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Techno-functional traits and safety aspects of coagulase-negative Staphylococcus saprophyticus isolated from traditional fermented food

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ABSTRACT

Ngari is a traditional-fermented fish food product of northeast India from which distinct bacteria were initially isolated and assessed for their antagonistic activities against wide spectrum of foodborne and enteric infection-causing pathogens. Among them, *Staphylococcus saprophyticus* strain AAS1 showed strong inhibitory activity against all the indicator pathogens, ranging from 81.14 ± 2.4 to 340.18 ± 2.1 AU/mL. *In vitro* techno-functional properties of strain AAS1 were further evaluated and confirmed by using standard methodologies. The AASI strain exhibited high tolerance at higher acidic conditions, simulated gastric juice of pH 2.0, and oxgall (0.5%, w/v). Furthermore, this strain showed noticeable hydrophobicity and auto-aggregation traits of 64.2 ± 1.3 and 44.3 ± 1.5%, respectively. The isolate depicted not only resistance to phenol and lysozyme but also exhibited 2,2-diphenyl-1-picrylhydrazyl degradation (16.6 ± 1.2–67.5 ± 1.1%), hydrogen peroxide tolerance (2.1 ± 0.04–1.1 ± 0.04), and hydroxyl radical scavenging (10.6 ± 1.2–57.5 ± 1.1%) properties. Strain AAS1 fermented varied carbohydrates and produced exopolysaccharide and lipase. The isolate exhibited significant rate of autolysis (48.6 ± 1.2%), catalase activity (18.14 ± 0.3 AU), and nitrate reductase production (26.32 ± 0.8 mM nitrite/mg dry weight). It has also showed negative results toward *in vitro* hemolytic, DNase, gelatinase, and biofilm formation tests, in addition to being susceptible to conventional antibiotics used. In a nutshell, strain AAS1 may be employed as quintessential candidate for its disparate applications in food and pharmaceutical industries if its probiotic function can be validated.

KEYWORDS

Anti-pathogenic; *Ngari*; safety aspects; *Staphylococcus saprophyticus*; techno-functional properties

1. Introduction

The implementation of probiotic bacteria as health-supporting system due to their nontoxic, anti-pathogenic, functional, and technological attributes has stimulated research interests worldwide. Probiotics are now known to exhibit

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several physiological characteristics such as retaining intestinal microbiota balance, inhibition of pathogens, boosting immunity, removal of carcinogen from intestinal tract, lactose tolerance, and biotransformation of nutrients availability to host (Parvez et al. 2006).

For being a potent probiotic strain, the isolate should exhibit diversified potentialities such as survival in the acidic environment of gastrointestinal tract, susceptibility toward conventional antibiotics, antagonism against pathogens, and adhesion to epithelial lining (Pisano et al. 2014). Aside from finding efficient strain with dormant probiotic traits, it is also imperative to consider its distinct technological properties for utilizing as starter cultures. The taste and aroma of fermented foods are attributed to varied biological factors, particularly its microbiota quality. Since the selection of autochthonous microbiota for starter formulation is an important tool to preserve the exemplary properties of fermented food products; hence, pervasive knowledge on the techno-functional characteristics of certain strains present in fermented foods would be a matter of interest with tangible benefits (Mauriello et al. 2004).

In recent times, coagulase-negative staphylococci (CNS) indigenous to traditional-fermented foods have emerged as the prevalent heterogeneous group of bacteria divulging cogent probiotic traits (Borah et al. 2016; Khusro et al. 2018). Interestingly, CNS viz. *Staphylococcus carnosus, S. equorum, S. hominis, S. cohnii, S. capitis, S. condimenti, S. succinus*, and *S. xylosus* are generally described as benign bacteria, and in fact, the infection due to fermented food associated CNS is subtle (Jeong et al. 2016). In spite of anti-pathogenic traits, CNS are known to enhance the sensory properties of fermented foods, by reducing nitrates to nitrite and then to nitrous oxide, by preventing rancidity through peroxide decomposition, and by producing flavor and aroma compounds through proteolysis and lipolysis (Khusro et al. 2018).

Ngari is one of the most widely consumed ethnic fermented non-salted dry fish product of Manipur (Northeastern state of India). *Ngari* is eaten daily as a side dish with cooked rice and sold in local markets of Manipur in earthen pots. *Lactobacillus* spp., *Bacillus* spp., *Saccharomyces* spp., *Micrococcus* spp., and *Tetragenococcus* spp., are well-known dominant probiotic microbiota of *Ngari* (Thapa 2016). However, there is paucity of study depicting the isolation and distinctive industrial applications of CNS, indigenous to *Ngari*. In consideration of this, the present study investigated the functional and technological attributes of autochthonous new potent strain of coagulase-negative *Staphylococcus* sp. isolated from *Ngari* for its beneficial role not only in as starter and/or adjunct culture in food industries but potentially in pharmaceutical applications improving antimicrobial solutions.

2. Materials and methods

2.1. *Preparation of* Ngari

Puntius sophore (non-salted dry fish) was procured from the local market of Imphal, Manipur, India and washed with clean water. The water was drained completely for few hours and then covered with gunny bags. The content was pressed hard and packed tightly in an old narrow-necked earthen pot/vat, locally called as '*chaphou*'. Later, the pot was sealed tightly using mud and kept for fermentation at room temperature for 6–8 months. After the fermentation period, the fermented food product obtained is called as *Ngari* (Fig. 1).

2.2. *Bacterial isolation and purification*

Bacteria were isolated from *Ngari* as per the methodology of Khusro et al. (2018). *Ngari* (1 g) was mixed with sterile distilled water and centrifuged at 2000 *g* for 15

