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Original scientific article

GENOMIC CHARACTERIZATION AND PHYTOSTIMULATIVE EFFECT OF A NOVEL *SERRATIA* SPECIES

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Some of non-pathogenic bacteria are effective biocontrol agents and plant growth inducers besides its degradative property on polycyclic aromatic hydrocarbons (PAH). Herein, we report a novel candidate *Serratia* species isolated in the purpose of PAH degradation, with its plant-growth-promoting and antifungal effect against *Phytophthora infestans*. Properties of bacterium determined by antifungal and phytostimulation assay under *in vitro* conditions displayed production of indole-3-acetic acid (IAA), chitinase and endoglucanase/cellulase activity. The identification of bacterium using whole-genome shotgun sequencing output also showed that the novel strain belongs to new *Serratia* species harboring the genes responsible for different secondary metabolites at the genomic level. Genome-wide analysis suggested a new candidate *Serratia* species (strain AGBY19) showing, in some extend, genetic relation with *Serratia fonticola* at molecular phylogeny level, which inhibits the growth of phytopathogenic fungi *Phytophthora infestans* by 73% compared to the control observed *in vitro* conditions. This strain colonised at the rhizosphere of tomato plant during *in vivo* host plant cultivation assay that remarkably enhanced the root growth. It causes the production of IAA hormone and cell wall degrading enzymes (chitinase, endoglucanase/cellulase). Further genome analyses of AGBY19 revealed different gene clusters comprising flanked regions associated with the production of secondary metabolites. These data eventually have provided its biocontrol properties and plant-growth inducer effect with globally potential to use for agricultural production.

Keywords: Biocontrol, Induced resistance, *Phytophthora infestans*, PGPR genes, *Serratia*, Whole genome sequencing

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INTRODUCTION

The genus *Serratia* belongs to the family *Enterobacteriaceae* within the *Gammaproteobacteria*. These bacteria are ubiquitous; can be found in water, soil, plants, and animals. Researchers have recognized most of them as the opportunistic human pathogen since the 1960s (KHANNA *et al*., 2013). Unusual *Serratia* species (STOCK *et al.,* 2003) i.e. *S. ficaria* (GRIMONT *et al.,* 1979), *S. fonticola* (GAVINI *et al.,* 1979), *S. odorifera* (GRIMONT *et al.,* 1978), *S. plymuthica*, *S. rubidaea* (EWING *et al*., 1973), *S. entomophila* (GRIMONT *et al.,* 1988) and *Serratia quinivorans* (ASHELFORD *et al.,* 2002) have no pathogenicity factors (DE VLEESSCHAUWER, 2008). Notably, *S. plymuthica* does not inflict disease in alternative animal model systems as reported in the *Caenorhabditis elegans* assay (ZACHOW *et al.,* 2009). *S. plymuthica* is an unusual species that have plant-growth inducer effect. This microorganism isolated from the rhizosphere of wheat (ASTROM and GERHARDSON, 1988), maize (LUCON and MELO, 2000), grape (CHERNIN *et al.,* 1995), melon (KAMENSKY *et al.,* 2003), onion (PARK and SHEN, 2002), sugar beet (TENNING *et al.,* 1987), and tomato (FROMMEL *et al.,* 1991) as previously reported. Additionally, BERG *et al.* (2005) have detected the bacteria in the endorhiza of potato as an endophyte besides edible parts of green onion, carrot, and lettuce (GRIMONT *et al.,* 1981).

Potato late blight caused by the oomycete *Phytophthora infestans* is the most destructive potato disease worldwide that causes annual loss around billions of dollars to the potato growers (COOKE and LEES, 2004). *P. infestans* is primarily a biotrophic pathogen, feeding on living host tissue. Zoospores are essential propagules in the pre-infection process, and they are potential target to control *P. infestans* and other oomycete pathogens in its disease cycle (DONALDSON and DEACON, 1993; ERWIN and ROBEIRO, 1996; VAN WEST *et al.,* 2002). Late blight is traditionally controlled by a combination of cultural practices and chemical applications but its control using fungicides and resistant varieties seems ineffective. This disease mainly has been controlled by the intensive application of fungicides (TOSUN *et al.,* 2007) but the introgression of fungicide resistant genes have caused another major problem (LEONARD and FRY, 1988; FRY *et al.,* 2015). The negative side effects of synthetic pesticides on environment and human health have also increased the attraction for organically food production. As an alternative measurement, growers use copper-based products instead of synthetic fungicides to protect their crops from late blight disease, but copper is not degradable and accumulates in the soil, then becomes toxic to the rhizosphere (DU PLESSIS *et al.,* 2005; EIJSACKERS *et al.,* 2005). Therefore, an environment-friendly method is necessary to control late blight in production areas. One of these alternatives is "use of natural enemies against pathogens causing disease" termed as biocontrol.

P. infestans is an aggressive pathogen and several biocompatible treatments have not been effective and are not able to reach the desired efficacious level (DORN *et al.,* 2007; AXEL *et al.,* 2012; ALAUX *et al.,* 2018). The effectiveness of the biocontrol agents relies on different mechanisms. Efficient strains successfully colonize in the root system of the host plant, promote plant growth, and activate plant defense mechanisms. Stimulating the systemic resistance reaction of the plant called as rhizobacteria-induced systemic resistance (ISR) is one of the alternative control measurements (ARGUELLES-ARIAS *et al.,* 2009). Moreover, the secretion of different secondary metabolites and enzymatic cell wall degrading enzymes with antibacterial or antifungal properties on phytopathogenic organisms contributes to plant defense (KÖHL *et al.,* 2019). Also, competition for iron between biocontrol strains and pathogens is an important issue.

Therefore, siderophore production provides advantages in favour of plant health by colonisation of the biological agent (ARGUELLES-ARIAS *et al.*, 2009; KRÖBER *et al.*, 2014).

Different plant growth strategies have attracted the attention of the researchers due to public concern about eco-friendly protection and alternative control strategies (ALABOUVETTE *et al.*, 2006). Several plant-associated bacterial biocontrol agents are commercially available such as *Bacillus* spp., *Pseudomonas* spp. (CHOUDHARY and JOHRI, 2009). Treatments of plants with plant-associated microorganisms are effective to diminish the loses cause of the plant pathogens (BAYSAL *et al.*, 2013). The development of biocontrol products based on isolates belonging to the Gram-negative genus *Serratia* could get attention of researchers due to their remarkable properties. For instance, studies on free-living and endophytic organism *Serratia plymuthica* recovered from rhizospheres have shown positive results with its broad-spectrum antimicrobial compounds (DE VLEESSCHAUWER and HÖFTE, 2007). These strains have also plant beneficial properties including PGPR effect, phosphate solubilization, ammonia production, antifungal activity, and synthesis of siderophores (DEVI *et al.*, 2013) that significantly increased crop yields under diversified controlled conditions (MIA *et al.*, 2005).

This study aims to characterize a novel bacterium, stimulating plant root growth, and its whole genes responsible for plant growth and antagonistic effect against *P. infestans* besides its PAH (Polycyclic aromatic hydrocarbon) degradation ability. In addition to the molecular phylogeny level, using whole-genome sequencing also allowed functional characterization of our strain showing biocontrol capacity related to chitinase, endoglucanase/cellulase activity and PAH degradation genes listed.

