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Original Article

Hypocholesterolemic activity of indigenous probiotic isolate *Saccharomyces cerevisiae* ARDMC1 in a rat model

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ABSTRACT

The aim of this study was to investigate probiotic attributes of *Saccharomyces cerevisiae* ARDMC1 isolated from traditional rice beer starter cake and its hypocholesterolemic effects on Wistar rats fed a high-cholesterol diet. The indigenous isolate ARDMC1 showed potential probiotic characteristics such as tolerance to simulated gastrointestinal stress conditions, autoaggregation properties, and adhesion to intestinal epithelium Caco-2 cell line. In addition, ARDMC1 isolate exhibited *in vitro* cholesterol assimilation properties in media supplemented with cholesterol. Furthermore, administration of probiotic isolate to rats fed a hypercholesterolemic diet resulted in significant reduction of serum total cholesterol, low-density lipoprotein cholesterol, and triglyceride at the end of 42 days. The present study envisages ARDMC1 as a promising starter culture for the preparation of functional foods with properties to combat cardiovascular diseases.

1. Introduction

Cardiovascular disease (CVD) and its related complications are triggered by elevated serum cholesterol levels and are considered as the leading causes of death worldwide. According to a report published by the World Health Organization, 17.5 million people died from CVDs in 2012, representing 31% of all global deaths and morbidity is expected to increase to 23.3 million by 2030 [1,2].

Several forms of therapy have been reported to prevent CVD; however, the resources available for its management in low and middle-income countries are limited. Statins are the most well-known hypolipidemic drugs, which act as inhibitors of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme reductase essential for the metabolic pathway producing cholesterol and other isoprenoids in the body [3]. However, side effects associated with statins, such as myalgia and muscle weakness, increased fatigue, reduced energy,

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deteriorating hyperglycemia, and risk of new-onset diabetes pose a greater threat to human health [4,5].

Many studies have shown that probiotics or products containing them impart various health benefits that include prevention of CVDs and enhancing general wellness of consumers [6,7]. Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [8]. Recent studies also have reported that the microbial communities for probiotics have revealed some interesting attributes of yeasts, and functional foods prepared with probiotic yeasts reduce the levels of lipids in the serum of rats fed a high-cholesterol diet [9]. Yeast cells from food and of animal origin are also reported to remove cholesterol from media, under simulated conditions that mimics the gastrointestinal tracts of monogastric animals [10,11].

To address the alarming burden of CVDs, a cost-effective and affordable alternative strategy is required to reduce the CVD-related risk factors. A recent approach for lowering cholesterol and hence minimizing the risk of CVDs is the use of probiotic- and prebiotic-based functional or health foods that modulate the gut microbial ecosystem or their metabolic products [12,13]. In recent studies, antiatherosclerotic effects of traditional fermented foods from Asia have gained much attention [14]. Keeping this in view, *Saccharomyces cerevisiae* ARDMC1 was investigated for *in vitro* probiotic attributes and cholesterol-lowering properties in rat fed a high-cholesterol diet. In the present study, *S. cerevisiae* ARDMC1 was isolated from starter culture cake of *Apong*, a traditional rice beer of the *Mishing* tribe of Assam, India. *Apong* has a sociocultural status as a popular alcoholic beverage inimitable to the *Mishing* community and reported to have various health promoting benefits [15].

2. Materials and methods

2.1. Yeast and bacterial strains, culture media, and growth conditions

In this study, *S. cerevisiae* ARDMC1 (out of 23 isolates) was isolated from rice beer starter culture of Assam, India and selected based upon promising probiotic attributes and their *in vitro* cholesterol-removal properties. The probiotic reference strain *Saccharomyces boulardii* was isolated from marketed probiotic drug (Lupin Laboratories, India). *Salmonella enterica typhimurium* MTCC 1252 was procured from Microbial Type Culture Collection (Chandigarh, India). The yeast strains, *S. cerevisiae* ARDMC1 and *S. boulardii* were grown in Yeast and Mould Broth (YMB; HiMedia, India) medium under shaking conditions at 30°C for 48 hours, whereas *S. enterica typhimurium* was grown in nutrient agar medium (HiMedia) at 37°C for 24 hours.

