Microbial Pathogenesis 118 (2018) 126–132

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Antagonistic trait of *Staphylococcus succinus* strain AAS2 against uropathogens and assessment of its *in vitro* probiotic characteristics

Ameer Khusro[a], Chirom Aarti[a], Abdelfattah Z.M. Salem[b,*], German Buendía Rodríguez[c], Raymundo Rene Rivas-Cáceres[d]

[a] Research Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai 600034, Tamil Nadu, India
[b] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Mexico
[c] Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, INIFAP, Ajuchitlán, Querétaro, 76280, Mexico
[d] Universidad Autónoma de Ciudad Juárez, Ave. Plutarco Elías Calles No. 1210, FOVISSSTE Chamizal Cd., Juarez C.P. 32310, Mexico

ARTICLE INFO

Keywords:
Antagonistic activity
Probiotic properties
S. succinus
Uropathogens

ABSTRACT

The desideratum aim of the present context was to isolate a promising antagonist probiotic bacterium from fermented food item as biocontrol agent against uropathogens. Among diversified isolates evaluated for antagonistic trait, *Staphylococcus succinus* strain AAS2 was found to be an auspicious candidate against urinary tract infection (UTI) causing bacterial pathogens, being the most active against *Staphylococcus aureus* with substantial activity of 352.5 ± 5.4 AU/mL. Further, the *in vitro* probiotic attributes of strain AAS2 were assessed using systematic methodology. The isolate exhibited tolerance to acidic condition (up to pH 3.0) and simulated gastric juice (at pH 3.0) with fairly high survival logarithmic cell counts of 5.3 ± 0.15 and 5.23 ± 0.02 log cfu/mL, respectively. Additionally, strain AAS2 showed capability to resist 0.5% w/v bile salt too. It also revealed significant values of auto-aggregation (32.5 ± 1.3–56.5 ± 1.4%) and cell surface hydrophobicity (38.35 ± 1.4%) properties. The isolate showed resistivity towards phenol (6.8 ± 0.08 log cfu/mL) and lysozyme (58.6 ± 1.6%). Further, the susceptibility trait of strain AAS2 to conventional antibiotics made this isolate a promising probiotic bacterium. Most importantly, the isolate depicted DPPH (2,2-Diphenyl-1-picrylhydrazyl) and hydroxyl radical scavenging activities in a concentration dependent manner, thereby exhibiting its propitious antioxidative properties. In a nutshell, the outcomes of this investigation divulge the plausible use of *S. succinus* strain AAS2 as biocontrol agent against uropathogens, and recommended further applications in pharmaceutics due to its pronounced probiotic traits.

1. Introduction

Fermented foods act as reservoirs for diversified groups of beneficial microorganisms. Probiotics are live microbes that confer a beneficial impact on the health of all age groups when supplemented in appropriate quantity [1]. In fact, probiotics are known to alter the microfloral balance of intestine, growth inhibition of pathogens, promotion of good digestion, boosting of immunity, and improved tolerance to various diseases [2].

Several bacterial species are regarded as a major group of probiotic bacteria due to their non-pathogenic, acid tolerance, bile tolerance, and antimicrobial substances production attributes [3], thereby classified under 'generally recognized as safe (GRAS)' category. Among them, *Lactobacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Bacillus* spp., *Bifidobacterium* spp., and *Escherichia coli* are predominant bacteria. However, in recent years, coagulase-negative staphylococci (CNS) have emerged as different group of fermented food associated bacteria revealing probiotic properties. Coagulase-negative staphylococci are opportunistic pathogens, gram positive, and catalase positive cocci bacteria. Lactic acid bacteria in combination with CNS are frequently used as starter cultures in fermented food products.

At present, the emergence of multidrug-resistant bacteria has attracted attention to attain effective prevention, durable cure, and treatment of the distending problems of growing infections [4]. Previously, Sung et al. [5] demonstrated the antagonistic property of *Staphylococcus hominis* (CNS bacteria) against *S. aureus* as biocontrol agent. Surprisingly, there is no report on the growth inhibition property of CNS against urinary tract infection (UTI) causing bacteria.

Urinary tract infection is the second most common bacterial infection and has a significant societal and economic burden worldwide. Pathogenic bacteria viz. *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus* spp., and *Enterococcus* spp. are

* Corresponding author.
E-mail addresses: salem@uaemex.mx, asalem70@yahoo.com (A.Z.M. Salem).

https://doi.org/10.1016/j.micpath.2018.03.022
Received 20 February 2018; Received in revised form 8 March 2018; Accepted 13 March 2018
Available online 14 March 2018

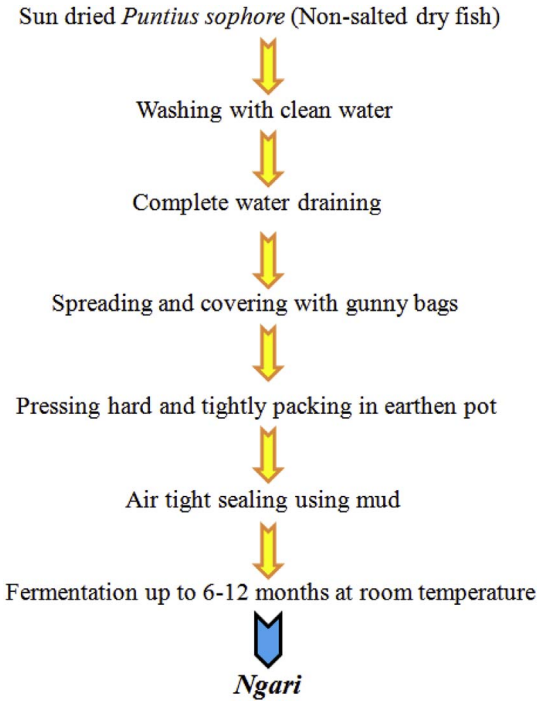

Fig. 1. Preparation of *Ngari*.