MATERIALS AND METHODS

Bacterial Strain and Cultivation

Bacterial strain AGBY19 (Agit-Baysal 2019) was isolated from the soil samples of crude petroleum pollutants contaminated seashore near petroleum refinements (Aliaga, İzmir, Turkey). The PAH degradation ability was determined in the modified Minimal Growth Medium A suggested by ERDOĞAN *et al.* (2013) with 50% (v/v) gasoline (per liter) as carbon source. The performed colonies were incubated for 72 h at 28˚C on a rotary shaker (180 rpm/ min) until the optical density at 600 nm (OD_{600}) reached to an absorbance of about 1.0 adjusted to 10^8 CFU / ml.

In vitro antifungal assay

Antimicrobial assay was performed using a modified double layer technique according to BAYSAL *et al.* (2013) (upper to lower layer, *P. infestans* on potato dextrose agar (PDA, Merck) and AGBY19 on nutrient broth agar (NBA, Merck) to test the antagonistic effect of the bacterium performed previously in MGA). Briefly, 100 µL of AGBY19 cultured liquid medium was spread onto sterile plates containing NBA. After spreading of the bacteria, PDA has been laid over once PDA solidified, a 5-mm mycelial plug of *P. infestans* from seven-day-old culture was placed at the center of the plate.

All the plates were incubated at 28ºC for 11 days to examine the growth of the fungus inhibited by the bacterium. A positive response was the visible zone of inhibition around the fungus (BAYSAL *et al.*, 2013). The diameter (mm) of the fungal colony was measured at 1, 2, 4, 7, 11 days after inoculation (DAI) with 5 replicates. The experiments were carried out according

to a completely randomized design, Duncan test was done for evaluations of the mean value differences ($P<0.05$) (DUNCAN, 1955).

Phytostimulation Assay / Pot Trials

Pot trials were carried out in our laboratory, to determine the colonization ability of AGBY19 on the rhizosphere of tomato plants (*Lycopersicum esculentum* Mill cv. Rio Grande). Before the cultivation tomato seeds were grown in alluvial loam (total N 112; P 32.3; K 17.4; and Mg 9.1 mg/100 g soil; pH 6.5) at 20/15°C (16/8 h, day/night cycle). Tomato plants were first inoculated with AGBY19 at the 2–3 leaf stage (1 week) before transplanting into the field beds. Seedlings were watered with 174 ml bacterial suspension (10^7 CFU/ml) of AGBY19 per seedling pot. The experiment was conducted with non-inoculated (control) and plants inoculated with AGBY19. Six weeks after planting of the tomato seedlings into the pots, 20 plants from each group with 5 replicates were randomly selected for measuring root growth and plant height. The experiments were carried out according to a completely randomized design and the data was statistically evaluated using the Duncan test.

Indol-3-Acetic Acid (IAA) production

To determine the amounts of IAA produced by our strain, a colorimetric technique was performed with Van Urk Salkowski reagent using the Salkowski's method (EHMANN, 1977). The strain was grown in yeast malt dextrose broth (YMD broth) (Himedia, India) and incubated at 28°C for 4 days. The broth was centrifuged after incubation. Supernatant (1 mL) was mixed with 2 mL of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ solution) and kept in the dark. The optical density (OD) was recorded at 530 nm after 30 and 120 min.

Chitinase activity

Colloidal chitin was prepared from the crust of a crab according to ROBERTS and SELITRENNIKOFF (1988) with a few modifications. 10 g crust dried for 3 days under room conditions was pulverized in mortar and 120 mL HCl (100%) added into solution and stirred for 1 h. Then ethanol (98%) dH_2O (1:1) mix was added and stirred for 2 min. The filtrated solution through filter paper rinsed with dH_2O was used to neutralize (pH 7.0) the solution. The extract centrifuged at 3000 rpm for 20 min at 4°C was re-centrifuged at 3000 rpm for 5 min to obtain colloidal chitin stored at 4°C for further use.

Chitinase detection medium consisted of a basal medium comprising (per liter) 0.3 g of $MgSO_4.7H_2O$, 3.0 g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 1.0 g of citric acid monohydrate, 15 g of agar, 200 µL of Tween-80, 4.5 g of colloidal chitin and 0.15 g of bromocresol purple; pH was adjusted to 4.7 and then autoclaved. Lukewarm medium was poured in petri-plates and allowed to solidify. Bacterial suspension (10 µl x 10^7 CFU/ml) of AGBY19 to be tested for chitinase activity were inoculated in the center of petri dishes and incubated at 25±2°C. They were observed for coloured zone formation (AGRAWAL and KOTASTHANE, 2012).

To confirm chitinase activity of strain AGBY19, another bacterium previously identified as *Enterobacter hormaechei* using 16S DNA analysis was the negative control. The results were compared depending on colour change on inoculated petri dishes at 7 days post inoculation.

Congo Red plate assay for endoglucanase/cellulase activity

This assay was used as an additional means of visual confirmation of the presence of cellulase activity in AGBY19. For this, wells were created in a plate of CM- cellulose agar (1% CM cellulose and 1.5% agar). Protein solutions were placed in these wells and allowed to diffuse into the substrate containing agar on the plates. The plates were incubated overnight at 37°C to allow the enzyme to act on the substrate. Plates were then stained with 0.1% (w/v) Congo Red for half an hour, followed by washing with 1 M NaCl. The zone of clearance thus obtained was an indicator of the amount of substrate hydrolyzed by the enzyme (SHARMA and GUPTASARMA, 2017).

DNA Extraction and Genome Sequencing

Sequencing Quality Analysis

Overnight culture of strain AGBY19 cultured in Luria broth (LB media) was centrifuged to obtain cellular pellets followed by genomic DNA purification by the CTAB method (NISHIGUCHI *et al.,* 2002). Purity of extracted genomic DNA was measured by agarose gel electrophoresis (1% agarose gel). QUBIT fluorimeter (Invitrogen Inc., USA) was used for measurement of concentration. In brief, 1 µL DNA was mixed with 199 µL double stranded DNA binding dye and incubated for 2 minutes in dark followed by concentration measurement by using default parameters of QUBIT fluorimeter. Purified DNA was then subjected to paired-end library preparation with an insert size of 500 bp. Prepared library was then sequenced by Illumina Hi-Seq 2500 sequencing system (Illumina Inc., San Diego, USA). The raw sequence data were deposited in the SRA database (SRA accession: PRJNA577011; Release date: 2020-04-12)

The quality of sequencing data was analyzed by the FASTQC tool (BROWN *et al.* 2017). Low-quality reads (i.e. Q-Score <20) were filtered using the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) followed by de-novo assembly of filtered reads into contigs using CLC genomics workbench 12.1. The contigs having length less than 500 bp were filtered. QUAST quality analysis pipeline was used for the analysis of resulting contigs (GUREVICH *et al.,* 2013).

Genome Annotation

The characterization of coding sequences and genome-level sequence alignment and re-ordering of contigs was performed by MAUVE and BRIG tools (ALEXEYENKO *et al.,* 2006; RISSMAN *et al.,* 2009; STOTHARD *et al.,* 2019; ALIKHAN *et al.,* 2011). The multiple genome alignment was carried out with genome sequences of isolate AGBY19, *Serratia fonticola*, *S. grimesii*, *S. liquifecians*, *S. marcescens*, *S. nematodiphila*, *S. odorifera*, *S. plymuthica*, *S. proteamaculans*, *S. rubidaea*, *S. symbiotica* and *S. urilytica* (https://www.ncbi.nlm.nih.gov/genome). Profiling of plant growth promoting genes and the genes encoding enzymes (chitinase, endoglucanase, celulase and protease genes) and antibiotics were identified using genome annotation pipelines (i.e. RAST and PROKKA) (AZIZ *et al.,* 2008; SEEMANN, 2014). Antibiotic production and bacteriocin genes were identified using BAGEL 4 server (DE JONG *et al.,* 2006).