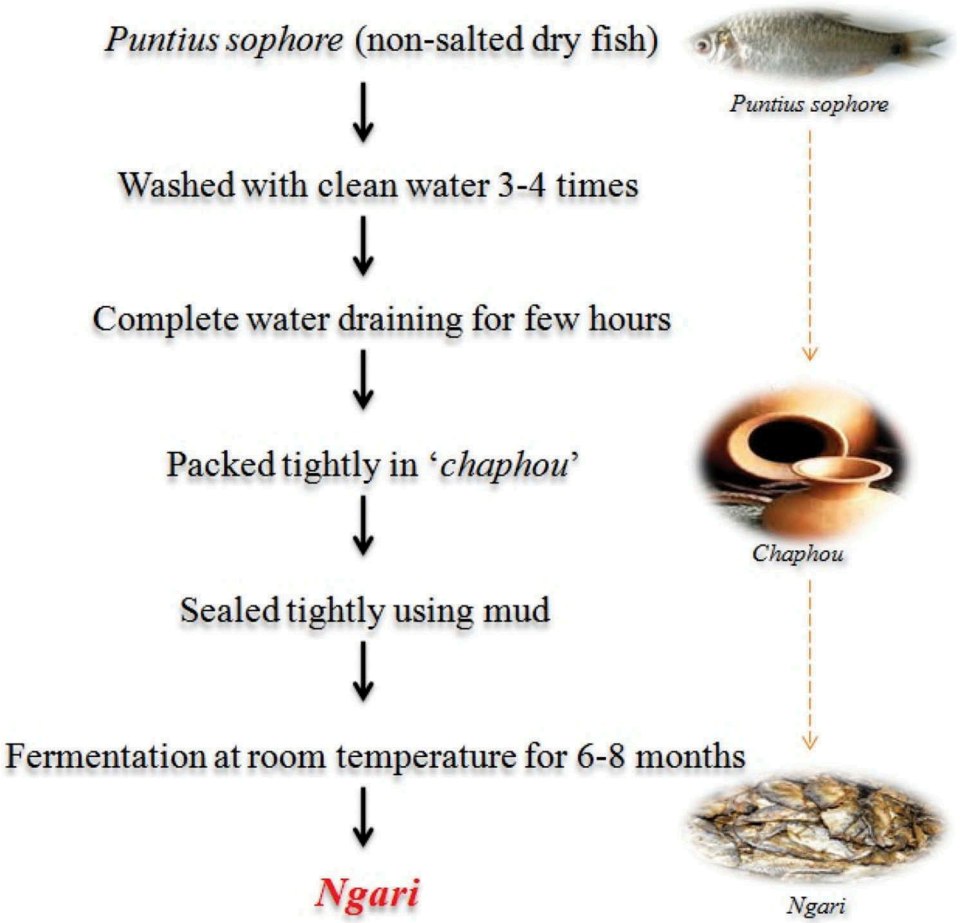

Figure 1. Schematic representation of *Ngari* preparation.

min for separating heavy particles. The supernatant was serially diluted up to 10^{-6} dilution and the suspension was poured onto sterilized De Man Rogose Sharpe (MRS) agar (g/L – proteose peptone 10.0, beef extract 10.0, yeast extract 5.0, dextrose 20.0, polysorbate 80 1.0, ammonium citrate 2.0, sodium acetate 5.0, magnesium sulfate 0.1, manganese sulfate 0.05, dipotassium phosphate 2.0, pH 6.5, and agar 18) plates. Plates were incubated at 30°C for 48 h and observed for distinct colonies appearance. Different colonies were picked and pure cultures were obtained by subsequent streaking on new sterilized MRS agar plates.

2.3. *Anti-pathogenic activities of isolates*

2.3.1. *Preparation of cell-free neutralized supernatant (CFNS)*

Isolates were inoculated into sterilized MRS broth and incubated for 48 h at 30°C. Bacterial cultures were centrifuged at 8000 *g* for 15 min, and the culture supernatant was filtered. The filtered cell-free supernatant was neutralized using 1 N NaOH, followed by its treatment individually with catalase, and incubated at room temperature for 2 h for eliminating the bactericidal property of hydrogen peroxide. The CFNS obtained was then assayed for anti-pathogenic activities against indicator pathogens.

2.3.2. *Anti-pathogenic activities*

Foodborne pathogens (*Staphylococcus epidermidis* MTTC 3615, *Staphylococcus aureus* MTCC 96, *Enterococcus faecalis* MTCC 439, and *Micrococcus luteus* MTCC 106) and enteric pathogenic bacteria (*Shigella flexneri* MTCC 1457, *Yersinia enterocolitica* MTCC 840, *Enterobacter aerogens* MTCC 111, and *Proteus vulgaris* MTCC 1771) were used as indicator bacteria in this investigation. All the indicator pathogenic bacteria were sub-cultured into Tryptone Soya Broth medium (g/L: pancreatic digest of casein –15.0, papaic digest of soyabean meal –2.0, dextrose –2.0; sodium chloride –5.0; dibasic potassium phosphate –2.0, and pH –7.0) and incubated at 37°C. After 24 h, indicator bacteria were swabbed onto sterile Mueller Hinton Agar (g/L: casein acid hydrolyzate –18.0, beef heart infusion –2.0, soluble starch –1.0, agar –18.0, and pH –7.0) plates. The anti-pathogenic activities were determined using agar well-diffusion assay by pouring 100 µL of CFNS of each isolate into the respective wells. Penicillin G was used as positive control. The anti-pathogenic activities of CFNS of isolates were determined using agar well-diffusion assay and expressed in arbitrary units (AU/mL) after 24 h of incubation as per the methodology of (Khusro et al. 2019).

2.4. *Characterization of potent isolate*

The potent isolate exhibiting broad-spectrum anti-pathogenic activity was subjected to morphological and biochemical characterization using standard tests. Gram staining test was performed to differentiate between Gram-positive and

Gram-negative bacterium. Endospore test was carried to determine sporulating or non-sporulating nature. Cellular morphology and colony characteristic of isolate were also examined on agar plate. Biochemical tests viz. indole, voges-proskauer, citrate utilization, ortho-nitrophenyl-β-D-galactopyranoside (ONPG), coagulase, nitrate reductase, arginine, malonate, catalase, and methyl red were performed aseptically. Further, the amplification of genomic DNA of the isolate was carried out using the universal primers 27F (5′-AGA GTT TGA TCG TGG CTC AG-3′) and 1492R (3′-GCT TAC CTT GTT ACG ACT T-5′). The 16S rRNA sequence was submitted to GenBank and accession number was assigned for the isolate.

2.5. *Functional properties of isolate*

2.5.1. *Tolerance to low pH*

The property of isolate to resist low pHs (6.0–2.0) was determined as per the methodology of Ramos et al. (2013) with slight modifications. The isolate was grown up to log phase in MRS broth and then centrifuged at 6000 *g* at 4°C for 10 min. pH of the freshly prepared MRS broth media was adjusted from 6.0 to 2.0. MRS broth of pH 6.5 represents control medium. The bacterium was resuspended in MRS broth of various pH ranges and incubated at 30°C up to 3 h. The suspension was serially diluted using phosphate buffer saline (PBS), plated on sterilized MRS agar plates, and incubated at 30°C for 48 h. Cell viability (log cfu/mL) was determined using plate count technique.

2.5.2. *Tolerance to simulated gastric juice*

The ability of isolate to tolerate simulated gastric juice was assessed as per the methodology of Aarti and Khusro (2019). Pepsin (3 mg/mL) and sodium chloride (0.5% w/v) were used to prepare simulated gastric juice, and pHs of the solution were adjusted to 2.0–4.0. The overnight grown isolate was centrifuged at 8000 *g* for 10 min. Cells were washed twice with 10 mL of 50 mM K_2HPO_4 solution and resuspended in 3 mL of 50 mM K_2HPO_4 solution. Simulated gastric juice was mixed with the cell suspension and incubated at 30°C for 3 h. Bacterial cell suspension was plated on the sterilized MRS agar plates after 3 h. Cell viability was calculated as log cfu/mL after 48 h.

2.5.3. *Tolerance to bile salt*

The viability of isolate in the presence of bile salt was determined according to the methodology of Khusro et al. (2018). Overnight grown isolate was inoculated into MRS broth supplemented with oxgall (0.5% w/v) and incubated at 30°C for 72 h. Aliquots of the suspension were withdrawn at regular time interval and cell viability was estimated with respect to control culture (without oxgall) by measuring absorbance at 600 nm.