the leading cause of UTI. These bacteria have various virulence factors that increase their pathogenesis. Urinary tract infections are causing an increased resistance to conventional antibiotics [6], and may result into end stage renal failure or hypertension if continued. Hence, there is an urgency to identify propitious alternative agents of existing antibiotics from un/less exploited sources in order to combat this infection globally. Considering this, the present context was investigated to isolate new strain of fermented food associated CNS for its antagonistic activity against UTI causing pathogenic bacteria, and to evaluate further its *in vitro* probiotic characteristics.

2. Materials and methods

2.1. Bacterial isolation from fermented food

Ngari (a fermented food item of North-East India) was prepared according to the methodology as described in Fig. 1. One gram of *Ngari* was mixed with 10 mL of autoclaved distilled water, followed by centrifugation at 2000 *g* for 10 min for settling the unmixed materials. The supernatant was collected and serially diluted. One millilitre of the suspension was spread 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, Agar 18) plates and incubated at 30 °C for the appearance of different colonies on the agar plates after 48 h. Different colonies were selected and streaked on the sterilized MRS agar plates. The pure form of cultures was maintained in 50% (v/v) glycerol stock at −80 °C for further experiments.

2.2. Inhibitory effect of bacteria on uropathogens

2.2.1. Indicator bacterial pathogens

Urinary tract infection causing bacteria viz. *Escherichia coli* (*E. coli*), *Proteus mirabilis* (*P. mirabilis*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Staphylococcus saprophyticus* (*S. saprophyticus*) were used as indicator pathogens. *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were grown in nutrient broth (g/L: peptone 5.0, yeast extract 1.5, beef extract 1.5, sodium chloride 5.0, pH 7.2) medium. *P. mirabilis* was grown in Mac Conkey broth (g/L: gelatin peptone 20.0, lactose monohydrate 10.0, dehydrated bile 5.0, bromo cresole purple 0.02, pH 7.2) medium. *S. aureus* and *S. saprophyticus* were sub-cultured in Mannitol Salt broth (proteose peptone 10.0, beef extract 1.0, sodium chloride 70.0, D-Mannitol 10.0, phenol red 0.025, pH 7.2) medium.

2.2.2. Antagonistic activity of isolates

Sterile MRS broth media were used for the inoculation of purified bacteria isolated from *Ngari*. The broth media were further kept in orbital shaker at 30 °C for 48 h of incubation period, and further centrifuged at 6000 g for 10 min. The culture supernatant was filtered using membrane filtration (0.22 μm). The well diffusion method was used for assaying the antibacterial activity. Mueller Hinton agar (g/L: beef heart infusion 2.0, casein acid hydrolysate 17.5, soluble starch 1.5, agar 20.0, pH 7.2) plates were punched, and 5 mm wells were created. Hundred microlitres of bacterial supernatant were poured into the wells for determining the antagonistic activity. Streptomycin was used as positive control for this assay. The clear zone of inhibition was measured after 24 h of incubation and the antagonistic property was represented in arbitrary units (AU/mL).

2.3. Identification of potent isolate

The Bergey's Manual of Systemic Bacteriology was implied to determine the morphological, physiological, and various biochemical characteristics of the most active isolate. Gram staining test was performed to identify Gram (+) or Gram (-) isolate. Biochemical tests such as indole, voges-proskauer, citrate utilization, nitrate reductase, coagulase, arginine, malonate, and methyl red were carried out under aseptic conditions. Bacterial DNA isolation and purification were carried out using NucleoSpin® Tissue Kit (Macherey-Nagel). Specific primers were used for the amplification process at optimum conditions. The amplicon obtained was purified and sequenced. The obtained sequences were submitted to Genbank and accession number was assigned.

2.4. Probiotic properties of isolates

2.4.1. Resistance to low pH

The low pH tolerance characteristic of isolate was evaluated based on the methodology of Ramos et al. [7] with minor changes. The exponentially phase grown isolate in MRS broth was centrifuged at 6000 g at 4 °C for 10 min. On the other hand, the pH of the freshly prepared sterilised MRS broth media was adjusted from 3.0 to 6.0 and bacterial cells were resuspended in it for 3 h. Bacterial suspensions were further serially diluted using phosphate buffer saline (PBS) and streaked on sterilized MRS agar plates. Total cell viability was estimated by plate count technique after 48 h in terms of log cfu/mL.

2.4.2. Tolerance to simulated gastric juice

The resistivity trait of isolate to simulated gastric juice was evaluated based on the methodology of Charteris et al. [8] with slight modification. Pepsin (3 mg/mL) and sodium chloride (0.5% w/v) were used for the preparation of simulated gastric juice, and pH of the solution was maintained to 3.0 and 4.0. The overnight grown isolate was centrifuged at 6000 g for 10 min. Bacterial cells were mixed into 3 mL of K_2HPO_4 solution (50 mM). Nine millilitres of simulated gastric juice were mixed with bacterial cell suspension and incubated at 37 °C for 3 h. Bacterial cells viability was estimated after 48 h as described earlier.