Genome-wide Phylogenetic Analysis

Digital DNA: DNA hybridization (dDDH) of strain AGBY19 was performed by Type Strain Genome Server (TYGS server) (MEIER-KOLTHOFF and GÖKER, 2019). Molecular phylogeny and Average Nucleotide Identity (ANI) analysis of isolate AGBY19 was carried out by multi-locus genotyping (MLST) method (THOMPSON *et al.,* 2003). Sequences of 100 housekeeping genes of strain AGBY19 were used to carry out MLST. Phylogenetic tree was constructed by using multi-locus sequence genotyping by AutoMLST webserver (ALANJARY *et al.*, 2019).

Antibiotic Resistome Profiling

To identify the antibiotic resistance potential in strain AGBY19, the contigs were aligned against the 'Comprehensive antibiotic resistance database (CARD)' (MCARTHUR *et al.,* 2013).

PAH Biodegration Profiling

PAH Biodegradation potential in strain AGBY19 by the contigs aligned was found with the KEGG database related to PAH degrading genes (KANEHISA and GOTO, 2000).

RESULTS

Antifungal Assay, Chitinase Activity and Endoglucanase/ Cellulase Activity

To test the antifungal ability of AGBY19, we directly inoculated bacteria in the same plate co-inoculated with fungi. Principally, if the bacteria have shown antifungal activity, the fungi will not growth in petri plates. In this point of view, the bacteria caused growth inhibition on the pathogenic fungi (73%) when co-inoculated and the results were statistically significant ($P<0.05$) compared to the control group.

We prepared agar plates where the only carbon source was chitin. These plates supplemented with changes color upon bacterial reproduction. The chitinase production was detected with visually changing purple colour on agar plates depending on chitin degradation as a carbon source. We observed no colour change in negative control plates inoculated with *Enterobacter hormaechei* (Figure 1A). AGBY19 produced chitinase and degraded chitin (Figure 1B). This result shows that AGBY19 produced chitinase and could utilize chitin (Table 1, S1, Figure 1B).

Table 1. Overview of biocontrol-related secondary metabolites production by AGBY19 strain depending on whole genome sequence analysis.

Metabolite	Production
Glucanases*	+
Chitinases*	+
Proteases	+
Siderophores	+
IAA*	+

**Astericks shows the results confirmed with in vitro assays.*

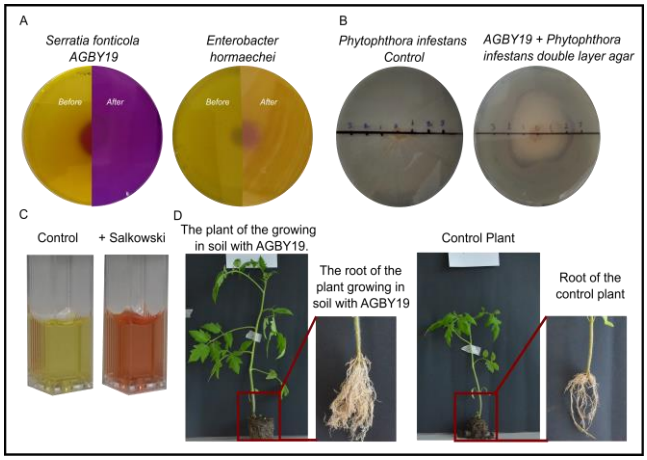

Figure 1. The effect of AGBY19 on fungal and plant growth. A) In vitro chitinase activity before and after bacterial growth for AGBY19 and *Enterobacter hormaechei*; purple colour represents chitinase activity on agar plates. Chitinase activity is observed for AGBY19 (left) but not for *Enterobacter hormaechei* (right). B) Antifungal

These data show that our strain has inhibitory effect by chitinase activity for the fungi with chitin in the cell wall. Nevertheless, the fungi belonging to Oomycetes do not harbour chitin. The inhibitory effect observed on *P. infestans* can be the reason for endoglucanase/cellulase activity. Our strain AGBY19 produces these enzymes as given in Figure S1.

Phytostimulation Assay / Pot Trials

To directly test the plant growth effect of AGBY19 by *in vivo* conditions, we have growth the tomato plants in the soil inoculated with AGBY19 and non-inoculated plots. These *in planta* assays showed significant difference ($P<0.05$) in plant height and root formation of the plants inoculated with bacteria compared to untreated (control) ones (Table S2). AGBY19 treatment gave rise to increased plant height and robust root growth (Figure 1D, Figure S2).

Indole-3-Acetic Acid (IAA) Production

To elucidate the reason for vigorous root formation by AGBY19 treatment, we checked the production of plant growth promoting hormone, IAA, in the secondary metabolites released by the bacterial strain. Salkowski reagent changed the colour of supernatant to pink due to detectable level of IAA. We have observed colour change by the supernatant of AGBY19 (Figure 1C).

Genomics Studies on Strain AGBY19

We confirmed that AGBY19 can produce these metabolites by genomics analysis. Next-generation sequencing (NGS) of strain AGBY19 resulted in 5,524,219 sequence reads with length of 150 nucleotides (total nucleotides sequenced = 828,632,850). Filtered NGS reads had a quality score of >Q20 which indicated that the NGS data was of appropriate quality to be

processed further. The NGS reads in both forward and reverse sequencing files were subjected to *de novo* assembly by Unicycler *de novo* genome assembler (WICK *et al.,* 2017). Consequently, 148 contigs were obtained. Contigs of <500 nucleotides were removed while downstream analysis was carried out on the remaining 96 contigs. Fasta format file of 96 contigs of length >500 nucleotides were subjected to the QUAST pipeline for the quality evaluation (GUREVICH *et al.,* 2013). Table 2 describes the statistics of contigs. Total length of 96 contigs was 5,165,459 nucleotides (length of 74 contigs was >1000 nucleotides). The N50 value of contigs was 132,933 nucleotides (length of the largest contig was 395,159 nucleotides). Additional data are given in Figure S3. The raw sequence data has also been deposited in the SRA database (SRA accession: PRJNA577011; Release date: 2020-04-12).

Table 2. Quality assessment parameters of assembled contigs.

QUAST Quality Parameters	Observations
Number of Contigs	96
Number of Contigs >1000 bp	74
Total Length of all Contigs	5,165,459 nucleotides
Size of Largest Contig	395,159 nucleotides
N50	132,933 nucleotides
N75	105,508 nucleotides
GC %	53.54 %

Taxonomic Characterization of Strain AGBY19

BLAST search of 96 contigs of strain AGBY19 against NCBI 16S rRNA database showed alignment of contig number 56 (length 1605 nucleotides) with 16S rRNA gene sequences of genus *Serratia* with 97% identity. This observation indicated that strain AGBY19 belonged to genus *Serratia*. Further characterization of strain AGBY19 was carried out at species level alignment of 11 *Serratia* species sequences with strain AGBY19 contigs by BRIG program (ALIKHAN *et al.,* 2011). Figure 2 describes circular representation of genome-level alignment of *Serratia* sp. with contigs of strain AGBY19. Among the 11 *Serratia* species, the contigs belonging to strain AGBY19 aligned with *S. fonticola* genome considering the highest sequence identity.