2.2. Molecular identification of isolates

The 5.8S internal transcribed spacer (ITS) rDNAs of yeast isolates ARDMC1 and *S. boulardii* were amplified using the primers ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′) [16]. The D1/D2 domain of the 26S rDNA gene was amplified using the primer pair NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) [17]. The polymerase chain reaction (PCR) amplification was performed in an Eppendorf Thermocycler according to a protocol developed in our laboratory with slight modifications [18]. Briefly, amplification parameters consisted of an initial denaturation step of 3 minutes at 95°C, followed by 30 cycles of 95°C for 30 seconds, primer annealing for 30 seconds at 58°C, elongation for 1 minute at 72°C, and final extension of 10 minutes at 72°C for one cycle. The amplified PCR product was purified and subjected to automated DNA sequencing using a 3130 Genetic Analyzer (Applied Biosystems, Rotkreuz, Switzerland). The phylogenetic tree was generated by the neighbor-joining method using MEGA version 5.05. The sequences obtained were submitted to Genbank (http://www.ncbi.nlm.nih.gov/genbank).

2.3. In vitro gastrointestinal stress tolerance test

Tolerance to simulated gastrointestinal conditions was evaluated according to a method developed by Maragkoudakis et al [19] with some modifications. Briefly, cells from a 48-hour culture were harvested by centrifugation at 6000 × *g* for 5 minutes at 4°C, washed once with phosphate-buffered saline (PBS) solution with pH 7.4, before being resuspended in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 10^8 colony forming units (CFU)/mL. SGF was prepared by supplementing sterilized PBS, pH 2, pH 3, and pH 4 with pepsin to a final concentration of 3 g/L. SIF was prepared by supplementing sterilized PBS, pH 6.8 and pH 8 with pancreatin (Sigma Aldrich, St. Louis, MO, USA) to a final concentration of 1 g/L and 0.3 g/L bile salt mixtures. The resistance of isolates was evaluated by counting viable colony on YMB agar plates after 0 hours, 1 hour, 2 hours, and 3 hours for SGF and 0 hours, 1 hour, 2 hours, 3 hours, and 4 hours for SIF experiments, respectively.

2.4. Hydrophobicity

The hydrophobicity of the isolates was assessed by following the method of Rosenberg [20]. Cells from a previously grown culture were harvested and washed twice with PBS, pH 7.4. Cell count was adjusted to ~10^9 CFU/mL. Two milliliters of cell suspension was mixed with an equal volume of *n*-hexadecane by vortexing for 2 minutes. The aqueous and the organic phases were allowed to separate by keeping the mixture undisturbed for 1 hour. After that, the aqueous layer was gently pipetted out and optical density at 600 nm (OD_{600}) was measured. The cell surface hydrophobicity was calculated as:

$$Hydrophobicity(\%) = \left(\frac{Abs_{initial} - Abs_{final}}{Abs_{initial}}\right) \times 100 \quad (1)$$

Where $Abs_{initial}$ represents initial absorption before mixing and Abs_{final} represents final absorption after mixing with *n*-hexadecane.

2.5. Autoaggregation and coaggregation

Autoaggregation capacity of the isolate was evaluated using previously described methods [21]. Briefly, 4 mL of cell suspension (10^9 CFU/mL) was vortexed for 10 seconds and incubated at 37°C. After 4 hours, a 100-μL aliquot was taken out from the upper surface, mixed with 900 μL PBS (pH 7.4) and OD_{600} was measured. Autoaggregation percentage was calculated as:

$$Autoaggregation\ (\%) = \left(1 - A_t/A_0\right) \times 100 \quad (2)$$

Where A_t = absorbance at 4 hours and A_0 = absorbance at 0 hours.

For the coaggregation assay, a mixed culture was prepared by mixing equal volume (2 mL) of the isolate and pathogenic indicator (*S. enterica typhimurium* MTCC 1252) cell suspension (~10^9 CFU/mL) by vortexing for 10 seconds. Control tubes were set up at the same time, containing 4 mL each bacterial suspension. The absorbance at 600 nm of the suspensions was measured followed by mixing and 4 hours of incubation at room temperature. Samples were taken in the same way as in the autoaggregation assay. The percentage of coaggregation was calculated using the equation of Handley et al [22].

$$Coaggregation\ (\%) = \frac{\{(Ax + Ay/2)\} - A(x+y)}{Ax + Ay/2} \times 100 \quad (3)$$

Where x and y represent each of the two strains in the control tubes, and (x + y) represents the mixture.

2.6. Probiotic adhesion to Caco-2 cells

The human colorectal adenocarcinoma Caco-2 cell line was procured from the National Centre for Cell Science (Pune, India). The cell line was routinely grown and maintained in minimal essential medium supplemented with 20% heat-inactivated fetal bovine serum [23]. Media and reagents were purchased from Sigma (India) and Gibco Life Technologies (Waltham, MA, USA).

