

THE FIRST REPORT ON THE ISOLATION, GENOMIC AND BIOCHEMICAL CHARACTERIZATION OF ENDOPHYTE *STAPHYLOCOCCUS HOMINIS* FROM OAT (*AVENA SATIVA L. CV. ALBATROS*)

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Abstract

Endophytes include microorganisms that live in/between the tissues of plants but do not cause any visible signs of disease in their host. In this study, *in silico* analyses were performed on the whole genome data obtained using Illumina HiSeq sequencing technology for *Staphylococcus* sp. G15S1, isolated as an endophyte from *Avena sativa* L. cv. Albatros. The isolate was also examined *In vitro* for its plant growth promoting (PGP) properties, biochemical characterization, resistance profile to some metals and antibiotics, and ability to synthesize hydrolytic enzymes. Detailed genomic analyses revealed that *Staphylococcus* sp. G15S1 genome consisted of a circular chromosome (2.21 Mbp; 31.4% G+C content). In the whole genome-based phylogenetic analysis, G15S1 was clustered together with *Staphylococcus hominis* NCTC 11320 with a digital DNA-DNA hybridization (dDDH) value of 92.9%. Annotation showed that the G15S1 genome consisted of 2199 protein-coding genes, 60 tRNAs, and 9 rRNA operons. Although genomic analyzes predicted that G15S1 encoded genes for phosphate solubility, siderophore, and indole acetic acid (IAA) synthesis, which were useful in promoting plant growth, *In vitro* tests were negative for phosphate solubility and IAA synthesis. Genes for production of glutathione peroxidase, superoxide dismutase and peroxidases stress regulator that confer resistance to oxidative stress in plants were also identified in G15S1. Moreover, genes for choline and glycine betaine biosynthesis were also found in the genome related with osmotic stress. The core genome data revealed 44 genes responsible for the defense properties of the G15S1 isolate, including virulence, disease, resistance to antibiotics and toxic compounds. Furthermore, genomic mining for pathogenicity revealed 14 gene clusters encoding proteins such as regulatory protein BlaR1, QacA, beta-lactamase, LysR-type transcriptional regulator, transposase and staphylococcal accessory regulator A proteins. These findings strongly suggested that G15S1 matched with *Staphylococcus hominis*, might also be a human pathogen. This is the first report of the isolation of *S. hominis* from *Avena sativa* L. as an endophyte, supporting the hypothesis that the internal tissues of plants can be a reservoir for opportunistic human pathogenic bacteria.

Key words: Endophyte, *Avena sativa* L. cv. Albatros, *Staphylococcus hominis*, whole genome sequencing (WGS)

Introduction

Endophytic microbial communities play crucial roles in the development and growth of a variety of host plants, whether in favorable conditions or under various stresses like heat, salinity, heavy metal contamination, and drought (Yaish *et al.*, 2015). Bacteria among these endophytes are particularly adept at colonizing internal plant tissues and providing beneficial effects that enhance host growth (Ryan *et al.*, 2008). These microorganisms contribute to the growth and developmental processes of the host by performing multiple functions, including facilitating both primary and secondary nutrient uptake through atmospheric nitrogen fixation, synthesizing siderophores, and solubilizing essential minerals such as phosphate, potassium, and zinc (Wang *et al.*, 1993; Iqbal *et al.*, 2010; Kang *et al.*, 2009). Endophytic bacteria (EB) promote plant growth through various mechanisms. These include the mineralization of inorganic substances from the soil into the roots of plants, as well as the production of enzymes and phytohormones within the host environment (Santoyo *et al.*, 2016). Also, these microbes help safeguard their host plants against pathogenic attacks by influencing host physiology and regulating phytohormone levels (Bach *et al.*, 2016).

On the other hand, opportunistic or facultative human pathogenic bacteria exclusively affect people who have a high propensity for sickness such as those who have HIV or cystic fibrosis (Steinkamp *et al.*, 2005). The ecology and pathology of these pathogens are still poorly understood. The rhizosphere, or region around roots that is impacted by the plant, is just one natural reservoir for opportunistic bacteria. This habitat is very useful for bacteria due to its high nutrient content. Root-associated strains of many bacterial genera, such as *Burkholderia*, *Enterobacter*, *Herbaspirillum*, *Ochrobactrum*, *Pseudomonas*, *Ralstonia*, *Staphylococcus*, and *Stenotrophomonas*, can interact bivalently with both plant and human hosts (Berg *et al.*, 2005). Several studies have shown that during pre- and post-harvest processing, packaging, and shipping, human pathogens can infect and colonize plant tissues (Berger *et al.*, 2010). Plants may therefore be a significant source of bacterial human diseases. Numerous soils and rhizospheric species are recruited as facultative endophytes, and bacteria that have adapted to live in plants may include opportunistic human/animal infections. For example, an endophyte related study of Alfalfa has yielded certain microorganisms that have been linked to human diseases (Pönkä *et al.*, 1995). A study conducted in India found that

the endophytic bacterial community in salad vegetables (carrot, cucumber, tomato, and onion) belonged to 5 classes covering 46 distinct species belonging to 19 genera. Human opportunistic pathogens were predominant in carrot and onion, whereas plant beneficial bacteria dominated in cucumber and tomato. Out of the 104 isolates, 16.25% were human pathogens and 26.5% were human opportunistic pathogens (Nithya & Babu, 2017).

Oats have various uses in human food. It is consumed in various baked goods such as oat cakes and oat bread; it is also used as an ingredient in many cereals, especially muesli and granola (Singh *et al.*, 2013). Oats are also used as a high-quality feed crop for livestock with high nutritional value (Andrzejewska *et al.*, 2019). Oats as feed for animals also have beneficial effects on growth and meat quality, favored for livestock and poultry farming (Su *et al.*, 2022). Considering that crops such as oats are used extensively in both human food and animal feed, it is an inevitable fact that knowledge about the natural reservoirs of infectious pathogens will be useful in the treatment and prevention of widespread disease outbreaks in humans and domestic animals. Understanding how infectious diseases spread and how to prevent infectious disease can be improved by recognizing the many kinds of disease reservoirs, such as rhizosphere and plant tissues themselves.

The EB isolate G15S1, isolated from *Avena sativa* L. cv. Albatros, was previously distinguished among 30 EB isolates in our laboratory by its siderophore production under cobalt (Co^{2+}), nickel (Ni^{2+}) and iron (Fe^{3+}) stress. (Atbaş & Taşkin, 2024). Annotation with other completely sequenced genomes will help delineating the unique and shared traits among different species, offering insights into the evolutionary changes that have occurred within the genus. Additionally, findings obtained from whole genome analysis and biochemical characterization of *S. hominis* G15S1 will help provide perspective on how a possible human opportunistic pathogenic bacterium may adapt to the endophyte life cycle with its plant growth-promoting properties. Therefore, the objectives of this study were as follows: (1) to reveal the metabolic potential of the G15S1 isolate by the genomic analysis through whole genome sequencing; (2) to investigate some important biochemical and PGP characteristics, extracellular enzyme production ability of the endophytic isolate G15S1, as well as the resistance of it to copper, zinc, manganese, mercury, lithium metals and selected antibiotics through *In vitro* tests.