Figure 3 demonstrates the MLST based phylogenetic relationship between strain AGBY19 and related *Serratia* species. AutoMLST webserver (MEIER-KOLTHOFF and GÖKER, 2019) was used which utilizes 100 housekeeping genes in order to carry out the multi-loccus sequence genotyping. AutoMLST grouped strain AGBY19 with *Serratia fonticola*. Average nucleotide identity (ANI) and digital DNA: DNA hybridization (dDDH) values for strain AGBY19 were observed to be 91.62% and 67%, respectively. dDDH value must be at least above 70% and preferably in the range between 70-90% for putting strains into the same species (AUCH *et al.,* 2010; LI *et al.,* 2015a). Since ANI and dDDH values for strain AGBY19 are less than 94% and 70%, respectively, therefore we consider this strain as a candidate new species of genus Serratia.

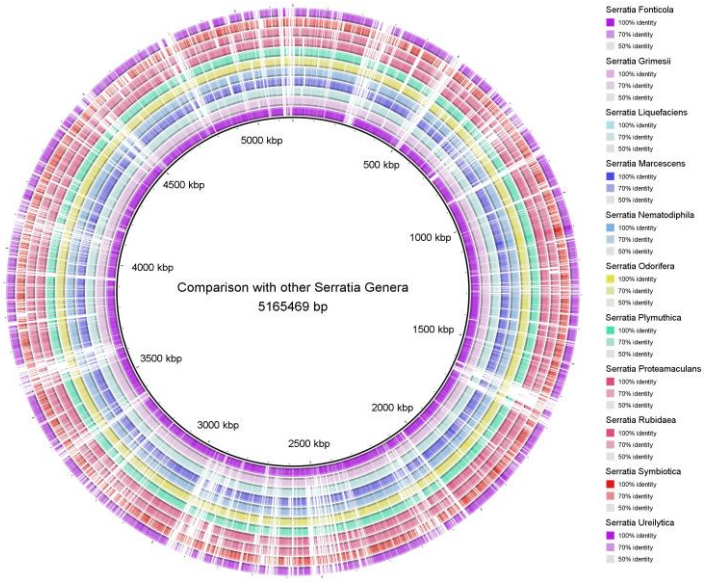

Figure 2. BRIG circular map demonstrating the percentage similarity of sequenced genome in comparison with *Serratia* reference genomes.

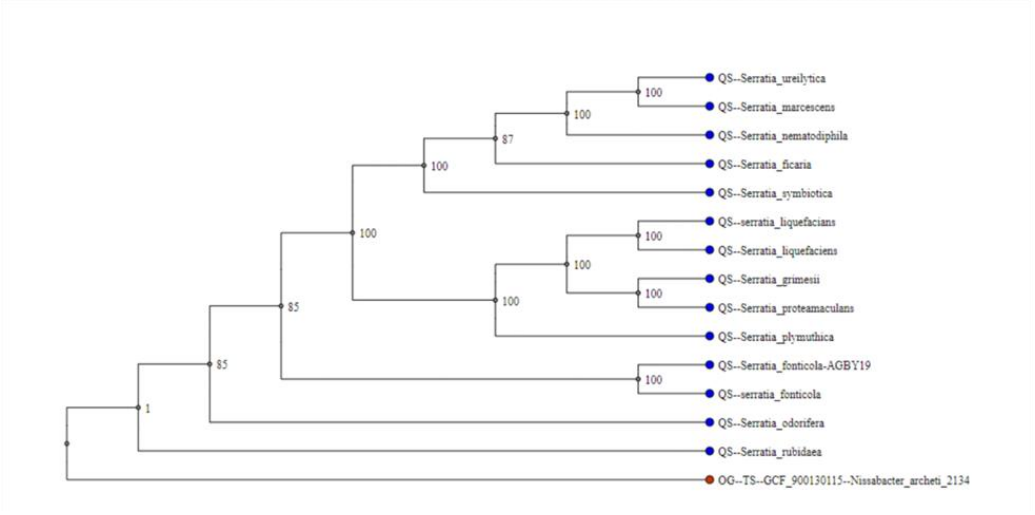

Figure 3. Neighbor-joining phylogenetic tree of *Serratia fonticola* AGBY19 with other *Serratia* species based on multi-locus sequence genotyping (MLST) method

Genome Annotation

Genome annotation pipelines revealed 4297 protein-coding genes. Figure 4 demonstrates the functional annotation of the studied genome. Most number of genes were associated with carbohydrate metabolism (10%) followed by amino acid metabolism (9%), transcription (9%), energy production and conservation (6%), cell wall biogenesis (6%) and translation, ribosomal structure biogenesis (6%). A substantial number of genes related to accessory functions such as inorganic transport and metabolism (6%), signal transduction (5%), coenzyme transport and metabolism (5%), etc have shown in Figure 4. Hypothetical proteins comprised of 7% of total genes were identified. Additional data demonstrated in Figure S4.

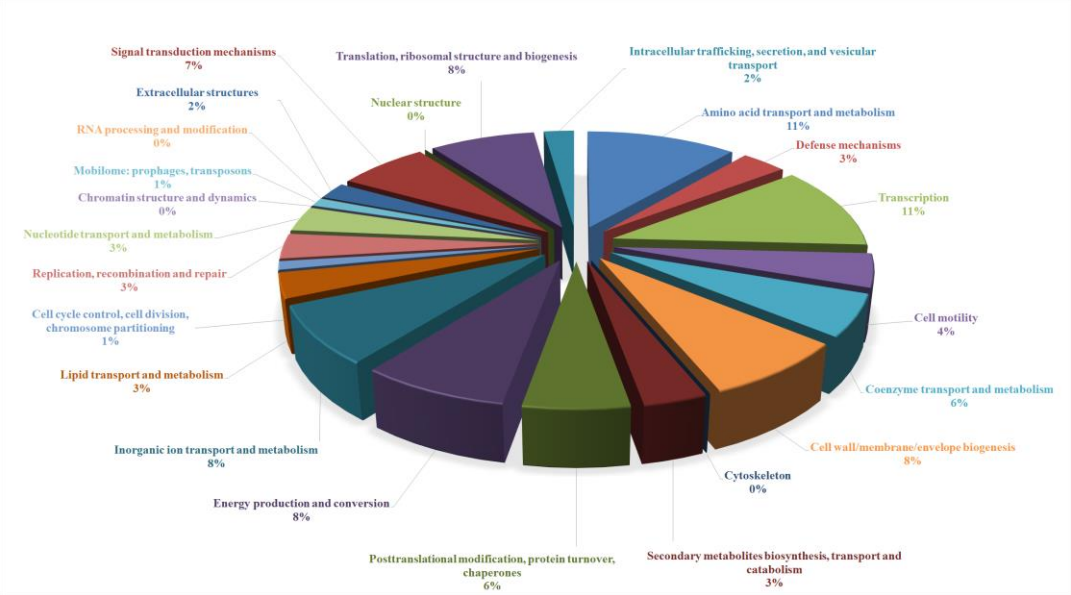

Figure 4. Pie chart representing the functional annotation of studied genome based on COG and KEGG ontology databases.

Plant Growth Promoting Genes in Strain AGBY19

We have identified genes associated with plant growth-promoting pathways in the genome of strain AGBY19. Table 3 represents the plant growth-promoting genes. Several genes were responsible of hydrogen sulfide production (9 genes) and phosphate solubilization (8 genes). Other related plant growth-promoting pathways included siderophore production, nitrogen fixation and metabolism, glycine betaine production, 4-hydroxybenzoate production, heat shock protein production, superoxide dismutase production, IAA production, and others.

Table 3. Plant growth promoting genes associated with studied genome with their respective e-values, metabolic pathway, number of detected ORF's and COG accession numbers.