The adhesion study was performed using previously described method of García-Cayuela et al [24]. Briefly, Caco-2 cells were seeded at a concentration of 10^4 cells/mL in 24-well tissue culture plates (NEST Biotechnology, New Jessey, 07065, USA) and grown for 14 days to achieve about 80% confluence at 37°C in a humidified atmosphere containing 5% CO_2. The yeast cells grown previously were harvested by centrifugation (2000×*g* for 5 minutes), washed twice with PBS 1× and resuspended in minimal essential medium without antibiotic supplementation at a concentration of ~10^8 CFU/mL. For adhesion assay, Caco-2 cell monolayers were washed to remove medium containing antibiotic and inoculated with fresh yeast cell suspensions (yeast cells: Caco-2 cells at a ratio of 10: 1). After an incubation period of 1 hour, the medium was discarded and the wells were gently washed three times with PBS buffer to remove nonadhering probiotic cells. Finally, Caco-2 monolayers were trypsinized with 0.25% trypsin–EDTA solution (Sigma) and the number of adherent isolates was determined by serial dilution plating on yeast and mould agar (YMA). All the experiments were performed in triplicate. Adhesion data were expressed as the percentage of yeast cells adhered compared to the total inoculum added (CFU yeast cells adhered/CFU yeast cells added). For visualization of adhesion, Caco-2 cell monolayers were washed three times with PBS, dried in air, and adherent yeast cells were observed in microscope (EVOS FL Cell Imaging System; ThermoFisher, USA) under 20× magnification after fixing with 3% paraformaldehyde.

2.7. Assimilation of cholesterol by isolates

The ability to assimilate cholesterol was determined following the method of Lye et al [25] with some modifications. Yeast isolates were inoculated into YMB supplemented with water-soluble cholesterol (polyoxyethanyl-cholesterylsebacate; Sigma) at a concentration of 50 μg/mL and 0.3% ox bile, followed by incubation at 37°C. The presence of cholesterol in the spent broth was determined using a colorimetric method [26]. The attachment of cholesterol particles onto the cell surface was visualized by scanning electron microscopy. The cell pellet obtained after centrifugation in the previous step was fixed with 2.5% glutaraldehyde for 6 hours. The samples were then centrifuged and the pellet was resuspended in 1× PBS, pH 7.4 for 1 hour containing 1% osmium tetroxide (Sigma–Aldrich, St. Louis, MO, USA). Further cells were dehydrated in graded concentrations of ethanol. Then, specimens were platinum coated using a Jeol JFC-1600 auto-fine coater and observed under scanning electron microscopy (JSM-6390 LV; Jeol, Akishima, Tokyo, Japan) at 20 kV.

2.8. Cholesterol-lowering ability of isolates in Wistar rat model

Cholesterol-lowering ability was tested following a method reported by Kumar et al [27], with some modifications. Thirty adult male Wistar rats (mean body weight 150 g) used in this study were maintained at the Defence Research Laboratory, Tezpur, India (Animal Ethics Approval No: 1AEC/01/2015). We housed six animals per cage under a constant 12-hour light–12-hour dark cycle with a controlled temperature at 25°C and a relative humidity of 56–60%. Both *S. cerevisiae* ARDMC1 and *S. boulardii* were separately mixed with high-fat diet (HFD) to achieve a final concentration of ~ 10^8 CFU/g. The CFU/g of the diet was counted by suspending 1 g diet in 9 mL PBS, and then the appropriate dilutions were made and plated on YMA to determine the exact CFU/g of the diet. All animals were acclimatized by feeding on a basal diet for 1 week prior to experiment. The animals were divided into five experimental groups: (1) normal diet, (2) HFD, (3) HFD with statin (atorvastatin, Macleods Pharmaceuticals Ltd., Mumbai, India), (4) HFD with *S. boulardii*, and (5) HFD with *S. cerevisiae* ARDMC1. All rats had free access to water and their specific diets (20 g/100 g of body weight/day). The body weights of the animals were measured weekly. The experimental HFD was formulated and fed to the animals for 42 days with slight modification of the method reported earlier [27]. The HFD contained cholesterol (0.5%), *dalda* (30%), refined soy oil (10%), and wheat flour (50%) as major constituents.

