Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(7): 23 – 39 (2020)
www.ejabf.journals.ekb.eg

Probiotic characterization and cholesterol assimilation ability of *Pichia kudriavzevii* isolated from the gut of the edible freshwater snail *"Pila globosa".*

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RTICLE INFO
Article History:
Received: Aug. 26, 2020
Accepted: Oct. 3, 2020
Online: Oct. 16, 2020

Keywords:
Probiotic yeast,
Pichia,
Snail's gut,
Cholesterol assimilation.

ABSTRACT

Excess cholesterol is one of the main causes of various metabolic diseases. Currently available therapeutic measures, lifestyle, and dietary interventions, as well as pharmaceutical agents, are inadequate for the regulation of cholesterol levels. Probiotic microorganisms have demonstrated the potential to lower cholesterol levels through various mechanisms, including the activity of bile salt hydrolase, production of enzyme-inhibiting compounds such as 3-hydroxy-3-methylglutaryl coenzyme A, and assimilation of cholesterol. In the present investigation a yeast culture designated as S-I, isolated from freshwater snail's gut (*Pila globosa*), fulfilled probiotic attributes. The culture was found to be non-hemolytic and could tolerate a bile concentration up to 1.2%, pH 1.5–10; and temperature 42ºC respectively. Additionally, 73.57% and 36.24% of S-I culture survived when exposed to the gastric environment for 90 and 180 min respectively, while 53% survived after 240 min incubation under the intestinal environment. The S-I culture could auto-aggregate (93.01%), as well as co-aggregate (77.89%) with gastrointestinal pathogens, an important property required for the probiotics to control the pathogens. The culture also inhibited *C. albicans* NCIM3557, *E. coli* NCIM3099, *E. faecalis* NCIM3040, *S. aureus* NCIM2408and *P. aeruginosa* NCIM2036 *in-vitro*. Other probiotic properties such as hydrophobicity (81%), bile salt hydrolase activity (86.30%), and tolerance to 8% NaCl (27.38%), as well as antibiotics, were found to be in favor of the S-I culture. Most importantly, S-I culture was found to assimilate 20.29% of cholesterol. Molecular characterization of this culture performed by 18S rRNA sequencing revealed that the S-I culture was *Pichia kudriavzevii* species with 97% similarity.

INTRODUCTION

High cholesterol is associated with an increased risk of developing cardiovascular disease. This may include cardiac disease, stroke, and peripheral artery disease. Diabetes and high blood pressure have also been linked to high cholesterol **(Shehata *et al.*, 2016)**. Probiotics, with cholesterol lowering ability, when supplemented through food can

provide many dietary benefits along with reduction in blood cholesterol levels **(Xie *et al.,* 2015)**. This desired activity might be underpinned by BSH in probiotics and their inherent ability to scavenge cholesterol **(Kowalczyk and Puchalski, 2008)**. While different probiotic strains such as *Lactobacillus reuteri, L. fermentum, L. rhamnosus, L. acidophilus* and few probiotic yeast which are also reported for their cholesterol-lowering potential have also been documented by (**Tomaro *et al.,* 2014)**. Bile salt hydrolase (BSH) responsible to reduce serum cholesterol level, has become an important selection criterion for the probiotic candidate **(Miremadi *et al.,* 2014)**.

Probiotics are the live microscopic organisms, which when administered in a specific amount, confers different health benefits to the hosts **(FAO and WHO, 2002)**. They have been reported for the production of precursors of aromatic, antimicrobial compounds, preventing autoimmunity, conferring resistance to food antigens, modulating the immune response, and assimilating cholesterol etc. **(Paraschiv *et al.,* 2010)**. Moreover, probiotics can also enhance digestion and absorption of nutrients **(Yirga, 2015).** Owing to various benefits, probiotics are increasingly used in commercial products such as the use of yeast in animal feed and as nutraceuticals **(Authority, 2004)**. Probiotic yeasts such as *Saccharomyces boulardii*, provides an advantage over bacterial counterpart as there is a minimum threat of transfer of resistance genes between pathogenic bacteria and yeasts in the gastrointestinal tract **(Sourabh, 2012; Saavedra, 2001)**. Similarly, along with antimicrobial compound production, probiotics are also being characterized for cholesterol removal potential **(Park *et al.,* 2007)**.