2.4.3. Resistance to bile salt

The resistance of isolate to bile salt was assessed according the modified methodology of Kumar et al. [9]. Overnight grown isolate was

inoculated into MRS broth supplemented with oxgall (0.5% w/v) and incubated at 37 °C for 48 h. After that, aliquots of this suspension were withdrawn, serially diluted using saline, and streaked on agar plates. Viable cell colonies were enumerated up to 48 h in a comparison with control culture (without oxgall).

2.4.4. Cell surface hydrophobicity

The adherence property of bacteria to hydrocarbons was analyzed by employing the methodology of Mishra and Prasad [10] with minor modifications. The isolate was inoculated in MRS broth for 18 h, and centrifuged at 6000 g for 10 min. The pellet was collected, suspended into PBS (pH 7.0), and the absorbance was read at 600 nm (A). Three millilitres of cell suspension were mixed with 1 mL of chloroform. The mixture was vortexed for 1 min, incubated at 37 °C for 3 h to separate carefully the aqueous phase from organic phase every 1 h, and decrease in optical density was measured at 600 nm (B). The cell surface hydrophobicity (%) was calculated according to the equation given below

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

2.4.5. Auto-aggregation

Cell auto-aggregation trait of bacteria was evaluated according to the modified methodology of Juarez Tomas et al. [11]. The isolate was grown in MRS broth for 18 h and pellets were collected after centrifugation at 6000 *g* for 10 min. Cells were suspended in PBS (pH 7.0) and the absorbance was adjusted to 0.5–0.6 at 600 nm (A), being further incubated at 37 °C for 24 h. Absorbance was measured at 600 nm (B) after separating 1 mL of the upper layer. Auto-aggregation (%) was estimated as follows

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

2.4.6. Tolerance to phenol and lysozyme

The resistant property of isolate to phenol solution was determined according to the modified methodology of Xanthopoulos et al. [12]. The overnight grown isolate was incubated in MRS broth constituting 0.5% phenol, and incubated at 37 °C for 24 h. After 0 h and 24 h, bacterial suspension was spread on MRS agar plates and the cell viability was enumerated in a comparison with control (without phenol).

The resistivity of isolate to lysozyme was evaluated according to the methodology of Zago et al. [13]. The isolate was grown for 18 h at 37 °C and centrifuged at 8000 g for 15 min. The pellet was suspended into PBS (pH 7.0) and re-suspended in Ringer solution [g/L: $CaCl_2$ 0.2; NaCl 6.0; KCl 2.0; $NaHCO_3$ 1.2) containing lysozyme (100 μg/mL). The sample was incubated at 37 °C for 2 h and percentage cell viability was calculated in a comparison with control (without lysozyme).

2.4.7. Proteolytic property

The log phase grown isolate was centrifuged at 6000 g for 10 min at 4 °C for the collection of supernatant. The skim milk agar medium was autoclaved and poured into sterile plate for solidification. After that, the bacterial supernatant was poured into the prepared wells for the diffusion process. The plate was incubated at 30 °C for 24 h and assessed for protease production by observing clear zone of hydrolysis.

2.4.8. Antibiotics susceptibility assay

The susceptibility assay of isolate to various antibiotics was evaluated by disc diffusion method. A homogeneous lawn of the exponentially phase grown isolate was prepared on MRS agar plates. Antibiotic discs viz. Streptomycin (10 μg), Penicillin G (10 μg), Chloramphenicol (30 μg), Kanamycin (30 μg), Gentamicin (10 μg), and Rifampicin (5 μg) were placed aseptically over the agar plates and incubated undisturbed at 37 °C for 48 h. The diameter of the zone of bacterial growth inhibition was measured after required period of incubation.

2.4.9. Antioxidant activity

2.4.9.1. DPPH scavenging property. The DPPH (2,2-Diphenyl-1-picrylhydrazyl) degrading property of isolate was estimated based on the modified protocol of Chen et al. [14]. The DPPH solution (1 mL; 0.05 mM) was mixed with varied doses (100–1000 μL) of bacterial cells (absorbance of 0.5–0.6) and kept in dark for 20–30 min at room temperature. The DPPH solution and ascorbic acid were used as control and standard, respectively. Cells along with methanol were used as blank during the assay and the absorbance was measured at 517 nm. The scavenging activity (%) was calculated according to formula given below-

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

2.4.9.2. Hydroxyl radical scavenging property. Hydroxyl radical degrading property of isolate was determined based on the modified methodology of He et al. [15]. Various concentrations (100–1000 μL) of overnight grown bacterial cells were inoculated into a reaction mixture constituting Brilliant Green (1 mL; 0.5 mM), $FeSO_4$ (2 mL; 0.5 mM), and H_2O_2 (1.5 mL). The suspension was kept at room temperature for 15 min and the optical density was read at 624 nm using ascorbic acid as standard. The hydroxyl radical scavenging property was estimated as follows-

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

where A - absorbance of sample, B - absorbance of the control, and C - absorbance devoid of the sample and the Fenton reaction system.

2.5. Statistical analysis

All the experimental sections of the study were performed in triplicate and data were represented as mean ± SD.

3. Results

3.1. Antagonistic trait of isolates against uropathogens

The antagonistic activity of isolates against UTI causing bacterial pathogens is shown in Table 1. The results reflected the strong growth inhibitory property of isolate AAS2 against all the indicator pathogens in the order of *S. aureus* (352.5 ± 5.4 AU/mL) > *S. saprophyticus* (250.12 ± 4.4 AU/mL) > *P. aeruginosa* (102.13 ± 3.3 AU/mL) > *K. pneumonia* (96.32 ± 2.3 AU/mL) > *P. mirabilis* (90.75 ± 1.9 AU/mL) > *E. coli* (80.15 ± 2.2 AU/mL). The isolate AAS1 exhibited comparatively lower growth inhibitory properties against the indicator bacteria. On the other hand, *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *K. pneumonia* showed resistance towards most of the other isolates tested.

