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

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35 **Abstract**

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

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

54

55 ## 1. Introduction

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

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

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

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

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

103 **2. Materials and Methods**

104 **2.1. Sample collection and processing**

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

111 2.2. **Isolation of GCC^{+}CNS strains**

112 Serial dilutions (10^{-1} to 10^{-9}) of 1 ml naturally fermented CW sample were prepared in distilled
113 water (DW) and then 100µl sample from different dilutions was spread plated over plates of
114 nutrient agar (NA) enriched with CW (CW and DW in 1:1 ratio) in triplicate and incubated at
115 37°C for 24-48 h. Gram-staining, catalase and coagulase tests were performed with
116 morphologically discrete colonies. Only Gram-positive, catalase-positive and coagulase-negative
117 colonies were propagated twice and restreaked on NA to obtain pure cultures, and the cultures
118 were stored at −20°C as 15% glycerol stock (15% glycerol supplemented nutrient broth) for
119 further analysis. All the media and chemicals for the above experiments were purchased from
120 Himedia, Mumbai, India.

121 2.3. **Biochemical and Morphological Characterization**

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

129 2.4. **Molecular identification of FCW1 strain**

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

150 2.5. ***In vitro* Evaluation of Probiotic Properties**

151 2.5.1. **Safety Assessment**

152 *2.5.1.1.* ***Antagonistic activity***

153 The screening of potential antagonistic activity was determined by the agar gel diffusion method
154 according to Qureshi et al. (2020). Cell-free supernatant was collected from the bacterial culture
155 grown overnight at 37°C in 50 ml Luria-Bertani broth (LB; Himedia, Mumbai, India) by
156 centrifugation at 5000 rpm for 10 min at 4°C and filtered through syringe filters (*0.2 µm*
157 *Acrodisc® Syringe Filters, Pall)*. Six pathogenic bacteria *Klebsiella pneumoniae, Streptococcus*
158 spp., *Escherichia coli*, (these clinical isolates belonging to K.A.P.V. Govt Medical Collage,
159 Tiruchirappalli), *Bacillus cereus* (NCIM 2156), *Staphylococcus aureus* (NCIM 5021),
160 and *Enterococcus faecalis* (MTCC 439) were cultured in LB broth, incubated at 37°C for 18 h
161 and the cultures were swabbed over the Muller Hinton agar (MHA; Himedia, Mumbai, India)
162 plates. On the swabbed MHA plate, 4 mm diameter wells were cut, and 100 µl of bacterial
163 culture supernatant was added. Tetracycline (10 mcg) disc was used as a control since it is a
164 broad-spectrum antibiotic which is used extensively in the prophylaxis and therapy of human and
165 animal infections. The inhibitory zone around the wells was measured after 24 h of incubation at
166 37°C. The assays were carried out in duplicate.

167 *2.5.1.2.* ***Antibiotic susceptibility test***

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

174 *2.5.1.3.* ***Antioxidant assay by DPPH method***

175 2,2-diphenylpicrylhydrazyl (DPPH; Himedia, Mumbai, India) assay was used to determine the
176 antioxidant activity of bacterial cell-free supernatant (CFS) as described by Bhukya and Bhukya
177 (2021). The reference standard was ascorbic acid (1 mg/ml). The following formula was used to
178 calculate antioxidant activity (%):

179 $$\text{Antioxidant activity (\%)} = (A0 - As/A0) \times 100 \quad (1)$$

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

181 ***2.5.1.4. Haemolytic and DNase activity***

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

187 2.5.2. **Tolerance to different stress conditions**

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

200 $$\text{Survivability \%} = \text{Survival rate (\%)} = \text{OD test}/\text{OD control} \times 100 \quad (2)$$

201 Following Liu et al. (2021), the tolerance to gastrointestinal conditions was determined.
202 Synthetic gastric juice was prepared using 3.0 g of pepsin dissolved in sterile PBS, pH 2.5
203 adjusted with hydrochloric acid. Trypsin (1.0 g/L) and bile salt (1.8%) were dissolved in sterile
204 PBS and adjusted to pH 8.0 with 0.1 mol/L NaOH to prepare artificial intestinal fluid. Before
205 use, the artificial gastric and intestinal fluids were filtered through a 0.22 µm filter membrane
206 (Millipore, Massachusetts, USA). The overnight culture (10 ml) of bacteria was centrifuged at
207 5000 rpm for 10 min; pellets were collected, washed thrice with PBS, and resuspended in sterile
208 PBS. To 4.5 ml of artificial gastric and intestinal fluid, 0.5 ml of bacterial suspension was added

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

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

215 To assess lysozyme resistance, overnight FCW1 culture (5 ml) was centrifuged at 5000 rpm for
216 10 min and resuspended in 10 ml LB broth with lysozyme (100 mg/l) and incubated for 3 h at
217 37°C. At different time intervals (0, 1, 2 and 3 h), the percentage of inhibition was calculated by
218 reading absorbance at 620 nm (Turchi et al., 2013).

219 Tolerance to phenol and NaCl was determined by growing the bacterial suspension (100 μl) in 5
220 ml LB broth containing various concentrations of phenol (0.1%, 0.4%, and 0.6%), and NaCl
221 (3%, and 6%) for 24 h at 37 °C (Shehata et al., 2016, Qureshi et al., 2020). LB broth without
222 phenol or NaCl was used as a control. The survival rate was determined by reading OD values
223 spectroscopically at intervals of 0, 1, 2, 3, 4 and 24 h and the inhibition percentage of was
224 calculated by using the following formula:

225 $$\text{Inhibition \%} = [(\text{OD control} - \text{OD test})/\text{OD control}] \times 100 \qquad (3)$$

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

230 2.5.3. **Adhesion assays**

231 *2.5.3.1.* ***Auto Aggregation***

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

236 600 nm at 0, 1, 2, 3, 4, 8 and 24 h. The auto aggregation percentage was measured using the
237 formula:

238 $$\text{Auto aggregation \%} = (1 - A_t / A_0) \times 100 \quad (4)$$

239 Where, At represents the absorbance for a particular incubation time and A0 is the absorbance at
240 0h incubation.

241 *2.5.3.2.* ***Cell Surface Hydrophobicity***

242 The hydrophobicity of FCW1 was assayed using the microbial cell adhesion to solvents (MATS)
243 method (Dlamini et al., 2018). The overnight bacterial cells were harvested by centrifugation at
244 5,000 rpm at 4°C for 10 min, washed twice with PBS, and resuspended in 3 ml PBS buffer,
245 followed by absorbance (A0) measurement at 600 nm. The cell suspension was added with 1 ml
246 of solvents (hexane, chloroform and ethyl acetate), vortexed for 1 min and incubated at 37°C for
247 1 h for the separation of aqueous and organic phases. The absorbance (A1) of the aqueous phase
248 (1 ml) was measured, and the percentage of hydrophobicity was calculated using the following
249 formula:

250 $$\text{\% cell surface hydrophobicity} = (1 - A1/A0) \times 100 \quad (5)$$

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

253 *2.5.3.3.* ***Biofilm formation assay***

254 A good biofilm-forming bacterium can adhere and colonize the intestinal epithelial cells. The
255 biofilm formation ability of the isolate FCW1 was determined as in Zayed et al. (2021) with
256 modifications. Briefly, the LB broth in test tubes was inoculated with FCW1 isolate and
257 incubated for 48 h at 37°C with shaking at 120 rpm. After incubation, the culture was decanted
258 and the tubes were washed with PBS buffer, which was then air-dried at room temperature. The
259 tubes were then stained with 3ml of 0.1% crystal violet, kept for 30 min, and washed with
260 distilled water thrice. The appearance of the violet colour on the test tube walls indicates biofilm
261 formation. The stain was released with ethanol and read OD at 595nm. Cut-off values were
262 calculated as the mean OD of the negative controls (ODc). The biofilm production was
263 determined based on the following classification: $OD \leq ODc$ = non-biofilm producer; $ODc < OD$
264 $\leq 2 \times ODc$ = weak biofilm producer, $2\times ODc < OD \leq 4 \times ODc$ = moderate biofilm producer, and
265 $OD > 4\times ODc$ = strong biofilm producer.

