ethylene is one of the most important plant hormones regulating plant growth and development, being involved in multiple physiological and developmental processes of plants (Van de Poel et al., 2015; Dubois et al., 2018), as well as in the regulation of plant-microbe interactions (Guinel, 2015; Nascimento et al., 2018a). Moreover, ethylene is also involved in plant responses to stress conditions, including those induced by biotic (e.g., pathogens, insects, and nematodes) and abiotic (e.g., salinity, low nutrient availability, low pH, heavy metals, and organic contaminants) stress factors (Lund et al., 1998; Broekaert et al., 2006; Gamalero and Glick, 2015; Tao et al., 2015; Keunen et al., 2016).

Bacteria presenting ACC deaminase activity decrease the deleterious levels of biotic and abiotic stress-induced ACC and ethylene that inhibit plant growth (Ma et al., 2004; Mayak et al., 2004; Belimov et al., 2009; Nascimento et al., 2013; Ali et al., 2014; Jaemsang et al., 2018). The beneficial and protective effect of ACC deaminase-producing bacteria often leads to the increased selection of these bacteria in bacterial communities associated with stressed plants (Timmusk et al., 2011; Croes et al., 2013; Truyens et al., 2013). Moreover, ACC deaminase genes (acdS) are positively selected in rhizobial symbionts associated with several leguminous plants worldwide (Nascimento et al., 2018b), further emphasizing the importance of this enzyme in beneficial plant-microbe interactions. Altogether, this data indicates that obtaining and selecting efficient ACC deaminase-producing bacteria is crucial for the development of successful biofertilizers with both increased plant growth promotion potential and an increased ability to protect plants from biotic and abiotic stress.

In a recent study, the direct isolation of several ACC deaminase-producing bacteria from various plants and locations was reported (Nascimento et al., 2019). One of the isolated strains, Pseudomonas thivervalensis SC5, presented very high levels of ACC deaminase activity suggesting an increased plant growth promotion potential (Nascimento et al., 2019).

The present work deals with the characterization, plant growth promoting activities and genomic properties of P. thivervalensis SC5.

MATERIALS AND METHODS

Pseudomonas thivervalensis SC5 Characterization

Pseudomonas thivervalensis SC5 was routinely grown in Tryptic Soy Broth (TSB, Casvi, Brazil) at 28°C, 150 rpm, overnight. The fresh bacterial suspensions were then used in the biochemical characterization assays, which were conducted in duplicate.

Briefly, strain SC5 was qualitatively tested for the ability to synthesize extracellular enzymes such as protease (in skim milk agar, Himedia), lipase (in rodamine B+ olive oil agar, Kouker and Jaeger, 1987), esterase (in Tributyrin agar, Himedia), amylase (Nutrient Broth supplemented with 5 g/l starch; Gopinath et al., 2017), and cellulase (carboxymethylcellulose-CMC-agar; Kasana et al., 2008; Vicente et al., 2012). The strain was also tested for phosphate (PO₄) and zinc oxide (ZnO) solubilization (de Freitas et al., 1997; Sharma et al., 2011); siderophores production in Chrome Azurol S Agar (Schwyn and Neillands, 1987) and in King’s B agar (Pseudomonas agar F, Himedia; presence of fluorescence); amino acid decarboxylase/polymamines production using Moeller Decarboxylase Broth supplemented with 2% L-Arginine, or L-Lysine, or L-Ornithine (Himedia); the biosynthesis of ammonia in proteose peptone broth using the Nessler reagent as indicator (Marques et al., 2010); the ability to produce H₂S and indole, as well as the ability to present motility were tested using Sulfide Indole Motility (SIM) media (Sigma-Aldrich); the biosynthesis of indole-3-acetic acid (IAA) in TSB supplemented with 500 μg/ml of tryptophan and posterior detection using the Salkowski reagent (Glickmann and Dessaux, 1995); nitrate/nitrite reduction (Buxton, 2011); resistance to high salinity (growth in TSB supplemented with 3 and 5% w/v NaCl); resistance to antibiotics (growth in TSB supplemented with ampicillin-100 μg/ml, chloramphenicol-30 μg/ml, kanamycin-50 μg/ml, and tetracycline-15 μg/ml); and the ability to use 4-aminoiminobutyrate (GABA), KNO₃, KNO₂, and phytohormones [IAA, benzoic acid (BA), salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), gibberellic acid-GA₃]- as sole carbon/nitrogen sources, as previously described (Nascimento et al., 2019).

Plant Growth Promotion Assays

Cucumber Plant Growth Promotion Assay Under Laboratory Conditions

Commercial cucumber “Straight eight” (Cucumis sativus) seeds (Isla, Brazil) were surface disinfected by immersion in 70% alcohol for 10 minutes and then rinsed thoroughly with sterile distilled water. The seeds were then planted in individually labeled Petri dishes containing sterile vermiculite and 3% sucrose (w/v) solution. After 5 days of incubation at 28°C, the seedlings were transplanted into the nutrient solution in 9 cm pots filled with a mixture of peat:vermiculite (7:3, v/v) and watered with a solution containing 300 μM FeSO₄·7H₂O, 200 μM MnCl₂·4H₂O, 50 μM CoCl₂·6H₂O, 500 μM CuCl₂·2H₂O, and 1000 μM ZnSO₄·7H₂O. The plants were grown in a growth chamber at 28°C, 150 µmol m⁻² s⁻¹, and 16 h photoperiod. At each harvest, plant parameters such as shoot length (SL), root length (RL), root dry weight (RDW), shoot dry weight (SDW), and total dry biomass (TDB) were measured. The dry biomass was determined by drying the samples at 80°C for 48 hours. The aboveground and belowground biomass was separated, dried, and weighed. The shoot and root dry weights were calculated as the difference between the fresh weight and the dry weight.