BSH is an important enzyme for the survival of probiotics in the stomach. BSH is considered a potential pharmacologically active enzyme, often known as an effective treatment to reduce serum cholesterol levels in patients with hypercholesterolemia, which is also an important reason for atherosclerosis results in cardiovascular diseases (CVD's) **(Chand *et al.,* 2017; Rajan *et al.,* 2019)**. Considering the wide applications and growing market, there is a lot of scope to isolate probiotic microorganisms from various source and screen them for applications **(Kathade *et al.,* 2020)**. Studies have revealed that many species of algae possess the ability to produce more than 50% oil and algae are one of the main foods of snails. Hence, there are more chances to find oil or lipid utilizing microorganisms from the gastrointestinal tract of snail.

Keeping these points, the present study aimed to isolate yeasts from the gut of edible freshwater snail *(Pila globosa*), characterize them for probiotic potential, and evaluate its ability to assimilate cholesterol.

MATERIALS AND METHODS

Sample collections and culture isolation

Snails (*Pila globosa*) were collected from the catchment area of Vishnupuri dam, Nanded, Maharashtra, India (19°07'13.6"N 77°17'01.4"E). Snails were dissected to open the alimentary canal **(Fig. 1).** Faeces were scratched from the intestinal part, serially diluted to 10^3 and 10^6 were streaked on yeast extract peptone dextrose (YPD) medium (Himedia, Pune, India), pH 6.5, and incubated at 37°C for 48 h for isolation of yeast cells.

Morphological distinct colonies were distinguished based on colony characterization and were screened microscopically for presence of yeast cells. Isolates were purified and stored at 4°C. The cultures were seeded in YPD broth and incubated for 24 h, centrifuged, and re-suspended in saline to get 10^7cfu/ml. This suspension (1%) was used as inoculum in all experiments. Marketed yeast culture of *Saccharomyces boulardii* (SB) was used as a positive control for each assay **(Khisti *et al*., 2019)**.

Probiotic characterization

Probiotic characterization of the isolates was performed as per the guidelines of ICMR-DBT and WHO-FAO **(FAO and WHO, 2002; Ganguly *et al*., 2011).**

Toxicity assay

For toxicity assay, cultures were surface spot inoculated on sheep blood agar plate and incubated at 37°C for 24 h to observe the pattern of haemolysis **(Pino *et al.*, 2019)**.

Bile, pH, and temperature tolerance

The pH tolerance was assessed by adjusting YPD medium pH from 1.5 to 10 using 1.0 HCl or 1.0 N NaOH before inoculating the culture. Similarly, cultures were inoculated in YPD broth containing different bile salt concentrations of 0.3, 0.6, 0.9, and 1.2% for assessing bile tolerance. For temperature tolerance, inoculated YPD broth were incubated at different temperatures of 28°C, 37°C and 42°C **(Angmo *et al*., 2016; Chanthala and Appaiah, 2014)**. The cultures were incubated for 24 h at 37°C and growth was observed by spectrophotometric analysis.

Gastric and intestinal juice tolerance

Survival under gastric and intestinal conditions was determined by suspending cultures in 0.2 ml phosphate buffer saline (PBS) that was added in a solution of 1.0 ml gastric juice, pepsin (pH 2) for gastric and pancreatin for intestinal (pH 8) along with (0.5% w/v) of 0.3 ml sodium chloride **(Chelliah *et al*., 2016)**. Viability of the cultures was assessed at 1, 90, and 180 min for gastric and for intestinal juice tolerance 1.0 min and 240 min by the method of spread plating the cultures on YPD agar plates. The survival in gastric and intestinal transit environment was determined by the total viable cell count (TVC) after the incubation period **(Sourabh, 2012).**

Auto-aggregation and co-aggregation

After 24 h of growth in YPD broth, cells were centrifuged, washed and suspended in 2 ml of PBS (1.0 M, pH 6.5). The suspension (1.0 ml) was mixed with 4 ml of sterile PBS (1.0 M, pH 6.5) in a test tube and vortexed for 10 seconds. The suspension was incubated for 2 h at 37°C and optical density was measured at 600 nm. For co-aggregation, culture pellets were suspended in 0.2 ml of PBS (1M, pH 6.5) and mixed with 0.2 ml pathogen (0.5 OD at 600 nm) and the final volume was adjusted to 5 ml using sterile PBS. This suspension was incubated for 24 h at 37°C and the absorbance were measured at 2, 4, and 24 h at 600 nm. **(Ogunremi *et al.*, 2015)**. The auto aggregation and co-aggregation percentage were calculated as (A_t / A_0) X 100, where A_t represents the absorbance at time t = 2 h and A_0 the absorbance at t = 0 h.