3.2. Identification of potential isolate

The potent isolate was observed as Gram positive cocci with small, smooth, round, and creamy white colonies. The biochemical tests revealed that the isolate was negative towards indole, voges-proskauer, citrate utilization, nitrate reductase, coagulase, arginine, malonate, and methyl red tests (Data not shown). The isolate was identified as coagulase-negative *Staphylococcus* sp. based on the morphological and biochemical reports. Further, 16S rRNA sequencing and BLAST, NCBI similarity search results identified the isolate as *Staphylococcus succinus* strain AAS2 (Accession no. - MG199187).

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

Fig. 2 shows the viability of strain AAS2 at low pH conditions. The strain consistently exhibited tolerance up to pH 3 with fairly high

Table 1
Antagonistic activity (AU/mL) of isolates against UTI causing bacterial pathogens.

Isolates	Indicator bacterial pathogens					
	E. coli	*P. mirabilis*	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*S. saprophyticus*
AAS1	76.14 ± 2.4	70.16 ± 2.1	81.14 ± 2.4	84.42 ± 2.4	220.12 ± 5.3	200.43 ± 4.5
AAS2	80.15 ± 2.2	90.75 ± 1.9	102.13 ± 3.3	96.32 ± 2.3	352.5 ± 5.4	250.12 ± 4.4
AAS3	NA	NA	NA	NA	88.32 ± 2.4	90.45 ± 2.6
AAS4	NA	72.14 ± 1.3	NA	NA	102.45 ± 3.3	100.56 ± 2.4
AAS5	NA	NA	NA	NA	122.43 ± 4.2	135.34 ± 3.5
AAS6	NA	80.14 ± 1.6	NA	NA	125.43 ± 4.1	121.34 ± 2.8
AAS7	70.14 ± 1.8	65.42 ± 1.7	NA	NA	90.23 ± 2.4	98.64 ± 2.3
AAS8	NA	75.43 ± 1.9	NA	NA	111.34 ± 3.2	118.76 ± 2.8
Streptomycin	NA	150.24 ± 2.5	162.14 ± 2.3	120.34 ± 2.6	450.34 ± 2.3	420.14 ± 2.1

NA – No activity.

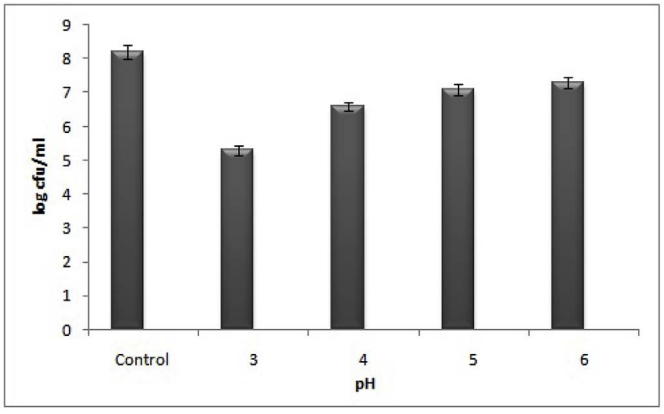

Fig. 2. Acidic pH tolerance property of strain AAS2.

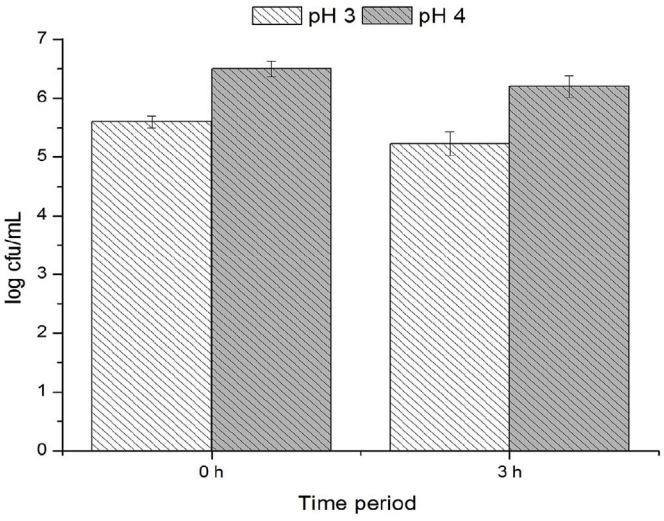

Fig. 3. Tolerance trait of strain AAS2 towards simulated gastric juice up to 3 h of incubation.

survival logarithmic cell counts of 5.3 ± 0.15 log cfu/mL in a comparison with control pH (8.2 ± 0.2 log cfu/mL). In like manner, strain AAS2 showed significant resistivity towards simulated gastric juice up to 3 h of incubation (Fig. 3). Strain AAS2 had potentiality to resist simulated gastric juice exposure and retained their viability at pH 3 and 4 with a logarithmic unit of 5.23 ± 0.02 and 6.2 ± 0.18 log cfu/mL, respectively. Strain AAS2 showed capability to resist oxgall (0.5% w/v) for 36 h with respect to control and a decrease in absorbance was observed afterwards (Fig. 4).