2.9. Analysis of serum lipid profile

For the collection of blood samples, animals were fasted overnight and on the next day, blood was collected from the retro-orbital sinus and preserved in prechilled tubes at −20°C. For serum lipid analysis, blood samples were collected at 21 and 42 days of feeding trial. The collected blood samples were centrifuged at 2000 × *g* for 15 minutes at 4°C. The serum obtained was analyzed for total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL)-cholesterol using

commercial enzymatic kits (Autopak; M/s Siemens Diagnostics, Mumbai, India). Friedewald's equation [28] was applied to analyze the following plasma lipid fractions: (1) low-density lipoprotein (LDL)-cholesterol = TC − HDL-cholesterol − (TG/5) and (2) very low-density lipoprotein (VLDL)-cholesterol = TG/5. Atherogenic index (AI) was calculated according to the formula AI = (TC − HDL-C)/HDL-C and LDL-C/HDL-C ratio was calculated as the ratio between plasma LDL-C and HDL-C as proposed by Harnafi et al [29]. All concentrations were expressed in mg/dL.

2.10. Statistical analysis

Results were expressed as mean ± standard deviation and the data were analyzed by GraphPad version 5.00 (San Diego, CA, USA). One-way analysis of variance with Tukey's multiple comparison tests was used to compare the differences among various groups. A value of $p < 0.05$ was considered to be statistically significant.

2.11. Ethical approval

All rat model experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India with institutional approval no: 1AEC/01/2015.

3. Results

3.1. Molecular identification of isolates

Figure 1A depicts the amplicons obtained from PCRs. The sizes of the amplicons were around 850 bp (5.8S-ITS) and 560 bp (D1/D2 domain) for ARDMC1, which corroborate with the expected amplicon size of *Saccharomyces* strain reported previously [30,31]. The sequences obtained from 5.8S-ITS rDNA and domain D1/D2 were used to construct the phylogenetic tree (Figures 1B and 1C) using the neighbor-joining method and showed maximum similarity to *S. cerevisiae* Sc19 and *S. cerevisiae* QWF, respectively. The gene sequences of 5.8S-ITS rDNA (KF414969) and the D1/D2 domain of the large subunit of 26S rDNA (KP233782) were submitted to NCBI GenBank. The probiotic reference strain *S. boulardii* used in this study was confirmed based on sequencing of 5.8S-ITS rDNA and 26S rDNA.

3.2. In vitro gastrointestinal stress tolerance test

Table 1 represents the survivability of ARDMC1 and probiotic reference strain *S. boulardii* after exposure to SGF and SIF