Antimicrobial assay

Antimicrobial activity of the isolates was carried against pathogenic microorganisms such as *Escherichia coli NCIM* 3099, *Staphylococcus aureus NCIM* 2408, *Enterococcus faecalis NCIM 3040, Candida albicans NCIM* 3557, and *Pseudomonas aeruginosa NCIM 2036.* The pathogens were spread plated on Muller Hinton agar plates (0.1 ml, 0. 5 OD at 600 nm) and incubated at 37°C for 10 min. Wells were made in agar using agar borer. Sample of 20 µl of 24 h old grown culture supernatant was added into the agar wells and incubated at 37°C for additional 24 h and antimicrobial activity was observed **(Chelliah *et al.,* 2016)**.

Hydrophobicity assay

The hydrophobicity assay demonstrates the probiotic culture's ability to form adhesive interactions with human epithelial cells. The cells (1.0 OD at A 600 nm) were suspended in phosphate buffer (pH 6.5) and treated with xylene in 5:1 ratio to detect culture hydrophobicity. For phase separation, the suspension was thoroughly mixed with xylene for 2 min and incubated at 37ºC. The decrease in the aqueous phase's optical density was estimated as a percentage of hydrophobicity (H %) and determined as H (%) = [(A0-A)/A0]*100, where A0 and A are aqueous-phase absorbances at 0 h and 2 h **(Chandran and Keerthi, 2018; Vinderola and Reinheimer, 2003)**.

Bile salt hydrolase (BSH) assay

The BSH assay was performed according to the method mentioned by **(Zheng *et al.,* 2013).** Cultures were spot inoculated on MRS agar plates containing 0.5 % (w/v) of taurodeoxycholic acid (Himedia) sodium salt and 0.37 % (w/v) of calcium chloride. Plates were incubated anaerobically at 37°C for 72 h to evaluate BSH activity, and the BSH activity was assessed by the presence of precipitation around colonies **(Toit *et al.,* 2003).**

NaCl tolerance

Tolerance to NaCl was determined by growing the cultures in MRS medium along with different concentration of NaCl (1-10%). After incubation period, tolerance was assessed by measuring spectroscopic readings at 0 h and 24 h of incubation **(Islam *et al.,* 2016)**.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed as per the method standardized by Kirby-Bauer **(Bauer *et al.,* 1959).** Accordingly, culture was plated on MRS agar and exposed to antibiotic disks (Himedia) containing ampicillin (10 mcg), chloramphenicol (25 mcg), penicillin-G (1.0 unit), streptomycin (10 mcg), sulphatried (300 mcg), and tetracycline (25 mcg). Susceptibility pattern was measured by calculating the clearance zone after incubation at 37°C for 24 h. Resistance and susceptibility of strains were considered according to the breakpoint proposed by European food safety authority **(Capozzi *et al.,* 2012; EFSA, 2009)**.

Cholesterol assimilation

Freshly prepared sterile YPD broth, containing 0.3% bile salt and filter sterilized water-soluble cholesterol (1.0 mg/ml), was inoculated with 1.0 OD culture and incubated under anaerobic conditions at 37°C for 48 h. Cell free supernatant of 24 h and 48 h old cultures

were removed by centrifugation at 10,000 rpm for 15 min and 1.0 ml was mixed with 3 ml KOH (33% w/v) and 3 ml absolute ethanol. The mixture was vortexed for 1.0 min and incubated at 65°C for 15 min to remove ethanol. Later, n-hexane (5 ml) and distilled water (2 ml) were added to this solution and were thoroughly mixed for 1.0 min. Hexane layer (1.0 ml) was transferred into a clean glass tube and evaporated till dryness in a water bath at 65°C. The dried residue was promptly dissolved in 1.5 ml of $FeCl_3$ reagent by mixing thoroughly. Concentrated sulphuric acid (1.0 ml) was added to the mixture, which was vortexed for 1.0 min and incubated in dark for 30 min. Cholesterol concentration from the mixture was measured using a spectrophotometer at 550 nm at 24 h and 48 h **(Ogunremi *et al.*, 2015)**. The cholesterol assimilation ability (mg/ml culture broth) was calculated as follows: Cholesterol assimilation A = 100-(B/C)*100
(A = % of cholesterol assimilation, B = Absorbance of the culture supernatant, C = absorbance of the control).