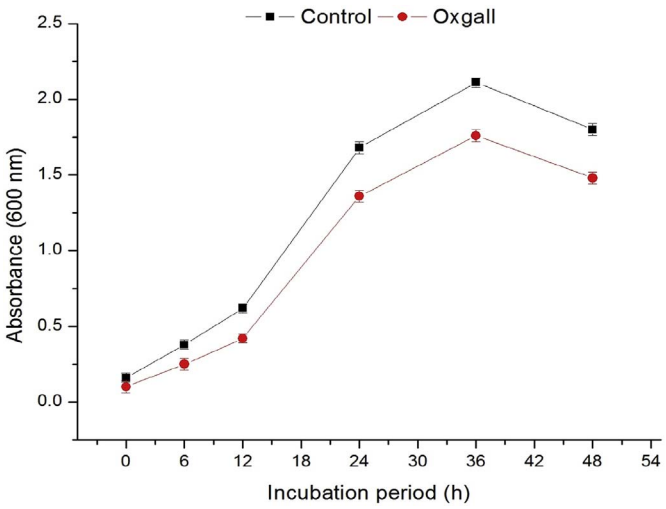

Fig. 4. Viability of strain AAS2 in the presence of bile salt up to 48 h of incubation.

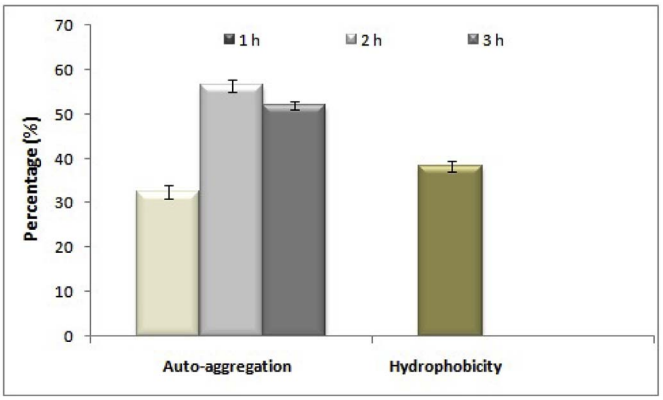

Fig. 5. Percentage auto-aggregation and hydrophobicity values of strain AAS2.

3.4. Auto-aggregation and cell surface hydrophobicity properties

Auto-aggregation and cell surface hydrophobicity values of strain AAS2 are mentioned in Fig. 5. The strain determined auto-aggregation property ranging from 32.5 ± 1.3 to 56.5 ± 1.4% with increase in incubation period. Furthermore, the isolate exhibited hydrophobicity value of 38.35 ± 1.4% in chloroform.

3.5. Tolerance to phenol and lysozyme

The effect of phenol on the growth of strain AAS2 with respect to control is shown in Fig. 6. The isolate showed resistivity towards phenol

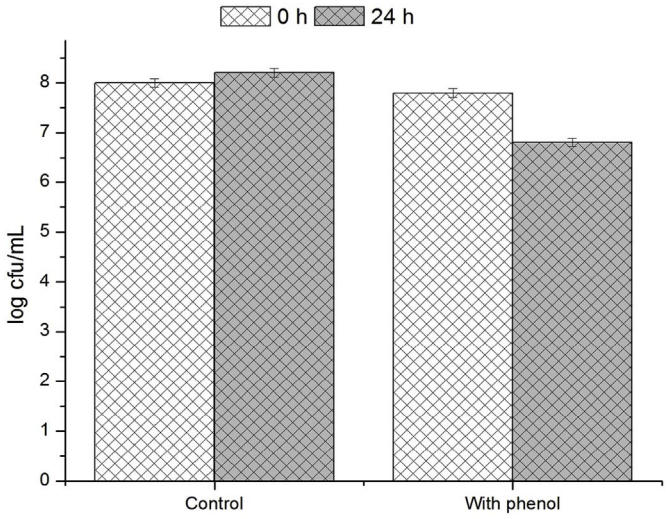

Fig. 6. Viability of strain AAS2 in the presence of phenol.

after 24 h of incubation, estimating viable counts of 6.8 ± 0.08 log cfu/mL with respect to control (8.2 ± 0.09 log cfu/mL). In like manner, strain AAS2 was found to be resistant to lysozyme with maximum cell viability of 58.6 ± 1.6% (Figure not shown).

3.6. Proteolytic and antibiotic susceptibility assay

Strain AAS2 demonstrated proteolytic property by showing hydrolytic zone of 14 ± 0.3 mm on the skim milk agar medium (Figure not shown). Likewise, the isolate was found to be sensitive to all the conventional antibiotics tested in this study (Fig. 7). Penicillin G exhibited maximum zone of inhibition of 32.5 ± 0.5 mm, followed by Gentamicin (28.5 ± 0.5 mm), Rifampicin (26.4 ± 0.5 mm), Chloramphenicol (24.42 ± 0.3 mm), Kanamycin (20.14 ± 0.4 mm), and Streptomycin (12.32 ± 0.6 mm).

3.7. Antioxidant property of isolate

The DPPH degrading property of strain AAS2 is illustrated in Fig. 8a. The isolate exhibited degradation values of DPPH in a dose dependent manner ranging from 18.6 ± 1.2 to 66.5 ± 1.1%. The results of hydroxyl radical scavenging property of strain AAS2 are shown in Fig. 8b. The study depicted the increased hydroxyl radical scavenging activity (8.6 ± 1.2 to 56.5 ± 1.1%) with increase in the bacterial cell density (100–1000 µL).

