Genomic and Metabolic Properties of *Staphylococcus gallinarum* FCW1 MCC4687 Isolated from Naturally Fermented Coconut Water towards GRAS Assessment

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Abstract

Staphylococcus gallinarum FCW1 was isolated from naturally fermented coconut-water and identified by biochemical and molecular methods. Probiotic characterization and safety assessment were conducted through a series of *in vitro* tests. A high survival rate was observed when the strain was tested for resistance to bile, lysozyme, simulated gastric and intestinal fluid, phenol, and different temperature and salt concentrations. The strain showed antagonism against some pathogens, was susceptible to all antibiotics tested except penicillin, and showed no hemolytic and DNase activity. Hydrophobicity, autoaggregation, biofilm formation, and antioxidation tests indicated that the strain possessed a high adhesive and antioxidant ability. Enzymatic activity was used to evaluate the metabolic capacities of the strain. In-vivo experiment on zebrafish was performed to check its safety status. The whole-genome sequencing indicated that the genome contained 2,880,305 bp with a GC content of 33.23%. The genome annotation confirmed the presence of probiotic-associated genes and genes for oxalate degradation, sulfate reduction, acetate metabolism, and ammonium transport in the FCW1 strain, adding to the theory that this strain may be helpful in treating kidney stones. This study revealed that the strain FCW1 might be an excellent potential probiotic in developing fermented coconut beverages and treating and preventing kidney stone disease.

Keywords: *Staphylococcus gallinarum*, Whole genome sequencing, Zebrafish, Safety assessment, Probiotic characteristics, Kidney stone.

1. Introduction

Growing consumer interest in probiotics and fermented foods has broadened their therapeutic and research opportunities. Over recent years, more focus has been shifted to searching for novel potential probiotics taxa from various sources and their therapeutic applications. Probiotics are beneficial microorganisms that provide health benefits to the host when consumed adequately (FAO/WHO, 2002; Cammarota et al., 2014). They are the core part of fermented foods that preserve and improve foods and maintain a healthy gut microbiome without adverse side effects

in humans and animals. Some traditional probiotics have marginal ameliorative effects on various diseases and are not disease-specific. Some exhibit safety issues also. An urgent need exists at this point for screening and characterizing of novel high-quality probiotics against specific diseases (Chang et al., 2019).

Naturally fermented foods are a good source of probiotics, which break down complex chemical matrices into simpler components that are more nutrient dense and improve their bioactivity, bioavailability and safety, as well as textural and sensorial characteristics (Tamang et al., 2016). Lactic acid bacteria (LAB) and Gram-positive catalase-positive cocci (GCC^+) are the predominant bacteria isolated from various naturally fermented foods. The most common and technologically relevant GCC^+ are non-pathogenic Coagulase-negative staphylococci (CNS), which are responsible for the flavour and aroma formation and colour stabilization of the fermented foods by their proteolytic and lipolytic activities and preventing rancidity by decomposition of peroxides (Talon et al., 2007). Besides improving the quality and sensory properties of the final product, GCC^+CNS also provides nitrate-reductase, catalase and antioxidant activities (Talon et al., 1999; 2007). Owing to this, food-derived GCC^+CNS have been widely used as a starter culture for the fermentation of sausages, meat, cheese, and soybean, suppressing the growth of poisoning and spoilage microorganisms. Numerous studies have reported the isolation and safety assessment of CNS from various fermented foods like meat (Landeta et al., 2013), milk (Irlinger, 2008), seafood (Jeong et al., 2014) and soybean (Jeong et al., 2016). However, the probiotic characterization and strain-specific safety evaluation of GCC^+CNS are limited.

Spontaneously or naturally fermented coconut water (CW), the reservoir and vehicle of beneficial bacteria, was investigated in the present study for the isolation and probiotic characterization of GCC^+CNS. Generally, CW is believed to be sterile when it is in the nut cavity. Nevertheless, a recent study by Sriram et al. (2020) proved the presence of endophytic bacteria in coconut endosperm. They isolated and identified *Staphylococcus cohnii* from coconut endosperm, which could be responsible for synthesizing secondary metabolites. Furthermore, sugars and minerals in CW provide a suitable environment for the survival and growth of autochthonous microbiota, which influences safety, sensory and nutritional properties. Prado et al. (2015) successfully isolated seven autochthonous LAB from naturally fermented CW with

probiotic properties and developed a potential fermented CW beverage. This evidenced the presence of probiotic bacteria in naturally fermented CW.

Probiotic strain identification and characterization are crucial for their practical use. According to "Guidelines for the Evaluation of Probiotics in Foods" the currently available *in vitro* tests are inadequate to characterize a probiotic microorganism and its functionality (FAO/WHO, 2002). Bacterial whole-genome sequence analysis is an advanced method for accurate phylogenetic and taxonomic profiling, determining health-promoting activities, and safety evaluation of the probiotic candidates. The main objective of the current study was isolation, identification, and *in vitro* and *in vivo* probiotic characterization along with the whole genome sequencing of autochthonous bacteria from naturally fermented CW. This study provides the data necessary to understand whether the isolated bacterial strain has probiotic traits and is safe for use.

2. Materials and Methods

2.1. Sample collection and processing

Fresh green tender coconuts were purchased from the local market in Tiruchirappalli, Tamil Nadu, India. CW was collected in a sterile condition in a laminar airflow chamber and filtered through Whatman grade 42 filter paper (2.5 µm) (Whatman, GE Healthcare, UK). An aliquot of CW was allowed for spontaneous or natural fermentation in aerobic conditions as described by Prado et al. (2015) with slight modifications (16-24 h at room temperature). After the fermentation procedure, the sample was subjected to microbiological analyses.

2.2. Isolation of GCC^{+}CNS strains

Serial dilutions (10^{-1} to 10^{-9}) of 1 ml naturally fermented CW sample were prepared in distilled water (DW) and then 100µl sample from different dilutions was spread plated over plates of nutrient agar (NA) enriched with CW (CW and DW in 1:1 ratio) in triplicate and incubated at 37°C for 24-48 h. Gram-staining, catalase and coagulase tests were performed with morphologically discrete colonies. Only Gram-positive, catalase-positive and coagulase-negative colonies were propagated twice and restreaked on NA to obtain pure cultures, and the cultures were stored at −20°C as 15% glycerol stock (15% glycerol supplemented nutrient broth) for further analysis. All the media and chemicals for the above experiments were purchased from Himedia, Mumbai, India.

2.3. Biochemical and Morphological Characterization

Preliminary identification of selected bacteria was done by morphological and biochemical characterization. Morphological characterization of the isolate FCW1 was performed by colony morphology, Gram-staining, and motility. Biochemical characterization was carried out using the IMVIC test, citrate utilization assay, catalase and oxidase test, urease test, nitrate reduction assay, triple sugar iron (TSI) test and hydrogen sulfide (H_2S) production (Cappuccino and Sherman, 2005). The Carbohydrate utilization test was performed using the HiIMViC Biochemical Test Kit (Himedia, Mumbai, India), following the manufacturer's guidelines.

2.4. **Molecular identification of FCW1 strain**

Molecular identification of isolate FCW1 was made by 16S rRNA gene sequencing. The genomic DNA of strain FCW1 was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel) as per the manufacturer's instructions. The quality of the DNA isolated was checked using agarose gel electrophoresis. The 16S rRNA gene amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using universal primers: 16S-RS-F (5′-CAGGCCTAACACATGCAAGTC-3′) and 16S-RS-R (5′-GGGCGGWGTGTACAAGGC-3′) (Selvin et al., 2019). The sequencing of 16S rRNA was done using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The 16S rRNA sequence was compared with the NCBI database through BlastN (basic local alignment search tool – http://www.ncbi.nlm.nih.gov/BLAST) as well as Ezbiocloud blast were performed at https://www.ezbiocloud.net/identify web server and also the sequence has been submitted in the GenBank data library with an accession number: MW453067. Phylogenetic analysis was done with MEGA X software using the Maximum Likelihood algorithm. The evolutionary distances were calculated using the Tamura-Nei model and are in the units of the number of base substitutions per site. The rate variation among sites was uniform. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value.The analysis involved 22 nucleotide sequences, 1000 bootstrap replications and Codon positions 1st + 2nd + 3rd + Noncoding.

2.5. ***In vitro*** **Evaluation of Probiotic Properties**

2.5.1. **Safety Assessment**

2.5.1.1. ***Antagonistic activity***

The screening of potential antagonistic activity was determined by the agar gel diffusion method according to Qureshi et al. (2020). Cell-free supernatant was collected from the bacterial culture grown overnight at 37°C in 50 ml Luria-Bertani broth (LB; Himedia, Mumbai, India) by centrifugation at 5000 rpm for 10 min at 4°C and filtered through syringe filters (*0.2 µm Acrodisc® Syringe Filters, Pall)*. Six pathogenic bacteria *Klebsiella pneumoniae, Streptococcus* spp., *Escherichia coli*, (these clinical isolates belonging to K.A.P.V. Govt Medical Collage, Tiruchirappalli), *Bacillus cereus* (NCIM 2156), *Staphylococcus aureus* (NCIM 5021), and *Enterococcus faecalis* (MTCC 439) were cultured in LB broth, incubated at 37°C for 18 h and the cultures were swabbed over the Muller Hinton agar (MHA; Himedia, Mumbai, India) plates. On the swabbed MHA plate, 4 mm diameter wells were cut, and 100 µl of bacterial culture supernatant was added. Tetracycline (10 mcg) disc was used as a control since it is a broad-spectrum antibiotic which is used extensively in the prophylaxis and therapy of human and animal infections. The inhibitory zone around the wells was measured after 24 h of incubation at 37°C. The assays were carried out in duplicate.

2.5.1.2. ***Antibiotic susceptibility test***

Antibiotic susceptibility profiles were determined by the Kirby-Bauer disc diffusion method according to CLSI guidelines (CLSI, 2022). The analysis was performed with eight antibiotics (Penicillin, Gentamycin, Tetracycline, Erythromycin, Ciprofloxacin, Chloramphenicol, Norfloxacin, Trimethoprim; Himedia, Mumbai, India), which were placed over the FCW1 inoculated MHA plates. The results were compared with the interpretative zone diameters described in Performance Standards for Antimicrobial Disc Susceptibility Tests.

2.5.1.3. ***Antioxidant assay by DPPH method***

2,2-diphenylpicrylhydrazyl (DPPH; Himedia, Mumbai, India) assay was used to determine the antioxidant activity of bacterial cell-free supernatant (CFS) as described by Bhukya and Bhukya (2021). The reference standard was ascorbic acid (1 mg/ml). The following formula was used to calculate antioxidant activity (%):

$$\text{Antioxidant activity (\%)} = (A0 - As/A0) \times 100 \quad (1)$$

A0 and As, respectively, represent the absorbance of controls and samples.

2.5.1.4. ***Haemolytic and DNase activity***

The Hemolytic assay was done by measuring the zone of hemolysis around FCW1 colonies on a blood-agar plate (Himedia, Mumbai, India) after 48 h incubation at 37°C as described by Zommiti *et al*. (2017). DNase agar plates (Himedia, Mumbai, India) were streaked with isolate FCW1 and the development of a pink halo or a clear zone around the bacterial colonies after 48 h incubation at 37°C indicates positive DNase activity (Shuhadha et al., 2017).