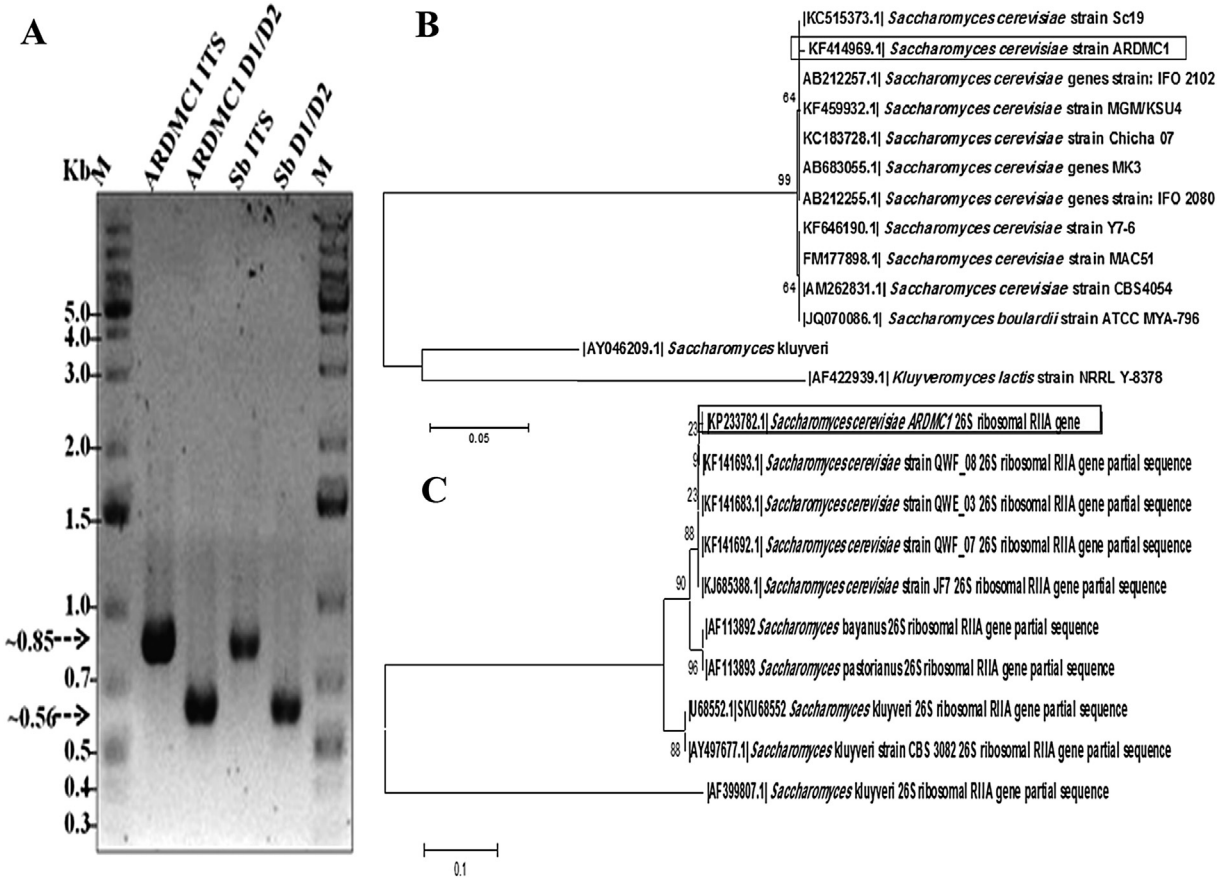

Figure 1 − (A) Molecular typing of *Saccharomyces cerevisiae* ARDMC1 and *Saccharomyces boulardii* by using ITS primer (Lanes 2 and 4) and 26S rRNA gene D1/D2 region (Lanes 3 and 5); Lanes 1 and 6 (M) indicate GeneRuler 1 Kb Plus DNA ladder (Fermentas, Thermo Scientific, USA). Phylogenetic tree showing *S. cerevisiae* ARDMC1 with closely related species based upon (B) 5.8S ITS rRNA and (C) D1/D2 26S rRNA sequences. Bootstrap values (1000 replicates) are indicated at branch nodes. ITS = internal transcribed spacer.

Table 1 – Simulated gastric fluid tolerance test and simulated intestinal fluid tolerance test

Gastric fluid tolerance						
Time (h)	pH 2.0 (SGF)		pH 3.0 (SGF)		pH 4.0 (SGF)	
	ARDMC1	Sb	ARDMC1	Sb	ARDMC1	Sb
0	7.63 ± 0.405^{a}	8.10 ± 0.54^{a}	7.86 ± 0.169^{a}	7.79 ± 0.34^{a}	7.78 ± 0.34^{a}	7.91 ± 0.172^{a}
1	6.13 ± 0.721^{b}	6.62 ± 0.359^{b}	6.06 ± 0.015^{bd}	6.176 ± 0.28^{b}	6.69 ± 0.42^{b}	6.24 ± 0.51^{b}
2	6.321 ± 0.45^{b}	6.28 ± 0.55^{c}	6.56 ± 0.412^{c}	6.193 ± 0.151^{b}	7.052 ± 0.6^{b}	6.68 ± 0.43^{b}
3	6.043 ± 0.06^{b}	5.94 ± 0.084^{c}	6.18 ± 0.413^{cd}	6.26 ± 0.345^{b}	6.27 ± 0.042^{b}	5.84 ± 0.34^{b}
Intestinal fluid tolerance						
Time (h)	pH 6.8 (SIF)			pH 8.0 (SIF)		
	ARDMC1	Sb		ARDMC1	Sb	
0	7.92 ± 1.43	7.48 ± 0.44		8.24 ± 0.75	8.07 ± 0.94^{a}	
1	6.62 ± 0.40	6.20 ± 0.20		6.27 ± 0.15	5.96 ± 0.26^{bc}	
2	6.90 ± 0.48	6.0 ± 0.19		6.77 ± 0.64	6.31 ± 0.34^{ac}	
3	6.49 ± 0.25	6.10 ± 0.2		6.12 ± 0.36	6.20 ± 0.19^{ac}	
4	6.58 ± 0.26	6.59 ± 0.17		6.47 ± 6.47	6.57 ± 0.47^{ac}	