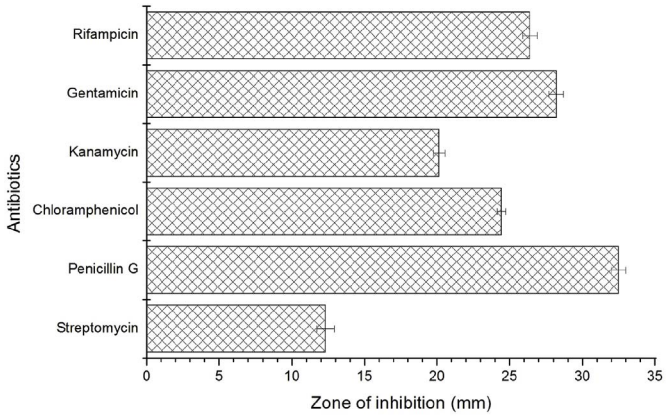

Fig. 7. Antibiotic susceptibility test of strain AAS2.

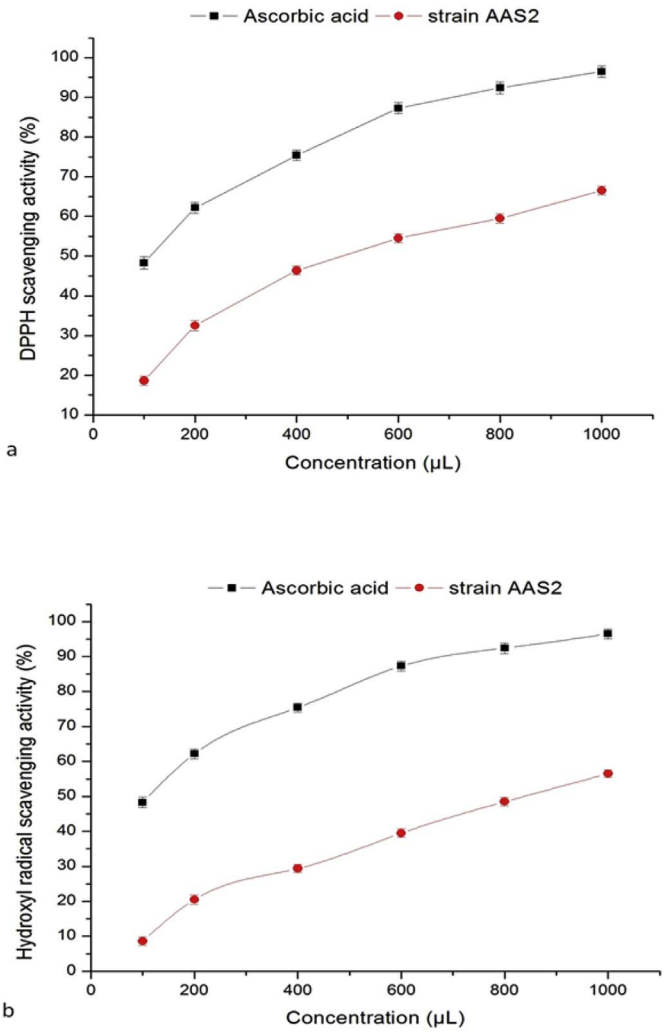

Fig. 8. (a) DPPH scavenging property and (b) hydroxyl radical scavenging activity of strain AAS2 at various concentrations.

4. Discussion

No doubt, UTI is one of the most prevalent bacterial infections worldwide whose morbidity rate is increasing each year. The problematic issue of dysuria is known to affect about 5% of women each year. However, at present, UTI is frequently treated with broad-spectrum antibiotics. As expected, it caused the rapid emergence of drug resistant bacteria. In view of this, there is an urgency to find cost-effective emphatic biocontrol agents from un/less exploited sources to overcome the increasing incidence of this infection by combating uropathogens without the development of drug resistant bacteria. In this investigation, a significant attempt was undertaken to isolate different group of bacteria from fermented food (*Ngari*) for evaluating its growth inhibitory traits against UTI causing bacterial pathogens. *S. succinus* strain AAS2 was identified as a potent candidate to inhibit the growth of indicator bacteria with high arbitrary unit values. The growth inhibitory property against uropathogens might be due to the release of antibacterial substances from strain AAS2 into the medium. Previously, Lim et al. [16] reported the use of probiotics and their metabolites as bactericidal agents against UTI causing bacterial pathogens. According to Liu et al. [17], the secretion of acid, short chain peptides, and other bactericidal substances from probiotics might be the effective reason to inhibit the growth of UTI causing pathogens. In another report, Borah et al. [18] successfully isolated fermented food derived *Staphylococcus* sp. and exhibited its antibacterial effect against *E. coli*. The findings of

our study revealed the first report on the antagonistic activity of traditionally fermented fish associated *S. succinis* against UTI causing bacterial pathogens and suggested its further exploitation for pharmaceutical purposes in future.

The survival of bacteria in the upper gastro-intestinal tract (GIT) is the baseline characteristics of probiotics. Gastric juice in stomach is a biological barrier where the pH varies between 1.5 and 3.0. In the present context, strain AAS2 was able to tolerate acidic condition (pH 3.0) and showed prominent resistivity towards simulated gastric juice up to 3 h of incubation with fairly high survival logarithmic cell counts, thereby proving the capability of this strain to transit through acidic conditions of GIT [19]. Bile is another biological barrier encountered by the bacteria in the upper GIT. The physiological range of human bile varies from 0.3 to 0.5% [20]. The bile salts hydrolyzing property of bacteria leads to the survival and sustainability of gut microflora [21]. Gu et al. [22] reported that several probiotic strains tolerated 0.3% oxgall, which is in good agreement with our results, as our strain resisted 0.5% oxgall. On the other hand, Simonova et al. [23] demonstrated the ability of staphylococci to tolerate 1% oxgall.