2.5.2. **Tolerance to different stress conditions**

Tolerance to low pH and bile salt was assessed as described by Somashekaraiah et al. (2019) and Nath et al. (2021), respectively, with slight modifications. Briefly, overnight culture FCW1 (10 ml) was centrifuged at 5000 rpm for 10 min before being twice rinsed with sterile phosphate-buffered saline (PBS). The pellets were resuspended in PBS to reach the initial volume. The ability of the strain to grow at low pH was evaluated by adding 100 µl bacterial suspension into 5 ml PBS solution after adjusting the pH to 2 with 1N hydrochloric acid (HCL) and pH 7 considered as control. For bile salt tolerance, bacterial suspension (100 µl) was added to 5 ml of LB broth with (0.3%) and without (control) bile salt (LobaChemie, India) and incubated at 37°C for 4 h. At every 1 h interval, the optical density (OD) was measured at 620 nm using a spectrophotometer (Lambda 35, Perkin Elmer) and the total viable cell count was determined by plating 100 µl of samples onto fresh LB agar plates. Percentage survivability was calculated by the formula given below:

$$\text{Survivability \%} = \text{Survival rate (\%)} = \text{OD test/OD control} \times 100 \quad (2)$$

Following Liu et al. (2021), the tolerance to gastrointestinal conditions was determined. Synthetic gastric juice was prepared using 3.0 g of pepsin dissolved in sterile PBS, pH 2.5 adjusted with hydrochloric acid. Trypsin (1.0 g/L) and bile salt (1.8%) were dissolved in sterile PBS and adjusted to pH 8.0 with 0.1 mol/L NaOH to prepare artificial intestinal fluid. Before use, the artificial gastric and intestinal fluids were filtered through a 0.22 µm filter membrane (Millipore, Massachusetts, USA). The overnight culture (10 ml) of bacteria was centrifuged at 5000 rpm for 10 min; pellets were collected, washed thrice with PBS, and resuspended in sterile PBS. To 4.5 ml of artificial gastric and intestinal fluid, 0.5 ml of bacterial suspension was added

and then incubated at 37°C for 5 h. The PBS solution at pH 7 was used as a control. Percentage survivability was calculated after measuring the OD values at 620 nm at every 1 h interval.

The ability of FCW1 to grow at different temperatures was tested according to Macías-Rodríguez (2008) with minor modifications. The overnight grown strain (100 µl) was inoculated in 5 ml LB broth and incubated at 4, 10, 25 and 37°C for 24 h and the growth rate was measured by reading absorbance at 620 nm spectroscopically.

To assess lysozyme resistance, overnight FCW1 culture (5 ml) was centrifuged at 5000 rpm for 10 min and resuspended in 10 ml LB broth with lysozyme (100 mg/l) and incubated for 3 h at 37°C. At different time intervals (0, 1, 2 and 3 h), the percentage of inhibition was calculated by reading absorbance at 620 nm (Turchi et al., 2013).

Tolerance to phenol and NaCl was determined by growing the bacterial suspension (100 µl) in 5 ml LB broth containing various concentrations of phenol (0.1%, 0.4%, and 0.6%), and NaCl (3%, and 6%) for 24 h at 37 °C (Shehata et al., 2016, Qureshi et al., 2020). LB broth without phenol or NaCl was used as a control. The survival rate was determined by reading OD values spectroscopically at intervals of 0, 1, 2, 3, 4 and 24 h and the inhibition percentage of was calculated by using the following formula:

$$\text{Inhibition \%} = [(\text{OD control} - \text{OD test})/\text{OD control}] \times 100 \quad (3)$$

Experiments were conducted in triplicate and results were expressed as mean + standard deviation. Statistical analysis was performed using a Paired two-sample t-test to compare the changes in stress tolerance of the isolate with the corresponding control. P value < 0.05 was considered statistically significant.

2.5.3. **Adhesion assays**

2.5.3.1. ***Auto Aggregation***

The auto aggregation ability of the FCW1 strain was tested as per Li et al. (2020). The overnight bacterial culture was centrifuged at 5,000 rpm for 10 min at 4°C and washed twice with PBS buffer. The pellets were resuspended in 5 ml PBS buffer to an OD_{620nm} of 0.33 ± 0.015, vortexed for 10 S, and incubated for 24 h at 37°C. The upper suspension was checked for absorbance at

600 nm at 0, 1, 2, 3, 4, 8 and 24 h. The auto aggregation percentage was measured using the formula:

$$\text{Auto aggregation \%} = (1 - At/A0) \times 100 \quad (4)$$

Where, At represents the absorbance for a particular incubation time and A0 is the absorbance at 0h incubation.

2.5.3.2. ***Cell Surface Hydrophobicity***

The hydrophobicity of FCW1 was assayed using the microbial cell adhesion to solvents (MATS) method (Dlamini et al., 2018). The overnight bacterial cells were harvested by centrifugation at 5,000 rpm at 4°C for 10 min, washed twice with PBS, and resuspended in 3 ml PBS buffer, followed by absorbance (A0) measurement at 600 nm. The cell suspension was added with 1 ml of solvents (hexane, chloroform and ethyl acetate), vortexed for 1 min and incubated at 37°C for 1 h for the separation of aqueous and organic phases. The absorbance (A1) of the aqueous phase (1 ml) was measured, and the percentage of hydrophobicity was calculated using the following formula:

$$\text{\% cell surface hydrophobicity} = (1 - A1/A0) \times 100 \quad (5)$$

Isolate with 50% and above MATS was considered strong hydrophobic (Garc´ıa-Hern´andez et al., 2016).

2.5.3.3. ***Biofilm formation assay***

A good biofilm-forming bacterium can adhere and colonize the intestinal epithelial cells. The biofilm formation ability of the isolate FCW1 was determined as in Zayed et al. (2021) with modifications. Briefly, the LB broth in test tubes was inoculated with FCW1 isolate and incubated for 48 h at 37°C with shaking at 120 rpm. After incubation, the culture was decanted and the tubes were washed with PBS buffer, which was then air-dried at room temperature. The tubes were then stained with 3ml of 0.1% crystal violet, kept for 30 min, and washed with distilled water thrice. The appearance of the violet colour on the test tube walls indicates biofilm formation. The stain was released with ethanol and read OD at 595nm. Cut-off values were calculated as the mean OD of the negative controls (ODc). The biofilm production was determined based on the following classification: $OD \leq ODc$ = non-biofilm producer; $ODc < OD \leq 2 \times ODc$ = weak biofilm producer, $2\times ODc < OD \leq 4 \times ODc$ = moderate biofilm producer, and $OD > 4\times ODc$ = strong biofilm producer.

2.5.4. **Enzymatic activity**

The isolate FCW1 was inoculated on an appropriate agar medium to detect the amylase, protease, lipase (Cappuccino and Sherman, 1983) and cellulase activity (Kasana et al., 2008). To detect amylase activity, a starch agar plate was inoculated with FCW1 and incubated for 24 h at 37°C. After adding Gram's iodine solution to the culture, the plate was observed for a clear zone around the colonies. The cellulolytic activity was detected by streaking the isolate on carboxy methyl cellulose agar, and the zone of clearance was recorded after 24 h incubation. Tributyrin agar was used for the detection of lipolytic activity. A cloudy zone around the colony shows lipolytic activity.

2.6. *In vivo* Bio-safety assay

Probiotic candidates were tested for pathogenicity and bio safety using the Immersion assay, which involves challenging zebra fish and looking at their health conditions. The animals were handled according to Bharathidasan University's (Tamil Nadu, India) Institution Animal Ethics Committee guidelines (BDU/IAEC/P11/2021). Experiments were carried out in triplicates in aquaria set at 25-30°C and a lighting schedule of LD 12:12. Ten *Danio rario* (mean body weight: 0.182g) were randomly distributed among the aquariums after an initial 10-day acclimation period. The strain FCW1 was inoculated into MRS broth and incubated for 24 h. Cells were extracted by centrifugation at 5000 rpm for 10 min at 4°C in a cooling centrifuge. Sterile PBS was used twice to wash and resuspend the cells. The concentrations of 10^5, 10^6, and 10^7CFU/ml were added to the fish tanks. Control aquariums were added with sterile PBS solution. Fish were fed 5% of their body weight twice daily (35% protein). Symptoms, abnormalities, and mortality rates were measured at the end of the experiment.

2.7. Whole Genome Sequencing of *S. gallinarum* FCW1

Purified *S. gallinarum*FCW1 culture broth was used for further DNA extraction usingQIAamp DNA Mini Kit (QiagenInc, USA) according to manufacturer's instructions. Illumina sequencing libraries were prepared and genome was sequenced on the Illumina NextSeq 2500 platform using 2 × 250 paired-end libraries. The quality control of raw reads was carried out in FastQC version 0.11.9 (Andrews, 2010) and low quality reads were trimmed using Trimmomatic (ver 0.35), with a phred cutoff of Q20 (Bolger et al., 2014). After quality filtering, the primary de novo assembly of the reads was performed using SPAdes genome assembler (ver 3.10) (Nurk et al., 2013). Genome annotation was performed using the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (Tatusova et al., 2013).

Overall genome relatedness index, measured as the Orthologous Average Nucleotide Identity (OrthoANI), was calculated using the OrthoANI application of EzBioCloud (Lee et al., 2016). The genes of probiotic characteristics were retrieved manually from the annotated genome and confirmed using BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against a non-redundant database of National Center for Biotechnology Information (NCBI). The Clusters of Orthologous Groups of proteins (COG) functional categories of protein coding genes were done using database WebMGA (http://weizhong-lab.ucsd.edu/webMGA/server/cog/). Genes involved in secondary metabolites biosynthesis were detected by Antismash 6.0 (Blin et al., 2021). Antibiotic resistance gene was detected by PATRIC's AMR classifier.

Strain Deposition and Complete Genome Sequence Data Accession Number

The whole genome sequence data of *S. gallinarum* FCW1 has been deposited at GenBank under the accession number CP086207. The strain has been deposited at National Centre for Microbial Resource, India (Accession no. MCC 4687).

3. Results

3.1. Isolation and screening of GCC^{+}CNS strains

A total of seven morphologically discrete colonies were isolated from the naturally fermented coconut water and tested for GCC^{+}CNS. Of these, only one isolate (FCW1) with Gram-positive, cocci-shaped (Fig. 1), catalase-positive and coagulase-negative properties was selected for further analysis. Scanning electron microscopic (SEM) analysis of *S. gallinarum* FCW1 confirms its spherical shape. The biochemical characterization revealed that isolate FCW1 does not utilize citrate as a carbon source, but could reduce the nitrate in nitrite. The Carbohydrate fermentation pattern showed that the isolate FCW1 was able to ferment glucose, sucrose, mannitol, adonitol, arabinose, and rhamnose, whereas lactose and sorbitol were not utilized (Table 1). According to the phenotypic and biochemical characterization, the isolate FCW1 was confirmed as GCC^{+}CNS.

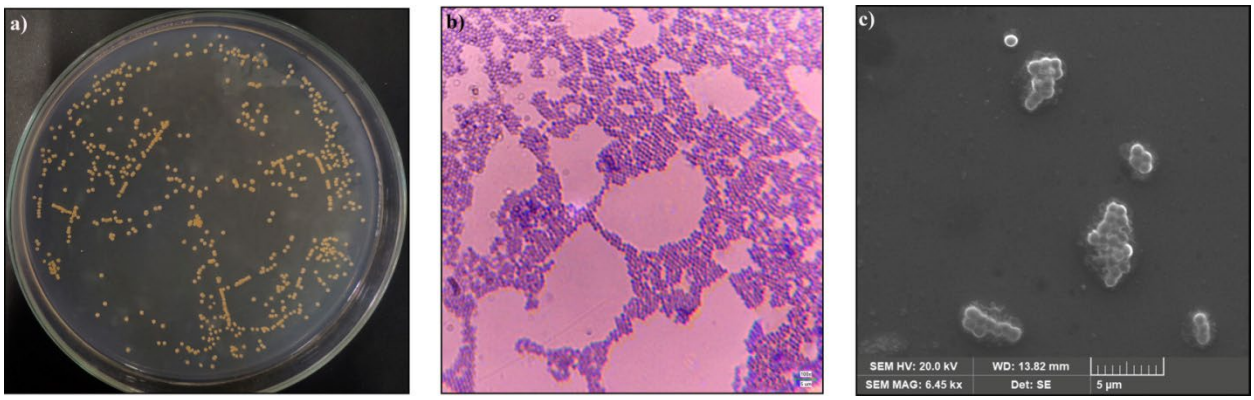


Fig. 1. Morphological view of GCC^{+}CNS strain FCW1. (a) Colony morphology of FCW1 on nutrient agar after 24 h incubation at 37℃, (b) Cell morphology of FCW1 based on Gram staining under the light microscope (100X magnification) and (c) SEM imaging of FCW1 with HV 20 kv and 6.45 kx magnification.