Viability of isolates under simulated gastric fluid at pH 2.0, pH 3.0, and pH 4.0 and SIF simulated intestinal fluid at pH 6.8 and pH 8.0. Values are represented as mean ± standard deviation, $n = 3$. Different letters along the same column represent significant differences ($p < 0.05$). ARDMC1 = *Saccharomyces cerevisiae* ARDMC1; Sb = *Saccharomyces boulardii*; SGF = simulated gastric fluid; SIF = simulated intestinal fluid.

conditions. When exposed to pepsin supplemented SGF of pH 2 for 3 hours, the yeast cell (ARDMC1) count was reduced to ~1.5 log units or 20.79%, whereas *S. boulardii* showed a decrease of 2.16 log units or 26.6% after exposure to the same conditions. The viability of ARDMC1 at pH 3 and pH 4 was found to be more than 6 log units which is comparable to that of *S. boulardii*. However, survivability of ARDMC1 was not inhibited significantly ($p < 0.05$) and showed considerable resistance under SIF conditions at pH 8.0, although its viability decreased from log 8.2 CFU/mL to about log 6.4 CFU/mL.

3.3. *Hydrophobicity, autoaggregation, and coaggregation*

Hydrophobicity indices of ARDMC1 and *S. boulardii* were found to be 61.4 ± 0.10% and 58.59 ± 0.2.25%, respectively. The autoaggregation capability of ARDMC1 and *S. boulardii* did not differ significantly ($p < 0.05$) and were 43.19 ± 0.90% and 40.40 ± 0.93%, respectively. Both ARDMC1 and *S. boulardii* could coaggreagate *S. enterica typhimurium* up to 54.47 ± 0.02 (Table 2).

3.4. *Microbial adhesion to Caco-2 cells*

Microscopic observation showed adhesion between Caco-2 cell culture and the isolated strain as depicted in Figures 2A–C. The ARDMC1 strain was also examined quantitatively for its capability to adhere to Caco-2 cells using *S. boulardii* as a reference strain. The adhesion ability of probiotic reference strain *S. boulardii* (38.34%) was found to be higher as compared with ARDMC1 (30.43%) as shown in Figure 2D.

3.5. *In vitro cholesterol assimilation and SEM of cholesterol binding to cellular surface*

The cholesterol assimilation efficiency of spent broth and cells of ARDMC1 and *S. boulardii* from culture media is presented in Figure 3. The concentration of cholesterol determined by spent broth decreased up to 20.76 ± 2 μg/mL that have been associated with the significantly ($p < 0.05$) increased amount of cholesterol (33.69 ± 1.0 μg/mL) detected in the resuspended cells of ARDMC1 after 72 hours incubation (Figure 3C). Contrary to this, *S. boulardii* could assimilate a small amount of cholesterol (8.62 μg/mL) after 72 hours incubation (Figure 3F). The cholesterol assimilation of ARDMC1 yeast cells was found to be higher as compared with *S. boulardii* isolated from the marketed probiotic drug, which is in accordance with the findings of Psomas et al [11]. Scanning micrographs also showed that cholesterol particles adhered to the cellular surface of yeast cells (Figures 3A, 3B, 3D, and 3E). The bounded cholesterol particles on the cell surfaces resulted in the roughness of cell wall. The capability of cholesterol-binding appeared to be strain specific [32].

3.6. *Cholesterol-lowering ability of probiotic strains in Wistar rat model*

S. cerevisiae ARDMC1 and *S. boulardii* were evaluated for their cholesterol-lowering capability under *in vivo* conditions using a Wistar rat model system. As shown in Table 3, the levels of TG, LDL-C, VLDL-C, and TC of the HFD group were found to be significantly higher ($p < 0.05$) than those of the HFD + statin, HFD + ARDMC1 and HFD + *S. boulardii* groups after 42-days feeding trial. In contrast to the control group (HFD), the probiotic supplemented group (HFD + ARDMC1) showed lower levels of TC (62.75 ± 1.34 mg/dL), TG (122.70 ± 10.04 mg/dL), and LDL-C (21.71 ± 0.18 mg/dL) at the end of 42 days. As anticipated, the HFD + statin group had significantly lower levels of serum TC, TG, and LDL-C levels of 65.83 mg/dL, 112.90 mg/dL, and 27.95 mg/dL, respectively. These values were not significantly different from those of the HFD + ARDMC1 and HFD + *S. boulardii* groups. Moreover, in probiotic-supplemented groups, AI and LDL-C/HDL-C ratios were significantly lower than in the HFD group. There is a dearth of

Table 2 – Autoaggrgation, coaggregation, and hydrophobicity of ARDMC1 and Sb

Isolates	Autoaggregation (%)	Coaggregation (%)	Hydrophobicity (%)
ARDMC1	43.19 ± 0.90	44.41 ± 0.005	61.4 ± 0.1
Sb	40.40 ± 0.93	54.47 ± 0.024	58.59 ± 0.225

Values are represented as mean ± standard deviation, $n = 3$. ARDMC1 = *Saccharomyces cerevisiae* ARDMC1; Sb = *Saccharomyces boulardii*.