Molecular identification

Total genomic DNA of S-I culture was isolated and purified using a genomic DNA isolation kit (Sigma) and used as a template for PCR. Each reaction mixture containing approximately 10 ng of DNA; 200 µM each dNTPs; 2 pmol of each, forward and reverse primers; 1x PCR buffer; and 2.5 mM $MgCl_2$; were loaded in Ankle-Branchial Index (ABI) Prism Big Dye Terminator Cycle Sequencing reaction kit for sequencing the PCR product. Combination of universal primers for amplification was used to sequence the nearly completed gene. The sequencing reaction and template were purified as per the manufacturer's instructions (Applied Biosystems, India). Samples were allowed to run on ABI prism 3100 Genetic analysers and output of sequencing was analysed using DNA sequence analyser computer software. The obtained sequence was compared and analysed with the National Centre for Biotechnology Information (NCBI) GenBank entries by using the BLAST algorithm **(Kathade *et al.*, 2020)**.

Statistical analysis

Each experiment was performed in triplicate and data were subjected to a one-way analysis of variance (ANOVA) and results are expressed as Mean ± SD. Statistical analysis was done by PRISM software. Differences were considered statistically significant when $p < 0.05$. ($p > 0.05$ = ns, $p < 0.05$ = *, $p < 0.01$ =**, $p < 0.001$ =***).

RESULTS

Isolation of yeast cultures

Spread plating of the snail faeces on YPD agar plates resulted in separation of 20 morphologically distinct colonies. Out of 20 isolates, three cultures designated as S-I, S-II and S-III showing yeast morphology.

Toxicity (haemolytic) assay

The haemolytic assay is one of the tests used for screening the toxicity of the isolates. In the present study, S-I, S-II and S-III isolates showed γ lysis on sheep blood agar plate

similar to standard culture SB **(Fig. 2),** and hence were eligible for further characterization.

Tolerance to pH and bile salt

Isolates S-I and S-II showed growth at a pH range of 1.5 to 10, with optimum growth at pH 7 and S-III could not able to survive in acidic pH. Similarly, these isolates could survive bile salt at a concentration up to 1.2%.

Effect of temperature

Yeast isolates S-I and S-II were able to grow at all three tested temperatures of 28°C, 37°C, and 42°C **(Fig. 3)**.

Gastric and intestinal tolerance

The experiment was aimed to find out the resistance of cultures to gastric and intestinal juices. When S-II isolate treated with gastric enzymes, it resulted in the decreased number of cells from 32.12% to 11.05% at 90 and 180 min, respectively. Similarly, the cell count decreased to 6% (p=0.012) in the presence of intestinal enzymes after 240 min. While isolate S-I showed good survival in gastric condition with 73.57%, 36.24% after 90 and 180 min (p=0.001, 0.0045), and in intestinal condition 53% culture tolerated after 240 min (p=0.01), whereas known probiotic SB had good tolerance in gastric condition with 51.8% and 24.27% after 90 and 180 min with p=0.048, 0.06, whereas in intestinal juice 75% of culture survived even after 240 min (p=0.067) **(Fig. 4 a and b).**

Auto-aggregation and co-aggregation

Aggregation ability of yeast isolates was measured by comparing the initial absorbance at 600 nm. The baseline auto-aggregation percentage for a good probiotic candidate recommended is more than 40%. The present study revealed, S-I isolate which showed 93.01% auto-aggregation after 2 h with significance p=0.09 and compared with standard SB 78.56% (p=0.1) after 2h **(Fig. 5 a).**