COG Database Accession	Gene Name	No of orfs	BLAST E-Value	Description	Plant Growth Promoting Pathway
COG3161	UbiC	1	1.89E-50	4-hydroxybenzoate synthetase (chorismate lyase)	
COG3161	UbiC	1	1.89E-50	4-hydroxybenzoate synthetase (chorismate lyase)	4-hydroxybenzoate production
COG1125	OpuBA	1	1.18E-161	ABC-type proline/glycine betaine transport systems, ATPase components	
COG1174	OpuBB	2	7.63E-79	ABC-type proline/glycine betaine transport systems, permease component	Glycine Betaine Production
COG1732	OpuBC	1	1.42E-124	Periplasmic glycine betaine/choline-binding (lipo)protein of an ABC-type transport system (osmoprotectant binding protein)	
COG2113	ProX	1	7.96E-82	ABC-type proline/glycine betaine transport systems, periplasmic components	
COG0484	DnaJ	2	5.83E-79	DnaJ-class molecular chaperone with C-terminal Zn finger domain	Heat shock proteins
COG0443	DnaK	3	1.32E-88	Molecular chaperone	
COG0529	CysC	1	5.37E-122	Adenylylsulfate kinase and related kinases	
COG0369	CysJ	1	0.00E+00	Sulfite reductase, alpha subunit (flavoprotein)	
COG0155	CysI	1	0.00E+00	Sulfite reductase, beta subunit (hemoprotein)	
COG2895	CysN	1	0.00E+00	GTPases – Sulfate adenylate transferase subunit 1	
COG0175	CysH	2	5.58E-86	3'-phosphoadenosine 5'-phosphosulfate sulfotransferase (PAPS reductase)/FAD synthetase and related enzymes	Hydrogen Sulphide Production
COG1648	CysG	2	4.51E-115	Siroheme synthase (precorrin-2 oxidase/ferrochelatase domain)	
COG0369	CysJ	1	0.00E+00	Sulfite reductase, alpha subunit (flavoprotein)	
COG0529	CysC	1	5.37E-122	Adenylylsulfate kinase and related kinases	
COG2895	CysN	1	0.00E+00	GTPases – Sulfate adenylate transferase subunit 1	
COG1251	NirB	1	0	NAD(P)H-nitrite reductase	
COG2146	NirD	2	7.87E-33	Ferredoxin subunits of nitrite reductase and ring-hydroxylating dioxygenases	Nitrogen Fixation and Metabolism
COG0004	AmtB	1	2.24E-170	Ammonia permease	

COG5013	NarG	1	0.00E+00	Nitrate reductase alpha subunit	
COG1104	NifS	1	0.00E+00	Cysteine 352ort he352r desulfinase/cysteine desulfurase and related enzymes	
COG1785	PhoA	1	1.58E-159	Alkaline phosphatase	
COG0406	phoE	5	1.66E-47	Broad specificity phosphatase PhoE and related phosphatases	
COG0704	PhoU	1	6.26E-70	Phosphate uptake regulator	Phosphate Solubilization
COG1702	PhoH	2	1.08E-118	Phosphate starvation-inducible protein PhoH, predicted ATPase	
COG0581	PstA	2	2.35E-81	ABC-type phosphate transport system, permease component	
COG1117	PstB	2	1.59E-176	ABC-type phosphate transport system, ATPase component	
COG0573	PstC	1	2.33E-104	ABC-type phosphate transport system, permease component	
COG0226	PstS	2	1.09E-51	ABC-type phosphate transport system, periplasmic component	
COG4615	PvdE	1	0.00E+00	ABC-type siderophore export system, fused ATPase, and permease components	Siderophore
COG0845	AcrA	9	1.12E-45	Membrane-fusion protein	Production
COG0841	AcrB	7	0.00E+00	Cation/multidrug efflux pump	
COG0806	RimM	1	1.42E-76	RimM protein, required for 16S rRNA processing	
COG0605	SodA	2	1.63E-107	Superoxide dismutase	Superoxide
COG2032	SodC	1	3.20E-73	Cu/Zn superoxide dismutase	Dismutase
COG0753	KatE	1	0.00E+00	Catalase	Catalase
COG1278	CspC	8	6.27E-38	Cold shock proteins	Cold shock proteins
COG4973	XerC	1	2.00E-07	Site-specific recombinase XerC	Effective rhizosphere colonizer
COG0160	GabT	2	0.00E+00	4-aminobutyrate aminotransferase and related aminotransferases	GABA production
COG0612	PqqL	1	1.11E-50	Predicted Zn-dependent peptidases	Pyrroloquinoline quinone biosynthesis
COG1854	LuxS	1	8.31E-102	LuxS protein involved in autoinducer AI2 synthesis	Quorum Sensing
COG1764	osmC	1	4.17E-62	Organic hydroperoxide reductase	Peroxidase
COG0079	HisC	2	3.02E-127	Histidinol-phosphate/aromatic aminotransferase and cobyric acid decarboxylase	Indole-3-Acetic Production

Bacteriocin, Chitinase, Endoglucanase, Celulase and Protease Genes in Strain AGBY19

To identify antibiotic mechanisms in strain AGBY19, genes involved in the production of bacteriocins, and proteases were characterized. Table 4 demonstrates genes associated with the

production of the aforementioned products. Four genes (i.e. kcp, cta, bmb and caa) were found to be involved in the production of bacteriocins. 20 genes encode proteases, while 6 genes were associated with chitin degradation activity.

Table 4. Genes associated with production of bacteriocins, proteases and chitinases in studied genome with their detection e-values and product.

Gene Symbol	BLAST E-value	Description	Gene Product
kcp	2.00E-137	Bacteriocin	Klebicin C phage associated protein
cta	1.00E-44	Bacteriocin	Colicin-10
bmb	0.0001	Bacteriocin	Bottromycin
caa	2.00E-57	Bacteriocin	Colicin A
chiA	3.07E-63	Chitinase	Chitinase
celF	0.00E+00	Galactosidase	Alpha-galactosidases/6-phospho-beta-glucosidases
celC	1.13E-40	Endoglucanase C	Cellulase Activity
celB	1.78E-145	Beta-galactosidase	Phosphotransferase system cellobiose-specific component IIC
celA	1.02E-33	Endoglucanase Y	Cellulase Activity
celF	0.00E+00	Galactosidase	Alpha-galactosidases/6-phospho-beta-glucosidases
Hslu	0.00E+00	Protease	ATP-dependent protease HslVU (ClpYQ), ATPase subunit
thiJ	5.90E-15	Protease	Putative intracellular protease/amidase
HslV	9.01E-111	Protease	ATP-dependent protease HslVU (ClpYQ), peptidase subunit
HflC	5.12E-60	Protease	Membrane protease subunits, stomatin/prohibitin homologs
Sms	0.00E+00	Protease	Predicted ATP-dependent serine protease
PtrB	0.00E+00	Protease	Protease II
ClpP	2.47E-129	Protease	Protease subunit of ATP-dependent Clp proteases
ClpX	0.00E+00	Protease	ATP-dependent protease Clp, ATPase subunit
HflB	0.00E+00	Protease	ATP-dependent Zn proteases
TldD	5.02E-169	Protease	Predicted Zn-dependent proteases and their inactivated homologs
Lon	0.00E+00	Protease	ATP-dependent Lon protease, bacterial type
TraF	5.55E-04	Protease	Type IV secretory pathway, protease TraF
DegQ	2.44E-90	Protease	Trypsin-like serine proteases, typically periplasmic, contain C-terminal PDZ domain
HtpX	1.74E-42	Protease	Zn-dependent protease with chaperone function
Prc	5.86E-04	Protease	Periplasmic protease
SppA	6.83E-99	Protease	Periplasmic serine proteases (ClpP class)
QRI7	0.00E+00	Protease	Metal-dependent proteases with possible chaperone activity
ArpD	0.00E+00	Protease	ABC-type protease/lipase transport system, ATPase, and permease components
SprT	1.49E-65	Protease	Zn-dependent metalloprotease, SprT family
TldD	2.39E-137	Protease	Predicted Zn-dependent proteases and their inactivated homologs