RESULTS

Growth Promotion Assays

Cucumber plants treated with P. thivervalensis SC5 exhibited an increased plant growth promotion potential compared to the control (Fig. 1). The shoot length (SL) and root length (RL) of the treated plants were significantly higher than those of the control plants. Similarly, the root dry weight (RDW) and shoot dry weight (SDW) of the treated plants were also significantly higher than those of the control plants. The total dry biomass (TDB) of the treated plants was also significantly higher than that of the control plants (Table 1). These results indicate that P. thivervalensis SC5 has the potential to promote plant growth and stress resistance without resorting to harmful chemical fertilizers and pesticides.
ethanol for 1 min, followed by 10 min in 1% sodium hypochlorite. The seeds were then rinsed five times in sterile deionized water. The seeds were distributed into Petri dishes containing 1% water agar and incubated in the dark at 25°C for 3 days. Each germinated seed was sown in a 300 ml (volume) pot filled with sterilized vermiculite and sand in a 1:1 (v/v) ratio. The experiment consisted of two treatments: the non-inoculated control, and SC5 inoculation. The inoculation consisted of soaking the germinating seeds for 1 h in an overnight grown bacterial suspension (TSB, 28°C, 150 rpm) adjusted to OD₆₀₀ = 0.3 in sterile 0.03 M MgSO₄, followed by the direct application of 3 ml of a bacterial suspension (OD₆₀₀ = 0.3) into the root-shoot junction of germinated plants. Six plants were used for each treatment. The assay was conducted under laboratory conditions with an average temperature of 25°C and a 12 h photoperiod. The plants received 10 ml Hoagland's nutrient solution (Hoagland and Arnon, 1950) whenever necessary, and were harvested 3 weeks after inoculation. The plant roots and shoots were excised and dried for 3 days at 60°C in order to record dry weights on an analytical scale.

Cucumber Plant Growth Promotion Assay Under Normal and Salt Stress Conditions in the Greenhouse
Salt stress is one the major inducers of ACC and ethylene production by several plant species, which ultimately leads to plant growth inhibition (root and shoot development, overall biomass; Tao et al., 2015). The fine-tuning of ACC and ethylene levels under salt stress conditions is therefore of importance for increased plant salt stress resistance (Riyazuddin et al., 2020). Since P. thivervalensis SC5 presented both ACC deaminase activity, salt stress resistance and overall plant growth promotion abilities, we hypothesized that this strain could be useful for increasing cucumber plant salt stress resistance. Cucumber is known for its high sensitivity to salt stress (Gamalero et al., 2010). Based on this hypothesis, P. thivervalensis SC5 was tested for its ability to help salt-sensitive cucumber plants to overcome some of the deleterious effects of salt stress (100 mmol/l NaCl).

The preparation of the experiment (seed germination, inoculation, and pots) was conducted as described above (see Section “Cucumber Plant Growth Promotion Assay Under Laboratory Conditions”).

The experiment consisted of four treatments: non-inoculated control (normal conditions); P. thivervalensis SC5 inoculation (normal conditions); non-inoculated control + 100 mmol/L NaCl (salt stress conditions); P. thivervalensis SC5 inoculation + 100 mmol/L NaCl (salt stress conditions). Six plants were used for each treatment. In the initial 5 days of growth the plants received 5 ml Hoagland's nutrient solution whenever necessary (dried top layer of vermiculite and sand). After this period, stress conditions were imposed (to the respective salt receiving treatments) by applying 10 ml Hoagland's nutrient solution containing 100 mmol/l NaCl 4 days per week. The non-inoculated control and P. thivervalensis SC5 inoculation treatments (normal conditions) received 10 ml Hoagland's nutrient solution 4 days per week.

The greenhouse experiment (average temperature 26.9°C) was conducted in the Universidade Federal de Santa Catarina, Florianópolis, Brazil. About 3 weeks after sowing, cucumber plants were harvested, washed with tap water, and root and shoot lengths (SLs) were measured. The plants were then dried at 60°C and root and shoot dry weights were measured.

Statistical Analysis
The SPSS Statistics v. 26 software (SPSS, IBM Company, United States) was used to perform a statistical analysis, which was conducted using an ANOVA and means comparison using Tukey's test.

Phytopathogen Antagonistic Activities
Strain SC5 antagonistic activities were tested against the fungal pathogen Botrytis cinerea and the bacterial pathogen Pseudomonas syringae DC3000 as previously described (Nascimento et al., 2016). The fungal antagonistic activities were detected by the inability of the fungal strain to grow over SC5 spots in potato dextrose agar plates. The bacterial antagonistic activities were detected by the inability of P. syringae DC3000 to grow in the presence of strain SC5.

Genome Sequencing and Analysis
The SC5 DNA library was constructed using Illumina TruSeq DNA Nano kit (automated) and sequenced in the Illumina MiSeq platform using the MiSeq V3 reagent kit (2×300 bp).

The complete 6,592,350 bp genome sequence of strain SC5 is a scaffold of 37 contigs (N50 = 313,629 bp), which were generated by a total of 1,480,168 reads (coverage of 67X) assembled using SOAPdenovo v2.04 (Luo et al., 2012), and constructed based on a guided assembly against the complete genome sequence of P. thivervalensis BS3779 (= LMG 21626 = NZ_LT629691.1) using Mauve (Darling et al., 2004). The contigs were joined by introducing 100 Ns in the assembly gap regions as indicated in the NCBI submission guidelines. The genome sequence of P. thivervalensis SC5 is available in the NCBI under the accession number CP022201.1.

The genome annotation was performed using the NCBI Prokaryotic annotation pipeline (Angiuoli et al., 2008). Functional genome annotation and analysis were conducted as described elsewhere (Nascimento et al., 2020). Genome circular view was created using CGview (Grant and Stothard, 2008).

Average nucleotide identity (ANI) was calculated using PyANI and the ANIb method (Pritchard et al., 2016).