Material and Methods

Bacterial isolate: In this study, the isolate with the code number G15S1 from the endophyte bacteria library created by isolating from some cultivated and wild cereal crops (Poaceae family) grown in and around Van province was used.

The endophytic bacteria stock library was built by procedure of Gao *et al.*, (2022) with minor modifications. The isolation procedure is as follows; Isolation studies were carried out from approximately 1 cm samples taken with the help of sterile scalpel from the shoot regions of the plant samples cleaned from soil wastes by washing with tap water. The samples were surface sterilized by soaking in 2% sodium hypochlorite ($NaOCl$) for 10 min and then in 70% ethyl alcohol for 10 min, followed by 3 rinses of 3 min each using sterile distilled water. After the surface sterilization

was completed, the samples were dried with the help of blotting papers, crushed with the help of sterile mortar and pestle and 1-2 mL sterile pure water was added. A 100 μ L sample of the extract obtained was spread on nutrient broth agar (NBA) (Merck) medium containing cycloheximide (100 μ g/L) using a sterile baguette and inoculated. The same amount of the final rinse water was taken and inoculated into the medium and the success of surface sterilization of the samples was checked. If any microbial growth was observed in the petri dishes of the rinse water at the end of the incubation period (25±2°C -48 h), the sterilization of the sample was considered unsuccessful, and the sample was discarded. The purified cultures were then inoculated onto media containing 8 g nutrient broth (NB) supplemented with 20% glycerol and stored in a deep freezer at -80°C for further studies.

Biochemical characterization: Rich media such as Luria Broth agar (LB), Nutrient Broth agar (NB), Tryptic Soy Broth agar (TSB) as well as King's B agar were used to establish the growth profiles and morphological characterizations of G15S1 isolate in different media. The growth profile of the isolate was tested at 3 different temperatures which were 25, 30 and 37°C. All experiments were performed in triplicate.

Phenotypic (biochemical) characteristics of G15S1 bacterial isolate cultures were determined by using Microgen and API commercial kits (GNA-GNB) for phenotypic identification. After incubation in the medium at 30°C for 48 h, the bacteria adjusted to McFarland optical density of 0.5 were inoculated into the pellets in the kit and incubated at 30°C for 48 h. After incubation, the results were evaluated as positive and negative according to the reading table of Microgen and API company. Protease, amylase, cellulase, lipase, xylanase and pectinase enzyme activities were carried out according to the procedures detailed in Dogan & Taskin, (2021). All experiments were performed in triplicate.

In vitro phosphate solubilization activity test: National Botanical Research Institute's phosphate growth medium (NBRIP) agar with pH value adjusted to 7 (Glucose; 10 g/L, $Ca_3(PO_4)_2$; 5 g/L, $(NH_4)_2SO_4$; 0.1 g/L, KCl; 0.2 g/L, $MgSO_4 \cdot 7H_2O$; 0.25 g/L, $MgCl_2 \cdot 6H_2O$; 5 g/L, Agar; 15 g/L) were inoculated with the isolates on points at equal distances and incubated for 14 days at 26-28°C. The formation of transparent zones around the colony at the end of this period was considered as an indicator of the bacteria's ability to produce phosphatase (Nautiyal, 1999).

In vitro indole acetic acid production test: The determination of Indole Acetic Acid (IAA) production of the isolate was carried out by modifying the colorimetric method described by Akbari *et al.*, (2007). Tubes containing 5 mL Nutrient Broth (NB) medium were sterilized in an autoclave. L-Tryptophan (0.05% w/v) dissolved in pure water and sterilized through filtration was added to the tubes containing NB. 100 μ L of the suspension prepared at a concentration of 10^8 CFU/mL from 48-hour cultures of EB isolates was inoculated into the prepared media. Tubes were incubated at 26-28°C for 24h in a vertical shaker. At the end of the incubation time, the samples were centrifuged at 5000 rpm for 10 min. The supernatant (3mL) was taken into a new tube and 2mL Salkowski indicator solution (0.5M

FeCl_3 2mL + 35% HClO_4 98mL) was added. After 30 min reaction time, it was measured in spectrophotometer at 535nm wavelength (Akköprü & Özaktan, 2018). The standard curve prepared by measuring IAA suspensions prepared at certain concentrations was used to convert absorbance values to ppm ($\mu\text{g/mL}$).

In vitro siderophore production test: Chrome Azurol S (CAS) test for determination of siderophore activities of G15S1 isolate was carried on according to Atbaş & Taşkın (2024).

Determination of resistance levels of G15S1 isolate to metals and antibiotics: The resistance level of the isolate for each metal ion was determined by gradually increasing the heavy metal concentrations in the solid medium until the isolate could not grow. For this purpose, stock solutions of manganese (Mn), copper (Cu), zinc (Zn), lithium (Li) and mercury (Hg) were prepared, sterilized by filtration method and added to nutrient agar solid medium and solid media containing different concentrations of heavy metals were prepared.

To prepare NA media containing the relevant concentrations of metals, the required volume of the relevant metal stock was added to the media sterilized by autoclaving, cooled to 60-70°C and mixed and poured into petri dishes under aseptic conditions. Suspensions of EB isolate was prepared at a density of 10^6 CFU/mL. From these suspensions, 20 μL of the isolates were inoculated by dropping into the prepared NA medium at equal distances. After 3 days of incubation at 30°C, when the isolates did not grow on the petri dishes, inhibitory values were noted. All measurement experiments were performed in four replicates.

Antibiotic susceptibility was assayed according to the protocol by Clinical and Laboratory Standards Institute (CLSI, 2020). Mueller Hinton Agar (MHA) plates are incubated for 24 h at 30°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The zone diameters of each drug were interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, 2020). Antibiotic disks (Oxoid™ antimicrobial susceptibility discs), infused with enrofloxacin (5 $\mu\text{g}/\text{disk}$), ampicillin (10 $\mu\text{g}/\text{disk}$), florfenicol (30 $\mu\text{g}/\text{disk}$), oxytetracycline (30 $\mu\text{g}/\text{disk}$), penicillin G (10 U/disk), ciprofloxacin (10 $\mu\text{g}/\text{disk}$), rifampin (5 $\mu\text{g}/\text{disk}$), erythromycin (15 $\mu\text{g}/\text{disk}$), kanamycin (30 $\mu\text{g}/\text{disk}$) and nitrofurantoin (300 $\mu\text{g}/\text{disk}$) were used to test antibiotic susceptibility.