266 2.5.4. **Enzymatic activity**

267 The isolate FCW1 was inoculated on an appropriate agar medium to detect the amylase,
268 protease, lipase (Cappuccino and Sherman, 1983) and cellulase activity (Kasana et al., 2008). To
269 detect amylase activity, a starch agar plate was inoculated with FCW1 and incubated for 24 h at
270 37°C. After adding Gram's iodine solution to the culture, the plate was observed for a clear zone
271 around the colonies. The cellulolytic activity was detected by streaking the isolate on carboxy
272 methyl cellulose agar, and the zone of clearance was recorded after 24 h incubation. Tributyrin
273 agar was used for the detection of lipolytic activity. A cloudy zone around the colony shows
274 lipolytic activity.

275 2.6. ***In vivo* Bio-safety assay**

276 Probiotic candidates were tested for pathogenicity and bio safety using the Immersion assay,
277 which involves challenging zebra fish and looking at their health conditions. The animals were
278 handled according to Bharathidasan University's (Tamil Nadu, India) Institution Animal Ethics
279 Committee guidelines (BDU/IAEC/P11/2021). Experiments were carried out in triplicates in
280 aquaria set at 25-30°C and a lighting schedule of LD 12:12. Ten *Danio rario* (mean body weight:
281 0.182g) were randomly distributed among the aquariums after an initial 10-day acclimation
282 period. The strain FCW1 was inoculated into MRS broth and incubated for 24 h. Cells were
283 extracted by centrifugation at 5000 rpm for 10 min at 4°C in a cooling centrifuge. Sterile PBS
284 was used twice to wash and resuspend the cells. The concentrations of 10^5, 10^6, and 10^7CFU/ml
285 were added to the fish tanks. Control aquariums were added with sterile PBS solution. Fish were
286 fed 5% of their body weight twice daily (35% protein). Symptoms, abnormalities, and mortality
287 rates were measured at the end of the experiment.

288 **2.7. Whole Genome Sequencing of *S. gallinarum* FCW1**

289 Purified *S. gallinarum*FCW1 culture broth was used for further DNA extraction usingQIAamp
290 DNA Mini Kit (QiagenInc, USA) according to manufacturer's instructions. Illumina sequencing
291 libraries were prepared and genome was sequenced on the Illumina NextSeq 2500 platform using
292 2 × 250 paired-end libraries. The quality control of raw reads was carried out in FastQC version
293 0.11.9 (Andrews, 2010) and low quality reads were trimmed using Trimmomatic (ver 0.35), with
294 a phred cutoff of Q20 (Bolger et al., 2014). After quality filtering, the primary de novo assembly
295 of the reads was performed using SPAdes genome assembler (ver 3.10) (Nurk et al., 2013).
296 Genome annotation was performed using the National Center for Biotechnology Information
297 (NCBI) Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (Tatusova et al., 2013).

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

307 ***Strain Deposition and Complete Genome Sequence Data Accession Number***

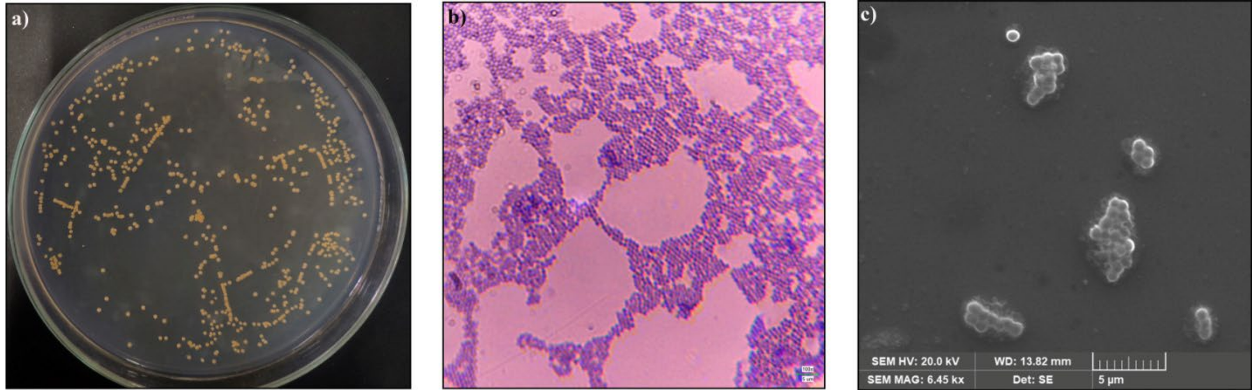
308 The whole genome sequence data of *S. gallinarum* FCW1 has been deposited at GenBank under
309 the accession number CP086207. The strain has been deposited at National Centre for Microbial
310 Resource, India (Accession no. MCC 4687).

311 **3. Results**

312 **3.1. Isolation and screening of GCC$^+$CNS strains**

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

323



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

328 **Table 1**. Phenotypic and Biochemical Characterization of Strain FCW1

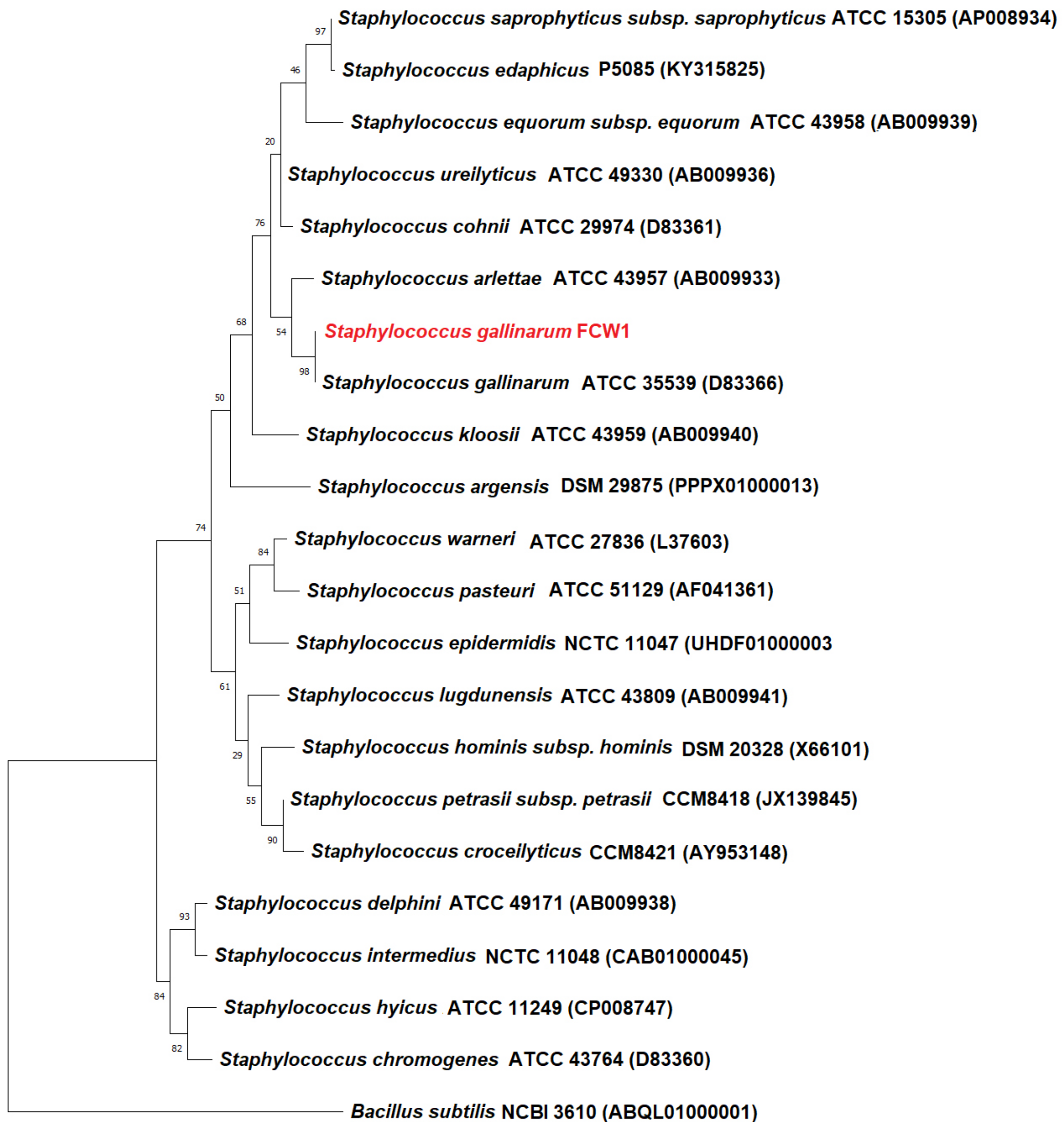
Tests	**FCW1**
Gram Staining	G^+
Shape	Cocci
Motility	Non-motile
Indole	Negative
MR	Positive
VP	Negative
Citrate	Negative
TSI	K/K
H_2S production	Negative
Nitrate Reduction test	Positive
Urease	Positive
Catalase	Positive
Oxidase	Positive
Coagulase	Negative
Carbohydrate Fermentation	
Glucose	Positive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