RESULTS AND DISCUSSION
Characterization and Plant Growth Promotion Properties of P. thivervalensis SC5
A general characterization of P. thivervalensis SC5 was conducted and is presented in Table 1. Strain SC5 is a Gram-negative, rod shaped, motile, and non-sporulating bacterium that can grow in temperatures ranging from 7 to 30°C and in NaCl

1https://www.ncbi.nlm.nih.gov/
TABLE 1 | Phenotypic characterization of *P. thivervalensis* SC5.

| Traits                        | Characteristics |
|-------------------------------|-----------------|
| Antibiotic resistance        | (+) Ampicillin (100 μg/ml), (+) Chloramphenicol (30 μg/ml) but found to be sensitive to kanamycin (50 μg/ml) or tetracycline (15 μg/ml). Strain SC5 produced siderophores, including a pyoverdine-like pigment (presents fluorescence when grown in King’s B medium); solubilized PO₄ and ZnO; produced polyamines when supplemented with arginine but not lysine or ornithine; produced low levels of IAA (or IAA-like compounds), ~4 μg/ml, in the presence of exogenous tryptophan (500 μg/ml); and reduced nitrate to N₂ and ammonia, which is consistent with its ability to use KNO₃ and KNO₂ as sole nitrogen sources. This bacterium is positive for the extracellular lytic enzyme lipase but negative for esterase, amylase, cellulase, and protease under the tested conditions. Strain SC5 is not able to use IAA, salicylic acid (SA), benzoic acid (BA), abscisic acid (ABA), jasmonic acid (JA), and gibberellins (GA) as sole carbon sources. On the other hand, it presented the ability to consume 4-aminobutyrate (GABA). |
| Extracellular lipase activity | (+) in Rhodamine B+ olive oil agar |
| Extracellular esterase activity | (+) in Tributyrin Agar |
| Extracellular protease activity | (+) in Skimmed milk agar |
| Extracellular cellulase activity | (+) in CMC agar |
| Extracellular amylase activity | (+) in NB + starch agar |
| Ability to grow in M9 minimal medium supplemented with: | (+) KNO₃ (N source) |
|                          | (+) KNO₂ (N source) |
|                          | (+) GABA (C source) |
|                          | (-) IAA (C source) |
|                          | (-) SA (C source) |
|                          | (-) BA (C source) |
|                          | (-) ABA (C source) |
|                          | (-) GA (C source) |

(+): positive (presence of halo, or growth, when applicable). 
(-): negative (absence of halo, or growth, when applicable).

*Nascimento et al. (2019).*

Promotes Cucumber Growth Under Normal and Salt Stress Conditions

The results obtained from the cucumber growth promotion assay conducted under greenhouse conditions showed that cucumber plants inoculated with *P. thivervalensis* SC5 presented a significant increased development when compared with non-inoculated plants, in both normal and salt stress conditions (0 and 100 mmol/l NaCl, respectively; Figures 1A–C).

Compared to the non-inoculated control, the application of strain SC5 led to a significant increase in cucumber SL (8.4 vs. 11.5 cm, 36.9%), root length (RL; 18.67 vs. 23.75 cm, 27.2%), root dry weight (RDW; 77.30 vs. 102.32 mg, 32.3%), shoot dry weight (SDW; 347.47 vs. 428.52 mg, 23.3%), and total dry biomass (TDB; 424.80 vs. 530.83 mg, 25%) in the absence of added salt (Figures 1A,B).

In the presence of salt there was a significant decrease in root and shoot growth (43% shoot, 22.3% RL) and in RDW (60.2%), SDW (34.6%), and TDB (39.3%) in non-inoculated cucumber plants (Figures 1A,B). Similar results were obtained with cucumber plants inoculated with *P. thivervalensis* SC5 under salt stress conditions, with decreases of 45.2, 20.35, 50.9, 23.3 and 28.6% in SL, RL, RDW, SDW, and TDB, respectively, when compared to SC5 inoculated plants under normal conditions. The effects of salt stress, especially those affecting biomass, were less pronounced in plants inoculated with *P. thivervalensis* SC5.

Compared to non-inoculated plants, the plants inoculated with *P. thivervalensis* SC5 showed a significant increase in both SL (4.80 vs. 6.25 cm, 30.2%), RL (14.5 vs. 18.92 cm, 30.5%), RDW (30.78 vs. 50.23 mg, 63.2%), SDW (227.36 vs. 328.88 mg, 44.7%), and TDB (258.14 vs. 379.12 mg, 46.9%), under salt stress conditions (Figures 1A–C). Moreover, the results showed that SC5-inoculated salt-stressed plants had similar biomass values to non-inoculated non-stressed control plants (Figure 1B).

The results obtained here are in agreement with previous reports demonstrating the important role of ACC deaminase-producing bacteria (especially *Pseudomonas*) in mitigating the effects of salt stress in several plants (Cheng et al., 2007; Saravanakumar and Samiyappan, 2007; Ali et al., 2014; Orozco-Mosqueda et al., 2019), including cucumber (Gamalero et al., 2010).

**Detailed Analysis of Pseudomonas thivervalensis SC5 Genomic Properties**

The genome of *P. thivervalensis* SC5 consists of a single circular chromosome of ~6.59 Mbp and an average GC content of 61.2% (Figure 2A). Genome analysis predicts a total of 5,884 open reading frames, of which 5,816 correspond to putative protein coding sequences (CDS). A total of 58 tRNA, seven rRNA, three ncRNA, and one tmRNA genes were also found.

BlastKoala analysis resulted in the functional annotation of 3,380 genes from a total of 5,816 CDS (58%).
The genome of strain SC5 contains 33 genomic islands (GI; Figure 2A). Two of these GIs correspond to phage sequences that were found by PHAST analysis. A total of two complete phage sequence clusters were found within the SC5 genome. CAZymes analysis identified 157 proteins belonging to the families of structurally related catalytic and carbohydrate-binding modules (or functional domains) of enzymes that degrade, modify, or create glycosidic bonds. A total of 42 proteins were predicted to belong to the Glycoside Hydrolase family, 38 to Glycosyl Transferases, 14 to Carbohydrate Binding Modules, 40 to Carbohydrate Esterases, 18 to Auxiliary Activities, and 5 to the Polysaccharide Lyases family.