Whole genome sequencing and analyses: The whole genome was sequenced using the Illumina HiSeq 2500 next generation sequencing platform (MicrobesNG, <https://microbesng.com/>). The whole genome sequence data was merged on the BV-BRC (Bacterial and Viral Bioinformatics Resource Centre) (<https://www.bv-brc.org/>) server. The complete genome annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline. This process involved predicting coding genes through an *ab initio* gene prediction algorithm combined with homology-based methods. The annotation helped clarify functional genomic units, including structural RNAs (5S, 16S, and 23S), tRNAs, and small noncoding RNAs (Angiuoli *et al.*, 2008). Furthermore, additional gene

prediction analysis and annotation were conducted using Rapid Annotation Subsystem Technology (RAST) version 3.0 (<https://rast.nmpdr.org/>) (Aziz *et al.*, 2008). We created a circular genomic map of the genome using Proksee (CGView program) (Stothard *et al.*, 2019) (Fig. 1). The whole genome data of isolate G15S1 were uploaded to GenBank under accession number PRJNA955416.

Prediction of biosynthetic gene clusters was performed in antiSMASH version 6.0 (Blin *et al.*, 2021). For a more comprehensive phylogenetic analysis, the genome sequences of the isolate were uploaded to the Type Strain Genome Server (TYGS) (<https://tygs.dsmz.de/>) (Meier-Kolthoff *et al.*, 2019) to construct a phylogenetic tree based on the whole genome sequence. Furthermore, using the whole genome sequence, the distance of the isolates from the microorganisms in the databases was determined by digital DNA-DNA hybridization (dDDH) (<https://ggdc.dsmz.de/>) analysis and average nucleotide identity (ANI) calculation (<https://jspecies.ribohost.com/jspeciesws/>). Analysis of the potential pathogenicity and related virulence gene analyses of the isolate was determined (<https://cge.food.dtu.dk/services/> Pathogen Finder/) (Cosentino *et al.*, 2013).

Results and Discussion

***S. hominis* G15S1 genome in comparison with related species:** Endophyte bacteria are microorganisms live inside or between various tissues of healthy plants (Hallman *et al.*, 1997). Whole genome-based and 16S rRNA gene-based phylogenetic trees were constructed using Type Strain Genome Server (TYGS) (<https://tygs.dsmz.de/>). In the whole genome-based phylogenetic tree, G15S1 clustered with *S. hominis* NCTC 11320, *S. hominis* subsp. *novobiosepticus* CCUG 42399 and *Plantactinospora veratri* CGMCC 4. 7143 (Fig 2). The 16S rRNA gene-based phylogenetic tree also revealed the same result. Digital DNA-DNA hybridization (dDDH) values between the isolate and its close phylogenetic neighbors were also determined using TYGS. The dDDH value given by the isolate with *S. hominis* strain NCTC 11320 was 92.9%, indicating that isolate G15S1 overlaps with *S. hominis*.

S. hominis is a Gram-positive nosocomial (hospital-acquired) pathogen and a member of the genus *staphylococcus*, which consists of spherical cells (Szczuka *et al.*, 2014). Although harmless to human and animal skin, it potentially causes bloodstream infections in immunocompromised patients. Among coagulase-negative staphylococci (CONs), *S. hominis* is one of the three most frequently identified isolates from the blood of hospitalized patients (Mendoza-Olazarán *et al.*, 2013). Although *S. hominis* is recognized as a member of the human and animal microbiota, many strains of this species have been isolated as endophytes from various plants. In a recent study, a novel antimicrobial peptide (homicordin) isolated from the endophyte *S. hominis* MBL_AB63, which was isolated from jute plant seeds from the linden family and identified by whole genome sequencing, was characterized (Aftab Uddin *et al.*, 2021). In another recent study, *S. hominis* 7E and *S. hominis* 9E strains isolated from soya and mung bean were tested for some plant growth-promoting properties and identified by 16s rRNA sequencing (Bakhtiyarifar *et al.*, 2021).

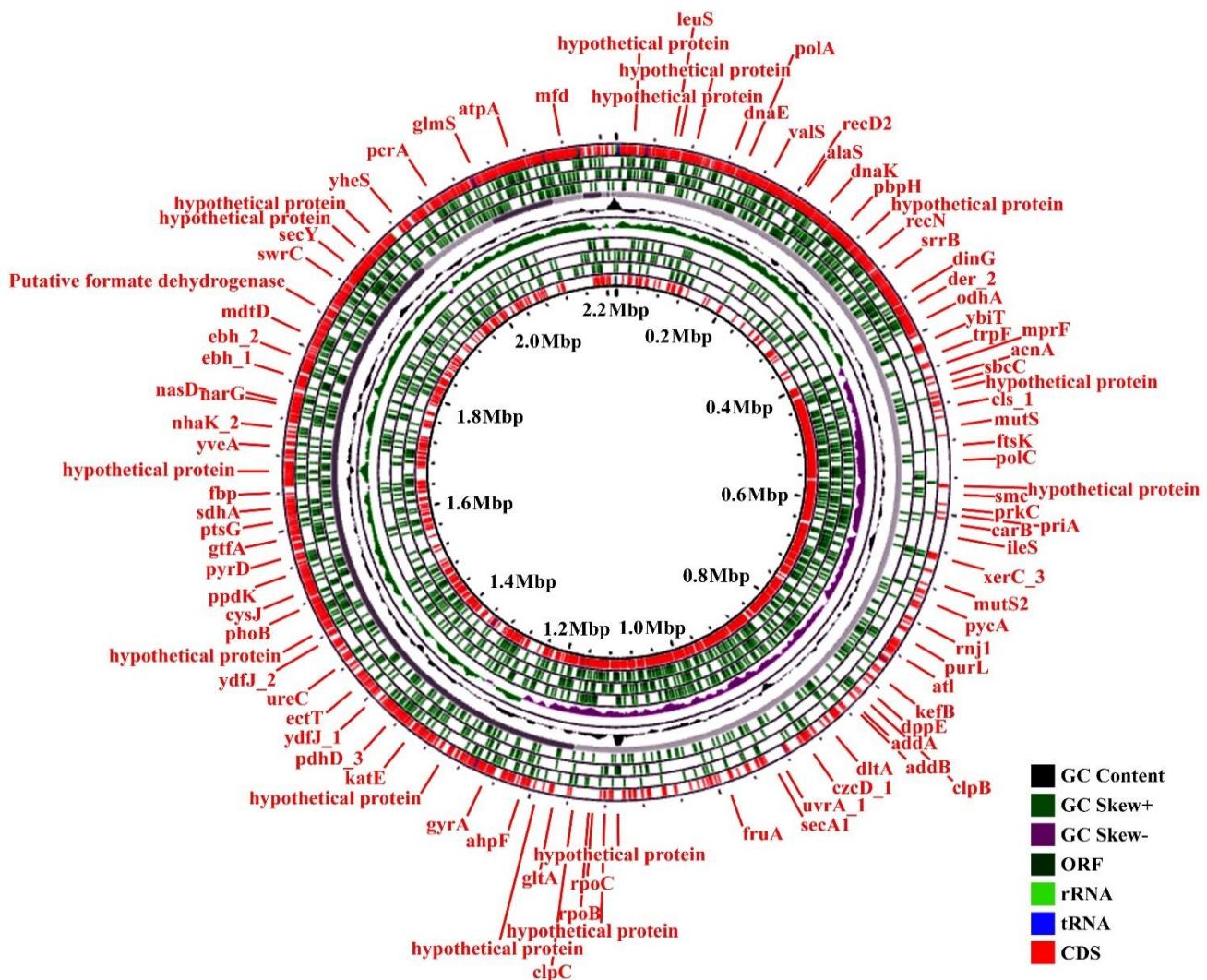


Fig. 1. Circular representation of the *S. hominis* G15S1 genome using Proksee (CGView program) (Stothard *et al.*, 2019).