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

347 ***In vitro* Evaluation of Probiotic Properties**

348 3.2.1. **Safety Assessment**

349 3.2.1.1. **Antagonistic activity**

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

353

354 **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

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

357 **3.2.1.2. Antibiotic Susceptibility Profiles**

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

361 **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

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

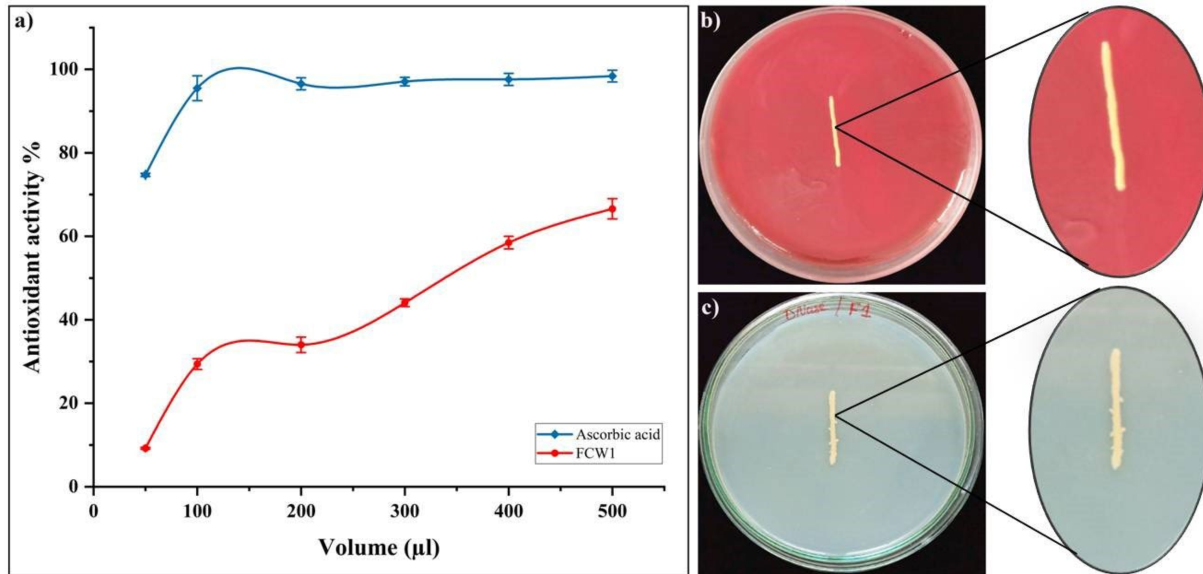
364 **3.2.1.3. Antioxidant Assay by DPPH Method**

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

371 **3.2.1.4. Haemolytic and DNase assay**

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

375



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

382 3.2.2. **Tolerance to different stress conditions**

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

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

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

392 The growth performance of isolate FCW1 at different temperatures was shown in Fig. 4d. At 0-4
393 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

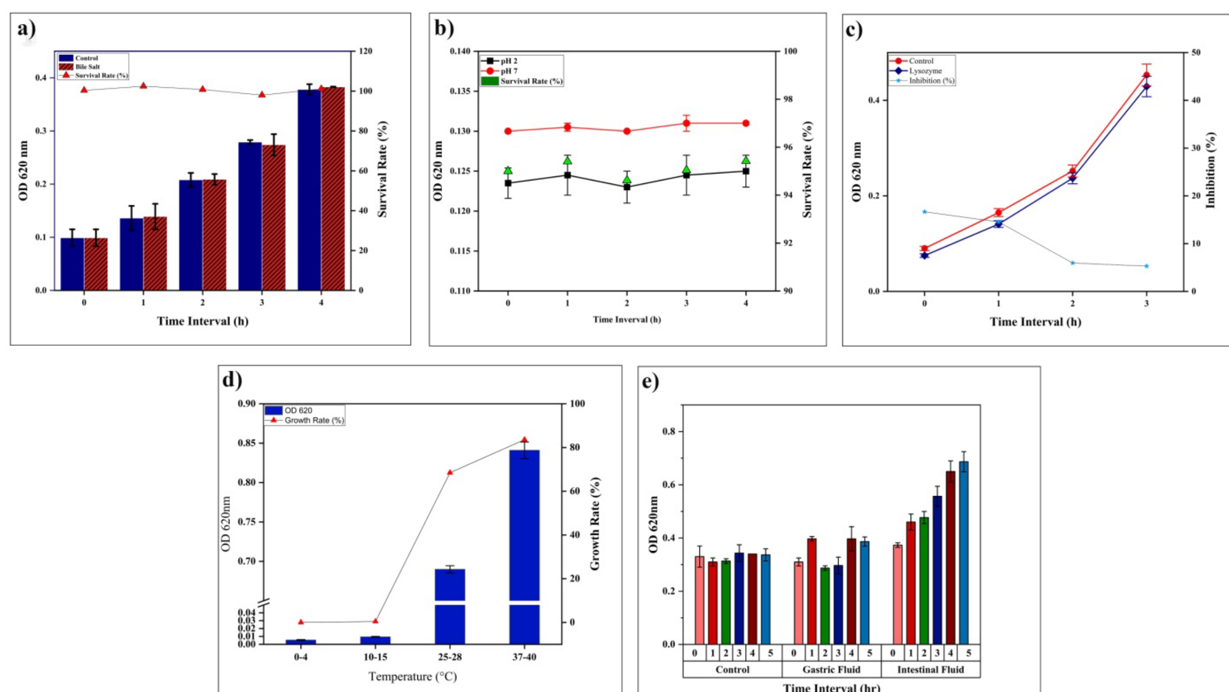


Fig. 4. Strain FCW1 tolerance to different stress conditions. (a) The growth of FCW1 was remained unaffected in the presence of 0.3 % bile salt ($P > 0.05$) when compared to control, (b) FCW1 showed stable growth at pH 2 and pH 7 ($P > 0.05$), (c) Significant growth was observed in the presence of lysozyme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

Table 4. Growth performance of FCW1 strain in the presence of different concentrations of 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.