The complete elements for sec and tat, type I (T1SS; Hemophore/metalloprotease transporter; Adhesin protein transporter; and AlgE-type Mannuronan C-5-Epimerase transporter), two type II (T2SS), a type III (T3SS), and two type VI (T6SS) secretion systems gene clusters were identified within the SC5 genome (Supplementary Table S1).

Genome alignment analysis revealed that the genome of *P. thivervalensis* SC5 is highly syntenic to the genomes of *P. thivervalensis* BS3779 and *P. thivervalensis* PITR2 genomes (Figure 2B), supporting the identification of strain SC5 as *P. thivervalensis*. Moreover, strain SC5 as well as other *P. thivervalensis* strains presented increased genomic identity when compared to members of the *Pseudomonas brassicacearum*, *Pseudomonas kilonensis*, and *Pseudomonas bijeensis* species (Figure 2B). Interestingly, these clades contain well known PGPB also presenting pathogen antagonistic activities (e.g., *P. brassicacearum* WCS 365, *P. kilonensis* F113, and *P. brassicacearum* LBUM300).

**Pseudomonas thivervalensis** SC5 Genomic Traits Involved in Soil Colonization, Plant-Growth Promotion, and Protection Nitrogen, Sulfur, and Phosphorous Metabolism

*Pseudomonas thivervalensis* SC5 reduces nitrate to produce N (gas) and ammonia, indicating that the dissimilatory nitrate reduction and denitrification pathways are active in this strain. These results are consistent with the presence of the genes responsible for the dissimilatory nitrate reduction (*nas*) and denitrification (*nir, nar, and nor*) pathways, as well as several...
genes involved in nitrate, nitrite, and ammonia transport (three \textit{amtB} genes) in the SC5 genome (\textit{Supplementary Table S2}). Additionally, genes involved in urea and allopheanate transport and degradation, namely the \textit{ureABC} cluster responsible for urease production and, \textit{atzF}, encoding the allopheanate hydrolase, were also found (\textit{Supplementary Table S2}).

Two nitrate monooxygenase genes (\textit{npd}) involved in the oxidative denitrification of toxic nitronates and nitroalkanes to their corresponding aldehydes and nitrates; and three copies of the \textit{azoR} gene, encoding azoreductase were found to be present in the genome (\textit{Supplementary Table S2}), and may account for strain's SC5 ability to degrade nitro-containing xenobiotics.

Strain SC5 possesses the complete set of genes responsible for the assimilatory sulfate reduction pathway (\textit{cis} genes) and several genes involved in sulfate transport (\textit{sulP}, \textit{cysPUWA}). The taurine transport and taurine dioxygenase genes (\textit{tauD}, three copies; \textit{Supplementary Table S3}) involved in the catabolism of taurine; sulfone and sulfonate transport and degradation genes involved in the degradation of sulfones (\textit{sfnG}) and alkanesulfonates (\textit{ssuABCD}); and \textit{dddP} and \textit{dmoA} genes involved in the catabolism of dimethylsulfiniopropionate and dimethylsulfide were also found in the genome, suggesting an ability to modulate plant and soil sulfur metabolism.

The genome of strain SC5 contains the pyrroloquinoline quinone (\textit{pqq}) operon and glucose dehydrogenase genes involved in the production of gluconate, a major organic acid involved in the solubilization of mineral phosphate and other compounds such as ZnO. In addition, genes encoding enzymes involved in organic phosphate solubilization, such as an extracellular phytase, several alkaline phosphatases, and some components of the phosphonate C-P lyase system were found in the genome of strain SC5 (\textit{Supplementary Table S4}). The strain SC5 extracellular phytase (CE140_04710) presents some similarity with \textit{Bacillus subtilis} extracellular 3-phytase involved in the catabolism of phytate a common plant metabolite found in plant tissues and soils, that serves as a storage source for phosphorus and inositol (Zeng et al., 2011). Two copies of the genes encoding the phosphate transport system (\textit{pstABCS}) were also detected in the genome (\textit{Supplementary Table S4}).

Overall, the data obtained indicates that \textit{P. thivervalensis} SC5 participates in N, S, and P soil cycles through several enzymatic activities, thus, increasing the availability of several key nutrients that are indispensable for plant growth.

Siderophore Production and Iron Acquisition
\textit{Pseudomonas thivervalensis} SC5 and its relatives, \textit{P. thivervalensis} LMG 21626\textsuperscript{T} (=BS3779) and \textit{P. thivervalensis} PITR2, synthesize siderophores. Recently, Matthijs et al. (2016) identified two siderophores produced by strain LMG 21626\textsuperscript{T}, as pyoverdine (\textit{PYO}_{\text{thi}}) and histocorrugatin, and characterized the genomic regions responsible for their biosynthesis. The genomic regions containing the \textit{pvd} and \textit{hcsABCDEFGHJKLM} genes were detected in the chromosome of \textit{P. thivervalensis} SC5 (\textit{Supplementary Table S5}), suggesting the production of pyoverdine (\textit{PYO}_{\text{thi}}) and histocorrugatin by this strain. In addition to pyoverdine and histocorrugatin biosynthetic clusters,
one other region containing a Non-Ribosomal Peptide Synthase (NRPS) and other siderophore biosynthesis related genes was detected in the strain SC5 chromosome (Supplementary Table S5). This genomic region (CE140_5180-5260) contained both isochorismate synthase (CE140_05240) and isochorismate pyruvate lyase (CE140_05235) gene homologs, which are involved in the production of SA, suggesting the production of a SA-containing siderophore. Moreover, the NRPS associated with this cluster (CE140_5180, CE140_5185) showed some homology (~43~46%) to NRPS’s from the coelobactin biosynthetic cluster of Streptomyces coelicolor A3(2). BLAST analysis revealed that this NRPS cluster is somewhat rare, being only detected in 20 other sequenced Pseudomonas strains (mostly synxantha, simiae, libanensis, and azotoformans).