2.5.4. *Cell surface hydrophobicity*

The adherence characteristic of isolate to hydrocarbons was evaluated as per the methodology of Khusro et al. (2018) with minor modifications. The isolate was grown in MRS broth for 48 h and centrifuged at 8000 *g* for 10 min. The pellet was washed twice with PBS (pH 7), resuspended in the same buffer, vortexed, and absorbance of the cell suspension was measured at 600 nm (A). The cell suspension (3 mL) was mixed with 1 mL of hydrocarbons viz. chloroform, toluene, and ethyl acetate. The mixture was vortexed for 1 min, and incubated at 30°C up to 3 h for separating the aqueous and organic phase. The aqueous phase was collected and decrease in absorbance was read at 600 nm (A_0). The percentage (%) cell surface hydrophobicity was calculated as follows:

$$\% \text{ Hydrophobicity} = [(A - A_0)/A] \times 100. \quad (1)$$

2.5.5. *Auto-aggregation*

Cell auto-aggregation characteristic of the isolate was determined according to the methodology of Juárez Tomás, Wiese, and Nader-Macías (2005) with slight modifications. The isolate was grown in MRS broth for 48 h and centrifuged at 8000 *g* for 10 min. The pellet was washed twice in PBS (pH 7.0) and adjusted to an absorbance of 1.5–1.8 at 600 nm (A). The cell suspension was further incubated at 30°C for 72 h. The upper phase (1 mL) was separated to measure the absorbance at 600 nm (A_0). The auto-aggregation % was calculated according to the following formula:

$$\% \text{ Auto-aggregation} = [(A - A_0)/A] \times 100. \quad (2)$$

2.5.6. *Tolerance to phenol and lysozyme*

The resistance trait of isolate toward phenol solution was determined according to the methodology of Khusro et al. (2018) with slight modification. The overnight grown bacterial culture was incubated in MRS broth supplemented with 0.5% phenol and incubated at 30°C for 24 h. Bacterial suspension was spread on MRS agar plates after 0 and 24 h of incubation and cell viability was calculated with respect to control (without phenol).

The tolerance of isolate to lysozyme was assessed according to the methodology of Zago et al. (2011). The isolate was allowed to grow for 24 h at 30°C and centrifuged at 8000 *g* for 15 min. The pellet was washed twice with PBS (pH 7.0) and resuspended in Ringer solution [g/L: $CaCl_2$ 0.2; NaCl 6.0; KCl 2.0; and $NaHCO_3$ 1.2) containing lysozyme (100 µg/mL). Sample was incubated at 30°C for 2 h and percentage cell viability was estimated against control (without lysozyme).

2.5.7. *Proteolytic activity*

The extracellular proteolytic property of log-phase grown isolate was determined as per the methodology of Khusro (2015). The log phase grown isolate was centrifuged at 8000 *g* for 15 min at 4°C and the supernatant was collected. The agar plate consisting of skim milk (% w/v: peptone 0.5; beef extract 0.3; skim milk 1.0 and agar 1.8) was prepared and allowed to cool aseptically. The agar medium was punched with sterilized cork borer, bacterial supernatant was poured into it, and incubated at 30°C. The proteolytic activity of the isolate was observed after 24 h of incubation.

2.5.8. *Antioxidant activity of isolate*

2.5.8.1. ***DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity.*** The DPPH free radical scavenging activity of isolate was assayed according to the methodology of Khusro et al. (2018) at different concentrations (100–1000 µL) of freshly prepared cells. DPPH scavenging capacity of isolate as well as ascorbic acid (standard) was read at 517 nm and the percentage scavenging capacity was calculated as follows:

$$\text{DPPH scavenging } (\%) = [(A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}] \times 100. \quad (3)$$

2.5.8.2. ***Resistance to H_2O_2.*** The tolerance characteristic of isolate to various concentrations of H_2O_2 (0.5, 1.0, 1.5, and 2 mM) was assayed based on the methodology of Khusro et al. (2018). Control culture was also maintained without the addition of H_2O_2 and cell viability was measured at 600 nm.

2.5.8.3. ***Hydroxyl radical scavenging assay.*** The hydroxyl radical scavenging activity of isolate was determined as per the method of He et al. (2004) at various concentrations (100–1000 µL) of bacterial cells. The reaction mixture was incubated at room temperature for 15 min and absorbance was measured at 624 nm. Ascorbic acid was used as standard. The hydroxyl radical scavenging activity was calculated as follows:

$$\text{Hydroxyl radical scavenging } (\%) = [(A_1 - A_0)/(A - A_0)] \times 100 \quad (4)$$

where A_1 is the absorbance of sample, A_0 is the absorbance of the control, and A is the absorbance without the sample and the Fenton reaction system.

2.6. *Technological traits of isolate*

2.6.1. *Fermentation capabilities*

Carbohydrate fermentation potentiality of isolate was determined according to the methodology of Delgado et al. (2015) using diversified carbohydrates viz. glucose, lactose, dextrose, maltose, fructose, and sucrose. The culture was

incubated at 30°C up to 48 h of incubation, and absorbance was read at 600 nm.

2.6.2. *Exopolysaccharide (EPS) production*

The potentiality of the isolate to produce EPS was qualitatively confirmed according to the methodology of Owusu-Kwarteng et al. (2015) in the presence of MRS-sucrose broth medium. An opaque link at the interface was observed for the confirmation of EPS production.

2.6.3. *Amylase and lipase production*

The potentiality of isolate to produce extracellular amylase was determined according to the methodology of Khusro and Aarti (2015). The extracellular lipolytic activity was determined as per the methodology of Mahmoudi et al. (2018).

2.6.4. *Cell autolysis*

Autolysis of cells was estimated according to the methodology of Mora et al. (2003). The isolate was harvested at the exponential phase, washed in potassium phosphate buffer (0.05 M, pH 6.5), resuspended in the same buffer, and incubated at 30°C. The degree of autolysis was expressed as the percentage decrease in absorbance at 630 nm after 6, 12, and 24 h.

2.6.5. *Catalase activity*

Catalase activity of isolate was determined according to the methodology of Aebi (1974) with slight modifications. Five milliliters of culture were grown up to 24 h and centrifuged at 6000 *g* for 10 min. The resulting pellet was mixed with 1.5 mL of 60 mM H_2O_2 in 20 mM phosphate buffer (pH 7.0). The reaction mixture was incubated for 3 min at room temperature. The activity was measured spectrophotometrically at 240 nm and results were expressed in arbitrary units: μmoles of degraded H_2O_2/min/mL of cells with OD_{600} = 1.0.

2.6.6. *Nitrate reductase activity*

Nitrate reductase activity of strain was estimated using spectrophotometric method (Casaburi et al. 2005). Nitrite production from nitrate was determined by reading absorbance at 540 nm. Relative activity was calculated as the rate: OD_{540} nm/mg dry weight.

2.7. In vitro *safety assessment tests*

2.7.1. *Biofilm formation*

The biofilm-forming trait of isolate was determined following the methodology of Wang et al. (2018) with slight modifications. The overnight grown

isolate (100 µL) was added in 96-wells microtiter plate and incubated for 24 h. After required period of incubation, the content of the well was discarded, and the well was washed gently twice with PBS (pH 7.0). Hundred microliters of methanol (99% v/v) was added into the well and kept undisturbed for 15 min. The methanol was discarded and the well was allowed to dry. After air dry, 100 µL of crystal violet stain solution was added into the well and held for 10–15 min. The stain was discarded after required incubation period and the well was washed gently with water. The well was air dried and absorbance was read at 620 nm using ELISA reader.

2.7.2. *Hemolytic, DNase, and gelatinase tests*

In vitro hemolytic, DNase, and gelatinase activities of strain AAS1 were determined according to the methodology of Khusro et al. (2019).