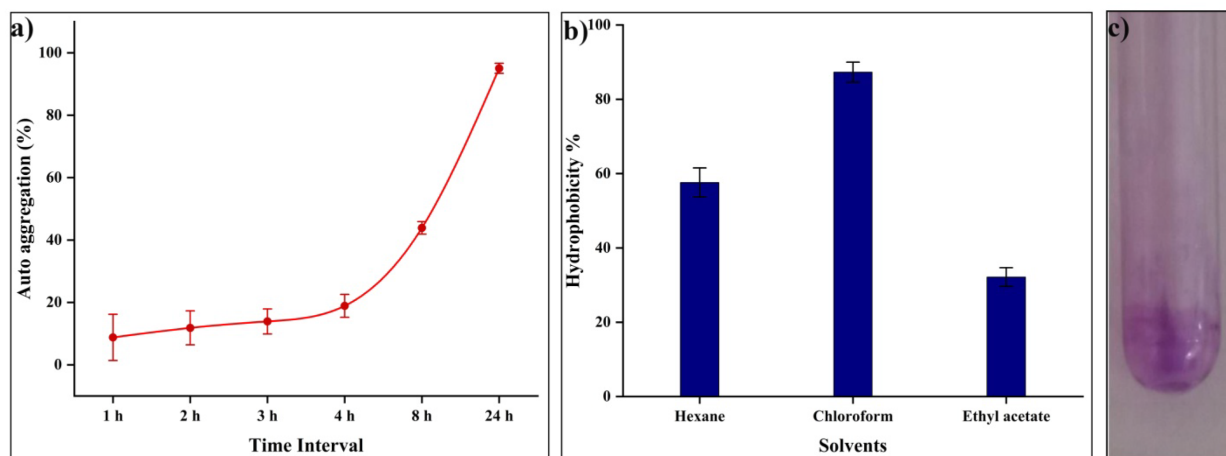
3.2.3.2. **Cell Surface Hydrophobicity**

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.

439



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

444 **Enzymatic activity**

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

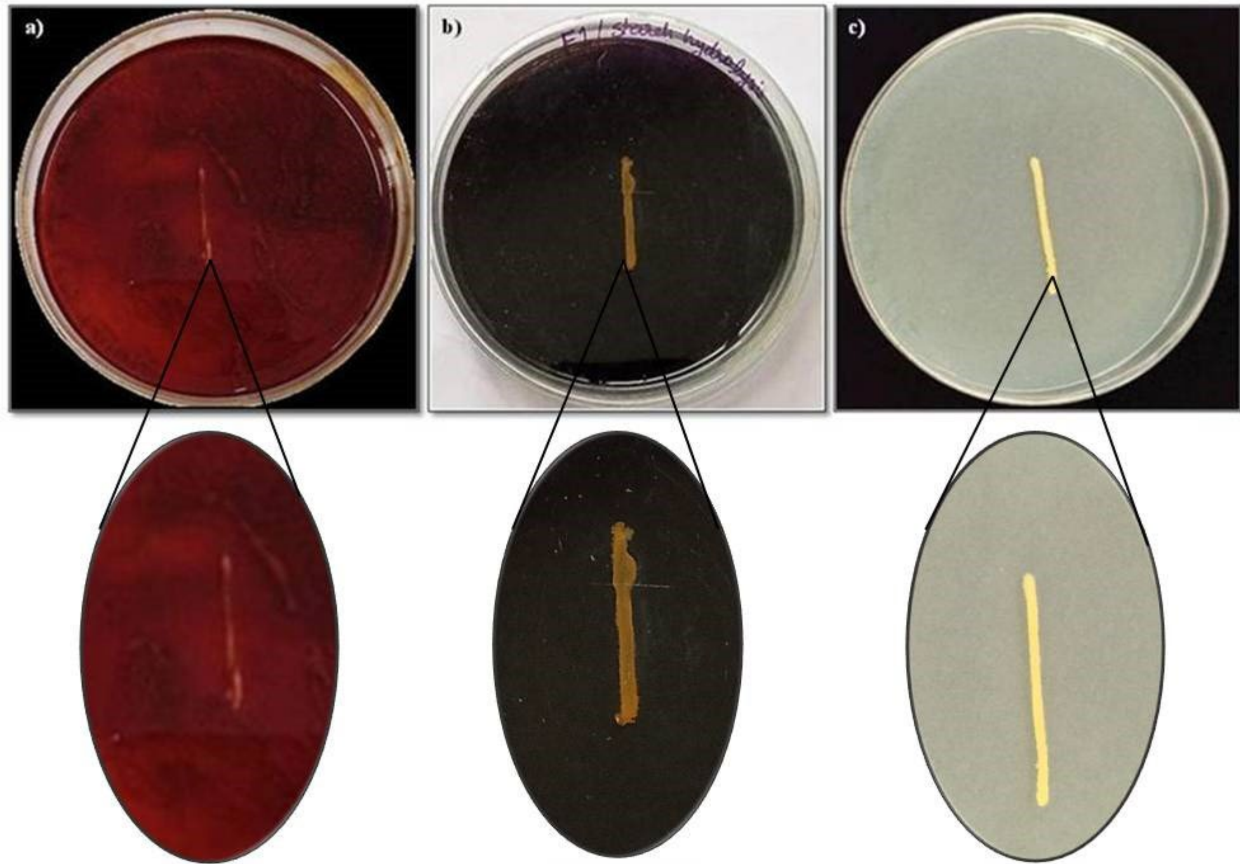


Fig. 6. Metabolic capacities through enzymatic activity of Isolate FCW1. No clear zone around the colonies indicates lack of a) cellulase, b) amylase, c) lipase activity. The zoomed image shows absence of enzymatic activity.

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).

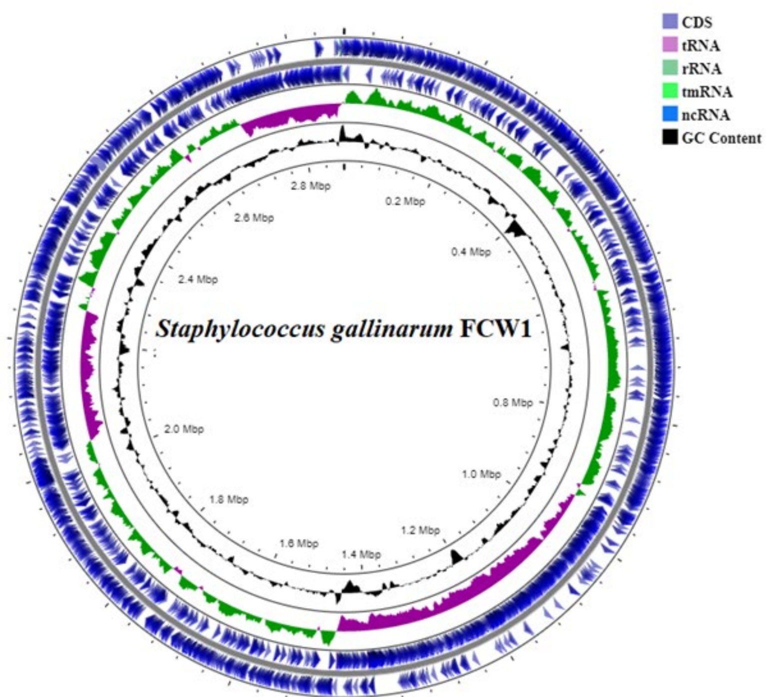


Fig. 7. Whole genome sequencing of *S. gallinarum* FCW1. The circular graphical representation refers to the genome annotations of FCW1 strain. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, and GC content. The circular genome was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