Iron and iron complex (including siderophore-iron complex) transport genes are extremely abundant in the genome sequence of strain SC5 (Supplementary Table S5), suggesting that iron acquisition and metabolism plays an important role in strain SC5 physiology and ecology.

Osmotic Stress Resistance

Strain SC5 was able to grow in the presence of high salt concentrations, which is consistent with the presence of multiple genes involved in the biosynthesis, metabolism and transport of osmoprotectants in its genome (Supplementary Table S6). In this regard, strain SC5 possesses the genes for the biosynthesis of compatible solutes such as proline (proABC), glutamate (gltBD), glutamine (seven gltA genes), carnitine (bodG, lcdH), N-acetylglutaminylglutamine amide (NAGGN; nglg), glycinebetaine (betABC), glycogen (gfg), trehalose (two treS genes, treYZ), ectoine, and hydroxyectoine (docABCD), as well as several of the transporters of these compounds (proP, proXWV, betT, opuABCD, thuEFGK, and ehuABCD; Supplementary Table S6). In addition, several genes involved in the transport of sodium and chloride (nhaAB, yfbK, glnT, and putP) were detected in the genome.

The SC5 genome also contains several genes involved in the biosynthesis, metabolism, and transport of polyanines and 4-aminobutyrate (GABA; Supplementary Table S7), which also play a role in salt stress resistance (Schiller et al., 2000). The strain SC5 chromosome contains the speA, aguA, aguB, and speC genes involved in putrescine biosynthesis; the hss, cansD, and nspC genes involved in homospermidine, carboxynorspermidine, and spermidine biosynthesis; as well as several genes involved in polyamine degradation (puuABCD, spuC, and prr) and conversion to GABA.

The results obtained indicate that P. thivervalensis SC5 can acquire, synthesize, accumulate, and metabolize wide range of osmolytes and polyanines that are known to play a role in osmotic stress tolerance, as well as in plant colonization and plant growth promotion. For example, the induction of osmotic stress and the presence of compatible solutes increased the plant colonization and biocontrol activities of P. fluorescens EPS62e (Bonaterra et al., 2007). The production of trehalose by the PGPR Pseudomonas sp. UW4, played an important role in the protection of tomato plants against salt stress (Orozco-Mosqueda et al., 2019). The biosynthesis of polyanines plays a role in plant growth promotion activities of plant-associated bacteria (Chen et al., 2017).

Motility, Chemotaxis, and Attachment to Plant Tissues

Pseudomonas thivervalensis SC5 chromosomal DNA contains the genes involved in the synthesis of flagella (fig, fil) and pilus (pil, cpa) systems involved in bacterial motility (Supplementary Table S8). The genome also harbors 38 genes encoding methyl-accepting chemotaxis proteins as well as multiple copies of chemotaxis related genes cheA, cheB, cheD, cheV, cheY, cheR, motA, and motB (Supplementary Table S8).

The SC5 genome harbors several genes involved in bacterial attachment to plant tissues, including the type IV pilus and fimbriae biosynthesis genes, the tight adherence (Tad) export apparatus and Flp (fimbrial low molecular-weight protein) pili, the bcs cellulose production operon, the alg operon responsible for alginate production, and several other gene clusters potentially involved in exopoly saccharide formation (Supplementary Table S8).

Resistance to Oxidative Stress

Pseudomonas thivervalensis SC5 harbors an increased number of genes involved in the response to oxidative stress, including four katE genes encoding catalase; katG, encoding a catalase-peroxidase; two sod genes that encode Fe-Mn family superoxide dismutases; two ahpC genes that encode an alkyl hydroperoxide reductase; a peroxiredoxin Q/BCP bcp gene; tpX, encoding an atypical 2-Cys peroxiredoxin; a cytochrome c peroxidase gene; three gpx genes encoding glutathione peroxidase; four non-heme chloroperoxidases (cpo); three osmC genes that encode a lipoyl-dependent peroxiredoxin; a dye-decolorizing peroxidase gene homolog, and a superoxide oxidase, cybB, gene. A gene cluster involved in the production of an aryl polyene, possibly involved in the protection against reactive oxygen species (ROS; Cimermancic et al., 2014), was also detected in the genome of P. thivervalensis SC5 (Supplementary Table S9). This data indicates that P. thivervalensis SC5 possess a significant ability to detoxify several ROS, which may impact several aspects of plant growth as well as plant-microbe interactions. For example, plants produce increased levels of ROS under stressful conditions (e.g., salt stress) that impact plant growth (Huang et al., 2019). The plant defense response against bacteria is mostly mediated by ROS (Bolwell and Wojtaszek, 1997). Ultimately, beneficial bacteria able to overcome the inhibitory effects of ROS are better able to colonize and protect plant hosts against stress (Kim et al., 2000; Alquéres et al., 2013; Tiepo et al., 2020).

Metabolism of Carbohydrates, Organic Acids, and Amino Acids

Genomic analysis revealed the presence of several carbohydrate (sugars, sugar alcohols, and sugar acids) catabolism and transport genes in strain SC5 chromosomal DNA (Supplementary Table S10). These included genes involved in the catabolism of sugars such as glucose (through gluconate), fructose, ribose, xylose, galactose, mannose, sucrose, maltose, trehalose, and beta-glucosides; sugar alcohols such as glycerol,
mannitol, galactitol/sorbitol, and inositol; and sugar acids such as glycolate, D-gluconate, L-gulonate, D-fructuronate, D-mannonate, D-lactate, galactarate, glyc erate, D-glucarate, L-tarlate, and D-galactonate degradation. The SC5 genome also contains the genes involved in the TCA cycle (citrate, fumarate, succinate,aconitate, isocitrate, and malate metabolism), the glyoxylate bypass (glyoxylate catabolism), and the metabolism of several organic acids such as acetate, oxaloacetate, formate, malonate, tartrate, propionate, and butyrate (Supplementary Table S11).