Co-aggregation indicates the ability of isolates to bind and inhibit the pathogen. Isolate S-I showed 77.89% (p=0.05), 91.76% (p=0.033), and 88.11% (p=0.041) co-aggregations with *E.coli, E. faecalis,* and *S. aureus, respectively*. In comparison standard culture S.B. showed 49.14% (p=0.067), 25.8% (p= 0.13), and 51.14% (p= 0.071) co-aggregation with respective cultures after 2 h incubation **(Fig. 5 b).**

Antimicrobial activity

Bioactive compounds present in supernatant were tested against both Gram-positive, as well as Gram-negative bacteria. The results showed that S-I culture was able to inhibit *E. coli NCIM* 3099, *S. aureus NCIM* 2408, *E. faecalis NCIM 3040, P. aeruginosa NCIM 2036,* and *C. albicans NCIM* 3557 with an inhibition zone of 0.9, 0.7, 0.5, 0.63, and 1.2 cm, respectively with p=0.032, 0.066, 0.05, 0.055, and 0.08. S-I culture had a wide range of bioactive compounds against these common pathogens than SB. Whereas SB

possessed inhibitory activity against *P. aeruginosa and C. albicans* with 0.55, 1.1 cm zone of inhibition with p=0.05, 0.071 **(Fig. 6).**

Hydrophobicity test

The present study showed that culture S-I when treated with xylene the optical density of the aqueous phase decreased from 1.142 to 0.264 with a hydrophobicity of 76.9% (p=0.0047), which is comparable to standard culture SB with hydrophobicity of 81% (p=0.01).

Bile salt hydrolase

BSH activity was determined by precipitation around colonies, after 72 h of incubation at 37°C. Precipitation was observed around S-I and SB colonies, indicating bile salt hydrolase activity of the culture **(Fig. 7) (Zheng *et al.*, 2013).**

NaCl tolerance

The present study showed S-I culture could tolerate salt concentration up to 8%. The survival of the culture was found to be 86.30% (p=0.021) at 6% NaCl but decreased to 27.38% (p=0.063) when the concentration of NaCl was increased to 8% and no growth was observed at 10%. Meanwhile, survival rate of SB was found to be 75.22% and 18.30% at 6% and 8% NaCl concentration with p-value 0.03, 0.004 respectively.

Antibiotic susceptibility test

The present study reported S-I culture when exposed with different antibiotics on MRS agar, was shown to be resistant to ampicillin (10 mcg), chloramphenicol (25 mcg), streptomycin (10 mcg), sulphatried (300 mcg), tetracycline (25 mcg), and penicillin-G (1 unit) **(Fig. 8)** as per the interpretation of zones of inhibition for Kirby-Bauer antibiotic susceptibility test as reported in **(Reynolds *et al.*, 2009)**.

Cholesterol assimilation

High blood cholesterol level is considered to be the main risk factor for the development of chronic diseases such as coronary heart disease. S-I isolate showed the ability to decrease cholesterol level from the media, with reduction of cholesterol by 12.5%, and 20.29% at 24 h and 48 h, respectively when compared with standard SB with reduction by 7.23%, and 11.8% at 24 h and 48 h, respectively **(Table 1).**

Molecular identification

After analysis of 18S rRNA sequencing, S-I culture was found to be *Pichia kudriavzevii* with 97% similarity. Sequence was deposited in DNA Data Bank of Japan (DDBJ) with accession no. LC528140.

Fig. 1. *Pila globosa* snail (a) and its dissected intestine (b).