Antibiotic Resistome Profiling

A total of 23 different genes observed in contigs possessing the potential to neutralize several antibiotics such as fluoroquinolones, rifampicin, fosfomycin and aminocoumarins are in Table S3. While twelve genes (mdtG, cpxA, emrR, mdtK, mdtB, emrB, H-NS, acrD, phoP, msbA, acrB, cpxR and CRP) identified that are involved in multi-drug resistance pathway, three of them (mfd, parC and ParE) were resistance genes against fluoroquinolone antibiotics (Table S3).

PAH Biodegration Profiling

Totally 27 genes responsible for PAH degradation listed in Table 5 are given, respectively. These genes are encoding the enzymes for benzoate, aminobenzoate, fluorobenzoate, chloralkane and chloralkene, toluene, xylene, nitrotoluene, ethylbenzene, styrene, caprolactam, dioxin, naphthalene, steroid degradation, and xenobiotics metabolism.

Table 5. List of PAH degrading genes with their respective metabolic pathway and KEGG identification numbers.

KEGG identificator	Gene Name	Description	Metabolic pathway
K00074	paaH, hbd, adB, mmgB	3-hydroxybutyryl-CoA dehydrogenase	Benzoate degradation
K01692	paaF, echA	Enoyl-CoA hydratase	
K00632	fadA, fadI	Acetyl-CoA acyltransferase	
K01782	fadJ	3-hydroxyacyl-CoA dehydrogenase / Enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase	
K01821	praC, xylH	4-oxalocrotonate tautomerase	
K01607	pcaC	4-carboxymuconolactone decarboxylase	
K01512	acyP	Acylphosphatase	
K16239	bsdC	4-hydroxybenzoate decarboxylase subunit C	Aminobenzoate degradation
K01692	paaF, echA	Enoyl-CoA hydratase	
K01061	tcbE	Carboxymethylenebutenolidase	Fluorobenzoate degradation
K13953	adhP	Alcohol dehydrogenase, propanol-preferring	Chloralkane and chloralkene degradation
K01061	tcbE	Carboxymethylenebutenolidase	Chlorocyclohexane and chlorobenzene degradation
K01061	tcbE	Carboxymethylenebutenolidase	Toluene degradation
K01821	praC, xylH	4-oxalocrotonate tautomerase	Xylene degradation
K10678	nfsA	Nitroreductase	Nitrotoluene degradation
K06281	hyaB, hybC	Hydrogenase large subunit	
K00198	cooS, acsA	Anaerobic carbon-monoxide dehydrogenase catalytic subunit	
K00622	nat	Arylamine N-acetyltransferase	
K00632	fadA, fadI	Acetyl-CoA acyltransferase	Ethylbenzene degradation
K00146	feaB	Phenylacetaldehyde dehydrogenase	Styrene degradation
K01692	paaF, echA	Enoyl-CoA hydratase	Caprolactam degradation
K00022	hadH	3-hydroxyacyl-CoA dehydrogenase	
K01821	praC, xylH	4-oxalocrotonate tautomerase	Dioxin degradation
K13953	adhP	Alcohol dehydrogenase, propanol-preferring	Naphthalene degradation
K16047	hsaA	3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione monooxygenase	Steroid degradation
K00799	gst	Glutathione S-transferase	Metabolism of xenobiotics
K13951	adh1_7	Alcohol dehydrogenase 1/7	

DISCUSSION

The ongoing commercial success of biocontrol agents depends on the identification of novel effective strains with multiple advantageous properties and their adaptation to different local conditions. Bioremediation gains importance on environmental contamination caused by pollutants in soil. Therefore, the investigations on multi-purposed, efficiently cost-effective methods have attracted the attention of many researchers. The current study focused on characterization of a novel bacterial strain at a genomic level that was isolated from petroleum-contaminated soil and its antagonistic potential to a major pathogen which causes late blight (*P. infestans*) on potato and tomato.

Most plant growth-promoting rhizobacteria (PGPRs) affects the plant growth indirectly and suppress diseases caused by major pathogens or by reducing the severity of minor pathogens. Additionally, PGPRs directly affect plant physiology and increased plant growth, seed emergence, or crop yield (WHIPPS, 2001). *Serratia* spp. species have plant growth-promoting effects in production fields (FALTIN *et al.*, 2004; KURZE *et al.*, 2001; BERG, 2000; BERG *et al.*, 2001). Our strain AGBY19 has significantly increased the plant height and root growth compared to control groups (Figure S2, Table S2).

The PGPR ability of our strain has been confirmed with the biochemical tests considering the synthesis of auxin phytohormone, IAA *in vitro* (Figure 1C). We have also observed IAA-mediated growth in pot trials and these results showed accordance with the gene HisC (Table 3) which is responsible for IAA production. In contrast to the finding of FALTIN *et al.* (2004) in *S. plymuthica* strain 3Re4-18, we have found a positive correlation between IAA production *in vitro* and the PGPR effect on tomato seedlings inoculated with AGBY19.

Previous studies showed that atmospheric N_2 fixation into soil enhances nutrient uptake and drought resistance (ARZANESH *et al.*, 2009; MIA *et al.*, 2009). The increase of plant height by AGBY19 treatment probably could be associated with bacterial genome harboring of the genes related with N_2 fixation (NirB, NirD, AmtB, NarG, NifS) (Table 3). The variable effects of PGPR inoculum density on legume growth has also shown with *Serratia fonticola* on nodule formation, or nitrogen fixation depending on different application of PGPR strains (LUCAS GARCIA *et al.*, 2004). Our genomic data and pot assays proved that strain AGBY19 partially belonging to *Serratia fonticola* has drastically increased root growth with the possible acceleration of nitrogen fixation on plants. The responsible genes present in our strain are in Table 3.

PGPR strains could ensure advantages with the use of less phosphate (P) fertilizer than estimated fertilization amount depending on soil nutrient analysis. Selection of new bacterial strains able to solubilize different forms of P is important in plant production that they could serve as alternative producers of uptake form of P sources as an eco-friendly option (KUMAR *et al.*, 2014; LAVAKUSH *et al.*, 2014; ANZUAY *et al.*, 2015; KAUR and REDDY, 2015). The strain AGBY19 possess these genes related to P solubilization (PhoA, phoE, PhoU, PhoH, PstA, PstB, PstC, PstS) (Table 3). Therefore, potentially it may help to solubilize different forms of P as other PGPRs, suggesting further studies necessary by qPCR analysis considering the genes responsible for P solubilization.

Many studies have reported increased GABA accumulation depending on environmental stresses on plants. GABA protects the plants to stress-related metabolism depending on

metabolic changes causing irreversible damage to plant tissue. Accumulation of GABA in stressed tissue may provide an early perception of environmental stresses to form of physiological responses and protection. GABA plays role on both stress regulation to abiotic and biotic stress factors (pest and disease) (KINNERSLEY and TURANO, 2000; GUPTA *et al.,* 2014; LIU *et al.,* 2016). In a recent study gabT has shown to have effect on GABA synthesis (CUI *et al.,* 2020). We also found the gene gabT present in AGBY19 genome (Table 3) responsible for γ-aminobutyric acid (GABA) pathways (CUI *et al.,* 2020).