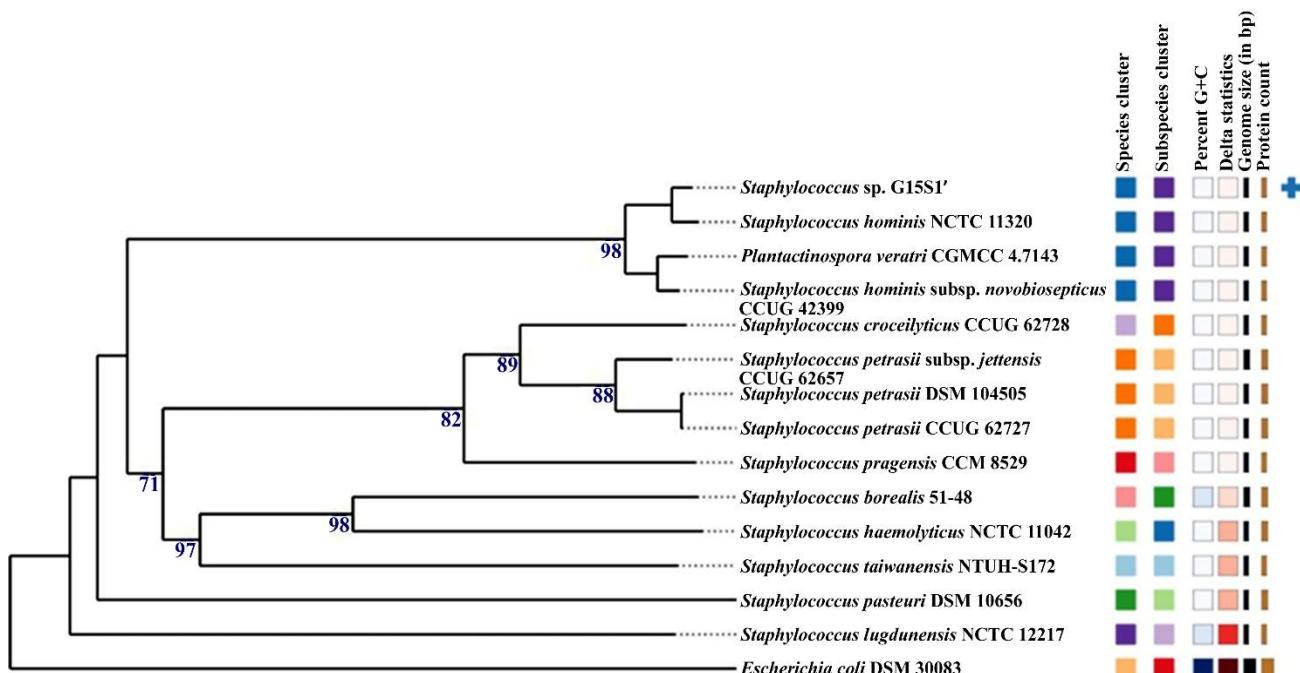


Fig. 2. The whole genome-based isolate phylogenetic tree constructed with the FastME 2.0 (Lefort *et al.*, 2015) algorithm from GBDP distances inferred from genome data. Branch lengths are scaled in terms of the GBDP distance formula d5. The numbers above the branches are GBDP pseudo-bootstrap values with an average branch support of 89.1% and greater than 60% (100 replicates) (<https://tvgs.dsrmz.de/>)

Table 1. Gene/protein prediction and annotation summary.

Annotation statistics	
Genome size (bp)	2,216,911
GC (%)	31.4
rRNA genes	9
tRNA genes	60
Number of CDSs	2199
Hypothetical CDSs	402
Proteins with functional assignments	1795
Hypothetical proteins	404
Proteins with Pathway assignments	495
Proteins with PATRIC genus-specific family (PLfam) assignments	2138

The annotation of the genome data performed using the NCBI Prokaryotic Genome Annotation Pipeline and the RAST server (<https://rast.nmpdr.org/rast.cgi>) determined the genome size as 2.2 Mbp (2216911 bp), N₅₀ value as 1161846 bp, L₅₀ value as 1 and G+C content as 31.4%. It was revealed that the completed genome of the isolate encoded 2199 proteins and 69 RNAs (Fig. 3, Table 1). These data are generally in parallel with the data of the study including the *S. hominis* strain isolated as a human pathogen and whole genome sequenced by Jiang *et al.*, (2012). Digging a little deeper into our draft genome data, we found 44 genes responsible for the defense properties of the G15S1 isolate, including virulence, disease, resistance to antibiotics and toxic compounds (Fig. 3). It was revealed that 6 of these 44 genes encode adhesin proteins responsible for cell adhesion, 4 of them encode ABC transport proteins used as part of the defense mechanism against bacitracin antibacterial peptides, and 25 of them encode resistance proteins against various antibiotics and toxic compounds. Among these 25 genes, there are 2 multidrug resistance genes, 1 beta lactamase gene, 2 fluoroquinolone resistance genes, 2 genes responsible for bile hydrolysis and cobalt, mercury, copper and zinc metal resistance genes.