Table 5. Genomic Statistics of FCW1 genome

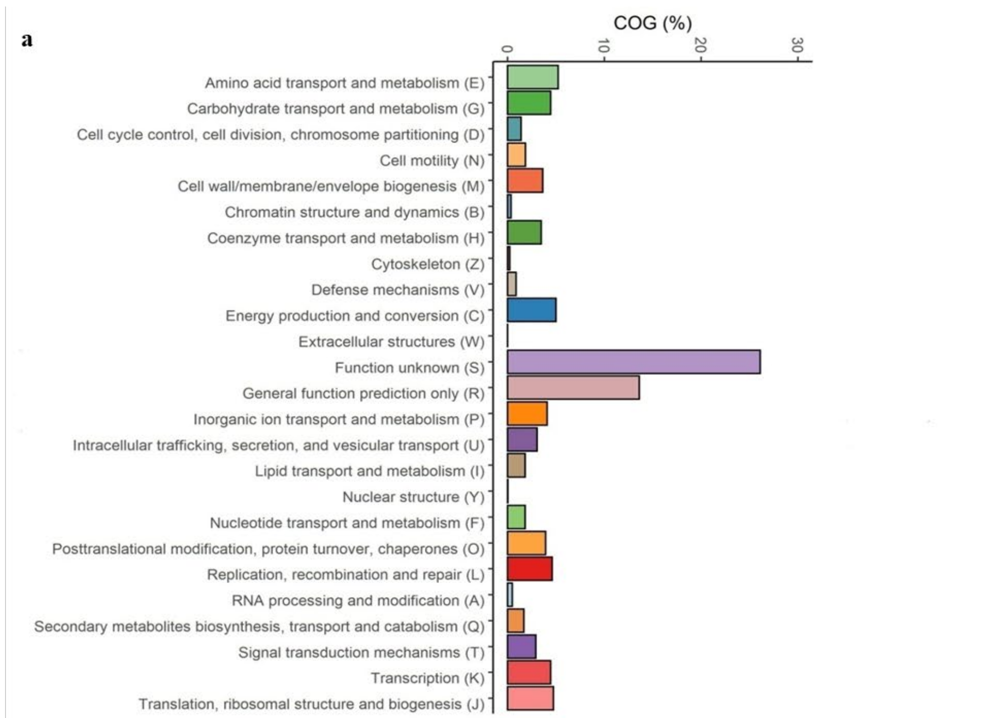
Species Attribute	
Genome size (bp)	2,880,305

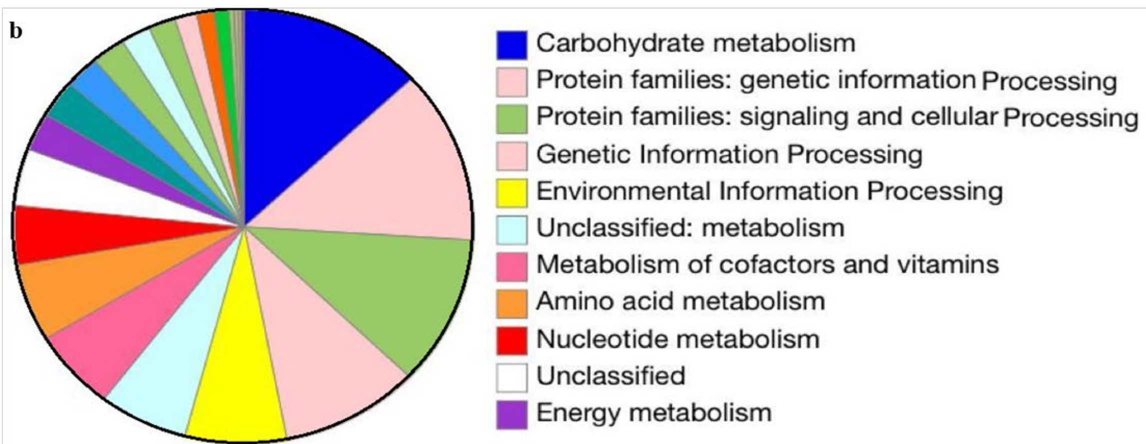
G + C %	33.23%
Contigs	1
Scaffolds	1
Total genes	2770
Protein coding genes	2708
RNA genes	62
Pseudo genes	11
CRISPR repeats	1

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).





491
492 **Fig. 8**. a) Number of genes associated with general COG functional categories b) Analysis of
493 KEGG distribution in *S. gallinarum* FCW1 using Blast KOALA algorithm.

494 **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

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

540 **3.4.3.4. Genes involved in Exopolysaccharide synthesis**

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

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

557 3.4.3.5. **Genes involved in the production of nutrients and other beneficial processes**

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

573 **3.4.3.6. Disease-specific genes**

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

583 **3.4.4. Secondary metabolite identification**

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

589 **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	-	-	-	-

590 "-" indicates not available.

591 **3.4.5. Antibiotic-resistant genes**

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

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

600 **Table 7**. Antibiotic-Resistant genes present in FCW1 genome based on PATRIC genome
601 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*

602

603 ## 4. Discussion

604 Fermented food products possess many health benefits, including antioxidant, anti-microbial,
605 anti-inflammatory, anti-diabetic, and anti-cancer properties. They are the large reservoir of
606 beneficial microorganisms or starter cultures which enhance the sensory properties and safety of
607 fermented foods by accelerating the acidification process of their matrix and producing
608 secondary metabolites. Food-derived CNS species are often used as starter cultures since they are
609 non-pathogenic and are native to fermented foods. CNS *S. gallinarum* is associated with
610 Japanese fermented foods, like miso (Onda et al., 2003), and African alkaline fermented foods,
611 like maari, dawadawa and soydawadawa (Parkouda et al., 2009). Considering the role of *S.*
612 *gallinarum* in food fermentation, this study aimed to evaluate its safety and probiotic
613 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

674 the bacteria's lifestyle and preferred environment (Merino et al., 2019). The isolate FCW1 has a
675 genome size with 2,880,305 bp and a GC content of 33.23%. However, the absence of plasmids
676 in FCW1 indicates it has a stable genome (Wang et al., 2021). FCW1 contains many cell surface
677 proteins, including *srtA* and *fbp* genes, as well as genes encoding pyruvate dehydrogenase and
678 lactate dehydrogenase, which contribute to adhesion, colonization, and biofilm formation. The
679 sortase (*srtA*) cleaves the cell wall sorting signal molecule (LPXTG motif) between threonine
680 and glycine and then covalently adhered to the cell wall peptidoglycan (Muñoz-Provencio et al.,
681 2012). The *fbp* gene encoding cell surface protein binds to fibronectin, a glycoprotein of the
682 extracellular matrix of epithelial cells (Azcarate-Peril et al., 2008). The biofilm protects bacteria
683 from the host immune system and antagonistic factors of enteropathogens (Toropov et al., 2020).
684 Bacteria should be tolerated in the harsh environment of the stomach. In order to resist such
685 stress conditions, the bacteria arise suitable responses in the expression of genes and protein
686 activity according to the environmental changes. Several bile, acid, and other stress-resistant
687 genes are identified in the FCW1 genome, including *nhap2*, *nhaK*, *clp*, *yveA*, *addAB*, *recDGQ*,
688 and *pcrA*, *cshAB*, and *dnaC*. In addition to the presence of bile salt hydrolase genes (*bshA*, *bshB*,
689 *bshC*), FCW1 is theoretically resistant to bile salts. The findings are consistent with our *in vitro*
690 results, which confirm that the bacteria are resistant to high salt, acid, and bile salt conditions.

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

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

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

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

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

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

739 5. **Conclusion**

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

752 **Ethics Statement**

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

756 **Authors Contributions**

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

760 **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.

763 **References**

764 1. Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data.
765 https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
766 2. Arena, M.P., Capozzi, V., Longo, A., Russo, P., Weidmann, S., Rieu, A., Guzzo, J., Spano,
767 G., Fiocco, D., 2019. The Phenotypic Analysis of Lactobacillus plantarum shsp Mutants
768 Reveals a Potential Role for hsp1 in Cryotolerance. Front Microbiol 10, 838.
769 https://doi.org/10.3389/fmicb.2019.00838
770 3. Azcarate-Peril, M.A., Altermann, E., Goh, Y.J., Tallon, R., Sanozky-Dawes, R.B., Pfeiler,
771 E.A., O'Flaherty, S., Buck, B.L., Dobson, A., Duong, T., Miller, M.J., Barrangou, R.,
772 Klaenhammer, T.R., 2008. Analysis of the Genome Sequence of Lactobacillus gasseri ATCC
773 33323 Reveals the Molecular Basis of an Autochthonous Intestinal Organism. Appl Environ
774 Microbiol 74, 4610–4625. https://doi.org/10.1128/AEM.00054-08
775 4. Bhukya, K.K., Bhukya, B., 2021. Unraveling the probiotic efficiency of bacterium
776 Pediococcuspentosaceus OBK05 isolated from buttermilk: An *in vitro* study for cholesterol
777 assimilation potential and antibiotic resistance status. PLoS One 16, e0259702.
778 https://doi.org/10.1371/journal.pone.0259702
779 5. Blin, K., Shaw, S., Kloosterman, A.M., Charlop-Powers, Z., van Wezel, G.P., Medema,
780 M.H., Weber, T., 2021. antiSMASH 6.0: improving cluster detection and comparison
781 capabilities. Nucleic Acids Res 49, W29–W35. https://doi.org/10.1093/nar/gkab335
782 6. Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina
783 Sequence Data. Bioinform. 30, 2114-20. https://doi.org/10.1093/bioinformatics/btu170
784 7. Borah, D., Gogoi, O., Adhikari, C., Kakoti, B.B., 2016. Isolation and characterization of the
785 new indigenous Staphylococcus sp. DBOCP06 as a probiotic bacterium from traditionally
786 fermented fish and meat products of Assam state. Egyptian J Basic Appl Sci 3, 232-240.
787 https://doi.org/10.1016/j.ejbas.2016.06.001