Table 1. Phenotypic and Biochemical Characterization of Strain FCW1

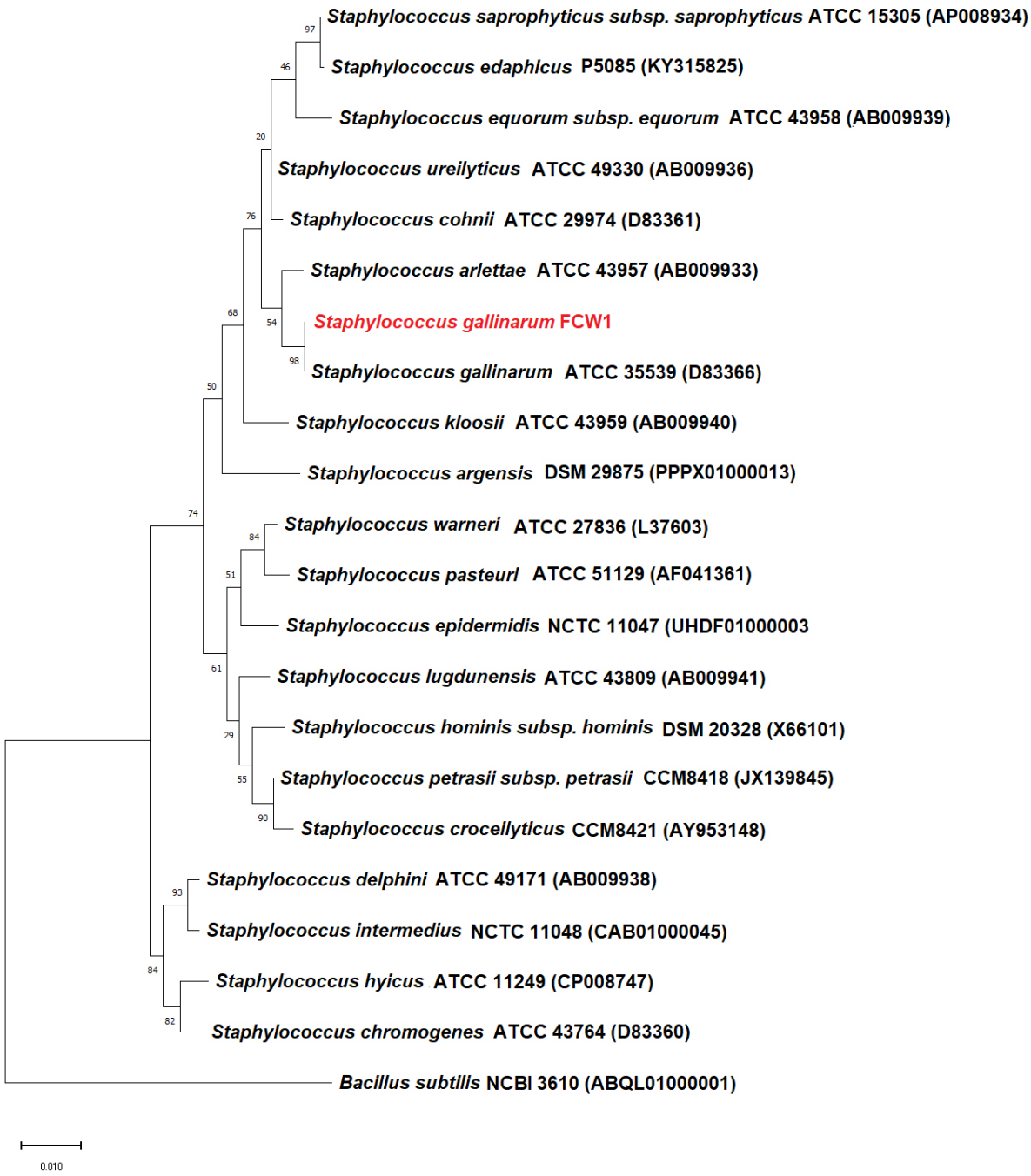
Tests	**FCW1**
Gram Staining	G^{+}
Shape	Cocci
Motility	Non-motile
Indole	Negative
MR	Positive
VP	Negative
Citrate	Negative
TSI	K/K
H_2S production	Negative
Nitrate Reduction test	Positive
Urease	Positive
Catalase	Positive
Oxidase	Positive
Coagulase	Negative
Carbohydrate Fermentation	
Glucose	Positive
Adonitol	Positive

Arabinose	Positive
Lactose	Negative
Sorbitol	Negative
Mannitol	Positive
Rhamnose	Positive
Sucrose	Positive

329 G^+ indicates Gram-positive, MR indicates methyl red, VP indicates Voges Proskauer

330 **3.2. Molecular Identification of FCW1 strain**

331 Based on the 16S rRNA sequencing comparison with NCBI BlastN as well as Ezbiocloud blast,
332 the FCW1 was identified as *S. gallinarum*. Using MEGA X software, a maximum likelihood
333 phylogenetic tree based on 16S rRNA gene sequences, including 1000 bootstrap replications,
334 was built to reveal the exact phylogenetic position of *S. gallinarum* FCW1 within 20
335 *Staphylococcus* species(Fig. 2). Moreover, we have used *Bacillus subtilis* as a outgroup . *S.*
336 *gallinarum* FCW1 is closely related to *S. gallinarum* D35539/ATCC 35539, isolated from
337 chicken skin (Devriese et al., 1983).



338

339 **Fig. 2**. Phylogenetic tree of *S. gallinarum* FCW1 based on Ezbiocloud database using MEGA X
340 software. This maximum likelihood tree illustrates the phylogenetic relationships of *S.*
341 *gallinarum* FCW1 and closely-related strains of the genus *Staphylococcus*. 1000 bootstrap
342 replications were used to generate the tree and *Bacillus subtilis* strain NCBI 3610 was used as an
343 out-group. The percentage of tree in which the associated taxa clustered together is shown next

to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Rate variations were uniform per sites. This analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding.

In vitro Evaluation of Probiotic Properties

3.2.1. Safety Assessment

3.2.1.1. Antagonistic activity

Antimicrobial activities of isolates are a suitable way of screening potential probiotic bacteria. The isolate FCW1 showed moderate inhibition of the growth of *E. coli*, *B. cereus*, and weak inhibition towards *K. pneumoniae* (Table 2).

Table 2. Antagonistic activity of *S. gallinarum* FCW1 against common human pathogens

Pathogens	Zone of Inhibition (mm)
Staphylococcus aureus	-*
Streptococcus spp.	-*
Escherichia coli	15 ± 0.71
Enterococcus faecalis	-*
Klebsiella pneumoniae	6 ± 0
Bacillus cereus	13 ± 0.71

The zone diameter values (mm) are the average of two experiments and ± indicates the standard deviation from the means; * No zone of inhibition

3.2.1.2. Antibiotic Susceptibility Profiles

Table 3 shows the antibiotic susceptibility profile of FCW1 using the disc diffusion method. Based on the findings, isolate FCW1 was susceptible to all the selected antibiotics except erythromycin (intermediated susceptible) and penicillin (resistant).

Table 3. Antibiotic Susceptibility Profile of Strain FCW1

Class	Antibiotics	Disc Content	Inhibition Zone (mm)*	Susceptibility Profile	CLSI Susceptibility breakpoints
Penicillin	Penicillin (P)	10 units	20 ± 0	Resistant	≥29
Aminoglycosides	Gentamycin (GEN)	10 mcg	24 ± 1	Susceptible	≥15
Tetracycline	Tetracycline (TE)	30 mcg	26 ± 1	Susceptible	≥19
Diaminopyrimidines	Trimethoprim (TR)	5 mcg	29 ± 1	Susceptible	≥16
Macrolides	Erythromycin (E)	15 mcg	20 ± 1	Intermediate	≥23
Fluoroquinolones	Ciprofloxacin (CIP)	5 mcg	31 ± 1	Susceptible	≥21
Chloramphenicol	Chloramphenicol (C)	30 mcg	26 ± 1	Susceptible	≥18
Quinolone	Norfloxacin (NX)	10 mcg	19 ± 1	Susceptible	≥17

*The zone diameter values (mm) are the average of two experiments and ± indicates the standard deviation from the means

3.2.1.3. Antioxidant Assay by DPPH Method

An *in vitro* antioxidant activity of cell-free supernatants FCW1 is shown in Fig. 3a. The DPPH radical scavenging activity increased from 9.22 ± 0.22% to 66.57 ± 2.43% with increasing volume of CFS from 50 to 500 µL. The radical scavenging activity percentage of ascorbic acid was 74.73 ± 0.39% at a concentration of 50 µg/L and 98.4 ± 1.4% at 500 µg/L. Various secondary metabolites, peptides, phenolic compounds, etc., may contribute to the radical scavenging activity.

3.2.1.4. Haemolytic and DNase assay

After 48 h incubation, strain FCW1 showed α haemolysis or no haemolysis on blood agar, indicating its weak or null ability to lyse blood cells (Fig. 3b). The strain demonstrated no zone of inhibition on DNase agar also indicates its safety for probiotic usage (Fig. 3c).

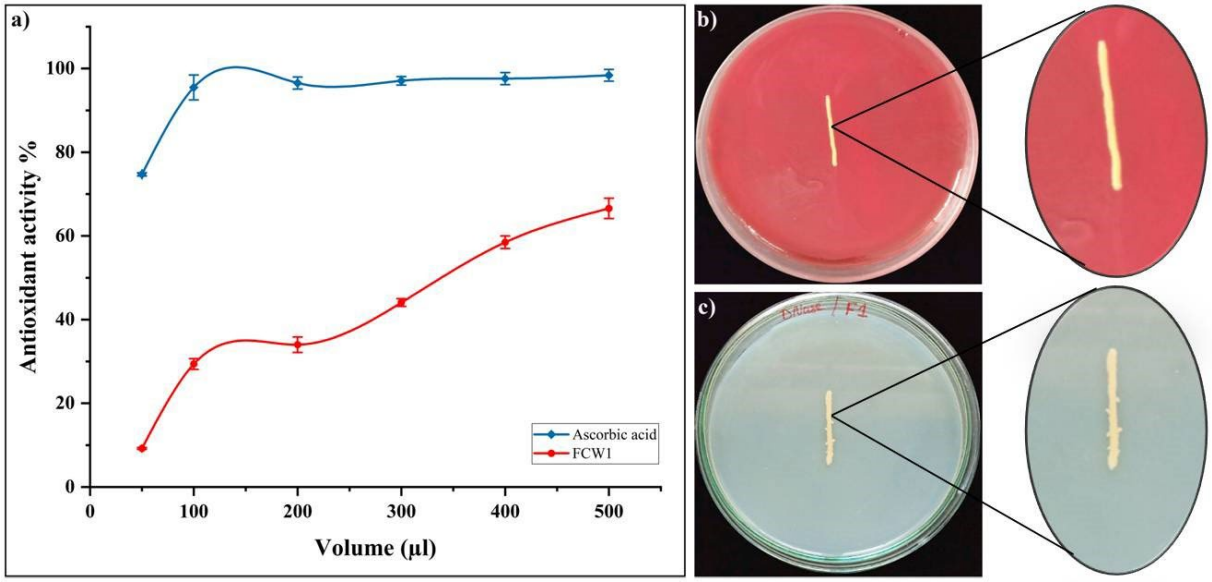


Fig. 3. Antioxidant activity and Safety assessment of Strain FCW1. a) DPPH radical scavenging activity of strain FCW1. Ascorbic acid used as positive control. The data were expressed as mean ± SD (n=3) b) Hemolytic activity of FCW1 shows α hemolysis on blood agar after 48 h incubation (zoomed imaged on right), c) DNase activity of FCW1 shows no pink or clear zone of inhibition around the colonies on DNase agar after 48 h incubation indicates no DNase enzyme activity (zoomed imaged on right).