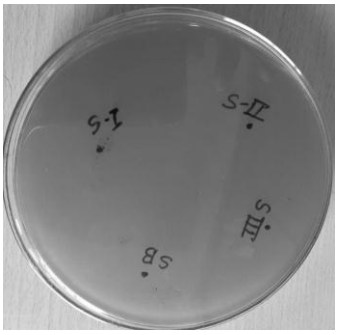

Fig. 2. Pattern of haemolysis were observed on sheep blood agar plate

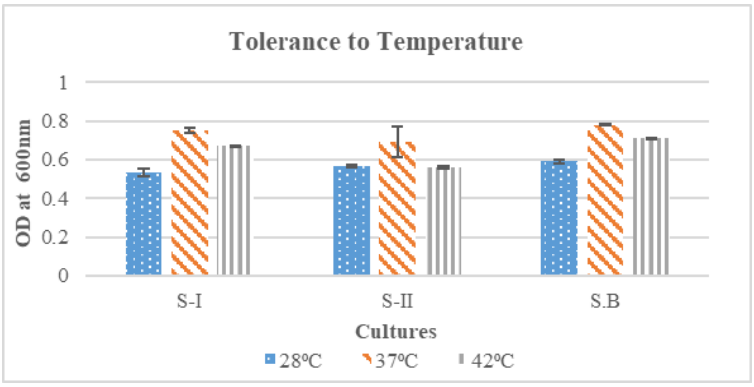

Fig. 3. Effect of temperature on isolates S-I, and S-II compared with standard SB

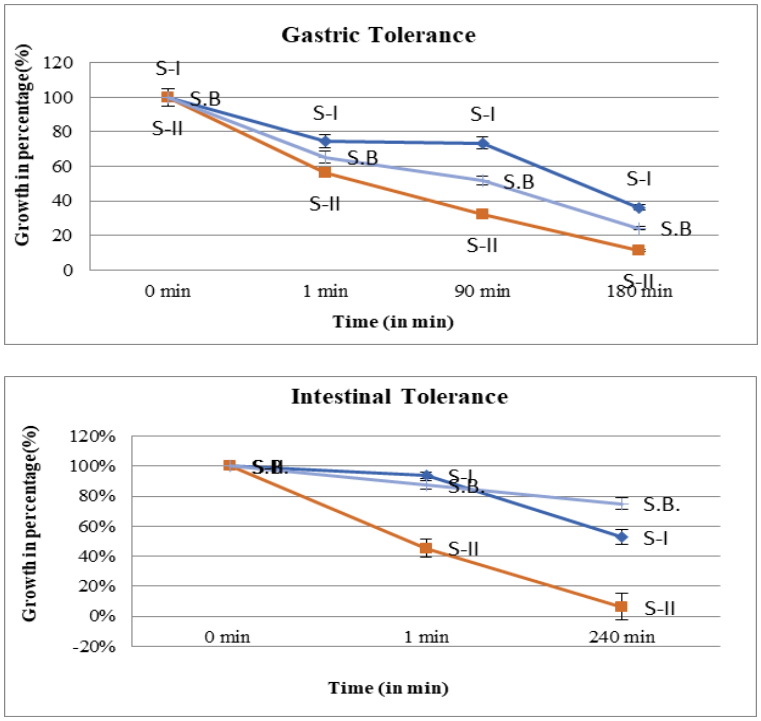

Fig. 4 a and b. Gastric and intestinal tolerance of S-I, S-II and S.B. culture

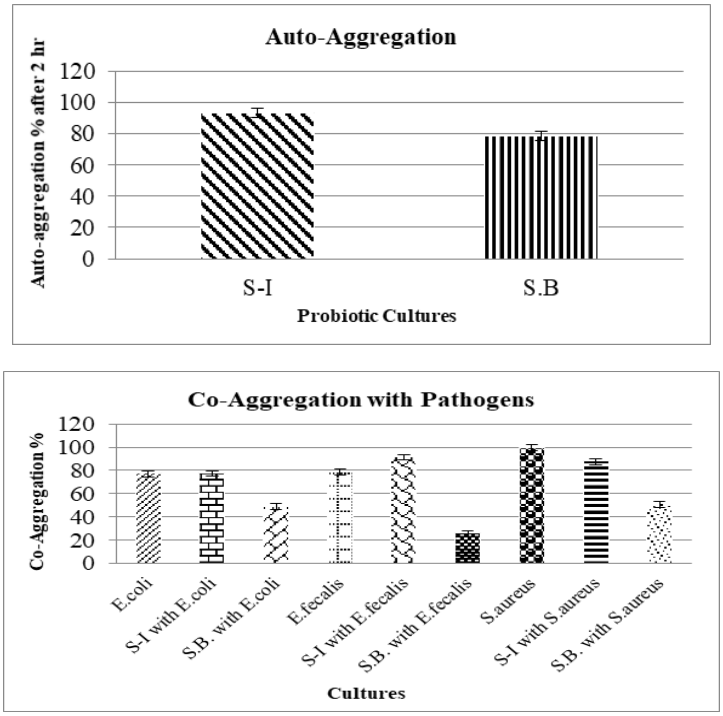

Fig. 5 a and b. Auto-aggregation of S-I and SB, and co-aggregation of S-I and SB with *E. coli*, *E. faecalis*, and *S. aureus.*