Plant growth-promoting potential of *S. hominis* G15S1: We examined genes classified by their capacity to improve nutritional availability, catabolize aromatic compounds, and withstand oxidative and other types of abiotic stress from the genomic sequence of *Staphylococcus hominis* G15S1. It has been demonstrated that G15S1 have the potential to increases plant growth via the IAA (Khan *et al.*, 2014). G15S1 core genome indicated the presence of several genes involved in IAA formation, including 2-oxoacid: ferredoxin oxidoreductase (locus QDP81_RS02655) and the tryptophan biosynthesis gene cluster (*trpA*, *trpB*, and *trpD*) (locus QDP81_RS02275) (Table 2). Tryptophan-related genes have long been known to be associated with IAA production and involved in biological processes in bacterial genomes (Gupta *et al.*, 2014). Additionally, we discovered that the G15S1 genome contained several phosphate metabolism genes, including as *phoH* (locus QDP81_RS01305), *phoU* (QDP81_RS02245), and *pstA-B-C* gene cluster (locus QDP81_RS02230) (Table 2). These genes may be able to solubilize inorganic mineral phosphates, which makes them a viable inoculant candidate for boosting phosphorous uptake in plants. By generating organic acids, primarily gluconic acid, and acid

phosphatases, certain bacteria have been shown to solubilize insoluble mineral phosphates (Achal *et al.*, 2007). The phosphate-specific transport (*pst*) system is used for free inorganic phosphate transport in *Bacillus subtilis* and *Escherichia coli* (Asaf *et al.*, 2018). Although the relevant genes for IAA production and phosphate solubilization ability appeared in genome analysis, isolate G15S1 gave negative results for these features in the relevant *In vitro* tests (Fig. 4). The microbial genome pathways frequently contain a large number of silent genes known as cryptic genes. Not all cryptic pathways are silent, although under certain culturing circumstances, some may have produced metabolites at a lesser rate (Subramaniam *et al.*, 2020).

Known for their ability to bind iron, siderophores are extracellular secondary metabolites that are present in fungi, plants, and microbes. They can have a wide variety of chemical configurations. To put it another way, siderophores are iron-binding chelating agents that organisms produce to extract iron from settings where iron scarcity is a possibility (Hussein & Joo, 2014). The isolate genome was found to have gene clusters encoding siderophore ABC uptake transport system (locus QDP81_RS04845) and *lucA/lucC* siderophore synthesis genes (locus QDP81_RS09710) (Table 2). The staphylococcal siderophore transporter (*sstABCD*) operon encodes the ABC transporter responsible for iron-catechol siderophores uptake (Ghssein & Ezzeddine, 2022). Through root-mediated chelate breakdown, microbial siderophores allow plants to absorb iron from Fe-siderophore complexes (Rajkumar *et al.*, 2009). In another study in which we investigated the ability of many endophytes isolates to produce siderophores in the presence of iron and different metals, isolate G15S1 showed significant siderophore production (Atbaş & Taşkin, 2024) (Fig. 4). Therefore, it is believed that bacterial siderophores are the main sources of phytoavailable iron for metal stressed plants.

Abiotic stressors like drought, metal pollution, and salinity are frequently encountered by plants. In these situations, plants have additional tools to fight stress by being inoculated with symbiotic, stress-regulating microorganisms (Khan *et al.*, 2015). Bacteria build up osmoprotective organic molecules, also known as compatible solutes, in their cytoplasm to defend themselves from high external osmolarity (Bremer & Krämer, 2019). They correlate to a small group of substances that bacteria accumulate through *de novo* synthesis or externally supplied osmoprotectants like choline, the precursor of glycine betaine (Alloing *et al.*, 2006). Numerous bacteria possess mechanisms that enable the effective transportation of osmoprotectants in stressful situations; a number of these osmoregulated mechanisms have been previously described. Most of these systems have been molecularly described as ATP-binding cassette (ABC) transporters and secondary transporters. ABC transporters discovered so far in nonhalophilic bacteria, including *Bacillus subtilis*'s OpuA, OpuB, and OpuC, OusB of *Erwinia chrysanthemi* (Kappes *et al.*, 1996; Choquet *et al.*, 2005). The G15S1 genome was found to contain several osmotic stress tolerance genes, *opuAA*, *opuAC*, *opuD*, *opuBA*, *opuBB*, *opuBC* and *opuBD*, which are mainly glycine and choline ABC transporters genes (locus QDP81_RS06995, locus QDP81_RS07410, locus QDP81_RS08395) (Table 2).

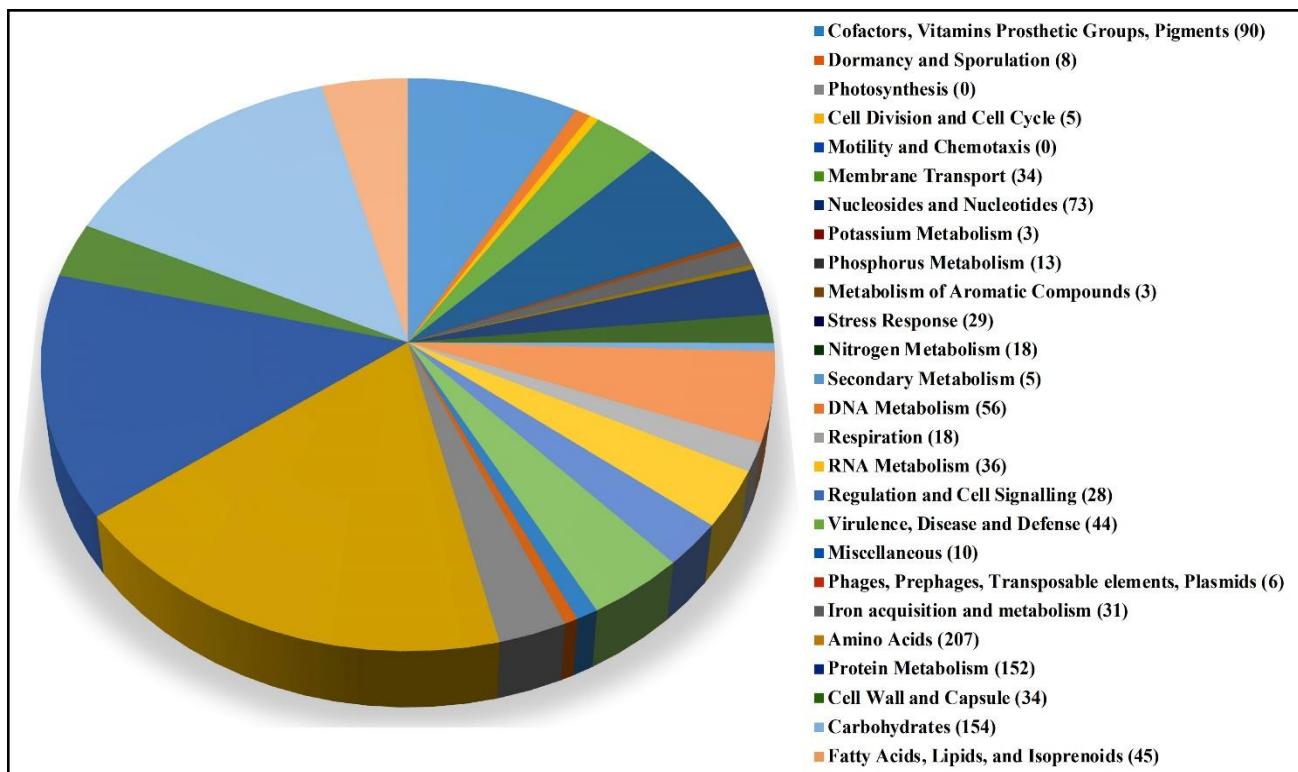


Fig. 3. Organism genome annotation subsystem overview for endophytic isolate G15S1 using Rapid Annotation Subsystem Technology (RAST) version 3.0 (<https://rast.nmpdr.org/>) (Aziz *et al.*, 2008).