3.2.2. **Tolerance to different stress conditions**

The strain FCW1 was resistant to 0.3% of bile salt ($P > 0.05$) and was viable even after 4 h incubation (Fig. 4a). In the presence of bile salt, FCW1 showed 101.15% survivability when compared to the control.

The isolate FCW1 tolerated to pH 2, which was be comparable to pH 7 (Fig. 4b) and showed stable growth ($P > 0.05$) after 4 h incubation without any significant loss of viability. The survival rate was calculated as 99.65%.

The lysozyme had less inhibitory effect on strain FCW1 (Fig. 4c) and significantly grew well in the presence of lysozyme ($P < 0.01$), with viable cell count 8.32 log CFU/ml after 3 h incubation, while the control having 9.04 log CFU/ml viable cells.

The growth performance of isolate FCW1 at different temperatures was shown in Fig. 4d. At 0-4 and 10-15°C the strain showed no or poor growth. However, the cells were viable after 24 h

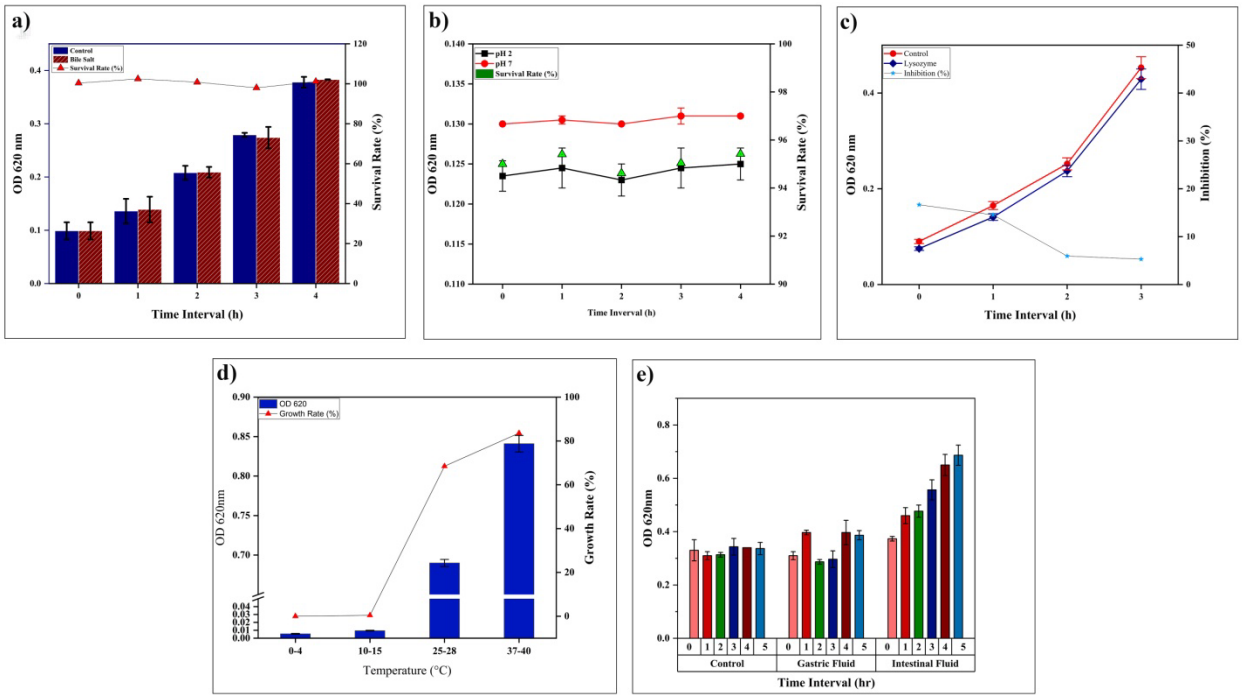
394 incubation. The best growth was seen at 37-40°C when compared to 25-28°C ($P > 0.05$). Based
395 on the results, the optimum temperature for FCW1 strain is 37°C.

396 The preliminary analysis of the isolate's tolerance to artificial gastric fluid and intestinal fluid
397 was shown in Fig. 4e. The isolate showed excellent growth in the gastric and intestinal
398 environments. The bacterial inoculation to gastric juice results in the rapid bacterial division
399 during the 1st hour, and then growth rates drop in the 2nd hour, which then gradually increases (P
400 > 0.05). The survival rate after 5 h exposure to gastric and intestinal fluid ($P < 0.005$) was
401 estimated as >100% compared to the control.

402 The ability of the FCW1 isolate to endure phenolic conditions was assayed in the presence of
403 0.1, 0.4 and 0.6% phenol. The bacterial growth is highly affected by 0.4% and 0.6% of phenol at
404 3 h of exposure with respect to corresponding control ($P < 0.05$). At these higher concentrations,
405 the inhibition rates at 3 h and 24 h are similar. However, the bacteria could grow well at 0.1%
406 phenol ($P > 0.05$). The percentage of inhibition is given in Table 4.

407 NaCl is an inhibitory substance which prevents the growth of probiotic bacteria. The NaCl
408 tolerance was performed at 3% and 6% NaCl concentrations. The isolate FCW1 was able to
409 grow at 3 and 6% NaCl concentrations ($P < 0.05$) and the inhibition percentage was given in
410 Table 4.

411



412

413 **Fig. 4**. Strain FCW1 tolerance to different stress conditions. (a) The growth of FCW1 was
414 remained unaffected in the presence of 0.3 % bile salt ($P > 0.05$) when compared to control, (b)
415 FCW1 showed stable growth at pH 2 and pH 7 ($P > 0.05$), (c) Significant growth was observed
416 in the presence of lysozyme (100 mg/l) with respect to corresponding control ($P < 0.01$), (d)
417 growth at different temperatures shows 37°C is the optimum temperature for FCW1, (e) FCW1
418 showed insignificant growth ($P > 0.05$) in the presence of gastric and highly significant ($P <$
419 0.005) growth in the presence of intestinal fluid tolerance.

420 **Table 4**. Growth performance of FCW1 strain in the presence of different concentrations of
421 NaCl and phenol.

Incubation time (h)	***Growth Inhibition %**				
	3% NaCl[a]	**6% NaCl[a]**	**0.1% Phenol[b]**	**0.4% Phenol[a]**	**0.6% Phenol[a]**
0	0	2.78	0	2.78	5.56
1	14.54	21.81	1.78	20	32.72

2	13.15	27.5	1.72	46.25	56.25
3	25.53	45.74	1.56	77.65	89.36
4	26.81	47.49	27.56	74.30	82.12
24	21.99	26.97	28.23	77.59	89.21

*The values (Inhibition %) are the mean of two experiments, 'a' indicates Significant ($P < 0.05$) and 'b' indicates Not significant ($P > 0.05$) with corresponding controls

3.2.3. Adhesion assays

3.2.3.1. Auto Aggregation ability

As shown in Fig. 5a, the auto aggregation percentage of isolate FCW1 increased as time progressed. Isolate FCW1 had a very low initial auto aggregation percentage of 8.77±7.38%, which increased gradually over time and reached 95.04±1.6% after 24 h incubation.

3.2.3.2. Cell Surface Hydrophobicity

The ability of probiotics to adhere to intestinal mucosal cells is associated with their hydrophobicity. In this study, the isolate FCW1 have moderate to strong adhesion capacity to all the tested solvents. The isolate showed high adherence to chloroform (87.31%), 57.62% hydrophobicity to hexane, and moderate adherence to ethyl acetate (32.16%) (Fig. 5b).

3.2.3.3. Biofilm formation assay

The biofilm formation property promotes adherence and colonization of bacteria on the host intestinal epithelium and thus prevents the colonization and reproduction of pathogens (Salas-Jara et al., 2016). The isolate FCW1 was found positive for biofilm formation (Fig. 5c) and based on the OD value, it was found that the isolate exhibited modest biofilm formation.

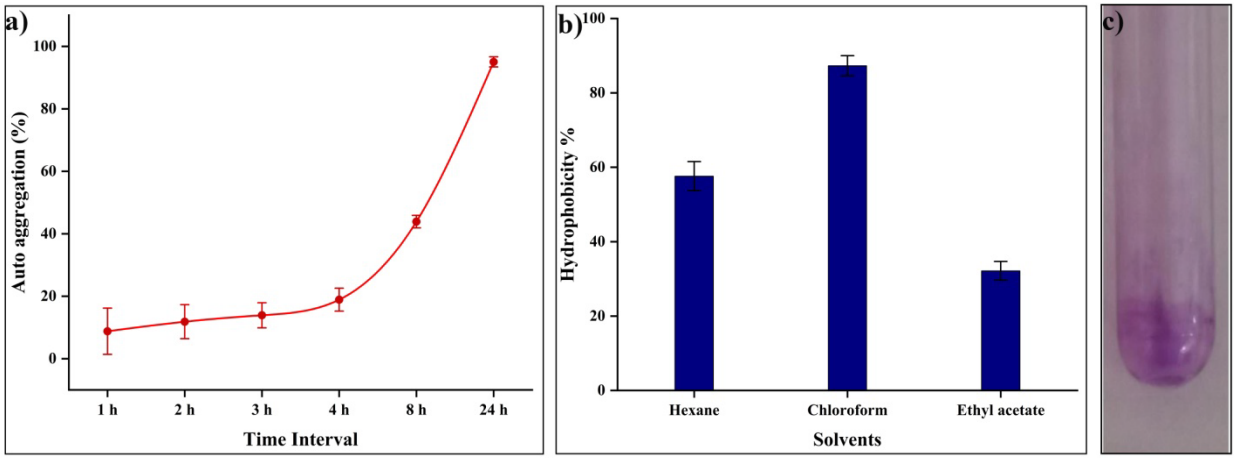


Fig. 5. Adhesion assays of strain FCW1. a) Auto aggregation. The auto aggregation % of FCW1 increased over time b) Hydrophobicity ability. FCW1 showed high hydrophobicity to chloroform and hexane whereas moderate to ethyl acetate. Error bar represents the mean value of three independent experiments ± SD, c) Stained tubes showing biofilm formation ability of FCW1.

Enzymatic activity

The enzyme-producing property of isolate FCW1 was tested by inoculating on specific media. The results showed that the isolate failed to produce the enzymes amylase, cellulase and lipase (Fig. 6).