2.7.3. *Antibiotics susceptibility assay*

The susceptibility profile of isolate to various conventional antibiotics viz. streptomycin, penicillin G, chloramphenicol, kanamycin, gentamicin, and rifampicin was determined by disc diffusion method (Khusro and Aarti 2015).

2.8. *Statistical analyses*

All the experimental sections of the study were carried out in triplicate and data were represented as mean±SD. Data were analyzed using one way ANOVA and values $P \leq 0.05$ were considered significant.

3. Results

3.1. *Anti-pathogenic activities of isolates*

A total of eight bacteria were isolated from *Ngari* and assessed for anti-pathogenic activity against indicator pathogens (Table 1). Isolate AAS1 indicated pronounced growth inhibitory trait ($P < .05$) against foodborne and enteric pathogens with maximum activity against *S. aureus* (340.18 ± 2.1 AU/mL), followed by *S. epidermidis* (200.15 ± 2.4 AU/mL), *M. luteus* (180.32 ± 2.4 AU/mL), *Y. enterocolitica* (160.43 ± 4.3 AU/mL), *E. aerogens* (150.23 ± 4.5 AU/mL), *S. flexneri* (140.14 ± 3.3 AU/mL), *P. vulgaris* (100.13 ± 4.5 AU/mL), and *E. faecalis* (81.14 ± 2.4 AU/mL).

3.2. *Identification of potent isolate*

Considering the broad-spectrum anti-pathogenic activity of isolate AAS1, this bacterium was selected for identification and further studies. Isolate AAS1 was

Table 1. Antagonistic activities (AU/mL) of isolates against foodborne and enteric bacterial pathogens.

	Indicator bacterial pathogens							
Isolates	*S. epidermidis*	*S. aureus*	*E. faecalis*	*M. luteus*	*S. flexneri*	*Y. enterocolitica*	*E. aerogens*	*P. vulgaris*
AAS1	200.15 ± 2.4^{b}	340.18 ± 2.1^{a}	81.14 ± 2.4^{g}	180.32 ± 2.4^{c}	140.14 ± 3.3^{e}	160.43 ± 4.3^{d}	150.23 ± 4.5^{de}	100.13 ± 4.5^{f}
AAS2	152.25 ± 2.2^{c}	220.25 ± 1.9^{a}	110.2 ± 3.3^{f}	90.4 ± 2.3^{e}	135.5 ± 5.2^{d}	85.3 ± 4.4^{g}	170.23 ± 4.5^{b}	92.6 ± 4.5^{e}
AAS3	98.43 ± 3.5^{b}	110.22 ± 3.5^{a}	NA	NA	80.32 ± 2.4^{d}	92.45 ± 2.6^{bc}	NA	NA
AAS4	NA	62.13 ± 1.3^{b}	NA	52.4 ± 2.5^{c}	NA	68.4 ± 3.3^{a}	NA	NA
AAS5	NA	NA	NA	NA	NA	102.34 ± 4.1	NA	NA
AAS6	96.6 ± 3.5^{a}	82.14 ± 1.6^{b}	NA	NA	68.53 ± 4.2^{c}	NA	NA	NA
AAS7	NA	NA	NA	NA	NA	68.54 ± 2.3	NA	NA
AAS8	75.5 ± 2.5^{a}	68.33 ± 2.9^{b}	60.32 ± 3.4^{c}	NA	NA	NA	NA	NA
Penicillin G	235 ± 3.4^{b}	420.34 ± 2.5^{a}	166.24 ± 2.3^{e}	220.44 ± 2.6^{bc}	210.44 ± 2.3^{bcd}	180.14 ± 2.1^{f}	168 ± 3.1^{e}	124.32 ± 3.4^{g}

NA – no activity; values are represented as mean±SD of experiments carried out in triplicate (n = 3).
^{abcdefg}Values with different superscript letters within the same row are significantly ($P \leq 0.05$) different.

observed as gram-positive cocci and non-spore forming based on microscopic study. Colonies were observed to be small, smooth, round, and creamy white in color. The isolate showed negative results toward indole, voges-proskauer, citrate utilization, coagulase, arginine, malonate, and methyl red tests. In contrast, the isolate showed positive results toward catalase, ONPG, and nitrate reductase (table not shown). Based on 16S rRNA sequencing and BLAST, NCBI similarity search reports, the isolate was identified as *Staphylococcus saprophyticus* strain AAS1 (Accession number – MN197708).

3.3. *Tolerance to low pH, simulated gastric juice, and bile salt*

The viability of strain AAS1 decreased with increase in the acidity and exhibited remarkable growth of 2.2 ± 0.16 log cfu/mL at pH 2.0 (Fig. 2a). No significant differences ($P > .05$) in the cell viability were observed at pH 6.5 (control) and pH 6.0. However, the cell viability decreased significantly ($P < .05$) with further increase in acidic condition up to pH 2.0. The strain revealed pronounced tolerance ($P < .05$) to simulated gastric juice of pH 2, 3, and 4 with viability of 2.2 ± 0.2, 4.2 ± 0.18, and 5.1 ± 0.18 log cfu/mL, respectively, after 3 h of incubation period (Fig. 2b). Further, the isolate showed high resistance to oxgall (0.5% w/v) with increase in incubation period up to 36 h. A gradual reduction in absorbance was observed afterward with respect to control (Fig. 2c).

3.4. *Adhesion and auto-aggregation traits*

Strain AAS1 exhibited significantly ($P < .05$) high hydrophobicity characteristic toward toluene (64.2 ± 1.3%). No significant differences ($P > .05$) in hydrophobicity traits were estimated in the presence of chloroform (48.5 ± 1.3%) and ethyl acetate (42.6 ± 1.3%) at all incubation periods (Fig. 3a). In like manner, strain AAS1 showed the highest auto-aggregation property (44.3 ± 1.5%, $P < .05$) at 48 h of incubation period (Fig. 3b).

3.5. *Tolerance to phenol and lysozyme*

Figure 4 shows the survival ability of strain AAS1 in the presence of phenol. No significant differences ($P > .05$) were observed in the viability of control (without phenol) and treated (with phenol) cells at 0 h of incubation. However, the treated cells showed slight significant ($P < .05$) reduction in total survival count (6.3 ± 0.12 log cfu/mL) with respect to control (8.4 ± 0.1 log cfu/mL) at 24 h of incubation. Likewise, strain AAS1 was found to be lysozyme resistance, thereby revealing maximum viability of 52.3 ± 1.6% with respect to control (Figure not shown).