Strain SC5 also contains several genes involved in the transport and metabolism of amino acids and peptides, as well as opines (Supplementary Table S12). Opines are compounds synthesized from the condensation of amino acids with ketoacids or sugars, and have been linked to the colonization abilities of rhizobia and Agrobacterium (Murphy et al., 1995; Dessaux et al., 1998). The genome of strain SC5 contains the mocC gene involved in the catabolism of rhizopine (Galbraith et al., 1998), several opine oxidase gene homologs (ooxAB) involved in octopine degradation, as well as the inositol/myo-inositol transport system and homologs of the octopine transport system, indicating the strain SC5 ability to catabolize opines and modulate its effects on plant-microbe interactions.

Phenolics and Lignin Metabolism

The P. thivervalensis SC5 genome contains the ech, vdh, and fcs gene cluster that encodes the enzymes enoyl-CoA hydratase/aldolase, vanillín dehydrogenase, and feruloyl-CoA synthetase, involved in the catabolism of hydroxycinnamic acids such as ferulic acid (conversion to vanillate), p-coumaric acid (conversion to 4-hydroxybenzoate) and caffeic acid (conversion to protocatechu ate). A mhpA gene homolog encoding the 3-(3-hydroxyphenyl)propanoate hydroxylase involved in 3-hydroxycinnamic acid (m-coumaric acid) catabolism, and the hpa operon encoding a 4-hydroxyphenylacetate 3-monoxygenase system involved in p-coumaric acid oxidation, are also present in the chromosome of strain SC5. Moreover, the vanillate catabolism genes, vanAB, responsible for the conversion of vanillate to protocatechu ate; the pobA gene responsible to the conversion of 4-hydroxybenzoate to protocatechu ate; and the pca operon containing the genes responsible for the conversion of protocatechu ate to acetyl-CoA (via 3-oxoaidpate) were also detected (Supplementary Table S13), consistent with the presence of a complete degradation pathway for hydroxycinnamic acids in strain SC5.

The ability to use hydroxycinnamic acids as carbon sources may play a role in P. thiveralensis SC5 rhizospheric colonization activities, as well as in soil and plant health maintenance by decreasing the long-term accumulation of these toxic allelochemical compounds (Turner and Rice, 1975; Batish et al., 2008; Singh et al., 2014; Ferro et al., 2015).

Two NADPH-dependent curcumin reductase genes (curA) were also detected in the SC5 genome (Supplementary Table S13) and may be involved in strain SC5 ability to metabolize the plant polyphenolic and antibacterial compound curcumin.

A gene encoding a Dyp type peroxidase enzyme (CE140_09525) with 84.5% identity to the P. fluorescens Pf-5 DypP1B enzyme, involved in the oxidation of lignin substrates (Rahman Pour and Bugg, 2015), was found in the genome of P. thivervalensis SC5, suggesting that this strain has the ability to degrade lignin.

Genomic analysis also revealed the presence a polyphenol oxidase/laccase encoding gene, responsible for the oxidation of several phenolics; calA and calB genes encoding the coniferyl-alcohol dehydrogenase and coniferyl-aldehyde dehydrogenase enzymes, respectively, involved in the catabolism of the monolignol coniferyl-alcohol and the respective coniferyl aldehyde (Supplementary Table S13); a 2,4'-dihydroxyacetophenone dioxygenase responsible for the degradation of 2,4'-dihydroxyacetophenone to 4-hydroxybenzoate; and the catechol 2,3-dioxygenase enzyme responsible for the catabolism of catechol. Coniferyl-alcohols and hydroxycinnamic acids are components of plant lignin, and 2,4'-dihydroxyacetophenone and catechol are known breakdown products of lignin. Overall, the acquired data suggests that strain SC5 may modulate plant lignin levels, which are an important determinant of plant growth, stress resistance, and defense (Liu et al., 2018).

Metabolism of Flavonoids

SC5 genomic analysis led to the identification of a cluster (CE140_08155-08295) containing several genes that present a high level of identity to the naringenin degradation genes of Herbaspirillum seropedicae SmR1 (Marin et al., 2013). In addition, three quercetin 2,3-dioxygenase genes, involved in the catabolism of the flavonoid quercetin were also detected in the genome of P. thivervalensis SC5 (Supplementary Table S13).

Flavonoid degradation is a trait described in several Pseudomonas species (Rao and Cooper, 1994; Pillai and Swarup, 2002) that may play an important role in the modulation of soil and plant flavonoid concentrations. The degradation of flavonoids leads to the production of p-coumaric acid, caffeic acid, phenylactic acid (PA), p-hydroxybenzoate, and protocatechu ate (B-ring products); and phloroglucinol, phloroglucinol carboxylate, resorcinol, and oxaloacetate (A-ring products; Rao and Cooper, 1994; Pillai and Swarup, 2002), which are then channeled to the TCA cycle. Most of the genes involved in these pathways were found in the SC5 genome, indicating the ability to completely use several flavonoids as carbon sources, a trait likely associated with the colonization activities of the bacterium.

ACC and Ethylene Modulation Genes

The acdS gene (CE140_07955) encoding ACC deaminase, as well as the acdR gene (CE140_07960) that encodes a leucine-responsive protein known to regulate acdS gene expression (Li and Glick, 2001), were found in the genome of P. thivervalensis SC5 (Supplementary Table S14). The AcdS and AcdR proteins present a high identity (97.6 and 86.4%, respectively) to those of the efficient ACC
deaminase-producing PGPB, *Pseudomonas* sp. UW4. Moreover, analysis of the *P. thivervalensis* SC5 acdS gene and its nearby regulatory regions indicated that this gene is stably present in the SC5 chromosome (with no transposase or mobile genetic elements in its vicinity), indicating a long-term acquisition and evolution of the acdS gene in strain SC5. The acdS gene is known to be positively selected in most legume-rhizobia symbioses (Nascimento et al., 2018b); hence, the presence of a stable acdS gene in *P. thivervalensis* SC5 suggests that this strain has evolved a long term mutualistic interaction with plant hosts, where the expression of ACC deaminase plays a valuable and positive role.