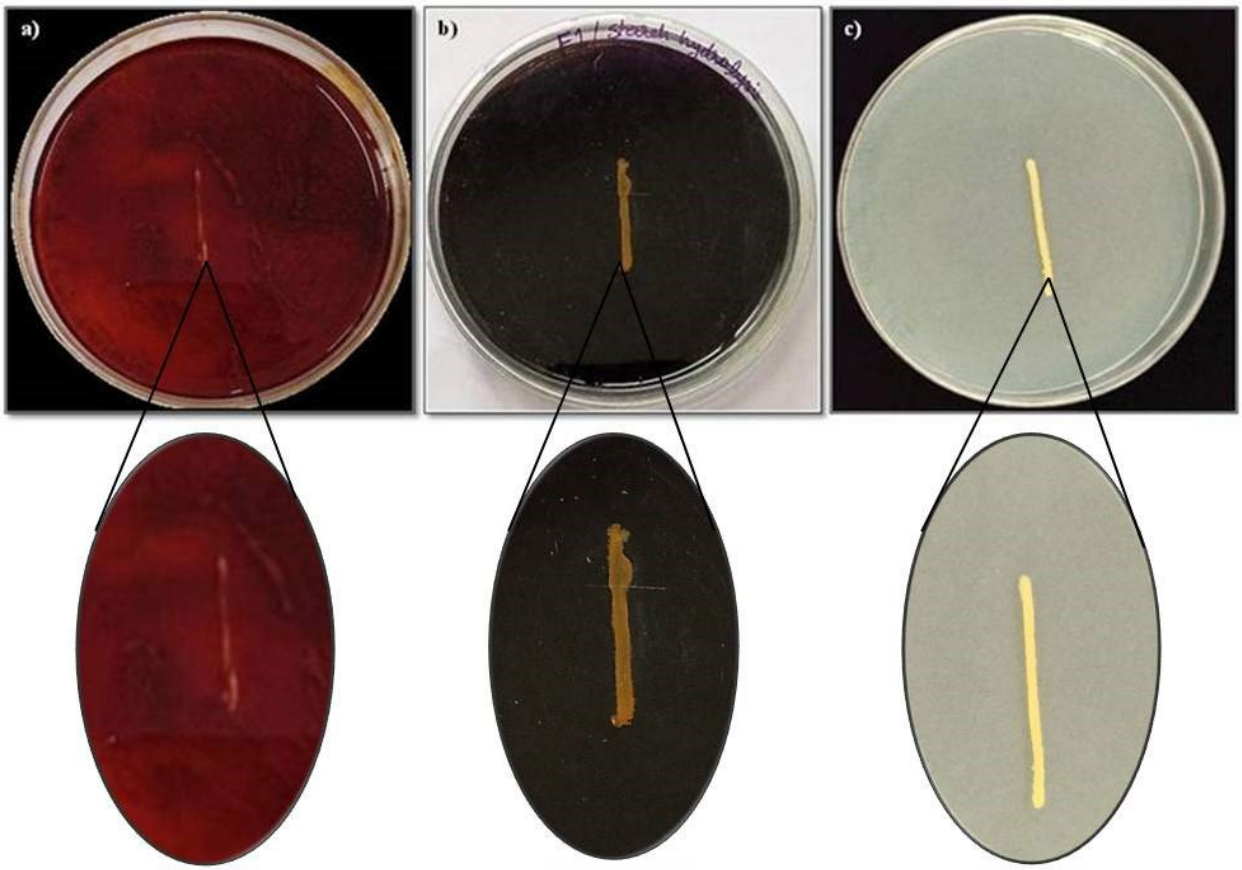


Fig. 6. Metabolic capacities through enzymatic activity of Isolate FCW1. No clear zone around the colonies indicates lack of a) cellulase, b) amylase, c) lipase activity. The zoomed image shows absence of enzymatic activity.

3.3. *In vivo* bio safety assay

The *in vivo* bio safety assay revealed no behavioural changes, disease symptoms such as external lesions, edema, haemorrhage, loss of scales or mucus and mortalities in either experimental or control groups after 10 days.

3.4. Whole Genome Sequencing

3.4.1. Genomic features of FCW1 strain

Based on the *in vitro* probiotic characteristics, the whole genome sequencing of strain FCW1 was performed to evaluate the probiotic properties at the genomic level. The complete genome of *S. gallinarum* FCW1 consisted of 2,880,305 nucleotides with a GC content of 33.23%. The circular genome map and its general genomic features are shown in Fig. 7 and Table 5. Plasmids

were not present in the FCW1 genome. The assembled reads consist of a single contig. The genome comprises a total of 2770 genes and 2708 protein-coding genes (CDS). Among the predicted CDS (an average length of 897.45bp), 1792 (66.17%) genes were functional genes, and 916 (33.83%) genes were hypothetical genes. The genome contains 62 RNA genes, including 57 tRNAs, 4 rRNAs and 1 tmRNA. It also contains one CRISPR repeat and 11 pseudogenes. The identified 57 tRNAs representing 19 amino acids: Arg (4), Asn (3), Ser (5), Glu (2), Gly (7), His (2), Phe (2), Asp (3), Met (4), Leu (5), Csy (1), Gln (2), Trp (1), Tyr (1), Thr (3), Ala (2), Pro (2), Lys (3), and Val (2).

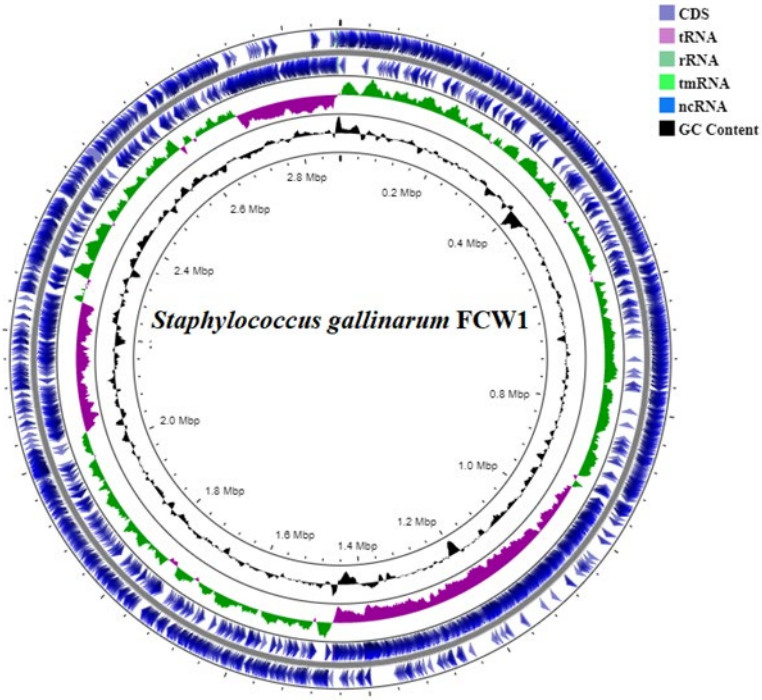


Fig. 7. Whole genome sequencing of *S. gallinarum* FCW1. The circular graphical representation refers to the genome annotations of FCW1 strain. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, and GC content. The circular genome was generated using CG server.

Table 5. Genomic Statistics of FCW1 genome

Species Attribute	
Genome size (bp)	2,880,305

G + C %	33.23%
Contigs	1
Scaffolds	1
Total genes	2770
Protein coding genes	2708
RNA genes	62
Pseudo genes	11
CRISPR repeats	1

476

477 **3.4.2. Functional classification**

478 The protein-coding genes involved in the major metabolic pathways were assigned to COG
479 categories (Wu et al., 2011) (Fig. 8a). In the FCW1 genome, major COGs were assigned into the
480 following categories i) amino acid transport and metabolism (5.23%), ii) energy production and
481 conversion (5%), iii) translation, ribosomal structure and biogenesis (4.75%), iv) replication,
482 recombination and repair (4.61%), v) transcription (4.47 %), vi) carbohydrate transport and
483 metabolism (4.45%), vii) Inorganic ion transport and metabolism (4.11%), viii) posttranslational
484 modification, protein turnover, chaperones (3,93%), ix) cell wall/membrane/envelope biogenesis
485 (3.64%), x) coenzyme transport and metabolism (3.47%). The summarized COG classification is
486 included in Supplementary Table 1. Based on the KEGG annotation results, a total of 1684
487 protein families were mapped in the KEGG database. This result reveals a higher number of
488 protein families classified into carbohydrate metabolism, genetic information processing,
489 signaling and cellular processing, environmental information processing, metabolism of
490 cofactors and vitamins and amino acid metabolism (Fig. 8b).

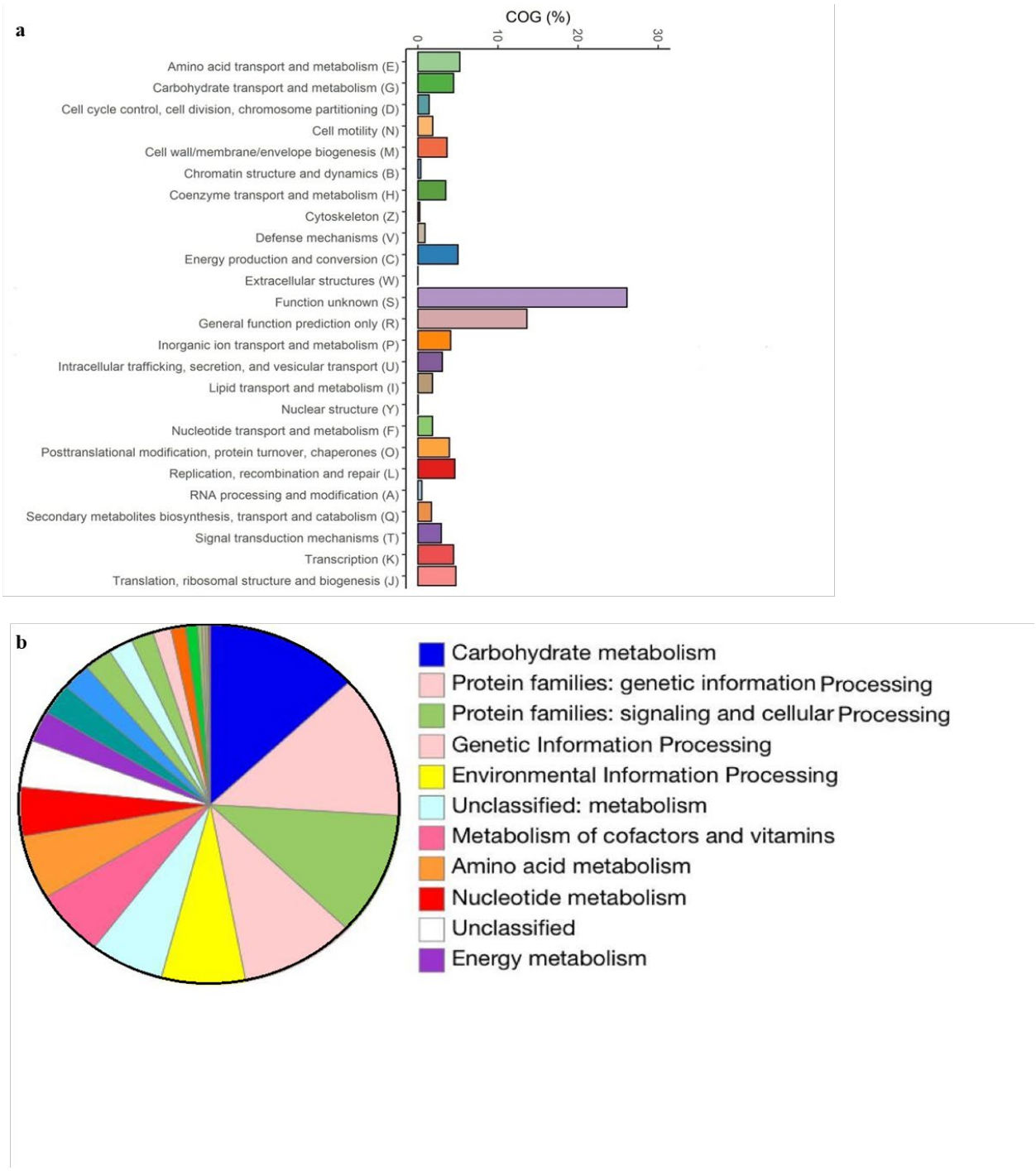


Fig. 8. a) Number of genes associated with general COG functional categories b) Analysis of KEGG distribution in *S. gallinarum* FCW1 using Blast KOALA algorithm.

3.4.3. Genome analysis for probiotic traits

We performed comprehensive genomic data analysis to evaluate stains FCW1's probiotic potency. The relevant genes, their function and locus tag in the genome are listed in Supplementary Table 2.

3.4.3.1. Genes encoding surface proteins

Genome analysis of FCW1 reveals the presence of different surface protein-encoding genes. For example, gene encoding sortase-dependent surface proteins (*srtA*) has been identified at Locus tag SG_01663. The adhesion gene (*fbp*), which encodes fructose-1,6-bisphosphatase class 3, has also been found at locus tag SG_01689. The *pdhABCD* genes (SG_00617 to SG_00620) encoding pyruvate dehydrogenase components are also responsible for fibronectin binding (Vastano et al., 2014). In addition, the analysis of the FCW1 genome reveals the presence of *gndA* gene encoding 6-phosphogluconate dehydrogenase, decarboxylating proteins (Locus tag SG_01015) and lactate dehydrogenase (*ldh1* and *ldhD*; Locus tag: SG_01280 and SG_01600) are responsible for the bacterial attachment to epithelial mucin (Qureshi et al., 2020).