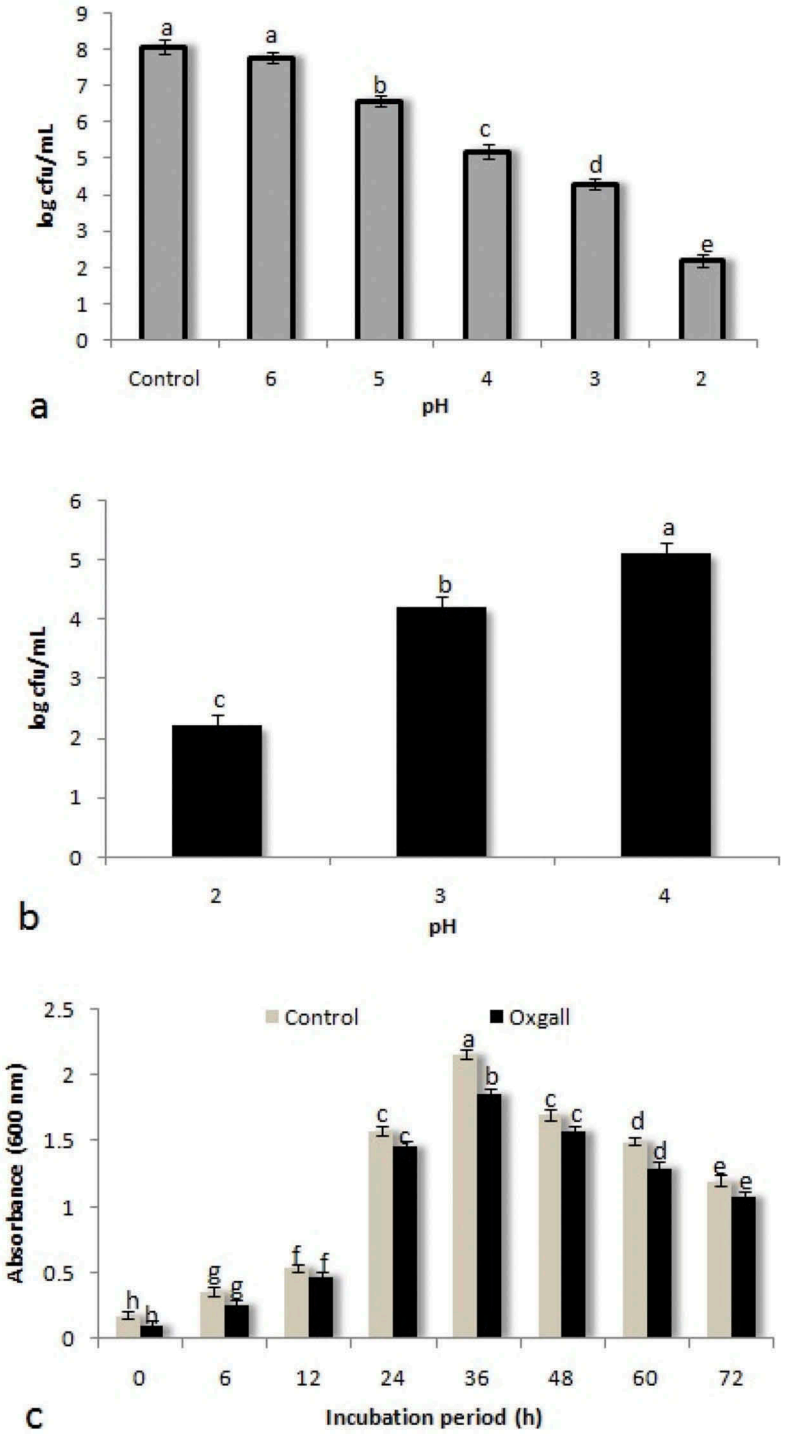

Figure 2. Tolerance of strain AAS1 toward (a) acidic condition ranging from pH 6.0 to 2.0, (b) simulated gastric juice of pH 2.0, 3.0, and 4.0, and (c) bile salt (0.5% w/v oxgall) up to 72 h. Data represent mean±SD. [abcdefgh]Values were compared to each other and distinct letters indicate significant differences ($P < .05$).

3.6. *Proteolytic activity and antioxidant properties*

Strain AAS1 indicated strong protease activity of 14 ± 0.6 mm by revealing clear zone of proteolysis on skim milk agar medium (figure not shown). Table 2 illustrates DPPH scavenging attribute of strain AAS1 at varied concentrations. The gradual increase in the degradation of DPPH, ranging from 16.6 ± 1.2 to 67.5 ± 1.1% was estimated in a concentration-dependent manner of strain AAS1 (100–1000 µL). Figure 5 shows the resistance

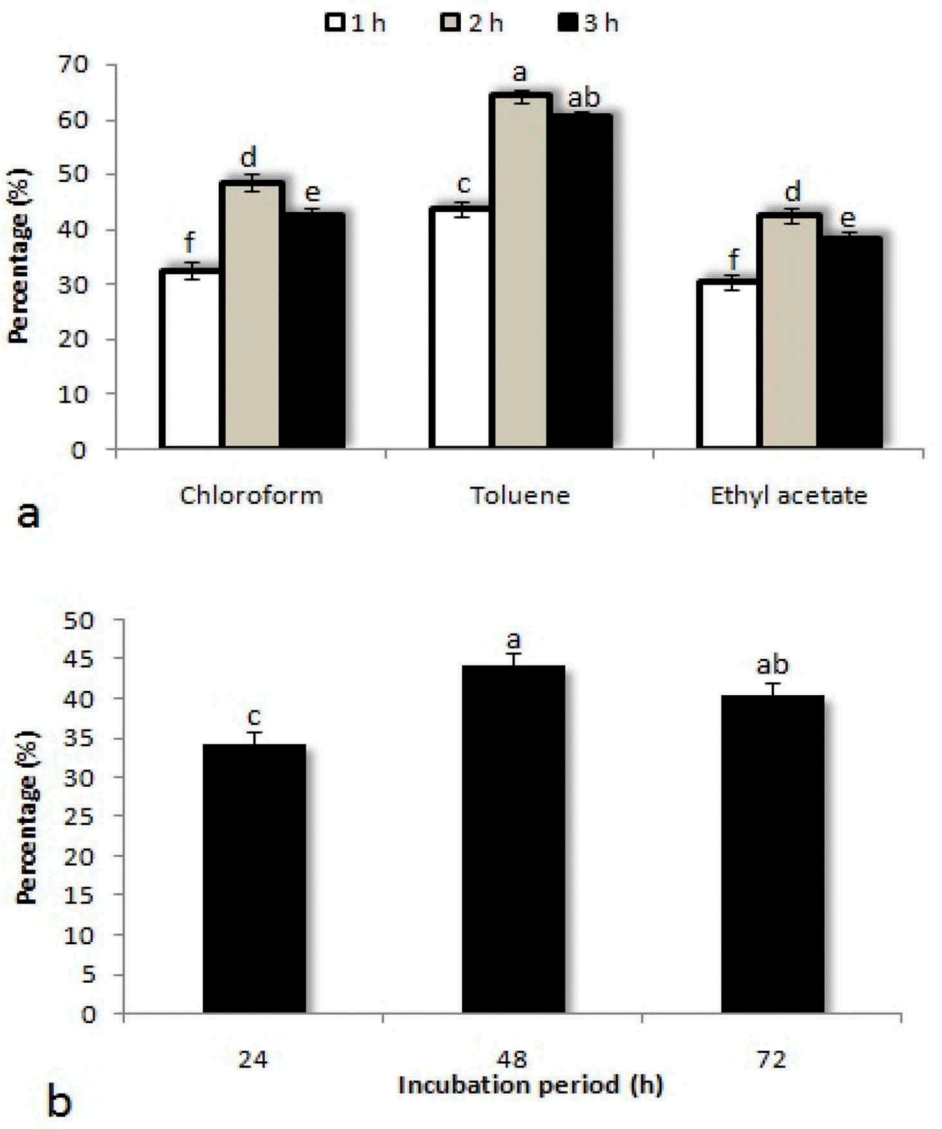

Figure 3. (a) Cell surface hydrophobicity toward chloroform, toluene, and ethyl acetate up to 3 h of incubation period and (b) auto-aggregation trait up to 72 h for strain AAS1. Data represent mean±SD. [abcdef]Values were compared to each other and distinct letters indicate significant differences ($P < .05$).