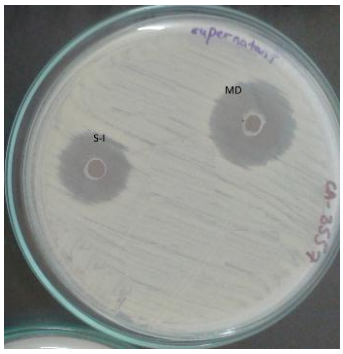

Fig. 6. Anti-microbial characteristics of S-I and S.B. culture against *Candida albicans NCIM* 3557.

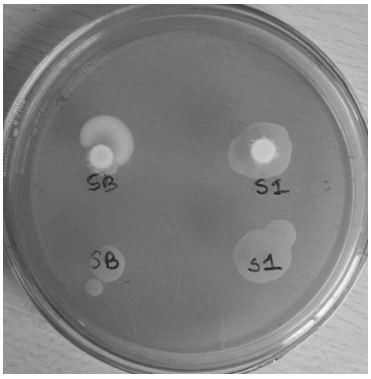

Fig. 7. Bile Salt Hydrolase (BSH) activity was evaluated by observing precipitation around colonies

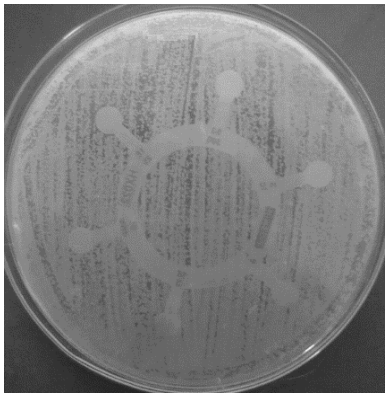

Fig. 8. Antibiotic susceptibility in presence of ampicillin (10 mcg), chloramphenicol (25 mcg), streptomycin (10 mcg), suphatried (300 mcg) tetracycline (25 mcg), and penicillin-G (1 unit)

Table 1 Cholesterol removal from the broth in percentage at 0 h, 24 h and 48 h with cultures S-I and SB.

Cultures	**Cholesterol removal from broth in percentage (%)**		
	0h.	24h.	48h.
S-I	0	12.5	20.29
SB	0	7.23	11.8

DISCUSSION

FAO/WHO has enlisted several tests for characterization of a microorganism as probiotics. These tests that offer an idea of the microorganism under study's ability to survive harsh gastrointestinal conditions as well as its ability to provide the host with certain benefits **(FAO and WHO, 2002; Ganguly *et al.*, 2011)**.

The S-I culture, a yeast, which was isolated from the freshwater edible snail, *Pila globosa* showed γ lysis pattern indicating a non-toxic nature, and hence eligible for further probiotic characterization **(Pino *et al.*, 2019)**. Probiotics are generally administrated through the oral route and they should survive the harsh acidic and alkaline environment in the alimentary canal **(Gupta and Sharma, 2017; Shehata *et al.*, 2016)**, as well as survive in presence of bile and NaCl condition, which can inhibit the growth of microorganisms **(Billah *et al.*, 2010; Shehata *et al.*, 2016)**. The culture under investigation could survive up to 1.2% bile acid and in the presence of 8% NaCl. The culture could also grow in a wide range of temperature and pH.

One of the important criteria for probiotic characterization is resistance to the gastric and intestinal juice. The S-I culture showed good tolerance to gastric and intestinal juice with 73 and 53% survivability respectively, which is comparable to other known probiotic cultures such as *Lactobacillus, Bifidobacterium, and Saccharomyces* spp **(Gupta and Sharma, 2017)**.