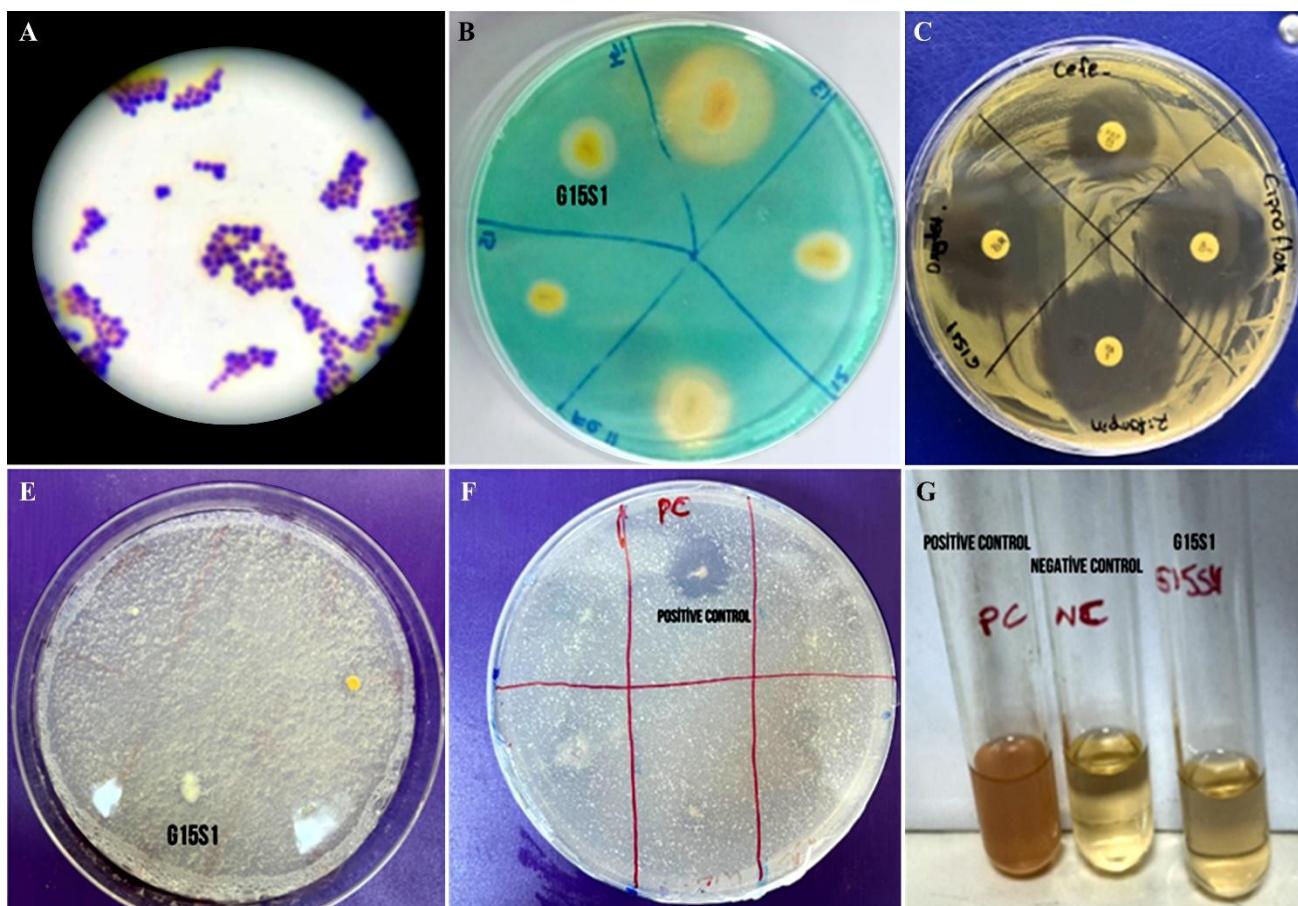


Fig. 4. *In vitro* photos showing some plant growth promoting (PGP) characteristics and antibiotic resistance tests of *S. hominis* G15S1 isolate. A) Gram staining image of *S. hominis* G15S1 cells under light microscope, B) The siderophore Chrome Azurol S (CAS) test of isolate G15S1, C) Antibiotic susceptibility test of isolate G15S1, E) Phosphate solubilization activity test of isolate G15S1, F) The petri plate including positive control for phosphate solubilization test, G) IAA production test of the isolate G15S1.

Table 2. Genes attributed to plant growth promoting traits in the G15S1 genome.

Plant growth promotion traits	Potential genes for PGP traits
Phosphorus metabolism	<i>phoH, phoU, pstA, pstB, pstC</i>
IAA production	Tryptophan synthase α chain (<i>trpA</i>), Anthranilate phosphoribosyl transferase (<i>trpD</i>), Tryptophan synthase β chain (<i>trpB</i>), Indole-3-pyruvate decarboxylase
Siderophore synthesis	ABC uptake transporter ATP-binding protein, <i>lucA/lucC</i> siderophore biosynthesis gene cluster
Osmotic Stress	Glycine betaine ABC transport system (<i>opuAA, opuAC</i>), Glycine betaine transporter (<i>opuD</i>), Choline ABC transport system (<i>opuBA, opuBB, opuBC, opuBD</i>)
Oxidative Stress	Glutathione peroxidase, Superoxide dismutase, Peroxidase stress regulator PerR
Heat shock Proteins	<i>dnaJ, dnaK, grpE, smpB</i>

Table 3. Proteins with known function among 29 proteins of the genus *Staphylococcus* identified by in silico pathogenicity analysis using the draft whole genome sequence of the isolate G15S1 (<https://cge.food.dtu.dk/services/PathogenFinder/>) (Cosentino *et al.*, 2013).