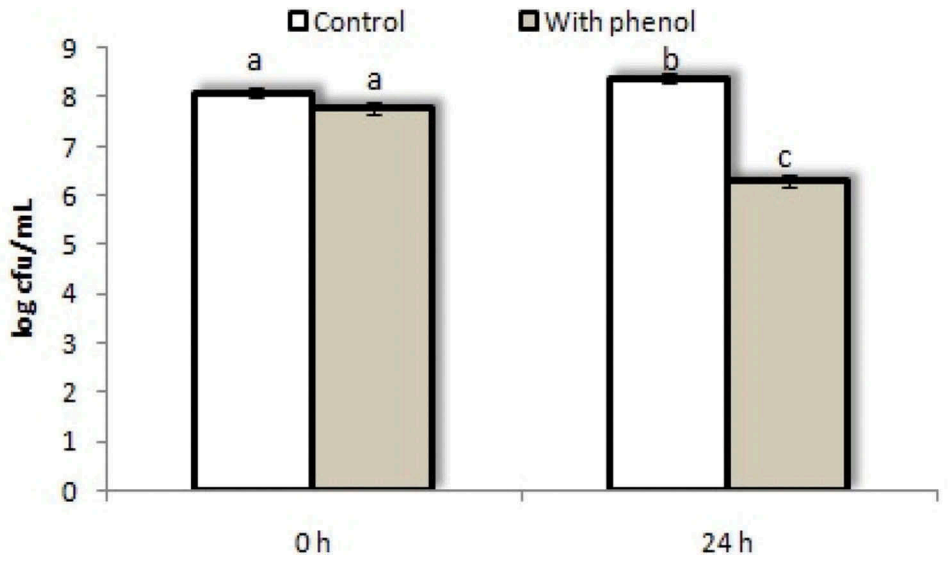

Figure 4. Survival capability of strain AAS1 in the presence of phenol at 0 and 24 h of incubation period. Data represent mean±SD. [abc]Values were compared to control and distinct letters indicate significant difference ($P < .05$).

Table 2. DPPH and hydroxyl radical scavenging activities of strain AAS1 and ascorbic acid at various concentrations.

	DPPH scavenging activity (%)		Hydroxyl radical scavenging activity (%)	
Concentration (µL)	Strain AAS1	Ascorbic acid	Strain AAS1	Ascorbic acid
100	16.6 ± 1.2^{e}	48.3 ± 1.5^{e}	10.6 ± 1.2^{d}	41.6 ± 1.2^{f}
200	34.5 ± 1.3^{d}	62.2 ± 1.4^{d}	22.5 ± 1.3^{c}	58.4 ± 1.4^{e}
400	48.4 ± 1.1^{c}	75.4 ± 1.3^{c}	29.4 ± 1.1^{c}	64.5 ± 1.1^{d}
600	58.5 ± 1.2^{b}	87.3 ± 1.4^{b}	42.5 ± 1.2^{b}	72.6 ± 1.3^{c}
800	62.5 ± 1.2^{a}	92.4 ± 1.5^{a}	51.5 ± 1.2^{a}	78.4 ± 1.2^{b}
1000	67.5 ± 1.1^{a}	96.5 ± 1.4^{a}	57.5 ± 1.1^{a}	84.4 ± 1.3^{a}

Values are represented as mean±SD of experiments carried out in triplicate (n = 3).
[abcdef]Values with different superscript letters within the same column are significantly ($P \leq 0.05$) different.

property of strain AAS1 in the presence of diversified concentrations of H_2O_2. Results showed that strain AAS1 had the potentiality to resist H_2O_2 (0.5–2 mM), exhibiting absorbance values of 2.1 ± 0.04 to 1.1 ± 0.04. In like manner, strain AAS1 showed increased hydroxyl radical scavenging property (10.6 ± 1.2 to 57.5 ± 1.1%) in a concentration-dependent manner (Table 2).

3.7. *Technological properties of strain AAS1*

Strain AAS1 exhibited pronounced growth in MRS medium constituting dextrose and sucrose with absorbance value of 2.4 ± 0.04 and 2.2 ± 0.04,

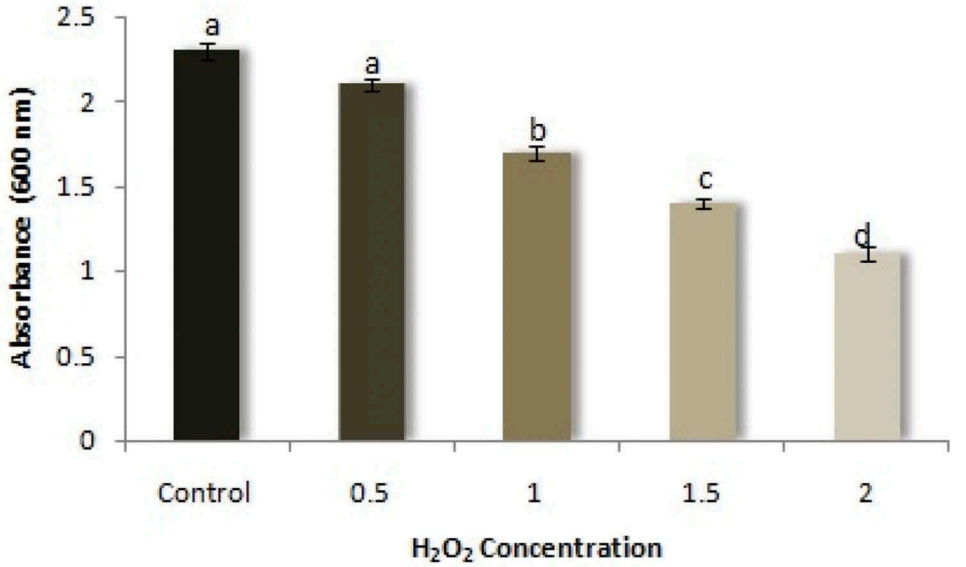

Figure 5. Resistance property of strain AAS1 in the presence of varying concentrations of H_2O_2 (0.5–2.0 mM). Data represent mean±SD. [abcd]Values were compared to control and distinct letters indicate significant difference ($P < .05$).

respectively, after 48 h of incubation period. Other carbohydrates caused comparatively lower growth of strain AAS1 with absorbance values ranging from 1.2 ± 0.03 to 1.6 ± 0.02 (Fig. 6a). The isolate showed slime formation or EPS production and moderate lipase activity after 48 h of incubation (figure not shown). On the other hand, amylolytic activity of strain AAS1 was not observed (figure not shown).

At 6, 12, and 24 h of incubation, the autolysis for strain AAS1 differed significantly ($P < .05$) and observed as 34.3 ± 1.2, 42.1 ± 1.3, and 48.6 ± 1.2%, respectively (Fig. 6b). Catalase activity of strain AAS1 increased significantly ($P < .05$) up to 24 h within the range of 10.34 ± 0.3 to 18.14 ± 0.3 AU (Fig. 6c). Likewise, the reduction of nitrate to nitrite by strain AAS1 was detected spectrophotometrically and showed significant differences ($P < .05$) in terms of nitrite production. The amount of nitrite released from the strain increased significantly up to 24 h with maximum yield of 26.3 ± 0.8 mM nitrite/mg dry weight (Fig. 6d).