The bacteria must be able to colonize themselves inside digestive tract of the host in order to categorized as probiotics [24]. In this study, strain AAS2 depicted promising auto-aggregation property, thereby indicating the specific binding ability in the GIT. Further, the isolate of this investigation exhibited reasonable range of cell surface hydrophobicity property. Bacterial surface properties lead to the attachment to several substrates, which in turn leads to hydrophobicity. In general, hydrophobicity is one of the physico-chemical characteristics that assist the first contact between the bacteria and the host's cells [25]. In fact, the increased hydrophobicity trait of the bacteria represents its potentiality to adhere to the intestinal epithelial cell lining and tolerate the digested food components movement [26].

The tolerance to phenol is a pivotal probiotic characteristic of bacteria because phenol is produced in the intestine due to the deamination of few aromatic amino acids [27]. In this context, strain AAS2 showed resistivity towards phenol after 24 h of incubation with significant viable cell counts. In like manner, strain AAS2 was found to be resistant to lysozyme, thereby indicating its unique characteristic to survive if it is administered orally because lysozyme is the first biological barrier in the human digestive system encountered by the consumed probiotic bacteria [28].

Strain AAS2 showed proteolytic activity on skim milk agar medium by secreting extracellular protease. The probiotics are solely dependent on the proteolytic process because of the reduced level of amino groups and peptidases. The antibiotics susceptibility test of strain AAS2 exhibited sensitivity to all the conventional antibiotics tested. Thus, this strain can be considered as an efficacious probiotic candidate due to the lack of resistant to all the antibiotics tested, as suggested by Resende et al. [29] too. Probiotics containing antibiotic resistance genes may have negative consequences to host's health.

Antioxidants play a decisive role in the prevention of the cellular damage caused by hydrogen atom transfer and electron transfer. In the present investigation, strain AAS2 depicted DPPH scavenging property at various concentrations. The DPPH scavenging trait of strain AAS2 would probably be due to the presence of surface active components involved in antioxidant property. Additionally, strain AAS2 also exhibited the hydroxyl radical degrading property at varied concentrations. Generally, hydroxyl radical causes the oxidative damage of biological molecules, and these originate particularly from the Fenton reaction in the presence of transition metal ions [30]. Our findings clearly represent the chelation of these ions, thereby indicating the hydroxyl radical scavenging attribute.

5. Conclusion

The present investigation extensively documented the strain specific antagonistic activity of *Ngari* associated probiotic bacteria against UTI causing bacterial pathogens. Among them, the most promising isolate was identified as *S. succinus* strain AAS2 based on 16S rRNA sequencing tool. Strain AAS2 exhibited satisfactory tolerance to low pH, simulated gastric juice, and oxgall with high cell survival counts. The isolate displayed good hydrophobicity value with increased percentage of auto-aggregation. Further, strain AAS2 showed resistance to phenol and lysozyme with high survival rate. In addition to this, the isolate exhibited proteolytic activity as well as susceptibility towards all the antibiotics tested. Most importantly, strain AAS2 revealed antioxidant trait by scavenging DPPH and hydroxyl radicals in a dose dependent manner. Considering the results obtained in the present context, strain AAS2 satisfied all the criteria for categorizing it a cogent probiotic candidate, and suggested its vast medicinal applications for inhibiting the growth of uropathogens.

Conflict of interest

None declared.

References

[1] Z. Liu, Z. Jiang, K. Zhou, P. Li, G. Liu, B. Zhang, Screening of bifidobacteria with acquired tolerance to human gastrointestinal tract, Anaerobe 13 (2007) 215–219.

[2] S. Handa, N. Sharma, *In vitro* study of probiotic properties of *Lactobacillus plantarum* F22 isolated from chhang - a traditional fermented beverage of Himachal Pradesh, India, J. Genet. Eng. Biotechnol. 14 (2016) 91–97.

[3] N. Mojgani, H.F. Fatimah, N. Vaseji, Characterization of indigenous *Lactobacillus* strains for probiotic properties, Jundishapur J. Microbiol. 8 (2015) 1–2.

[4] A. Khusro, C. Aarti, A. Barbabosa-Pliego, A.Z.M. Salem, Neoteric advancement in TB drugs and an overview on the anti-tubercular role of peptides through computational approaches, Microb. Pathog. 114 (2018) 80–89.

[5] C. Sung, B.G. Kim, S. Kim, H.S. Joo, P.I. Kim, Probiotic potential of *Staphylococcus hominis* MBBL 2–9 as anti-*Staphylococcus aureus* agent isolated from the vaginal microbiota of a healthy woman, J. Appl. Microbiol. 108 (2010) 908–916.

[6] S. Kurutepe, S. Surucuoglu, C. Sezgin, H. Gazi, M. Gulay, B. Ozbakkaloglu, Increasing antimicrobial resistance in *Escherichia coli* isolates from community-acquired urinary tract infections during 1998-2003 in Manisa, Turkey, Jpn. J. Infect. Dis. 58 (2005) 159–161.

[7] C.L. Ramos, L. Thorsen, R.F. Schwan, L. Jespersen, Strain-specific probiotics properties of *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus brevis* isolates from Brazilian food products, Food Microbiol. 36 (2013) 22–29.

[8] W.P. Charteris, P.M. Kelly, L. Morelli, J.K. Collins, Development and application of an *in-vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract, J. Appl. Microbiol. 84 (1998) 759–768.