788 8. Cammarota, G., Ianiro, G., Bibbò, S., Gasbarrini, A., 2014. Gut microbiota modulation:
789 probiotics, antibiotics or fecal microbiota transplantation? Intern Emerg Med 9, 365–373.
790 https://doi.org/10.1007/s11739-014-1069-4
791 9. Cappuccino, J.G., Sherman, N., 1983. Microbiology: a laboratory manual. Addison-Wesley,
792 Readings. Mass.
793 10. Cappuccino, J.G., Sherman, N., 2005. Microbiology: A Laboratory Manual, The Benjamin
794 Cummings Publishing Co., Inc., San Francisco, CA
795 11. Chang, C.-J., Lin, T.-L., Tsai, Y.-L., Wu, T.-R., Lai, W.-F., Lu, C.-C., Lai, H.-C., 2019. Next
796 generation probiotics in disease amelioration. J Food Drug Anal 27, 615–622.
797 https://doi.org/10.1016/j.jfda.2018.12.011
798 12. Clinical and Laboratory Standards Institute (CLSI). 2022. Performance standards for
799 antimicrobial susceptibility testing, 32nd ed. CLSI supplement M100 (ISBN 978-1-68440-
800 134-5 [Print]; ISBN 978-1-68440-135-2 [Electronic]). Clinical and Laboratory Standards
801 Institute, Wayne, PA, USA.
802 13. Devriese, L., Poutrel, B., Kilpper-Bälz, R., Schleifer, K., 1983. *Staphylococcus*
803 *gallinarum* and *Staphylococcus caprae,* two new species from animals. Int J Syst Bacteriol
804 33: 480-486, 1983. https://doi.org/10.1099/00207713-33-3-480
805 14. Dlamini, Z. C., Langa, R. L. S., Aiyegoro, O. A., &Okoh, A. I. (2018). Safety Evaluation and
806 Colonisation Abilities of Four Lactic Acid Bacteria as Future Probiotics. Probiotics and
807 Antimicrobial Proteins.https://link.springer.com/article/10.1007/s12602-018-9430-y
808 15. Ferreira, A.B., De Oliveira, M.N.V., Freitas, F.S., Alfenas-Zerbini, P., Da Silva, D.F., De
809 Queiroz, M.V., Borges, A.C., De Moraes, C.A., 2013. Increased expression of clp genes in
810 Lactobacillus delbrueckii UFV H2b20 exposed to acid stress and bile salts. Benef Microbes
811 4, 367–374. https://doi.org/10.3920/BM2013.0022
812 16. Fujisawa, M., Kusumoto, A., Wada, Y., Tsuchiya, T., & Ito, M. (2005). NhaK, a novel
813 monovalent cation/H+ antiporter of Bacillus subtilis. *Archives of microbiology*, *183*(6), 411–
814 420. https://doi.org/10.1007/s00203-005-0011-6
815 17. García-Hernández, Y., Pérez-Sánchez, T., Boucourt, R., Balcázar, J.L., Nicoli, J.R., Moreira-
816 Silva, J., Rodríguez, Z., Fuertes, H., Nuñez, O., Albelo, N., Halaihel, N., 2016. Isolation,
817 characterization and evaluation of probiotic lactic acid bacteria for potential use in animal
818 production. Res Vet Sci 108, 125–132. https://doi.org/10.1016/j.rvsc.2016.08.009

819 18. Hamon, E., Horvatovich, P., Marchioni, E., Aoudé-Werner, D., Ennahar, S., 2014.
820 Investigation of potential markers of acid resistance in Lactobacillus plantarum by
821 comparative proteomics. J Appl Microbiol 116, 134–144. https://doi.org/10.1111/jam.12339
822 19. Irlinger, F., 2008. Safety assessment of dairy microorganisms: coagulase-negative
823 staphylococci. Int J Food Microbiol 126, 302–310.
824 https://doi.org/10.1016/j.ijfoodmicro.2007.08.016
825 20. Jeong, D.-W., Han, S., Lee, J.-H., 2014. Safety and technological characterization of
826 Staphylococcus equorum isolates from jeotgal, a Korean high-salt-fermented seafood, for
827 starter development. Int J Food Microbiol 188, 108–115.
828 https://doi.org/10.1016/j.ijfoodmicro.2014.07.022
829 21. Jeong, D.-W., Lee, B., Her, J.-Y., Lee, K.-G., Lee, J.-H., 2016. Safety and technological
830 characterization of coagulase-negative staphylococci isolates from traditional Korean
831 fermented soybean foods for starter development. Int J Food Microbiol 236, 9–16.
832 https://doi.org/10.1016/j.ijfoodmicro.2016.07.011
833 22. Jin, J., Zhang, B., Guo, H., Cui, J., Jiang, L., Song, S., Sun, M., Ren, F., 2012. Mechanism
834 analysis of acid tolerance response of bifidobacterium longum subsp. longum BBMN 68 by
835 gene expression profile using RNA-sequencing. PLoS One 7, e50777.
836 https://doi.org/10.1371/journal.pone.0050777
837 23. Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., Gulati, A., 2008. A rapid and easy method for
838 the detection of microbial cellulases on agar plates using gram's iodine. CurrMicrobiol 57,
839 503–507. https://doi.org/10.1007/s00284-008-9276-8
840 24. Khusro, A., Aarti, C., Dusthackeer, A., Agastian, P., 2018. Anti-tubercular and probiotic
841 properties of coagulase-negative staphylococci isolated from Koozh, a traditional fermented
842 food of South India. MicrobPathog 114, 239–250.
843 https://doi.org/10.1016/j.micpath.2017.11.054
844 25. Koponen, J., Laakso, K., Koskenniemi, K., Kankainen, M., Savijoki, K., Nyman, T. A.,
845 Varmanen, P. (2012). Effect of acid stress on protein expression and phosphorylation in
846 Lactobacillus rhamnosus GG. Journal of Proteomics, 75(4), 1357–1374.
847 http://dx.doi.org/10.1016/j.jprot.2011.11.009
848 26. Kosin, B., Rakshit, S., 2006. Microbial and processing criteria for production of probiotics: a
849 review. Food Technol Biotechnol 44, 371–379.