3.8. In vitro *safety evaluation tests*

The isolate was observed for their safety for applications by screening *in vitro* biofilm formation trait. Strain AAS1 showed absorbance value <1, thereby indicating lack of *in vitro* biofilm formation characteristic (data not shown). Further, the isolate showed negative results toward

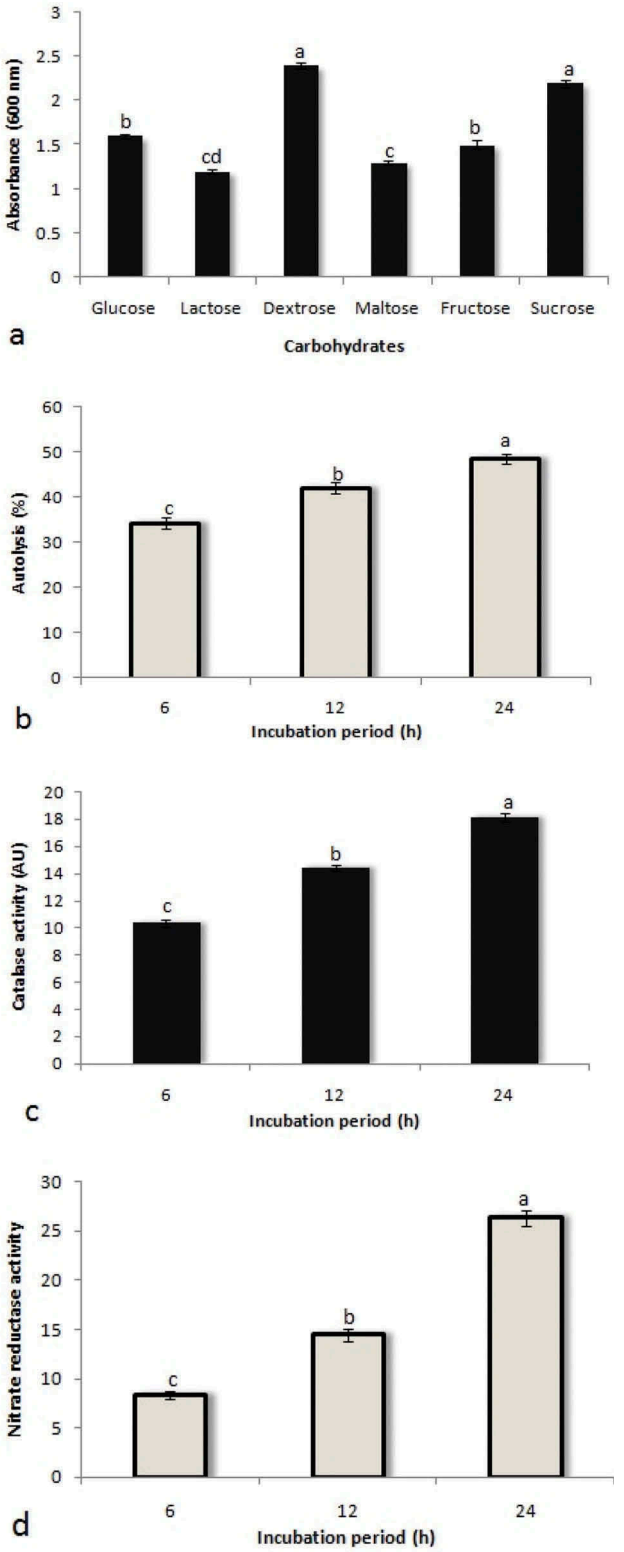

Figure 6. (a) Carbohydrates fermentation, (b) rate of autolysis, (c) catalase production, and (d) nitrate reductase activity of strain AAS1. Data represent mean±SD. [abcd]Values were compared to each other and distinct letters indicate significant differences ($P < .05$).

in vitro hemolytic, DNase, and gelatinase tests, as confirmed by lack of zone surrounding the bacterial culture (figure not shown). Additionally, the isolate was found susceptible to all the conventional antibiotics tested (Fig. 7). The growth inhibitory activity of antibiotics was observed in the order of penicillin G (30.5 ± 0.5 mm) > rifampicin (28.4 ± 0.5 mm) > chloramphenicol (26.4 ± 0.3 mm) > gentamicin (25.2 ± 0.5 mm) > kanamycin (22.1 ± 0.4 mm) > streptomycin (16.3 ± 0.6 mm).

4. Discussion

In the present investigation, the CFNS of strain AAS1 exhibited broad-range of antagonistic activities against foodborne and enteric pathogens. Thapa, Pal, and Tamang (2004) affirmed antibacterial activities of *Ngari* associated probiotic bacteria against distinct indicator pathogens. In another report, Khusro et al. (2018) demonstrated antagonistic traits of *Ngari* associated *S. succinus* strain against urinary tract infection causing bacteria. In general, probiotics are known to possess anti-pathogenic properties by secreting diverse group of active components such as reuterin, diacetyl, D-isomers of amino acids, bacteriocins, and acetaldehyde (Khusro et al. 2018). The secretion of these bioactive constituents into the medium may potentially be the cause of anti-pathogenic attributes of CFNS of strain AAS1 against foodborne and enteric pathogens tested.

In the present study, strain AAS1 disclosed its potential to resist various ranges of acidity and simulated gastric juice with high survival logarithmic

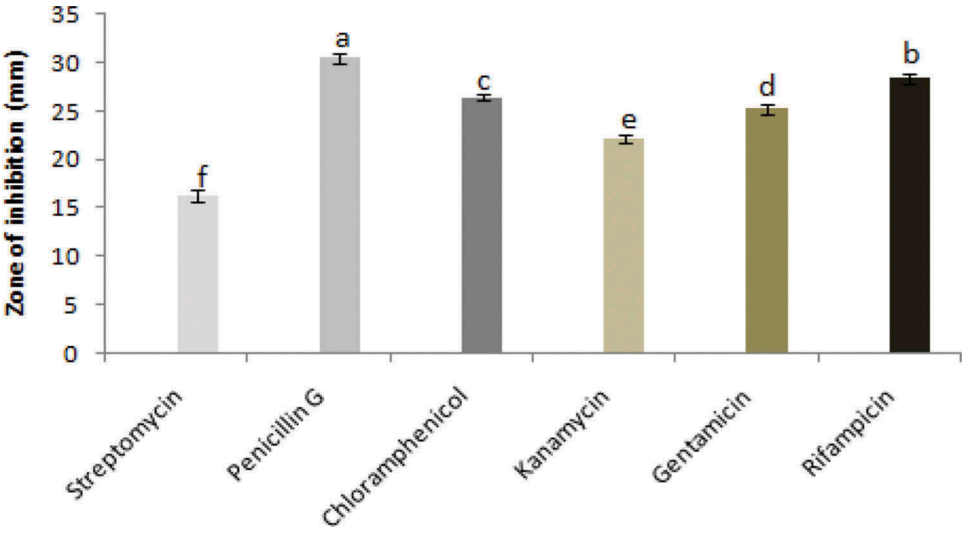

Figure 7. Diameter of zone of inhibition (mm) of conventional antibiotics against strain AAS1. Data represent mean±SD. [abcdef]Values were compared to each other and distinct letters indicate significant differences ($P < .05$).

cell counts. Previously, Borah et al. (2016) and Sathyabama et al. (2012) also demonstrated the significant viabilities of staphylococci at low pHs and in the presence of simulated gastric juice, respectively. Likewise, resistance to bile salt is a prerequisite for the colonization of probiotic bacteria in small intestine of host. In fact, duodeno-gastric biliary reflux mechanism needs the bacteria to tolerate bile salt (Dunne et al. 2001). The physiological concentrations of bile salt in human vary from 0.3% to 0.5% and are produced because of the catabolism of cholesterol. In the present context, strain AAS1 showed resistance toward high concentration of bile salt (0.5% w/v) for a longer period. Simonova et al. (2006) also reported promising tolerance rate of staphylococci to higher (1% w/v) concentration of bile salt.