Auto-aggregation of the culture **(Bao *et al.*, 2010)** can be related to the cell surface components and its adhesion ability to intestinal epithelial cell **(Kos *et al.*, 2003)**. The S-I culture showed 93% auto-aggregation property, which is higher than the standard SB culture. Co-aggregation indicates the ability of the culture to aggregate with other cultures **(Soleimani *et al.*, 2010),** and more than 40% co-aggregation with pathogenic organisms is considered to be as good activity. In the present study S-I and positive control, SB culture showed more than 50% co-aggregation against different food pathogens. At the same time, the culture also inhibited common pathogens *in-vitro*. Hydrophobicity activity gives a clear idea of cell surface and ability of adherence to the

surface of the intestinal wall by the probiotic microorganisms. The hydrophobicity is due to the presence of cell surface molecules such as glycolipids, glycoproteins, and complex carbohydrates on the microbial cell **(Chandran and Keerthi, 2018; Kos *et al.,* 2003).** In the present study, S-I and SB cultures showed more than 75% hydrophobicity.

Bile salt hydrolysis is a method to detoxify the bile salt by producing BSH enzyme by probiotic microorganisms, and it has become one of the important criteria of probiotic characterization **(Noriega *et al.,* 2006)**. BSH enzyme catalyse the deconjugation of bile into free primary bile acids **(Gilliland and Speck, 1977)**. It is also important because BSH activity help to reduce the blood cholesterol level of the host **(Noriega *et al.,* 2006)**. Both the cultures, S-I and SB showed precipitation around the colony showing BSH activity.

Usually, probiotic supplements are prescribed along with antibiotics to restore the intestinal microflora since antibiotics disturb the normal flora of the gut and the beneficial microorganisms along with pathogen. This leads to abdominal pain and antibiotic associated diarrhoea. Hence, one of the criteria for probiotic microorganisms is to test antibiotic resistance pattern **(Czerucka *et al.,* 2007; Viswanathan, *et al.,* 2015)**. The antibiotic resistance observed for *S. boulardii* and *Lactobacillus* strains were considered to be intrinsic or natural resistance and therefore, non-transmissible **(Hickson, 2011)**. The study showed that S-I and SB cultures showed resistance to most of the antibiotics tested. The S-I culture was then identified as *Pichia kudriavzevii* with 97% similarity using 18sR RNA techniques.

High blood cholesterol level is considered a major risk factor for the development of coronary heart disease, and it is a leading cause of deaths worldwide. Current therapeutic measures, lifestyle and dietary interventions as well as pharmaceutical agents are inadequate for the regulation of cholesterol levels **(Miremadi *et al.,* 2014; Duchesneau *et al.,* 2014; Wang *et al.,* 2012)** Probiotic microorganisms have demonstrated potential to lower cholesterol levels through different mechanisms, but the exact mechanisms responsible for the cholesterol-lowering activity by probiotic remains unclear **(Ma *et al.,* 2019)**. Three main possible mechanisms have been proposed, which include removing intestinal cholesterol by probiotic cells, inhibiting small-intestinal cholesterol absorption by the down-regulation of intestinal NPC1L1 protein levels, and increasing faecal bile acid excretion levels by bile salt deconjugation that is catalysed by bile salt hydrolase (BSH) of probiotic cells **(Ma *et al.,* 2019)**. These mechanisms were found to be strain dependent and conditions produced under laboratory conditions **(Shehata *et al.,* 2019)**. In this study we have partially assessed the potential of cholesterol assimilation property of probiotic microorganism.

In the present study, S-I culture demonstrated the ability to reduce 20.29% cholesterol from media compared with standard SB with 11.8%. This property of the culture could extrapolate towards controlling the cholesterol from blood serum. *Pichia kudriavzevii* has been reported as probiotic previously **(Chelliah *et al.,* 2016)**, however, it is the first report of the culture isolated from fresh water snail and also its ability to assimilate cholesterol. Hence it can be an ideal probiotic for lowering the blood cholesterol in human.

CONCLUSION

Probiotics are one of the most sorted out organisms as supplements due to various health benefits they provide to hosts. Many bacterial and yeast species are identified for their probiotic use and are available over the counter. Due to the huge microbial diversity and functionality still, there is large scope to isolate microorganisms and evaluate them for probiotic potential. In the present study culture isolation from gut of a *Pila globosa* was identified as *Pichia kudriavzevii*. The culture showed all the probiotic properties as described by WHO/FAO. Along with probiotic properties, the culture was found to assimilate cholesterol from the medium. These properties make the present isolate *Pichia kudriavzevii,* an excellent probiotic candidate to control blood cholesterol with further characterization.

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