In addition to acdS, strain SC5 also contains a tplQ gene homolog (CE140_07340), involved in the chemotactic response to ethylene, which acts as an attractant to several plant-associated *Pseudomonas* strains (Kim et al., 2007); and several genes involved in polyamine (putrescine, spermidine) production and transport (Supplementary Table S14). Bacterial spermidine inhibits plant ethylene biosynthesis, leading to an increase in plant growth promotion (Xie et al., 2014).

**Auxin Biosynthesis**

A tryptophan 2-monoxygenase gene (iaaM) homolog, possibly involved in the biosynthesis of indole-3-acetamide (IAM), and several amidase genes (amiE, yafV) that may convert IAM to IAA, were found in the genome of *P. thivervalensis* SC5 (Supplementary Table S14), thus suggesting that strain SC5 produces IAA through the IAM pathway (Duca and Glick, 2020). The production of IAA through the IAM pathway has also been described in other beneficial *Pseudomonas* strains, such as *Pseudomonas chlororaphis* O6 (Dimkpa et al., 2012).

Curiously, two fcaB genes encoding the phenylacetaldehyde dehydrogenase enzyme involved in phenylacetate (PAA) production were found in the genome of strain SC5 (Supplementary Table S14), thus suggesting its ability to synthesize PAA. PAA is also considered an auxin with significant impact in plant growth (Cook, 2019). Moreover, PAA production by beneficial plant-associated bacteria also acts as an inducer of the plant defense response, leading to increased plant resistance against fungal pathogens (Sumayo et al., 2013; Akram et al., 2016).

**Cytokinins Biosynthesis and Metabolism**

The genome of strain SC5 contains several genetic elements involved in the production and transformation of cytokinins (CKs; Supplementary Table S14), such as the miaA, miaB, and miaE genes involved in the production of N6-((Δ2-isopentenyl)adenosine, 2-methylthio-N6-(dimethylallyl) adenosine, and 2-methylthio-cis-ribozatin, respectively. The SC5 genome also contain several genes encoding RNases, which can be involved in the dissociation of tRNA-associated CKs. Additionally, two log genes, which encode the cytokinin riboside 5′-monophosphate phosphoribohydrolase responsible for the conversion of CK-nucleotides to free-base forms (Samanovic et al., 2015); and the xanthine dehydrogenase genes, xdhABC, responsible for the biotransformation of CKs (Taylor et al., 2006), were also found in the genome sequence of *P. thivervalensis* SC5 (Supplementary Table S14).

The abundance of genetic elements involved in the production and transformation of CKs suggests that *P. thivervalensis* SC5 is able to synthesize CKs, a trait also found in other *Pseudomonas* strains (de Salamone et al., 2001; Karnwal and Kaushik, 2011; Großkinsky et al., 2016). Moreover, *P. fluorescens* G20-18 CK production abilities also play an important role in the biocontrol of *P. syringae* infection in *Arabidopsis* (Großkinsky et al., 2016); hence, the production of CKs by *P. thivervalensis* SC5 may impact not only its plant growth promotion activities but also its biocontrol activities.

**Salicylic Acid Biosynthesis**

Several genes involved in the production of SA, namely, one isochorismate synthase (*pchA*), which converts chorismate into isochorismate (the building block for SA biosynthesis) and two isochorismate-pyruvate lyase gene homologs (*pchB*), which are responsible for the transformation of isochorismate to SA, were identified in the SC5 chromosomal DNA (Supplementary Table S14). The *pchA* and one copy of the *pchB* gene were found in an operon responsible for siderophore biosynthesis (described above), suggesting a role in the synthesis of a SA-containing siderophore. One additional copy of *pchB* (*pchB2*) was found in a genomic region not related to siderophore production; *pchB2* (CE140_03015) is clustered with a gene encoding an isochorismatase-like protein. These results suggest the production of SA by *P. thivervalensis* SC5, a trait that has also been described in other *Pseudomonas* spp. (Bakker et al., 2014).

Salicylic acid is one of the main plant hormones involved in plant defense responses, and, consequently, has important functions in regulating plant-microbe interactions (Lebes et al., 2015). Moreover, SA also plays an important role in plant growth and abiotic stress resistance (Rivas-San Vicente and Plasencia, 2011; Khan et al., 2015). The production of SA by *P. thivervalensis* SC5 may play an important role in its ability to interact with plant hosts, to promote plant growth as well as in its biocontrol activities against pathogens.

**Phytopathogen Antagonistic Activities**

ANTISMASH analysis revealed the presence of several gene clusters involved in the biosynthesis of antagonistic secondary metabolites in the genome of strain SC5. These included a 2,4-diacytethylphloroglucinol (DAPG) biosynthesis cluster constituted by the *phiHGF* and *phlACBDE* genes; a cluster involved in hydrogen cyanide (HCN) production (*hcnABC*); a gene cluster involved in the biosynthesis of a fragin homolog; three gene clusters involved in bacteriocin production, and one cluster involved in the production of a lantipeptide (Supplementary Table S15).

Strain SC5 DAPG biosynthesis cluster (*phlACBDE* and *phiHGF*) presents high identity to the functional DAPG biosynthesis gene cluster of *P. thivervalensis* PITR2 (~99% identity).
identity; Keel et al., 1996; Molina et al., 2003), and other studied biocontrol Pseudomonas strains such as P. kilonensis F113 (~93% identity; Redondo-Nieto et al., 2013; Vacheron et al., 2018), P. brassicacearum BIM B-446 (~93% identity; Mandryk-Litvinkovich et al., 2017), P. brassicacearum 3Re2-7 (~93% identity; Nelkner et al., 2019), P. brassicacearum LBUM300 (~93% identity; Lanteigne et al., 2012; Novinscak et al., 2016), among others. Moreover, BLAST analysis revealed that the hcnABC gene cluster of P. thivervalensis SC5 also presented high identity to the functional hcnABC genes of several HCN-producing Pseudomonas, including P. thivervalensis PITR2 (~99% identity; Ramette et al., 2003), P. brassicacearum 3Re2-7 (~92% identity; Nelkner et al., 2019) and P. brassicacearum LBUM300 (~92% identity; Lanteigne et al., 2012; Novinscak et al., 2016).