Pyrroloquinoline quinone (PQQ), also called methoxatin, is a redox cofactor. The biochemical pathways of PQQ protein biosynthesis is known as precursors of Glu and Tyr (UNKEFER *et al.,* 1995). Genes involved in PQQ biosynthesis have been identified in various bacteria including *Klebsiella pneumoniae* (PUEHRINGER *et al.,* 2008), *Gluconobacter oxydans* (YANG *et al.,* 2010), and *Pseudomonas aureofaciens* (UMEZAWA *et al.,* 2015). The pqqABCDEF genes are conserved in bacteria; but the biochemical functions of encoded proteins are largely unclear. MAGNUSSON *et al.* (2004) reported PqqC is a final catalyst in the production of pqq and CHOI *et al.* (2008) reported pqq synthesized by *P. fluorescens* B16 is a key factor involved in growth promotion in tomato (*Solanum lycopersicum*), cucumber, Arabidopsis (*Arabidopsis thaliana*), and hot pepper (*Capsicum annuum*). They also reported four previously unidentified pqq genes and demonstrated that expression of the pqq genes is regulated by a transcriptional activator, PqqH. We found that our strain AGBY19 is harboring PqqL gene (COG0612) related to transcriptional activator which could be associated with the increased plant height (Table 3).

Several ecological behaviour and interdependent key characters of bacteria i.e. antibiotic, siderophore, or enzyme secretion, virulence factors of phytopathogens, as well as plant-microbe communications are coordinated through quorum sensing (QS). QS regulates various gene expressions in a cell density-dependent manner through the production and recognition of small molecules known as autoinducer. Rhizosphere is a competitive environment where microbe-microbe and microbe-plant interaction is the highest level due to existence of the rich exudates and uptake form of nutrients. The genome of strain AGBY19 contains a gene (LuxS) for QS that can be related to microbe-microbe and microbe-plant interaction (ALTAF *et al.,* 2017) (Table 3).

The strain AGBY19 is harboring the genes (OpuBA, OpuBB, OpuBC, ProX) related to production of glycine betaine (Table 3). Antioxidant production and osmolyte accumulation are physiological marker responses against salt and other abiotic stresses (MUNNS, 2002). Under such circumstances, biosynthesis of osmolytes including proline, trehalose, and glycine betaines by PGPR is most likely being faster than their associated host plants. Application of PGPR inoculants (such as our strain) seems a new tool to combat with salinity in agricultural fields for increasing food production in problematic areas.

Iron-chelating ability of *Serratia* species results limited supply of available iron in the rhizosphere therefore, pathogenic fungi starve for iron. Under limited iron conditions, bacteria produce siderophores to competitively acquire ferric iron (KAMENSKY *et al.,* 2003; BERG *et al.,* 2005; OVADIS *et al.,* 2004; FALTIN *et al.,* 2004). Even many studies have declared the iron chelator capacity of *Serratia* species, we did not find any report demonstrated at the genomic level. We have determined 4 genes that one of them PvdE related to siderophore production (SUZUKI *et al.,* 2018) and the other three genes (AcrA, AcrB, RimM) are playing role in solvent resistance and colonisation capability (WIKSTRÖM and BJÖRK, 1988; KOBAYASHI *et al.,* 2001).

These findings could be correlated with characteristic behaviour of our strain cause of the genes related to siderophore production (Table 3). These genes could be considered as the marker property for the biocontrol efficiency.

On H_2S production, if the bacteria express an effector protein recognized by the host-plant resistance protein, the hypersensitive response (HR) activates, and the plant defends itself against the invasion of the bacteria. On the other hand, disease occurs if the plant lacks the resistance gene. Therefore, the effector gene in the bacterial genome determines the virulence of bacteria causing disease on certain plants. Once the plant defense system does not respond to the existence of bacteria then, this effect has determined the realizing of disease in the plant. Plant apoplastic content is rich in a variety of nutrient components and different proteins that alters bacteria-plant interaction (PETRICCIONE *et al.*, 2014; O'LEARY *et al.*, 2016). The content of the apoplastic fluid contains many signaling molecules such as reactive oxygen species (ROS) and gasotransmitters. These gasotransmitters and plant pathogen interaction affect the bacterial pathogen which increases pathogenic virulence. Our strain AGBY19 has a variety of genes encoding H_2S production being one of the gasotransmitter (CysC, CysJ, CysI, CysN, CysH, CysG, CysJ, CysC, CysN) that may positively affect plant defense mechanism and induce plant resistance genes (Table 3). These signal molecules are inturn within plant-associated non-pathogenic bacteria and they could be a key role for activation of plant defense mechanism related to nitrite oxide production following antioxidative protection system. On this subject, the results of a previous study showed five enzymatic systems contributing to H_2S biosynthesis in plant cells (LI, 2015) related to the activity of L-cysteine desulfhydrase (L-DES) (ROMERO *et al.*, 2013) which converts into L-cysteine to pyruvate with the release of H_2S and NH_4^+ (ÁLVAREZ *et al.*, 2010; LI, 2015). Under salt-stressed conditions, L-DES stimulation on plants resulted in increased salicylic acid or H_2O_2 production on heat-stressed maize (*Zea mays*) (LI *et al.*, 2015b). Correspondingly, a postulated H_2S production by AGBY19 could be as a deceptive molecule in favour of plant, which could result in activation of plant defense through the induction of antioxidative pathways and resistance formation occurring longer period during infection within interaction between pathogen and host plant.

As a precursor of the important electron carrier, 4-hydroxybenzoate (p-hydroxybenzoate) produced from chorismate by the enzyme chorismate lyase ubiquinone is the intermediate component for the biosynthesis of amino acids, quinones, catechols and some vitamins in bacteria and fungi (NICHOLS and GREEN, 1992; SIEBERT *et al.*, 1994). The gene (UbiC) involved in 4-hydroxybenzoate synthesis has been identified in the strain AGBY19 which is accordance with findings on PGPR produced compounds such as 4-hydroxybenzoate with antibiotic property and suppressive effect on plant pathogenic microbes (DUAN *et al.*, 2013; GUPTA *et al.*, 2014). The existence of the gene region encoding 4-hydroxybenzoate production shown in AGBY19 has also suggested the present potential for cosmetic industry and pharmacological production (KOPALLI *et al.*, 2013; BŁĘDZKA *et al.*, 2014; ITO *et al.*, 2015; LENZEN *et al.*, 2019) besides its the plant growth inducer effect (Table 3).