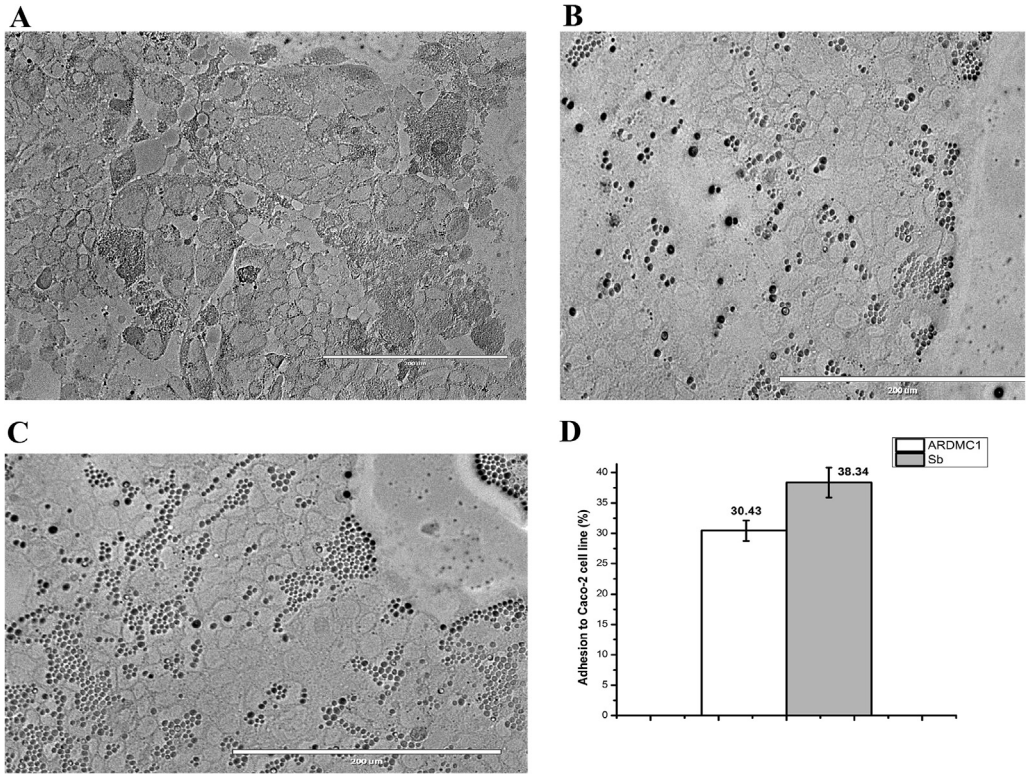

Figure 2 – Adhesion to Caco-2 cell line as observed under inverted microscope (20×). (A) Control Caco-2 cells before addition of yeast cells, (B) adhesion of *Saccharomyces cerevisiae* ARDMC1, (C) adhesion of *Saccharomyces boulardii* and (D) percentage adhesion as calculated by plate count method.

published information on hypocholesterolemic effects of yeast cells and few attempts have been made to assess the possible cholesterol-lowering mechanisms based on *in vivo* experiments [27,33]. At the end of the study, the mean body weight of the normal diet group was found to be significantly lower than the other treatment groups. The mean body weights of probiotic-treated groups were similar to the statin-treated group.

4. Discussion

The use of yeast as potential probiotics has gained interest in the food pharmaceutical industry. Yeast contributes a significant role in the production of some cheeses and fermented milk [34,35]. They have the capability to significantly enhance the aroma of the final product through the generation of free amino acids and free fatty acids [36]. The low pH of the stomach and intestinal fluids (e.g., bile and pancreatic juice) towards the distal part of the gastrointestinal tract is inhibitory to most of the microbes. The probiotic candidate must withstand these harsh conditions to exhibit health benefits on the host. In the present study, ARDMC1 yeast isolate exhibited considerable tolerance to gastrointestinal stress conditions, proving its suitability as putative probiotics. Previous studies also have reported that probiotic yeasts can survive under gastrointestinal stress conditions [37].