Several genes involved in acyl homoserine lactone catabolism (pvdQ, quiP) and homoserine lactone efflux were detected in the genome of strain SC5 (Supplementary Table S15), suggesting the ability to modulate quorum-sensing signals, a trait of relevant importance for biocontrol activities (Alymanesh et al., 2016).

Based on the increased prevalence of genes involved in the biosynthesis of antagonistic secondary compounds it was decided to test P. thivervalensis SC5 for its ability to antagonize the phytopathogens B. cinerea (fungal) and P. syringae DC3000 (bacterial). These assays revealed that P. thivervalensis SC5 inhibited the growth of both pathogens, confirming the active production of pathogen antagonistic traits (Figure 3). The obtained results indicate that strain SC5 presents the ability to act as a biocontrol agent, a trait also observed in its close relative, P. thivervalensis PITR2, a bacterium isolated from a disease suppressive soil known to actively produce the antifungal metabolites DAPG and HCN [73].

CONCLUSION

Pseudomonas thivervalensis SC5 acts as a versatile and efficient PGPB, presenting both plant growth promoting properties as well as antagonistic activities against phytopathogens. Although, this strain was originally isolated based on its ACC deaminase activity, a thorough genomic analysis revealed the presence of several other elements involved in plant colonization and growth promotion. Thus, the success of P. thivervalensis SC5 as a PGPB results from a web of complex mechanisms and actions that modulate several aspects of plant growth, resistance, and plant-microbe interactions (Figure 4; Table 2).

Ultimately, understanding the functions of successful PGPB, such as P. thivervalensis SC5 is of importance for the selection and development of next generation inoculants to be applied in a wide range of agricultural and biotechnological applications, and to gain more knowledge regarding beneficial plant-microbe interactions.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/genbank/, CP022201.1.

AUTHOR CONTRIBUTIONS

FN performed the plant experiments, bacterial characterization assays, genomic analysis, and wrote the manuscript. PU performed the plant experiments (greenhouse assays) and participated in

![FIGURE 3](image-url) | Antagonistic activities against phytopathogens. (A) Botrytis cinerea control; (B) B. cinerea + P. thivervalensis SC5; (C) Pseudomonas syringae DC3000 (incorporated in agar) + Pseudomonas sp. MS8 (negative control); (D) P. syringae DC3000 (incorporated in agar) + P. thivervalensis SC5. The arrow in panel (B) points to a P. thivervalensis SC5 colony. The inhibition of fungal growth and the presence of halos (P. syringae DC3000 assay) indicate the antagonistic activities of strain SC5.
TABLE 2 | Features encoded in the *P. thivervalensis* SC5 genome and their roles in plant growth promotion, plant-microbe interactions, and phytopathogen antagonism. Detailed analysis of genes can be found in Supplementary Tables S1–S15.

| Genome encoded features                                                                 | Major roles                                                                 |
|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Metabolism of: NO$_3$, NO$_2$, N, NO, urea, and NH$_4$                                    | • Modulation of soil and plant N levels                                    |
| Inorganic and organic (phytase) PO$_4$ solubilization; Zn solubilization                | • Induction of plant defense response and systemic resistance               |
| Metabolism of: H$_2$S, DMS, DMSP, taurine, and sulfonates                                | • Modulation of soil and plant P levels                                     |
| Siderophore biosynthesis (Pyoverdine, histocompragatin, and SA-containing siderophore); Siderophore/ion acquisition | • Availability of important co-factors (Zn)                                |
| Chemosensory activity, Flagella, Pili, Fimbriae, Cellulose, Alginate, EPS, and LPS      | • Modulation of soil and plant S levels                                     |
| Biosynthesis and metabolism of: proline, glutamate, glutamine, carnitine, NAGGN, glycin-betaine, glycollen, trehalose, ecorine, hydroxysteine, putrescine, homospermidine, carboxyxspermidine spermidine, and GABA Catalases, SODs, peroxygen, and aryl polyene | • Molulation of soil and plant Fe levels                                     |
| Metabolism of fructose, ribose, xyllose, galactose, mannose, sucrose, maltose, trehalose, beta-glucosides; glycerol, mannitol, galactitol/sorbitol,inositol; glycollate, D-gluconate, L-gulonate, D-fructuronate, D-mannonate, D-lactate, galactarate, glycerate, D-glucarate, L-talarate, and D-galactonate; citrate, fumarate, succinate, aconitate, isocitrate, malate, acetate, oxaloacetate, formate, malonate, tartrate, propionate, butyrate; amino acids, and peptides, opines. | • Antagonism to phytopathogens (biocontrol)                                 |
|                                                                                       | • Motility, chemotaxis and attachment to plant tissues                      |
|                                                                                       | • Resistance to osmotic stress                                             |
|                                                                                       | • Plant protection against osmotic stress                                  |
|                                                                                       | • Modulation of ethylene levels (by spermidine)                           |
|                                                                                       | • Resistance to ROS                                                       |
|                                                                                       | • Resistance to plant defenses (endophytic colonization)                   |
|                                                                                       | • Plant protection against stress (e.g., osmotic, drought)                |
|                                                                                       | • Soil nutrient recycling                                                 |
|                                                                                       | • Catabolism of root exudates                                             |
|                                                                                       | • Plant colonization                                                      |
|                                                                                       | • Resistance to plant defenses (peptides)                                 |

(Continued)
manuscript writing. BG, AG, and MR were responsible for advising, writing, and revising this work. All authors contributed to the article and approved the submitted version.

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