3.4.3.2. Mucin secreting genes

The *adh* genes clusters at locus tag: SG_02307, SG_02050, and SG_00095, encode alcohol dehydrogenase, which is involved in the adhesion and mucin secretion. The genes responsible for the phosphotransferase (PTS) system and ABC transporters (SG_02068; SG_02485) will get induced in the presence of mucin and help the bacteria to colonize the human gastrointestinal tract.

3.4.3.3. Stress related genes

The probiotic bacteria have to face a harsh environment in the stomach and intestine. The presence of $Na(^{+})/H(^{+})$ antiporter subunit ABCDEF, which is encoded by *nhaC* gene (SG_01628) and *mrpABCDEF* genes (SG_00161 to SG_00166 and SG_00482 to SG_00488) play a major role in Na^{+}-resistance, pH homeostasis, and osmoregulation. The genes *nhaP2* and *nhaK* (SG_01688 and SG_00169) encoding Na^{+}, K^{+}, Li^{+}, Rb^{+} /H^{+} antiporters are also present, which help to survive in acidic conditions (Fujisawa et al., 2005). Heat shock proteins such as *dnaK*, *groS*, *groL*, and *grpE* are present at locus tag SG_01085, SG_02502, SG_02503 and SG_01086. Genes encoding ATP-dependent *clp* protease (*clpBCPX*; Locus tag: SG_00513; SG_00008; SG_00378; SG_01193) are also expressed by acid and bile stress and refold or

degrade the denatured proteins (Ferreira et al., 2013). The main function of *yveA* gene encoding asparate proton symporter (SG_01520) and *bsaA* gene encoding glutathione peroxidase (SG_00822) is to protect the bacterium from the acid stress response. Genes encoding DNA repair proteins such as *uvrABC* system proteins (Locus tag: SG_00297; SG_00296; SG_00665), ATP-dependent helicase/nuclease subunit (*addAB* genes at Locus tag: SG_00504, SG_00505), ATP-dependent DNA helicase (*recDGQ* and *pcrA* at Locus tag: SG_00739, SG_01120, SG_00257, SG_02538), DEAD-box ATP-dependent RNA helicase (*cshAB*at SG_00994; SG_01063), replicative DNA helicase (*dnaC* at Locus tag: SG_02179) are present which upregulated under acid stress and involved in DNA repair mechanisms (Jin et al., 2012; Petit et al., 1998; Lehnik-Habrink et al., 2013). The gene *luxS* encoding S-ribosylhomocysteine lyase located at Locus tag: SG_02650 contributes acid stress resistance through quorum sensing. The presence of *fab* genes (*fab BDFHIGZ*) responsible for fatty acids and cell envelop biosynthesis will have a specific role in acid stress tolerance. The *bsh* genes (*bshABC*) at locus tag SG_00973, SG_00107 and SG_00686 encode the enzyme bile salt hydrolase, which deconjugates bile salts like glycine and taurine. Another gene involved in the deconjugation of bile salt is *betA* gene encoding oxygen-dependent choline dehydrogenase is present at SG_01510.

3.4.3.4. Genes involved in Exopolysaccharide synthesis

The *glm* genes involved in the biosynthesis of UDP-GlcNAc, the building blocks of peptidoglycan, glucosamine-6-phosphate synthase (*glmS*), phosphoglucosamine mutase (*glmM*), and *N*-acetylglucosamine-1-phosphate uridyltransferase (*glmU*) are present at Locus tag: SG_02672, SG_02677 and SG_02714. The *dapA* genes encoding 4-hydroxy-tetrahydrodipicolinate synthase present at Locus tag SG_00908, SG_00073, and SG_02241 are also involved in peptidoglycan biosynthesis. Acetyl-CoA carboxylase genes (SG_01216, SG_01032, SG_01108, SG_01107, SG_01217) encode biotin carboxyl carrier proteins which participate in lipid metabolism and fatty acids biosynthesis. The *eps* genes encode putative regulatory proteins such as putative glycosyl transferase *epsD* (SG_00046), putative sugar transferase *epsL* (SG_00047), putative acetyl transferase *epsM*(SG_00048), and putative pyridoxal phosphate-dependent amino transferase *epsN* (SG_00049) are involved in the biosynthesis of exopolysaccharide and lipoteichoic acids. The gene *ftsW* is the SEDS-family protein putative peptidoglycan glycosyltransferase located at SG_00638 and SG_02369 are also

involved in cell wall or peptidoglycan biosynthesis. The *dlt* genes (*dltACD*) encode D-alanine-D-alanyl carrier protein ligase at SG_00468, SG_00470, and SG_00471, involved in the lipoteichoic acid biosynthetic process.

3.4.3.5. Genes involved in the production of nutrients and other beneficial processes

The presence of genes involved in the production of vitamins, Biotin, and other cofactors synthesis evince the ability of probiotics to produce bioactive compounds. The vitamin B12 import ATP-binding protein encoding gene *btuDF* are present at locus tag: SG_00258, SG_00928, SG_01061, SG_02367, SG_02511, SG_02513 and SG_00148, which aids in the *In situ* production of important nutrients. The genes (*bioBDY*) responsible for Biotin synthase (SG_01536, SG_02354) and Biotin transporter (SG_01901) play a major role in biotin and cofactor biosynthesis. The gene responsible for molybdenum cofactor biosynthesis such as *moeA* encoding molybdopterin molybdenum transferase (SG_01915), *moaA* encoding GTP 3',8-cyclase (SG_01920), *moaB* encoding molybdenum cofactor biosynthesis protein B (SG_01913), *moaC* encoding cyclicpyranopterin monophosphate synthase (SG_01914), *moaD* encoding molybdopterin synthase sulfur carrier subunit (SG_01918), *moaE* encoding molybdopterin synthase catalytic subunit (SG_01917) and *mobA* gene for putative molybdenum cofactor guanylyl transferase (SG_01919). The catabolite control protein A gene (*ccpA*) is present at locus tag SG_00866, SG_01254, which plays a major role in cholesterol reduction. The gene *ccpN* is a transcriptional repressor (SG_01069) that controls the carbon catabolite repression.

3.4.3.6. Disease-specific genes

Our bacterial genome contained a unique *frc* gene encoding Formyl-CoA: oxalate CoA-transferase protein at Locus tag SG_02283. This gene has been associated with oxalate metabolism. Additionally, an ammonium transporter gene (*nrgA*) is present at SG_02455, which will facilitate passive ammonium uptake in low pH environments. This genome also contains genes involved in sulfate reduction, such as sulfate adenylyl transferase encoding gene (*sat*) at locus tag SG_02342, and genes for phosphoadenosine phosphosulfate reductase (*cysC*, *cysH*) at locus tags SG_02343 and SG_02335. Phosphotransacetylase (*pta_1*, *pta_2*) and acetate kinases (*ack*) involved in acetate metabolism are also found at locus tags SG_00128, SG_01023, and SG_01229, respectively.

3.4.4. Secondary metabolite identification

According to the antiSMASH tool, seven metabolites' regions were detected by FCW1 secondary metabolites (Table 6). These include a biosynthetic siderophore cluster with high overall similarity to staphyloferrin A and two T_3PKS (Type III polyketide synthases) and NRPS (Non-ribosomal peptide synthetases) clusters resembling capsular polysaccharide and rhizocticin A, respectively.

Table 6. Genes responsible for Secondary metabolites by AntiSMASH tool

Region	Type	Location	Metabolites	Gene cluster	Similarity %	Function
Region 1	Terpene	902,072-921,889	-	-	-	-
Region 2	Terpene	1,461,570-1,481,615	-	-	-	-
Region 3	T_3PKS	1,624,274-1,665,443	Capsular polysaccharide	Exopolysaccharide	3%	Antimicrobial activity
Region 4	Siderophore	2,012,549-2,027,535	Staphyloferrin A	Other:Non-NRP siderophore	100%	Antimicrobial activity
Region 5	Terpene	2,201,938-2,222,819	-	-	-	-
Region 6	NRPS	2,451,92-2,508,725	Rhizocticin A	Other	6%	Antifungal activity
Region 7	Cyclic-lactone-autoinducer	2,600,285-2,620,994	-	-	-	-

"-" indicates not available.

3.4.5. Antibiotic-resistant genes

The PATRIC's AMR classifier module revealed that *S. gallinarum* FCW1 contained 46 resistant genes categorized into different strategies such as Antibiotic target in susceptible species, Antibiotic target modifying enzymes, Antibiotic resistance gene cluster, cassette, or operon, Efflux pump conferring antibiotic resistance, Protein altering cell wall charge conferring antibiotic resistance, Regulator modulating expression of antibiotic resistance genes (Table 7). The genome contained *blaZ* gene responsible for penicillin which supports our *in vitro* antibiotic

susceptibility results. The list of genes responsible for antibiotic resistance based on PATRIC annotation is provided in the supplementary Table 3.

Table 7. Antibiotic-Resistant genes present in FCW1 genome based on PATRIC genome annotation

AMR Mechanisms	Genes
Antibiotic target in susceptible species	*alr, ddl, EF-G, EF-Tu, folA, dfr, folP, gyrA, gyrB, inhA, fabI, Iso-tRNA, kasA, murA, rho, rpoB, rpoC, S10p, S12p*
Antibiotic target modifying enzymes	*blaZ*
Antibiotic resistance gene cluster, cassette,or operon	*tcaB, tcaB2, tcaR*
Efflux pump conferring antibiotic resistance	*norA, ykkCD*
Gene conferring resistance via absence	*gidB*
Protein altering cell wall charge conferring antibiotic resistance	*gdpD, mprF, pgsA*
Regulator modulating expression of antibiotic resistance genes	*bceR, bceS, liaF, liaR, liaS*

4. Discussion

Fermented food products possess many health benefits, including antioxidant, anti-microbial, anti-inflammatory, anti-diabetic, and anti-cancer properties. They are the large reservoir of beneficial microorganisms or starter cultures which enhance the sensory properties and safety of fermented foods by accelerating the acidification process of their matrix and producing secondary metabolites. Food-derived CNS species are often used as starter cultures since they are non-pathogenic and are native to fermented foods. CNS *S. gallinarum* is associated with Japanese fermented foods, like miso (Onda et al., 2003), and African alkaline fermented foods, like maari, dawadawa and soydawadawa (Parkouda et al., 2009). Considering the role of *S. gallinarum* in food fermentation, this study aimed to evaluate its safety and probiotic capabilities. In recent studies, CNS isolated from various fermented foods exhibited probiotic

614 characteristics (Khusro et al., 2017; Sung et al., 2010; Mangrolia et al., 2020). However, only
615 limited information available on the safety of *S. gallinarum* and its probiotic properties.

616 In the current study, of the 7 bacterial isolates recovered from naturally fermented coconut water,
617 only one was confirmed as $GCC^{+}CNS$ bacteria and was processed further. The isolate was
618 susceptible to most of the antibiotics tested except penicillin, a crucial characteristic for probiotic
619 selection. In the antagonistic study, the isolate showed activity against *E. coli*, *K. pneumoniae*,
620 and *B. cereus*. This is due to the organic acids or secondary metabolites produced by the isolate,
621 which suppress the pathogen growth (Kosin and Rakshit, 2006). Researchers have previously
622 reported CNS's antagonistic role against *Mycobacterium tuberculosis* (Khusro et al., 2017), *S.*
623 *aureus* (Sung et al., 2010), *E. coli* (Mangrolia et al., 2020), *Salmonella* spp. (Sathyabama et al.,
624 2012). In addition, antioxidant properties were used to confirm the probiotic's potential and
625 safety. The antioxidants protect cells from oxidative damage. FCW1 showed antioxidant activity
626 in a concentration-dependent manner, which is in par with previous study by Khusra et al.
627 (2017). Although hemolysins and DNase are major virulence factors, many other factors may
628 contribute to the virulence of a strain (Yasmin et al., 2020; Somashekaraiah et al. 2019). The
629 isolate FCW1 was found to be non-hemolytic and DNase negative, which indicates its non-
630 pathogenic nature. Our findings were consistent with those of Somashekaraiah et al. (2019), who
631 found that LAB isolated from naturally fermented coconut palm nectar lacked hemolytic and
632 DNase activity. Um et al. (1996) also reported similar observations with *Staphylococcus* spp.
633 isolated from fermented fish.