[9] H. Kumar, A. Rangrez, K. Dayananda, A. Atre, M. Patole, Y. Shouche, *Lactobacillus plantarum* (VR1) isolated from an Ayurvedic medicine (Kutajarista) ameliorates *in vitro* cellular damage caused by *Aeromonas veronii*, BMC Microbiol. 11 (2011) 152 https://doi.org/10.1186/1471-2180-11-152.

[10] V. Mishra, D. Prasad, Application of *in vitro* methods for selection of *Lactobacillus casei* strains as potential probiotics, Int. J. Food Microbiol. 103 (2005) 109–115.

[11] M.S. Juárez Tomás, B. Wiese, M.E. Nader-Macías, Effects of culture conditions on the growth and auto-aggregation ability of vaginal *Lactobacillus johnsonii* CRL 1294, J. Appl. Microbiol. 99 (2005) 1383–1391.

[12] V. Xanthopoulos, E. Litopoulou-Tzanetaki, N. Tzanetakis, Characterisation of *Lactobacillus* isolates from infant faeces as dietary adjuncts, Food Microbiol. 17 (2000) 205–215.

[13] M. Zago, M.E. Fornasari, D. Carminati, P. Burns, V. Suàrez, G. Vinderola, et al., Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeses, Food Microbiol. 28 (2011) 1033–1040.

[14] Y.C. Chen, Y. Sugiyama, N. Abe, R. Kuruto-Nima, R. Nozawa, A. Hirota, DPPH radical scavenging compounds from Dou-Chi, a soybean fermented food, Biosci. Biotechnol. Biochem. 69 (2005) 999–1006.

[15] Z.S. He, H. Luo, C.H. Cao, Z.W. Cui, Photometric determination of hydroxyl free radical in Fenton system by brilliant green, Am. J. Clin. Med. 6 (2004) 236–237.

[16] I.S. Lim, H.S. Lee, W.Y. Kim, The effect of lactic acid bacteria isolates on the urinary tract pathogens to infants *in vitro*, J. Kor. Med. Sci. 24 (2009) S57–S62.

[17] Y.H. Liu, C.Y. Ho, C.C. Huang, C.C. Tsai, Inhibitory effect of lactic acid bacteria on uropathogenic *Escherichia coli*-induced urinary tract infections, J. Prob. Health 4 (2016) 2 https://doi.org/10.4172/2329-8901.1000144.

[18] D. Borah, O. Gogoi, C. Adhikari, B.B. Kakoti, Isolation and characterization of the new indigenous *Staphylococcus* sp. DBOCP06 as a probiotic bacterium from traditionally fermented fish and meat products of Assam state, Egypt, J. Basic Appl. Sci. 3 (2016) 232–240.

[19] G. Corzo, S.E. Gilliland, Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*, J. Dairy Sci. 82 (1999) 472–480.

[20] E. Vlkovál, L. Kalous, V. Bunešová, K. Rylková, R. Sveˇtlíková, V. Rada, Occurrence of *Bifidobacteria* and *Lactobacilli* in digestive tract of some freshwater fishes, Biologia. Sect. Zool. 67 (2012) 411–416.

[21] M.P. Taranto, H.A.P. de Ruiz, G.F. de Valdez, Bile salt hydrolase activity in *Enterococcus faecium* strains, Microbiol. Aliment. Nutr. 13 (1995) 375–379.
[22] R.X. Gu, Z.Q. Yang, Z.H. Li, S.L. Chen, Z.L. Luo, 2008. Probiotic properties of lactic acid bacteria isolated from stool samples of longevous people in regions of Hotan, Xinjiang and Bama, Guangxi, China, Anaerobe 14 (2008) 313–317.
[23] M. Simonova, V. Strompfova, M. Marciňáková, A. Lauková, S. Vesterlund, M.L. Moratalla, et al., Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak meat products, Meat Sci. 73 (2006) 559–564.
[24] Y. Rivera-Espinoza, Y. Gallardo-Navarro, Non-dairy probiotic products, Food Microbiol. 27 (2010) 1–11.
[25] P. Shobharani, R. Agrawal, A potent probiotic strain from cheddar cheese, Indian J. Microbiol. 51 (2011) 251–258.
[26] G. Chauviere, M.H. Coconnier, S. Kerneis, A. Darfeuille-Michaud, Competitive exclusion of diarrheagenic *Escherichia coli* (ETEC) from human enterocyte-like Caco-2 cells by heat killed *Lactobacillus*, FEMS Microbiol. Lett. 70 (1992) 213–217.
[27] S. Sathyabama, R. Vijayabharathi, P.B. Devi, M.R. Kumar, V.B. Priyadarisini, Screening for probiotic properties of strains isolated from feces of various human groups, J. Microbiol. 50 (2012) 603–612.
[28] B. Turchi, S. Mancini, F. Fratini, F. Pedonese, R. Nuvoloni, F. Bertelloni, et al., Preliminary evaluation of probiotic potential of *Lactobacillus plantarum* strains isolated from Italian food products, World J. Microbiol. Biotechnol. 29 (2013) 1913–1922.
[29] J.A. Resende, V.L. Silva, T.L.R. De Oliveira, F.S. De Oliveira, J.C. Carneiro, M.H. Otenio, et al., Prevalence and persistence of potentially pathogenic and antibiotic resistant bacteria during anaerobic digestion treatment of cattle manure, Bio Technol. 153 (2014) 284–291.
[30] T. Kao, B. Chen, Functional components in soybean cake and their effects on antioxidant activity, J. Agric. Food Chem. 54 (2006) 7544–7555.