850 27. Ku, S., Park, M.S., Ji, G.E., You, H.J., 2016. Review on Bifidobacterium bifidum BGN4:
851 functionality and nutraceutical applications as a probiotic microorganism. Int J Mol Sci 17,
852 E1544. https://doi.org/10.3390/ijms17091544
853 28. Landeta, G., Curiel, J.A., Carrascosa, A.V., Muñoz, R., de las Rivas, B., 2013.
854 Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat
855 products. Meat Science 93, 387–396. https://doi.org/10.1016/j.meatsci.2012.09.019
856 29. Lee, I., Ouk Kim, Y., Park, S.-C., Chun, J., 2016. OrthoANI: An improved algorithm and
857 software for calculating average nucleotide identity. Int J Syst EvolMicrobiol 66, 1100–1103.
858 https://doi.org/10.1099/ijsem.0.000760
859 30. Lehnik-Habrink, M., Rempeters, L., Kovács, Á.T., Wrede, C., Baierlein, C., Krebber, H.,
860 Kuipers, O.P., Stülke, J., 2013. DEAD-Box RNA helicases in Bacillus subtilis have multiple
861 functions and act independently from each other. J Bacteriol 195, 534–544.
862 https://doi.org/10.1128/JB.01475-12
863 31. Li, M., Wang, Y., Cui, H., Li, Y., Sun, Y., Qiu, H.-J., 2020. Characterization of Lactic Acid
864 Bacteria Isolated From the Gastrointestinal Tract of a Wild Boar as Potential Probiotics.
865 Front Vet Sci 7, 49. https://doi.org/10.3389/fvets.2020.00049
866 32. Liu, C., Han, F., Cong, L., Sun, T., Menghe, B., Liu W.,2022. Evaluation of tolerance to
867 artificial gastroenteric juice and fermentation characteristics of Lactobacillus strains isolated
868 from human. Food Sci. Nutr.10, 227–238. https://doi.org/10.1002/fsn3.2662
869 33. Macías-Rodríguez, M.E., Zagorec, M., Ascencio, F., Rojas, M., 2008. Potential probiotic
870 Lactobacillus strains for piglets from an arid coast. Ann. Microbiol. 58, 641–648.
871 https://doi.org/10.1007/BF03175569
872 34. Mangrolia, U., Osborne, W.J., 2020. Staphylococcus xylosus VITURAJ10: Pyrrolo [1,2α]
873 pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (PPDHMP) producing, potential probiotic
874 strain with antibacterial and anticancer activity. MicrobPathog 147, 104259.
875 https://doi.org/10.1016/j.micpath.2020.104259
876 35. Merino, N., Zhang, S., Tomita, M., Suzuki, H., 2019. Comparative genomics of Bacteria
877 commonly identified in the built environment. BMC Genomics 20, 92.
878 https://doi.org/10.1186/s12864-018-5389-z

879 36. Muñoz-Provencio, D., Rodríguez-Díaz, J., Collado, M.C., Langella, P., Bermúdez-Humarán,
880 L.G., Monedero, V., 2012. Functional analysis of the Lactobacillus casei BL23 sortases.
881 Appl Environ Microbiol 78, 8684–8693. https://doi.org/10.1128/AEM.02287-12
882 37. Nath, S., Roy, M., Sikidar, J., Deb, B., Sharma, I., Guha, A., 2021. Characterization and in-
883 vitro screening of probiotic potential of novel Weissellaconfusa strain GCC_19R1 isolated
884 from fermented sour rice. Current Research in Biotechnology 3, 99–108.
885 https://doi.org/10.1016/j.crbiot.2021.04.001
886 38. Nurk, S., Bankevich, A., Antipov, D., Gurevich, A.A., Korobeynikov, A., Lapidus, A.,
887 Prjibelski, A.D., Pyshkin, A., Sirotkin, A., Sirotkin, Y., Stepanauskas, R., Clingenpeel, S.R.,
888 Woyke, T., McLean, J.S., Lasken, R., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2013.
889 Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J
890 Comput Biol 20, 714–737. https://doi.org/10.1089/cmb.2013.0084
891 39. Onda, T., Yanagida, F., Tsuji, M., Shinohara, T., Yokotsuka, K., 2003. Time series analysis
892 of aerobic bacterial flora during Miso fermentation. Lett Appl Microbiol 37, 162–168.
893 https://doi.org/10.1046/j.1472-765x.2003.01371.x
894 40. Parkouda, C., Nielsen, D.S., Azokpota, P., Ouoba, L.I.I., Amoa-Awua, W.K., Thorsen, L.,
895 Hounhouigan, J.D., Jensen, J.S., Tano-Debrah, K., Diawara, B., Jakobsen, M., 2009. The
896 microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa
897 and Asia. Crit Rev Microbiol 35, 139–156. https://doi.org/10.1080/10408410902793056
898 41. Parlindungan, E., Lugli, G.A., Ventura, M., van Sinderen, D., Mahony, J., 2021. Lactic acid
899 bacteria diversity and characterization of probiotic candidates in fermented Meats. Foods 10,
900 1519. https://doi.org/10.3390/foods10071519
901 42. Petit, M.A., Dervyn, E., Rose, M., Entian, K.D., McGovern, S., Ehrlich, S.D., Bruand, C.,
902 1998. PcrA is an essential DNA helicase of Bacillus subtilis fulfilling functions both in repair
903 and rolling-circle replication. Mol Microbiol 29, 261–273. https://doi.org/10.1046/j.1365-
904 2958.1998.00927.x
905 43. Prado, F.C., Lindner, J.D., Inaba, J., Thomaz-Soccol, V., Brar, S.K., Soccol, C.R.,
906 2015. Development and evaluation of a fermented coconut water beverage with potential
907 health benefits. J Funct Food. 12, 489–497. https://doi.org/10.1016/j.jff.2014.12.020

908 44. Prasad, J., McJarrow, P., Gopal, P., 2003. Heat and osmotic stress responses of probiotic
909 Lactobacillus rhamnosus HN001 (DR20) in relation to viability after drying. Appl Environ
910 Microbiol 69, 917–925. https://doi.org/10.1128/AEM.69.2.917-925.2003
911 45. Qureshi, N., Gu, Q., Li, P., 2020. Whole genome sequence analysis and *in vitro* probiotic
912 characteristics of a Lactobacillus strain Lactobacillus paracasei ZFM54. J Appl Microbiol
913 129, 422–433. https://doi.org/10.1111/jam.14627
914 46. Raaska, L., Mattila-Sandholm, T., 1995. Effects of iron level on the antagonistic action of
915 siderophores from nonpathogenic Staphylococcus spp. J Ind MicrobiolBiotechnol 15, 480-
916 485. https://doi.org/10.1007/BF01570018
917 47. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of
918 Probiotics in Food, 2002. Guidelines for evaluation of probiotics in food.
919 48. Saadat, Y.R., Khosroushahi, A.Y., Gargari, B.P., 2019. A comprehensive review of
920 anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria
921 exopolysaccharides. CarbohydrPolym 217,79–89.
922 https://doi.org/10.1016/j.carbpol.2019.04.025
923 49. Salas-Jara, M.J., Ilabaca, A., Vega, M., García, A., 2016. Biofilm Forming Lactobacillus:
924 New Challenges for the Development of Probiotics. Microorganisms 4, 35.
925 https://doi.org/10.3390/microorganisms4030035
926 50. Sathyabama, S., Vijayabharathi, R., Bruntha Devi, P., Ranjith Kumar, M., Priyadarisini,
927 V.B., 2012. Screening for probiotic properties of strains isolated from feces of various human
928 groups. J Microbiol 50, 603–612. https://doi.org/10.1007/s12275-012-2045-1
929 51. Selvin, J., Lanong, S., Syiem, D., De Mandal, S., Kayang, H., Kumar, N.S. and Kiran, G.S.,
930 2019. Culture-dependent and metagenomic analysis of lesser horseshoe bats' gut microbiome
931 revealing unique bacterial diversity and signatures of potential human pathogens.
932 MicrobPathog 137, 103675. https://doi.org/10.1016/j.micpath.2019.103675
933 52. Shehata, M.G., El Sohaimy, S.A., El-Sahn, M.A., Youssef, M.M., 2016. Screening of
934 isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt
935 hydrolase activity. Ann Agric Sci 61, 65–75. https://doi.org/10.1016/j.aoas.2016.03.001