Moreover, studies on a homolog of gene encoding chitinase degrading the cell wall of pathogenic fungal and insect have reported its beneficial effect on controlling of the different pathogens (GUPTA *et al.*, 2014; LOPER *et al.*, 2012; LIU *et al.*, 2016). On this concept, chitinolytic enzymes produced by another bacteria *Enterobacter agglomerans* has shown suppressive effect

on *Rhizoctonia solani* which is one of the damping-off pathogens (CHERNIN *et al.,* 1995; SILME and BAYSAL, 2016). Consequently, sequence analysis of AGBY19 showed the existence of gene (chiA, Table 4) encoding chitinase enzyme confirmed with our enzymatic assays carried out *in vitro* conditions. However, the remarkable inhibitory effect of AGBY19 on *P. infestans* is not cause of chitinase enzymes, due to no existence of chitin in the cell wall of Oomycetes. Therefore, the positive results on endoglucanase/cellulase activity of our strain AGBY19 could be concerned with the harboring of the celA,C genes because we have observed inhibition of *P. infestans in vitro* assays (Table 4). On the other hand, some of the secreted proteins of AGBY19 are proteases ClpP, ClpX (Table 4). Lipopeptides from strain AGBY19 could be another prominent micromolecules playing role in biocontrol agent-pathogen interactions. Cyclic lipopeptides (CLPs) as a new class of natural compounds has strong activities against Oomycetes. The previous studies reported to have induction of plant resistance by exogenously CLP introduction against oomycete pathogens *P. infestans* (ONGENA *et al.,* 2007; TRAN *et al.,* 2007; VAN DE MORTEL *et al.,* 2009; BAYSAL *et al.,* 2011).

Bacteriocins are extracellular substances produced by bacteria having distinctive morphological and biochemical characteristics. They are natural preservatives, and as therapeutic agents against pathogenic bacteria, these antimicrobial peptides have been a major area of scientific research due to their commercial importance (DE LA FUENTE-SALCIDO *et al.,* 2013). The genes encoding bacteriocin synthesis by AGBY19 has potential in agriculture and veterinary or human medicine. The genes related to production of bacteriocins are listed in Table 4 as kcp (Klebicin C phage associated protein), caa (Colicin A), cta (Colicin-10) and bmb (Bottromycin).

Another study has reported to have Klebicin C phage associated protein encoded by an operon consisting of a phage-associated open reading frame related with immunity genes in *Klebsiella pneumoniae* for the generation of bacteriocins being a novel toxin. They are most closely related to the rRNase group of colicins (such as colicin E4), while the others belong to tRNase group of colicins (such as colicin D) with similar genetic and regulatory organizations (CHAVAN *et al.,* 2005). Our genomic data has postulated the presence of similar cases for AGBY19. Colicin A is also a type of Colicin which acts by forming a pore in the membrane, leading to depolarisation of the membrane to kill the bacterial cell. This Colicin is a channel-forming type in the class of transmembrane toxins depolarizing the cytoplasmic membrane, and results in impairment of cellular energy (BATY *et al.,* 1987). Additionaly, Colicin10 is a new component due to its cognate receptor role conferring antibiotic production in genome, which provides additive property in order to enhance the higher suppressive effect of Colicin A much more than its alone (PILSL and BRAUN, 1995). Bottromycin is a macrocyclic peptide which shows antibiotic activity to inhibit methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) among other Gram-positive bacteria and mycoplasma (KOBAYASHI *et al.,* 2010). Bottromycin binds to the A site of the ribosome and blocks the binding of aminoacyl-tRNA, and inhibits bacterial protein synthesis (OTAKA and KAJI, 1976). The production of the antibiotics by AGBY19 could ensure the use of its formulation targeted not only for plant protection purpose but also pharmaceutical industry that further studies will show its probability to be put into practice.

Multidrug resistance with other known antibiotic resistance genes identified in sequence analysis of bacteria by comparison with databases are in attached file (Table S3). Results showed

remarkable competence of AGBY19 in the soil microflora, which provide possible positive population dynamism and stability after its administration.

Previous studies have shown degradability of the compounds by PAH-degrading single or various bacterial species in contaminated areas. PAHs are the carbon and energy sources of these bacteria belonging to *Pseudomonas*, *Sphingomonas*, *Brevibacterium*, *Arthrobacter*, *Enterobacter*, *Nocardioides*, and *Mycobacterium* (YAKIMOV *et al.*, 2007; SILME and BAYSAL, 2016). Our strain AGBY19 is also harboring the genes responsible for degradation of PAHs, particularly naphthalene (NAP) as reported by GHOSAL *et al.* (2016). The listed genes of AGBY19 (Table 5) detected in genome analysis shows PAH degradation capacity of our strain for contaminated soils besides its plant stimulative property and antagonistic activity against the plant pathogen.

CONCLUSION

Genome-level comparison with other *Serratia* strains reveals that strain AGBY19 partialy belongs to the *S. fonticola* group even it is not close to this species that proved with MLST and phylogency analysis. Genome analysis revealed that our strain AGBY19 acts as a PGPR agent due to confirmed studies related to chitinase and IAA biosynthesis by *in vitro* assays besides the existence of the genes potentially involved in plant growth promotion such as, phosphate solubilization, siderophore production, N_2 fixation, 4-hydroxybenzoate, H_2S synthesis, heat shock proteins, and superoxide dismutase production. Some conserved regions indicated to have common PGP characteristics observed among *Serratia* PGPR strains. AGBY19 genome also contain sets of catabolic genes which are able to degrade of PAHs. Moreover, the bacterial genome harboring the genes responsible for high salinity resistance including glycine-betaine synthesis could be a potential candidate as stress regulator agent for the cultivation of the crops under salt and water stress conditions in problematic fields.

As a model study, the completed in-depth analysis on the genome of this strain will help also for our understanding on the antifungal activity and stress management by using other PGPR strains, of which their mode of action remained unclear. We believe that these findings will rise to development of innovative biocontrol products.

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AVAILABILITY OF DATA AND MATERIAL

The raw sequence data are available in SRA database (SRA accession: PRJNA577011; Release date: 2020-04-12) (https://trace.ncbi.nlm.nih.gov/Traces/sra/?study=SRP225159). 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 in the article/supplementary material.

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GENOMSKA KARAKTERIZACIJA I FITOSTIMULATIVNI EFEKAT NOVE VRSTE *SERRATIA*

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Izvod

Neke od nepatogenih bakterija su efektivni agensi biokontrole i induktori rasta biljaka, pored svog degradativnog dejstva na policiklične aromatične ugljovodonike (PAH). U ovom radu predstavljamo kandidata za novu vrstu *Serratia*, izolovanu u svrhu degradacije PAH, sa sposobnošću stimulacije rasta biljaka i antifungalnim efektom na *Phytophthora infestans*. Svojstva bakterije, utvrđena antimikotičkim i ispitivanjem fitostimulacije u *in vitro* uslovima, predstavljena su prdukcijom indol-sirćetne kiseline i aktivnošću enzima hitinaze i endoglukanaze/celulaze. Identifikacija bakterije na osnovu rezultata dobijenih iz shotgun sekvencioniranja celog genoma takođe je pokazala da novi soj pripada novoj vrsti *Serratia* sa sposososbnošću produkcije odabranih sekundarnih metabolita potvrđenom na genomskom nivou. Analiza celokupnog genoma ukazala je na kandidata za novu vrstu *Serratia* (soj AGBY19) koji je suprimirao rast fitopatogene gljive *Phytophthora infestans* 73% u poređenju sa kontrolom u *in vitro* uslovima. Ovaj soj je kolonizovao rizosferu biljke paradajza kao biljke domaćina u *in vivo* uslovima, stimulišući rast korena. To je uzrokovano proizvodnjom hormona indol-sirćetne kiseline i enzima koji razgrađuju ćelijski zid (hitinaze, endoglukanaze/celulaze). Dalje analize genoma AGBY19 ukazale su na različite genske klastere koji uključuju dodatne regione povezane sa proizvodnjom sekundarnih metabolita koji potvrđuju osobine neophodne za biološku kontrolu i indukciu rasta biljaka. *Serratia* AGBY19 ima globalni komercijalni potencijal za upotrebu u poljoprivrednoj proizvodnji.

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