634 Probiotics ought to survive the harsh digestive environment for at least 3-5 h before reaching the
635 colon, where they colonize and confer benefits. The high gastric pH, bile salt, and lysozyme pose
636 extreme hindrances for probiotic microorganisms. In the present study, FCW1 showed a higher
637 survival rate (99.65%) in gastric acidity (pH 2) even after 3 h of incubation. According to Borah
638 et al. (2016), *Staphylococcus* spp. can survive up to pH 2, which supports our findings. The next
639 obstacle for probiotics is bile salt tolerance, which is secreted by cholesterol catabolism.
640 Probiotics must resist the high concentration of bile (0.3%) in the small intestine for at least 4 h.
641 Despite being resistant to 0.3% bile salt, FCW1 grew well in the presence of bile salt. In a study,
642 the researchers found that *Staphylococcus* spp. isolated from Slovak meat products, could
643 tolerate 1% bile salt. (Simonova et al., 2006). In another study, Khusro et al. (2017) also

644 confirmed that six CNS from koozh could survive 0.5% bile salt. These reports support our
645 findings that bile salt boosted FCW1's survivability (101.15%). Another critical property of
646 probiotics is their ability to tolerate gastric and intestinal conditions, such as the presence of
647 lysozyme, acid pH, pepsin, trypsin, and bile salt. We found that isolate FCW1 was remarkably
648 resistant to pepsin and trypsin in gastric and intestinal fluids. As well, FCW1 showed a high level
649 of resistance to lysozyme, indicating that it can tolerate lysozyme in saliva. Previously, Borah et
650 al. (2016) and Kushra et al. (2017) reported similar results for *Staphylococcus* spp. in the
651 presence of lysozyme. Phenol is a toxic metabolic byproduct of the deamination of aromatic
652 amino acids by gut bacteria during digestion. At a low phenol concentration (0.1%), the isolate
653 FCW1 remained viable even after 24 h of incubation, and the growth inhibition rate was 28.23%,
654 while 0.4% and 0.6% of phenol caused a higher inhibition rate, 77.58% and 89.21%,
655 respectively. A similar result was reported by Parlindungan et al. (2021), the
656 strains *Lactobacillus plantarum*, *Pediococcusacidilactici* and *Lactobacillus coryniformis*
657 exhibited a greater growth rate at 0.2% phenol and decreased at 0.5% phenol. They also found
658 that some strains, such as *Lactobacillus curvatus* and *Lactobacillus sakei* exhibited a low growth
659 rate even in the presence of 0.2% phenol. We found that FCW1 displayed a high tolerance level
660 to 3% and 6% NaCl concentrations, with a growth inhibition rate of 21.99% and 26.97%. In
661 addition, the strain showed considerable growth at 4°C, 15°C and 25°C, with 37°C being the
662 optimum temperature. These findings coincide with a previous report by Qureshi et al. (2020).
663 Probiotics should possess hydrophobicity and auto aggregation properties, which aid in adhesion
664 and biofilm formation, thereby hindering enteropathogens (Ku et al., 2016). FCW1 exhibits high
665 hydrophobicity to chloroform (87.31%) and hexane (57.62%) and moderate hydrophobicity to
666 ethyl acetate (32.16%), indicating its ability to adhere to epithelial surfaces. The auto aggregation
667 rate of FCW1 was 95.04 ± 1.6% after 24 h incubation, and biofilm formation ability was
668 moderate, indicative of potential probiotic activity.

669 An *in-vivo* testing of strain FCW1 on zebrafish was conducted for safety evaluation and no
670 mortality or disease symptoms were observed during the 10 days study period, confirming the
671 non-pathogenic nature of the strain.

672 Genomics provides in-depth knowledge of bacteria's physiology, metabolism, functions, and
673 ability to adapt to varying environmental conditions. Genome size and GC content may reflect

the bacteria's lifestyle and preferred environment (Merino et al., 2019). The isolate FCW1 has a genome size with 2,880,305 bp and a GC content of 33.23%. However, the absence of plasmids in FCW1 indicates it has a stable genome (Wang et al., 2021). FCW1 contains many cell surface proteins, including *srtA* and *fbp* genes, as well as genes encoding pyruvate dehydrogenase and lactate dehydrogenase, which contribute to adhesion, colonization, and biofilm formation. The sortase (*srtA*) cleaves the cell wall sorting signal molecule (LPXTG motif) between threonine and glycine and then covalently adhered to the cell wall peptidoglycan (Muñoz-Provencio et al., 2012). The *fbp* gene encoding cell surface protein binds to fibronectin, a glycoprotein of the extracellular matrix of epithelial cells (Azcarate-Peril et al., 2008). The biofilm protects bacteria from the host immune system and antagonistic factors of enteropathogens (Toropov et al., 2020). Bacteria should be tolerated in the harsh environment of the stomach. In order to resist such stress conditions, the bacteria arise suitable responses in the expression of genes and protein activity according to the environmental changes. Several bile, acid, and other stress-resistant genes are identified in the FCW1 genome, including *nhap2*, *nhaK*, *clp*, *yveA*, *addAB*, *recDGQ*, and *pcrA*, *cshAB*, and *dnaC*. In addition to the presence of bile salt hydrolase genes (*bshA*, *bshB*, *bshC*), FCW1 is theoretically resistant to bile salts. The findings are consistent with our *in vitro* results, which confirm that the bacteria are resistant to high salt, acid, and bile salt conditions.

Furthermore, the presence of chaperones and proteases, such as *dnaK*, *groS*, *groL*, *grpE*, and *clp* proteins, enables FCW1 to withstand acid stress for an extended time. These molecular chaperone proteins get induced under acid stress, tolerate heat and osmotic shock, and repair damaged proteins (Prasad et al., 2003; Hamon et al., 2014). Furthermore, Arena et al. (2019) and Skinner et al. (2001) also reported upregulation of these chaperones and proteases in response to heat and cold shock. The gene *luxS* encoding quorum sensing will also get expressed under oxidative stress and acidic conditions and enhanced quorum sensing to tolerate the stress conditions. Also play an important role in the induction of anti-inflammatory cytokines adhesion, bacterial growth and biofilm formation (Koponen et al., 2012). The presence of *glm* and *dapA* genes for exopolysaccharide synthesis protects bacteria from environmental damage by producing a polysaccharide capsule. In addition, these exopolysaccharides stimulate the host's immune system, are involved in cell adhesion and biofilm formation, act as antioxidants and anti-inflammatory agents, promote auto aggregation and enhance the sensory properties of fermented foods (Saadat et al., 2019; Stergiou et al., 2021). The PTS system is a distinctive

method used by bacteria to acquire sugar, which uses phosphoenol pyruvate as an energy source and phosphoryl donor. The PTS system for maltose/fructose/glucose was found in the FCW1 strain, which is involved in sugar uptake. Besides regulating carbohydrate metabolism, PTS also governs colonization, biofilm formation, stress response, chemotaxis, and virulence (Wanna et al., 2021). The strains also contain genes that regulate the synthesis of vitamin B12, biotin, and other beneficial nutrients.

Antibiotic-resistant genes associated with fluoroquinolone resistance (*gyrA, gyrB*) and trimethoprim resistance (*dfrc*) were identified in the PATRIC annotation. Due to the absence of plasmids in the FCW1 genome, plasmid-mediated horizontal gene transfer may not be possible. Also an *in vitro* antibiotic susceptibility test revealed that the strain was susceptible to ciprofloxacin (fluoroquinolone) and trimethoprim. A preliminary antibiotic sensitivity assay showed that the strain is resistant to penicillin and the *blaz* gene responsible for penicillin resistance is also detected in the genome. In addition to genome screening, *in vitro* antibiotic tests, hemolytic assays, and DNase tests, the strain was confirmed to be safe.

An *in vitro* anti-microbial assay has shown that FCW1 can inhibit some pathogens. The FCW1 genome analysis by antiSMASH software showed that it produces Staphyloferrin A, an iron-chelating siderophore that suppresses the growth of pathogenic bacteria through iron chelation, as iron is essential in virulence and bacterial interactions. Siderophore-producing non-pathogenic *Staphylococcus* strains are generally considered as safe (GRAS), are promising candidates for probiotics that fight pathogens with low iron uptake capabilities, and are often used as starter cultures for fermented meat (Raaska and MattilaSandholm, 1995). Moreover, the KEGG and COG annotation results showed that our strain possesses many beneficial characteristics.

The *frc* gene-bearing bacterial pool is also identified as Oxalate Metabolizing Bacterial Species (OMBS), which prevents oxalate toxicity in the gastrointestinal tract (GIT) by converting it to formic acid and CO_2. It is also possible for bacteria like acetogenic, methanogenic, and sulfate-reducing bacteria (AMS) to use formic acid and CO_2 as carbon and energy sources. As FCW1 contains sulfate-reducing genes *sat*, *cysC*, and *cysH*, as well as acetate-metabolizing genes such as *ppa* and *ack*, the bacterium can reduce oxalate toxicity by utilizing formic acid. FCW1 genome also contains ammonium transporter genes (*nrgA*), which enhance passive ammonium

uptake as an energy source. It has been hypothesized that bacteria may oxidize ammonium in struvite stones, releasing hydrogen ions that enhance the dissolution process. Based on the presence of these genes in FCW1, this strain might be beneficial in treating and preventing kidney stone disease through its role as OMBS, AMS and Ammonia oxidizing bacteria (AOB).

5. Conclusion

This study has highlighted the probiotic strain of *S. gallinarum* FCW1, which is convincing by the presence of crucial probiotic genes as annotated on the draft genome sequence. The strain FCW1 meets the probiotic selection criteria, including antibiotic susceptibility, survivability to GI conditions, and non-hemolytic properties. In addition, we found the frc genes responsible for oxalate degradation, sulfate-reducing genes, acetate-metabolizing genes and ammonium transporter genes in *S. gallinarum* FCW1, which adds to the idea that this strain can contribute to treating kidney stones. Taking into account *in vitro* probiotic capabilities, *in vivo* bio safety and probiotic-associated genes, the strain FCW1 offers a variety of potential applications in the medical and nutritional industries. In this context, in future studies, the FCW1 strain of *S. gallinarum* will be studied for its potential as a starter culture for developing coconut water beverages since it was isolated from fermented coconut water. Further investigation of strain FCW1's role in preventing and treating kidney stones will be conducted.

Ethics Statement

Animal experiment was conducted in accordance with the Institutional Animal Ethics Committee (IAEC) guidelines of Bharathidasan University. The study was approved by IAEC, Bharathidasan University (Ref No: BDU/IAEC/P11/2021, dated 04.09.2021).

Authors Contributions

Conceptualization - DRCT, RAJ; Formal analysis - DRCT; Investigation and Methodology - DRCT; Resources - RAJ, MVS; Software; MVS, DRCT; Supervision; RAJ; Roles/Writing - original draft - DRCT, KPR; Writing - review & editing- SK, KPR, RRS, RAJ.

Declaration of Competing Interest

761 The authors declare that they have no competing interests or personal relationships that could
762 have appeared to influence the work reported in this paper.