936 53. Shuhadha, M.F.F., Panagoda, G. J., Madhujith, T., and Jayawardana, I.A., 2017. Evaluation
937 of probiotic attributes of Lactobacillus sp. isolated from cow and buffalo curd samples
938 collected from Kandy. Ceylon 159, 159–166. http://dx.doi.org/10.4038/cmj.v62i3.8519
939 54. Simonová, M., Strompfová, V., Marciňáková, M., Lauková, A., Vesterlund, S., Moratalla,
940 M.L., Bover-Cid, S., Vidal-Carou, C., 2006. Characterization of Staphylococcus xylosus and
941 Staphylococcus carnosus isolated from Slovak meat products. Meat Sci 73, 559–564.
942 https://doi.org/10.1016/j.meatsci.2006.02.004
943 55. Skinner, M.M., Trempy, J.E., 2001. Expression of clpX, an ATPase subunit of the Clp
944 protease, is heat and cold shock inducible in Lactococcus lactis. J Dairy Sci 84, 1783–1785.
945 https://doi.org/10.3168/jds.S0022-0302(01)74615-2
946 56. Somashekaraiah, R., Shruthi, B., Deepthi, B.V., Sreenivasa, M.Y., 2019. Probiotic Properties
947 of Lactic Acid Bacteria Isolated FromNeera: A Naturally Fermenting Coconut Palm Nectar.
948 Front Microbiol 10, 1382. https://doi.org/10.3389/fmicb.2019.01382
949 57. Sriram, K.P., Mangrolia, U., Osborne, W.J., 2020. Isolation and characterization of
950 culturable indigenous endophytic bacteria in the tender coconut. Food Biotechnology 34,
951 228–242. https://doi.org/10.1080/08905436.2020.1789872
952 58. Stergiou, O.S., Tegopoulos, K., Kiousi, D.E., Tsifintaris, M., Papageorgiou, A.C., Tassou,
953 C.C., Chorianopoulos, N., Kolovos, P., Galanis, A., 2021. Whole-Genome sequencing,
954 phylogenetic and genomic analysis of Lactiplantibacilluspentosus L33, a potential probiotic
955 strain isolated from fermented sausages. Front Microbiol 12, 746659.
956 https://doi.org/10.3389/fmicb.2021.746659
957 59. Sung, C., Kim, B.-G., Kim, S., Joo, H.-S., Kim, P.I., 2010. Probiotic potential of
958 Staphylococcus hominis MBBL 2-9 as anti-Staphylococcus aureus agent isolated from the
959 vaginal microbiota of a healthy woman. J Appl Microbiol 108, 908–916.
960 https://doi.org/10.1111/j.1365-2672.2009.04485.x
961 60. Sweeney, M.T., 2018. CLSI Performance Standards for Antimicrobial Disk and Dilution
962 Susceptibility Tests for Bacteria Isolated from Animals. Clinical and Laboratory Standards
963 Institute, CLSI standard VET01, Wayne, PA.
964 61. Talon, R., Leroy, S., Lebert, I., 2007. Microbial ecosystems of traditional fermented meat
965 products: The importance of indigenous starters. Meat Science, 53rd International Congress

966 of Meat Science and Technology (53rd ICoMST) 77, 55–62.
967 https://doi.org/10.1016/j.meatsci.2007.04.023
968 62. Talon, R., Walter, D., Chartier, S., Barrière, C., Montel, M.C., 1999. Effect of nitrate and
969 incubation conditions on the production of catalase and nitrate reductase by staphylococci.
970 Int J Food Microbiol 52, 47–56. https://doi.org/10.1016/s0168-1605(99)00127-0
971 63. Tamang, J.P., Watanabe, K., Holzapfel, W.H., 2016. Review: Diversity of Microorganisms
972 in Global Fermented Foods and Beverages. Front Microbiol 7, 377.
973 https://doi.org/10.3389/fmicb.2016.00377
974 64. Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Ciufo, S., Li, W., 2013.
975 Prokaryotic Genome Annotation Pipeline. In The NCBI handbook, 2nd ed. National Center
976 for Biotechnology Information, Bethesda, MD.
977 http://www.ncbi.nlm.nih.gov/books/NBK174280.
978 65. Toropov, V., Demyanova, E., Shalaeva, O., Sitkin, S., Vakhitov, T., 2020. Whole-Genome
979 Sequencing of Lactobacillus helveticus D75 and D76 Confirms Safety and Probiotic
980 Potential. Microorganisms 8, E329. https://doi.org/10.3390/microorganisms8030329
981 66. Turchi, B., Mancini, S., Fratini, F., Pedonese, F., Nuvoloni, R., Bertelloni, F., Ebani, V.V.,
982 Cerri, D., 2013. Preliminary evaluation of probiotic potential of Lactobacillus plantarum
983 strains isolated from Italian food products. World J MicrobiolBiotechnol 29, 1913–1922.
984 https://doi.org/10.1007/s11274-013-1356-7
985 67. Um, M.N., Lee, C.H., 1996. Isolation and Identification of *Staphylococcus* sp. from Korean
986 Fermented Fish Products. J MicrobiolBiotechnol, 6, 340-346.
987 68. Vastano, V., Salzillo, M., Siciliano, R.A., Muscariello, L., Sacco, M., Marasco, R., 2014. The
988 E1 beta-subunit of pyruvate dehydrogenase is surface-expressed in Lactobacillus plantarum
989 and binds fibronectin. Microbiol Res 169, 121–127.
990 https://doi.org/10.1016/j.micres.2013.07.013
991 69. Wang, Y., Liang, Q., Lu, B., Shen, H., Liu, S., Shi, Y., Leptihn, S., Li, H., Wei, J., Liu,
992 Chengzhi, Xiao, H., Zheng, X., Liu, Chao, Chen, H., 2021. Whole-genome analysis of
993 probiotic product isolates reveals the presence of genes related to antimicrobial resistance,
994 virulence factors, and toxic metabolites, posing potential health risks. BMC Genomics 22,
995 210. https://doi.org/10.1186/s12864-021-07539-9

996 70. Wanna, W., Surachat, K., Kaitimonchai, P., Phongdara, A., 2021. Evaluation of probiotic
997 characteristics and whole genome analysis of Pediococcuspentosaceus MR001 for use as
998 probiotic bacteria in shrimp aquaculture. Sci Rep 11, 18334. https://doi.org/10.1038/s41598-
999 021-96780-z
1000 71. Wu, S., Zhu, Z., Fu, L., Niu, B., Li, W., 2011. WebMGA: a customizable web server for fast
1001 metagenomic sequence analysis. BMC Genomics 12, 444. https://doi.org/10.1186/1471-
1002 2164-12-444
1003 72. Yasmin, I., Saeed, M., Khan, W.A., Khaliq, A., Chughtai, M.F.J., Iqbal, R., Tehseen, S., Naz,
1004 S., Liaqat, A., Mehmood, T., Ahsan, S., Tanweer, S., 2020. *In vitro* probiotic potential and
1005 safety evaluation (hemolytic, cytotoxic activity) of Bifidobacterium Strains isolated from raw
1006 camel milk. Microorganisms 8, E354. https://doi.org/10.3390/microorganisms8030354
1007 73. Zayed, S.M., Aboulwafa, M.M., Hashem, A.M., Saleh, S.E., 2021. Biofilm formation by
1008 Streptococcus mutans and its inhibition by green tea extracts. AMB Express 11, 73.
1009 https://doi.org/10.1186/s13568-021-01232-6
1010 74. Zheng, J., Du, M., Jiang, W., Zhang, J., Shen, W., Ma, X., Liang, Z., Shen, J., Wu, X., Ding,
1011 X., 2021. In Vitro Probiotic Characteristics and Whole Genome Sequence Analysis of
1012 Lactobacillus Strains Isolated from Cattle-Yak Milk. Biology (Basel) 11, 44.
1013 https://doi.org/10.3390/biology11010044
1014 75. Zommiti, M., Connil, N., Hamida, J.B., Ferchichi, M., 2017b. Probiotic Characteristics of
1015 Lactobacillus curvatus DN317, a Strain Isolated from Chicken Ceca. Probiotics &Antimicro.
1016 Prot. 9, 415–424. https://doi.org/10.1007/s12602-017-9301-y

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1018 **Supplementary Materials**

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1020 **Supplementary Table 1**: Number of genes associated with general COG functional categories in
1021 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

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1023 **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

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1025 **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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