Organisms	Protein function	Protein Id	% Identity
<i>S. epidermidis</i> RP62A	regulatory protein BlaR1	AAW52778	99.83
<i>S. aureus</i> subsp. <i>aureus</i>	QacA protein	BAB47540	100
<i>S. epidermidis</i> ATCC 12228	conserved hypothetical protein	AAO05951	94.86
<i>S. epidermidis</i> RP62A	beta-lactamase	AAW52777	100
<i>S. epidermidis</i> ATCC 12228	LysR-type transcriptional regulator	AAO05952	97.07
<i>S. epidermidis</i> ATCC 12228	transposase	AAO03698	94.67
<i>S. aureus</i> subsp. <i>aureus</i> Mu50	transcriptional regulator	BAB47539	98.92
<i>S. aureus</i> subsp. <i>aureus</i> Mu50	hypothetical protein	BAB47541	100
<i>S. haemolyticus</i> JCSC1435	transposase for IS1272	BAE04193	93.59
<i>S. aureus</i> subsp. <i>aureus</i> Mu50	conserved hypothetical protein	BAB47512	99.27
<i>S. haemolyticus</i> JCSC1435	staphylococcal accessory regulator A	BAE05590	94.35
<i>S. aureus</i> subsp. <i>aureus</i> JH9	hypothetical protein	ABQ47866	97.7
<i>S. epidermidis</i> ATCC 12228	hypothetical protein	AAO03738	95.24
<i>S. aureus</i> subsp. <i>aureus</i> JH1	conserved hypothetical protein	ABR50913	100

Plant defenses against abiotic stress-induced reactive oxygen species (ROS) generation may be strengthened by endophytic bacteria, which have the ability to reduce oxidative stress (Khan *et al.* 2017). The G15S1 genome encodes genes to protect itself during the activation of plant defense mechanisms; such genes encode, peroxidase stress regulator PerR (locus QDP81_RS10305) and superoxide dismutase (locus QDP81_RS01395) (Table 2). Furthermore, the G15S1 genome contains heat shock protein genes *dnaJ* (locus QDP81_RS01265), *dnaK* (locus QDP81_RS01260), *grpE* (locus QDP81_RS01255) and *smpB* (locus QDP81_RS04600) to the modulation heat adaptive functions (Table 2).

Pathogenicity potential of the *S. hominis* G15S1: Potential pathogenicity analysis revealed that the isolate was predicted to be a human pathogen (<https://cge.food.dtu.dk/services/PathogenFinder/>) (Cosentino *et al.*, 2013). The analysis detected a total of 29 virulence gene clusters in the G15S1 genome, which are found in *S. haemolyticus*, *S. epidermidis* and *S. aureus* pathogenic strains, and calculated the probability of the tested isolate being a human pathogen as 91.8%. Among these genes, 14 gene clusters include genes encoding proteins including regulatory protein BlaR1 (locus QDP81_RS11135), QacA (locus QDP81_RS11030), beta lactamase (*blaZ*) (locus QDP81_RS11130), LysR-type transcriptional regulator (locus QDP81_RS05125), transposase (locus QDP81_RS06510, locus QDP81_RS11125) and staphylococcal accessory regulator A proteins (AgrA, AgrC, AgrD) (locus QDP81_RS09890, locus QDP81_RS09895, locus QDP81_RS09900) (Table 3). Functions of the rest of the protein remained unknown (Table 3). Beta-lactamase mediated antibiotic resistance

has been thoroughly studied, especially in *S. aureus* (Fuda *et al.*, 2005). The beta-lactamase is encoded by *blaZ* gene. The transcription of *blaZ* is controlled by the BlaZ-BlaR1-BlaI system. This system is clustered together, either on a plasmid or within the bacterial chromosome. In the absence of beta-lactam exposure, the repressor BlaI represses *blaZ* by binding to the conserved DNA motif, located in the promoter region of *blaZ*. The detection of beta-lactam molecules by BlaR1 initiates a signaling cascade, ultimately resulting in expression of *blaZ* and resistance to beta-lactams (Pence *et al.*, 2015). In *Staphylococcus* spp., *qacA* encodes a 514-amino-acid, transmembrane efflux pump belonging to major facilitator superfamily (MFS) with the capacity to efflux of a broad range of mono- and divalent cations, including dyes and quaternary ammonium compounds such as pentamidine, benzalkonium, chlorhexidine and ethidium bromide (Hassanzadeh *et al.*, 2019). LysR-type transcriptional regulators (LTTRs) are highly prevalent in bacteria including *Pseudomonas* sp., *Acinetobacter* sp. and *Staphylococcus* sp. The presence of this regulator in bacteria is not associated with any specific lifestyle or phylogeny. LTTRs control diverse functions and regulate many genes. For example, LeuO from *E. coli* activates and/or represses more than 100 targets affecting functions such as pathogenicity, CRISPR-Cas immunity and biofilm formation (Baugh *et al.*, 2023). Accessory gene regulator (*agr*) system has numerous biological functions. The most common are regulating the expression of staphylococcal virulence factors such as α -, β -, γ -hemolysin, toxic shock syndrome toxins (TSST), some cell wall-associated proteins (e.g., coagulase, fibronectin binding protein and biofilm formation). These functions are important for pathogenicity of highly virulent *S. aureus* (Tan *et al.*, 2018).

Table 4. Antibiotic susceptibility tests. Mean differences of zone diameters (in mm) measurements as determined by the disk diffusion susceptibility test in accordance with Clinical and Laboratory Standards Institute for *Staphylococcus* spp. (CLSI 2020).

Antibiotics	Inhibition zone diameter (in mm)	Interpretation
Ampicillin	36	Susceptible (≥ 29 mm)
Enrofloxacin	35	Susceptible (≥ 21 mm)
Penicillin G	30	Susceptible (≥ 29 mm)
Florfenicol	30	Susceptible (≥ 29 mm)
Ciprofloxacin	32	Susceptible (≥ 21 mm)
Rifampin	35	Susceptible (≥ 20 mm)
Tetracycline	30	Susceptible (≥ 19 mm)
Erythromycin	20	Intermediate Resistant (14-22 mm)
Kanamycin	17	Intermediate Resistant (14-17 mm)
Nitrofurantoin	25	Susceptible (≥ 17 mm)

Table 5. Phenotypic (biochemical) test results using API and Microgen commercial kits for bacterial isolate G15S1. (+): Positive Activity, (-): Negative Activity.

Biochemical Tests	Results	Biochemical Tests	Results
Lysine	-	Inositol	-
Ornithine	-	Sorbitol	-
H ₂ S	-	Rhamnose	-
Glucose	+	Sucrose	+
Mannitol	-	Lactose	-
Xylose	-	Arabinose	+
ONPG	-	Adonitol	-
Indole	-	Raffinose	-
Urease	+	Salicin	-
VP	-	Arginine	-
Citrate	+	Tryptophane	+
TDA	-	Melibiose	-
Gelatinase	+	Amygdalin	-
Malonate	-	Nitrate reduction	-
Amylase	-	Xylanase	-
Protease	-	Pectinase	-
Cellulose	-	Lipase	-