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Supplementary Materials

Supplementary Table 1: Number of genes associated with general COG functional categories in FCW1

Class	No_families	Coverage	Abundance	Description
J	245	0.542857	0.068502	Translation, ribosomal structure and biogenesis
A	25	0	0	RNA processing and modification

K	231	0.341991	0.084429	Transcription
L	238	0.352941	0.046236	Replication, recombination and repair
B	19	0.052631	0.000444	Chromatin structure and dynamics
D	72	0.194444	0.00815	Cell cycle control, cell division, chromosome partitioning
Y	2	0	0	Nuclear structure
V	46	0.347826	0.012928	Defense mechanisms
T	152	0.276315	0.031664	Signal transduction mechanisms
M	188	0.393617	0.053083	Cell wall/membrane/envelope biogenesis
N	96	0.052083	0.001684	Cell motility
Z	12	0	0	Cytoskeleton
W	1	0	0	Extracellular structures
U	158	0.132911	0.010017	Intracellular trafficking, secretion, and vesicular transport
O	203	0.270935	0.030244	Posttranslational modification, protein turnover, chaperones
C	258	0.317829	0.065648	Energy production and conversion
G	230	0.465217	0.105124	Carbohydrate transport and metabolism
E	270	0.585185	0.114633	Amino acid transport and metabolism
F	95	0.663157	0.034228	Nucleotide transport and metabolism
H	179	0.502793	0.052684	Coenzyme transport and metabolism
I	94	0.478723	0.035889	Lipid transport and metabolism
P	212	0.415094	0.067791	Inorganic ion transport and metabolism
Q	88	0.272727	0.02196	Secondary metabolites biosynthesis, transport and catabolism
R	702	0.282051	0.146984	General function prediction only
S	1347	0.146993	0.103742	Function unknown

Supplementary Table 2: Genes responsible for probiotic properties

S No	Category	Gene	Product	Locus Tag	Function
1	**Cell Surface Proteins**	*srtA*	Sortase A	SG_01663	Cleaves the signal molecule between threonine and glycine and then attaches the covalent residue to peptidoglycan
		gnd	6-phosphogluconate dehydrogenase, decarboxylating	SG_01015	Promote bacterial adhesion to mucin and epithelial cells
		ldh1	L-lactate dehydrogenase 1	SG_01280	Promote bacterial adhesion to mucin and epithelial cells
		ldhD	D-lactate dehydrogenase	SG_01600	Cleaves the signal molecule between threonine and glycine and then attaches the covalent residue to peptidoglycan
		fbp	Fructose-1,6-bisphosphatase class 3	SG_01689	Fibronectin binding protein
		lspE	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	SG_02718	Large surface protein, Putative mucus-binding
2	**Active removal of stressors**	*copA*	Copper-exporting P-type ATPase	SG_01602	
		copZ	Copper chaperone CopZ	SG_01601	
		bshA; bshB2 ; bshC	putative N-acetyl-alpha-D-glucosaminyl L-malate deacetylase	SG_00107; SG_00686	Deconjugation of bile salts
3	**Stress related genes**	*dnaK*	Chaperone protein	SG_01085	Tolerate heat and osmotic shock, Repair of damaged proteins
		groS	10 kDa chaperonin	SG_02502	Repair of damaged proteins
		groL	60 kDa chaperonin	SG_02503	Repair of damaged

					proteins
		grpE	Protein	SG_01086	Repair of damaged proteins
		clpB; clpC; clpP; clpX	ATP-dependent Clp protease ATP-binding subunit	SG_00513; SG_00008; SG_00378; SG_01193	Refold or degrade the denatured proteins
		gltT_1 ,2; gltP	Proton/sodium-glutamate symport protein	SG_01792; SG_02213 SG_01418	Help the bacterium to survive in acidic environment of gastrointestinal tract
		*acp_1 ; acp_*	Sodium/proton-dependent alanine carrier protein	SG_02247; SG_00540	"
		yveA	Asparate proton symporter	SG_01520	Protect bacteria from acid stress
		bsaA	Glutathione peroxidase	SG_00822	Protect bacterium from Acid Stress response
4	**Cell Envelope and Lipoteichoic acids**	*dltA; dltC; dltD*	D-alanine--D-alanyl carrier protein ligase	SG_00468; SG_00470; SG_00471	d-Alanylation of LTA
		glmU	Bifunctional protein	SG_02714	Peptidoglycan biosynthesis
		epsM	Putative acetyltransferase	SG_00048	Cell wall biogenesis/ degradation, Teichoic acid biosynthesis
		mgtE	Monofunctional glycosyltransferase	SG_00536	Cell wall biogenesis/ degradation, Teichoic acid biosynthesis
		rodA	Peptidoglycan glycosyltransferase	SG_02600	Cell wall biogenesis/ degradation, Teichoic acid biosynthesis
		crtQ	4,4'-diaponeurosporenoate glycosyltransferase	SG_02154	Cell wall biogenesis/ degradation, Teichoic acid biosynthesis
		ftsW	putative peptidoglycan glycosyltransferase	SG_02369	Cell wall biogenesis/ degradation, Teichoic acid biosynthesis
5	**Protection**	*uvrA;*	UvrABC system	SG_00297;	DNA repair

	and repairDNA and proteins	*uvrB; uvrC*	protein	SG_00296; SG_00665	
		dps	Protein	SG_02656	DNA protection during starvation
		msrB	Peptide methionine sulfoxide reductase	SG_00945	DNA repair
		addA ;addB	ATP-dependent helicase/nuclease subunit	SG_00505; SG_00504	DNA repair
		recG	ATP-dependent DNA helicase	SG_00739	DNA repair
		cshA_ 1; cshA_ 2; cshB	DEAD-box ATP-dependent RNA helicase	SG_00994; SG_01063	DNA repair
		recD2	ATP-dependent RecD-like DNA helicase	SG_01120	DNA repair
		ruvA ; ruvB_ 1; ruvB_ 2	Holliday junction ATP-dependent DNA helicase RuvB	SG_01143; SG_01142	DNA repair
		dnaC	Replicative DNA helicase	SG_02179	DNA repair
		pcrA	ATP-dependent DNA helicase PcrA	SG_02538	DNA repair
		recQ	ATP-dependent DNA helicase RecQ	SG_00257	DNA repair
6	**Quorum sensing and Antipathogenic effects**	*luxS*	S-ribosylhomocysteine lyase	SG_02650	Induction of anti-inflammatory cytokines, Adhesion and competitive exclusion of pathogens; Direct role in the production of AI-2; indirect in the production of AI-3-like agonist molecules
7	**Immunomodulation**	*clpB*	Chaperone protein	SG_00513	Potential immunogenic proteins

		serA	D-3-phosphoglycerate dehydrogenase	SG_01243	Inhibition of elastases
		serS	Serine--tRNA ligase	SG_02186	Inhibition of elastases
8	**Degradation of Mucin**	*adh*	Alcohol dehydrogenase	SG_02050	Adhesion and stimulation of mucin secretion
9	**Protein metabolism**	*metK*	S-adenosylmethionine synthase	SG_01300	Putative vitamin B12 - independent methionine synthase
10	**Production of nutrients and other beneficial process**	*btuD_1 to 6 &btuF*	Vitamin B12 import ATP-binding protein	SG_00928; SG_01061	In situ production of important nutrients
		ytrE, yheS, ylmA	Putative ABC transporter permease and ABC transporter ATP-binding protein	SG_02068; SG_02485	High production of acetate and protection from enteropathogenic infection
		accB_1, accC	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	SG_01032; SG_01107	Fatty acid biosynthesis and lipid metabolism
		bioB, bioD	Biotin synthase	SG_01536	Biotin and cofactor biosynthesis
		bioY	Biotin transporter	SG_01901	interacts with the energy-coupling factor (ECF) ABC-transporter complex
		moaA to E	Molybdenumcofactorbiosynthesisprotein B	SG_01920; SG_01913; SG_01914; SG_01918; SG_01917	Biosynthesis of molybdopterin
		mobA	putative molybdenum cofactor guanylyltransferase	SG_01919	Mo-molybdopterin co-factor biosynthesis
		ccpA_1	Catabolite control protein A	SG_00866	Influencing blood cholesterol
		fosB	Metallothiol transferase	SG_01676	Processing of health-promoting fructoligosaccharides

12	**Carbohydrate metabolism**	*malP; mtlA; fruA; ptsG*	PTS system for (maltose/fructose/ mannitol/glucose)	SG_01860; SG_02673; SG_00237; SG_01529	Sugar uptake
		gtfA	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyl transferase	SG_01821; SG_02748	Glucosyltransferase
		treP; treA; treR	PTS system trehalose-specific EIIBC component	SG_02462; SG_02463; SG_02464	Trehalose

Supplementary Table 3: AMR genes of *S. gallinarum*FCW1

Source	Gene	Product	Identity	E-value
PATRIC	*S12p*	SSU ribosomal protein *S12p* (S23e)	-	-
	rho	Transcription termination factor *Rho*	-	-
	bceS	Two-component sensor histidine kinase *BceS*	-	-
	kasA	3-oxoacyl-[acyl-carrier-protein] synthase, *KASII* (EC 2.3.1.179)	-	-
	liaR	Cell envelope stress response system *LiaFSR*, response regulator *LiaR*(*VraR*)	-	-
	inhA, fabI	Enoyl-[acyl-carrier-protein] reductase [NADH] (EC 1.3.1.9)	-	-
	tcaB2	Teicoplanin resistance transporter, *TcaB* family => TcaB2	-	-
	gdpD	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)	-	-
	tcaR	Teicoplanin-resistance associated HTH-type transcriptional regulator *TcaR*	-	-
	S10p	SSU ribosomal protein *S10p* (S20e)	-	-
	alr	Alanine racemase (EC 5.1.1.1)	-	-
	tcaB	Teicoplanin resistance transporter, *TcaB* family =>*TcaB*	-	-
	bceR	Two-component response regulator *BceR*	-	-
	norA	MFS-type transporter quinolone resistance protein *NorA*	-	-
	gyrB	DNA gyrase subunit B (EC 5.99.1.3)	-	-
	liaF	Membrane protein *LiaF*(*VraT*), specific inhibitor of *LiaRS*(*VraRS*) signaling	-	-

		pathway		
	EF-Tu	Translation elongation factor Tu	-	-
	mprF	L-O-lysylphosphatidylglycerol synthase (EC 2.3.2.3)	-	-
	bla	Class A beta-lactamase (EC 3.5.2.6)	-	-
	ddl	D-alanine--D-alanine ligase (EC 6.3.2.4)	-	-
	liaS	Cell envelope stress response system *LiaFSR*, sensor histidine kinase *LiaS*(*VraS*)	-	-
	murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase (EC 2.5.1.7)	-	-
	ykkCD	Broad-specificity multidrug efflux pump *YkkC*	-	-
	folP	Dihydropteroate synthase (EC 2.5.1.15)	-	-
	Iso-tRNA	Isoleucyl-tRNA synthetase (EC 6.1.1.5)	-	-
	gidB	16S rRNA (guanine(527)-N(7))-methyltransferase (EC 2.1.1.170)	-	-
	EF-G	Translation elongation factor G	-	-
NDARO	*dfrc*	Dihydrofolate reductase (EC 1.5.1.3)	82	7e-74
CARD	*sav1866*	Efflux ABC transporter, permease/ATP-binding protein *YgaD*	84	1e-283
	pgsA	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase (EC 2.7.8.5)	87	6e-89
	rpoB	DNA-directed RNA polymerase beta subunit (EC 2.7.7.6)	94	0.0
	arlR	Putative response regulator *ArlR*	81	2e-100
	pare	DNA topoisomerase IV subunit B (EC 5.99.1.3)	90	0.0
	tuf	Translation elongation factor Tu	87	1e-180
	mgrA	Transcriptional regulator *MgrA* (Regulator of autolytic activity)	85	4e-63
	rpoC	DNA-directed RNA polymerase beta' subunit (EC 2.7.7.6)	94	0.0
	gyrA	DNA gyrase subunit A (EC 5.99.1.3)	89	0.0

1026 "-" indicates not available

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