Surface properties of cells are critical essential factors for probiotic strains that have the ability to adhere to the mucosal surface in the intestinal tract and resulting in health benefits to the host (Kos et al. 2003). In this investigation, strain AAS1 indicated high hydrophobicity characteristics toward toluene, followed by chloroform and ethyl acetate. Our results were observed to be in complete concurrence with the findings of Borah et al. (2016) who showed significant hydrophobicity trait of *Staphylococcus* sp. in the presence of toluene. The environmental conditions affecting the expression of surface protein might be the cause of high variations in the cell surface hydrophobicity trait among probiotic strains (Khusro et al. 2018). Likewise, strain AAS1 showed reliable auto-aggregation property (34–44%) too, thereby indicating its specific binding abilities in gastro-intestinal tract. The capability of strain AAS1 to adhere to different hydrocarbons and to exhibit auto-aggregation signifies its capacity to colonize intestinal epithelia effectively.

The resistance of bacteria to phenol is a crucial probiotic characteristic because phenol is produced in the intestine due to the deamination of few aromatic amino acids (Khusro et al. 2018). Similarly, tolerance to lysozyme represents the unique trait of strain AAS1 in order to survive in the human mouth if it is administered orally because lysozyme is the first biological barrier of the human digestive system (Turchi et al. 2013). In agreement with our findings, previous studies also reported the significant viabilities of staphylococci in the presence of phenol and lysozyme (Borah et al. 2016; Sathyabama et al. 2012).

The proteolytic characteristics of probiotics are known to improve organoleptic properties of fermented food products. In this study, strain AAS1 revealed proteolytic trait by showing the zone of hydrolysis on skim milk agar medium. Extracellular protease production from probiotics hydrolyzes the milk protein and induces the peptidases as well as free amino groups (Khusro et al. 2018).

A unique property shown by strain AAS1 is its unique potential as an antioxidant agent by degrading DPPH, tolerating higher concentrations of H_2O_2, and scavenging hydroxyl radical in a concentration-dependent manner. Similar observation regarding prominent antioxidant trait of probiotic

bacteria was previously reported by Lee et al. (2005) and Khusro et al. (2018). The antioxidant attribute of strain AAS1 might be mainly because of the presence of surface-active components that degrade radicals and chelate transition metal ions (Kao and Chen 2006).

Strain AAS1 showed clear variations in the fermentation abilities of various carbohydrates and had remarkable growth in MRS medium supplemented with dextrose and sucrose. In fact, CNS contributes to the enhancing of the sensory qualities of fermented foods via the catabolism of carbohydrates (Jeong et al. 2016).

The qualitative evaluation of EPS production from strain AAS1 reflected its role not only as a potential bio-thickener but also indicated the potential for adapting to various harsh conditions in food environments. Fanning et al. (2012) demonstrated that EPS helps the producing bacterium tolerate dehydration and the adverse conditions of acid and bile availability. In like manner, according to Owusu-Kwarteng et al. (2015), EPS production enriches the aggregation characteristics of probiotics.

The isolation of amylase producing probiotic bacteria could be of economic values due to their capability to diminish the viscous nature of bulky, starchy, and weaning porridges (Owusu-Kwarteng et al. 2015). Surprisingly, in this study, strain AAS1 did not show amylolytic activity. However, apart from few lactobacilli strains indicating significant amylase activity in previous reports (Adesulu-Dahunsi, Jeyaram, and Sanni 2018; Tallapragada et al. 2018), the high prevalence of amylolytic probiotic bacteria has not been reported yet. Likewise, the flavor and texture of fermented food are consistently influenced by lipase activity of its autochthonous microbiota. In this investigation, strain AAS1 showed moderate production of lipase, thereby supporting the previous findings of Jeong et al. (2016) who reported moderate yield of lipase from few CNS strains.

The dexterity of probiotics to exhibit autolysis and subsequently producing intracellular enzymes is a desirable trait as starter culture preparation (Piraino et al. 2008). Strain AAS1 revealed potent autolysis trait, suggesting its potential applications in advancing the texture, aroma, and flavor of foods during the fermentation process. On the other hand, strain AAS1 showed increased catalase activity at regular time intervals. Similar observations were reported by Mauriello et al. (2004) and Casaburi et al. (2005) who estimated the lowest to the highest levels of catalase yield from diversified CNS strains, and hence, suggested catalase activities of probiotic bacteria a strain-specific trait. Furthermore in this study, strain AAS1 exhibited the potentiality to reduce nitrates to nitrites, supporting previous studies of Mauriello et al. (2004) and Casaburi et al. (2005) who demonstrated nitrate reductase activity of distinct CNS strains indigenous to fermented sausages. As a matter of fact, nitrate reductase activity is considered to be the most decisive trait of CNS for utilizing as adjunct cultures during the preparation of multifarious fermented foods.

The biofilm-forming ability of probiotics is not a direct virulence property, but it is a negative trait in food industries as starter culture because it induces pathogenicity and antibiotics resistance pattern in bacteria (Khusro et al. 2018). In this context, strain AAS1 showed lack of *in vitro* biofilm formation ability. As per the report of Jeong et al. (2016), biofilm-forming characteristics of CNS are strain-dependent mechanism, regardless of similar species. The lack of *in vitro* hemolytic, DNase, and gelatinase activities is recommended as important selection criterion for starter strains in fermented food preparation (Aarti and Khusro 2019). In this context, *in vitro* safety assessment tests of strain AAS1 represented the absence of hemolytic, DNase, and gelatinase activities.

Antibiotics sensitivity pattern of strain AAS1 exhibited susceptibility to all the conventional antibiotics tested, thereby suggesting the safe utilization of the bacterium because of the lack of resistance to the antibiotics used. Results of our study are in agreement with the recent report of Leite et al. (2015) who observed wide sensitivity pattern of Brazilian kefir grains associated probiotic bacteria to all the antimicrobial agents analyzed.

5. Conclusion

To the best of our knowledge, the current investigation ascertained the first report on the isolation and identification of *S. saprophyticus* strain AAS1 from *Ngari*. Strain AAS1 showed anti-pathogenic activities against diversified foodborne and enteric infection causing bacterial pathogens. The isolate indicated efficiency to endure unfavorable environmental conditions such as high acidity, simulated gastric juice, and bile salt. Furthermore, strain AAS1 exhibited pronounced cell surface properties, resistance toward phenol and lysozyme, and susceptibility to common conventional antibiotics analyzed. Most importantly, the isolate showed antioxidants characteristics, favorable distinct technological traits, and revealed *in vitro* safety aspects, thereby supporting its wide applications as an ideal adjunct culture in targeted food fermentation and potentially in processing applications.

Disclosure statement

No potential conflict of interest was reported by the authors.

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