Determination of resistance levels of *S. hominis* G15S1 to selected metals and antibiotics: The isolate could not grow on media containing 1.5 mM CuSO₄, 1.0 mM MnCl₂, 0.2 mM ZnSO₄, 0.04 mM HgCl₂, 6.9 mM LiCl or higher concentrations. To differentiate metal resistivity, bacteria that can grow at metal ion concentrations of 1.0 mM and above can be regarded as resistant to the relevant metal (Tomova *et al.*, 2015). According to this inference, the isolate G15S1 were resistant to copper, manganese and lithium. According to the annotation results, G15S1 has *copC* (locus QDP81_RS08710), *copZ* (locus QDP81_RS11100) genes related to copper resistance and ABC transporter solute-binding protein, Zn/Mn family (locus QDP81_RS06680) related to manganese and zinc resistance. Although specific genes for lithium resistance could not be detected, it should be considered that *norA* (locus QDP81_RS05035) and *sdrM* (locus QDP81_RS09765) multidrug resistance genes in the isolate genome may play a role in the development of resistance to these metals. It was determined that G15S1 isolate is susceptible to antibiotics which are ampicillin, enrofloxacin, penicillin G, florfenicol, rifampin, oxytetracycline, ciprofloxacin and nitrofurantoin. On the other hand, according to criteria of CLSI, it showed intermediate resistance to erythromycin and kanamycin (Table 4). Silent antimicrobial and heavy metal resistance

genes, also known as cryptic genes, are those that bacteria carry but do not show symptoms of phenotypic antibiotic resistance. The presence of such silent genes in important pathogens has been demonstrated in many studies. Human pathogens identified as priorities by the World Health Organization, such as *Klebsiella pneumoniae*, *Salmonella* spp. and *Acinetobacter baumannii*, are known to harbor silent resistance genes for different classes of antimicrobial agents (Stasiak *et al.*, 2021; Deekshit & Srikumar, 2022). For instance, 16% of *A. baumannii* strains were still imipenem-sensitive while carrying a silent *bla*_{OXA-23} (Carvalho *et al.* 2011). Similarly, some clinical *Klebsiella pneumoniae* bacteria did not develop cephalosporin resistance despite having the *bla*_{CTX}, *bla*_{SHV}, and *bla*_{TEM} genes (Xu *et al.*, 2014). Several mechanisms associated with the lack of gene expression have been identified. Therefore, possessing of resistance-related genes in the genome may not be enough to confer resistance for the bacteria (Stasiak *et al.*, 2021; Deekshit & Srikumar, 2022).

Biochemical characterization of *S. hominis* G15S1: The experiments showed that G15S1 isolate grew most efficiently and rapidly on TSB and NB agar media. These media were followed by King's B agar and LB agar media, respectively. The isolate was incubated at 25, 30 and 37°C and showed a similar and good growth profile in each temperature. Also, in this study, the bacterial isolate was qualitatively analyzed for its ability to produce protease, lipase, amylase, pectinase, xylanase and cellulase enzymes. Comparison was made according to the positive control. It was observed that it could not produce any of the tested enzymes (Table 5). Verma *et al.*, (2001) reported that hydrolytic enzymes such as cellulase and pectinase, which are synthesized and released at different rates, may have an important role in the colonization of endophytic bacteria in plants. However, the fact that our isolate G15S1 did not synthesize these, and other hydrolytic enzymes suggests that our isolate entered and colonized the inner parts of the plant tissue using other mechanisms (aperture, wound tissue, etc.). In addition, the commercial kits used in the biochemical characterization of G15S1 bacterial isolate in this study showed positive results only for glucose, urease, citrate, gelatin, sucrose and arabinose tests and negative results for the remaining 18 tests (Table 5). When these results were compared with the literature, although they were generally in parallel, there were also some tests that showed differences. Considering the great diversity of endophyte bacteria, it is quite normal to encounter different phenotypic characteristics. Endophyte bacterial diversity can include large variations in samples taken from different regions, different plants, and even from soils of the same plant in different regions (Cho *et al.*, 2007).

Plants as reservoir for opportunistic human pathogenic bacteria: Are plants reservoirs for opportunistic human/animal pathogenic bacteria? The answer to this question has been "yes" especially in the last 20-30 years. Berg *et al.*, (2005) state that one of the natural reservoirs of opportunistic pathogens is the rhizosphere, the region around the roots affected by the plant. Due to its high nutrient content, this habitat is a "microbial hotspot" where the abundance of bacteria, including those with strong antagonistic properties, increases. Several bacterial genera, including *Burkholderia*,

Enterobacter, Herbaspirillum, Ochrobactrum, Pseudomonas, Ralstonia, Staphylococcus and *Stenotrophomonas*, contain root-associated strains that can encounter bivalent interactions with both plant and human hosts (Berg *et al.*, 2005). The mechanisms responsible for colonization of the rhizosphere and antagonistic activity against plant pathogens are similar to those responsible for colonization and pathogenicity of human organs and tissues (Cao *et al.*, 2001; Berg *et al.*, 2005). Some human pathogens such as *Salmonella* spp. have also been isolated as endophytes (Rosenblueth & Martínez-Romero, 2006). In some studies, endophytes have been found to be closely related to human pathogens or to be human/opportunistic human pathogens. Endophytic *Salmonella* strains (Guo *et al.*, 2002), which cause outbreaks and pose a health risk to consumers of raw fruit and vegetables, are an example of this situation. As Parke & Gurian-Sherman, (2001) stated, "It is perhaps no coincidence that many of the most effective biocontrol agents of plant diseases are also opportunistic human pathogens".

The ability to transfer genetic information within the family and to other microorganisms in different environments and to have a wider host network for survival and propagation leads to the conclusion that knowledge of the natural reservoirs of pathogens is essential in the treatment and prevention of widespread disease outbreaks in humans and domestic animals. Understanding the biology of endophytic colonization of human/animal pathogenic bacteria will result in a safer food supply and more efficient food production.

Conclusion

This study presents the first report on the isolation, genome sequencing, and biochemical characterization of *Staphylococcus hominis* strain G15S1 from *Avena sativa* L. cv. Albatros as an endophyte. Genomic and phylogenetic analyses confirmed the close identity of the isolate with *S. hominis*, a known opportunistic human pathogen. Despite possessing genes associated with plant growth-promoting (PGP) traits such as IAA synthesis, phosphate solubilization, and siderophore production, only siderophore production was confirmed *In vitro*. The isolate also exhibited genes for resistance to oxidative and osmotic stress, suggesting potential adaptive mechanisms to the plant endospheric environment.

Importantly, the genome of G15S1 harbored several virulence and antibiotic resistance genes, including those associated with beta-lactamase production and efflux pump systems, highlighting its potential pathogenicity. However, phenotypic assays indicated susceptibility to most tested antibiotics, implying the presence of cryptic resistance genes. Clinical studies such as animal or cell culture experiments to test the degree of pathogenicity of the isolate will be the next phase of this work. The identification of *S. hominis* as an oat endophyte underscores the role of plants as possible reservoirs for opportunistic human pathogens, raising concerns about food and feed safety, particularly for crops extensively used in both human consumption and animal nutrition.

Overall, this work contributes to the understanding of the dual nature of certain endophytes that possess both plant-beneficial and potentially harmful human pathogenic traits, emphasizing the need for careful evaluation of endophytes for agricultural applications and public health implications.

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