The adhesion to and colonization of the intestinal mucosa is an essential criterion for probiotic microorganisms to enhance the immune system and impart health benefits on the host. The adhesion assay was performed using the well-established Caco-2 intestinal cell line from human colonic adenocarcinoma. The differences in the adhesion abilities of yeast cells might be strain specific as reported earlier [38]. Our results suggest that both the yeast strains have significant adhesive properties, but adhesion of *S. boulardii* to Caco-2 cells was found to be similar to *S. cerevisiae* ARDMC1, in spite of showing less autoaggregation and hydrophobic properties than the latter. These findings imply that the adhesion properties of probiotic yeasts are not correlated to hydrophobicity. This finding is in accordance with the work of Martins et al [39].

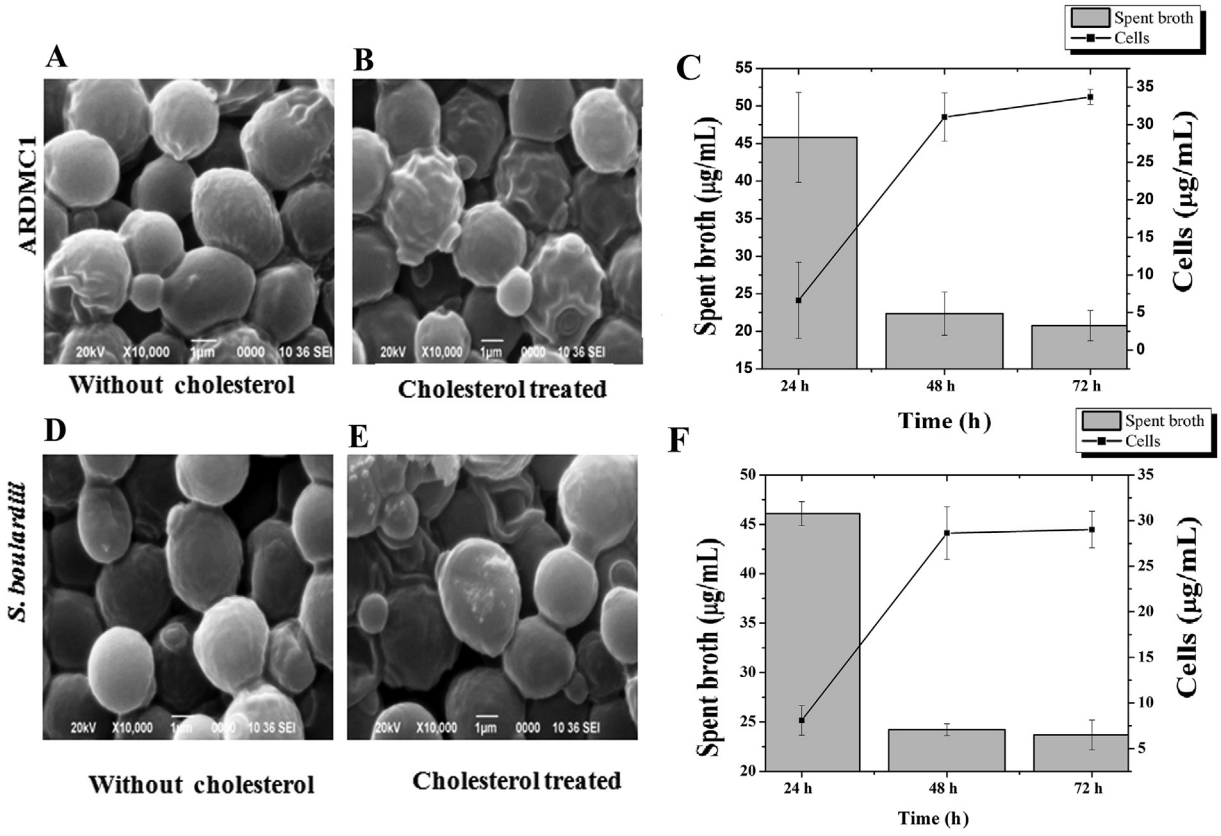

Figure 3 – (A) SEM image of *Saccharomyces cerevisiae* ARDMC1grown in YMB without cholesterol. (B) *S. cerevisiae* ARDMC1 grown with cholesterol. (C) Cholesterol assimilation by *S. cerevisiae* ARDMC1 spent broth and resting cells. (D) SEM image of *Saccharomyces boulardii* grown in YM broth without cholesterol, (E) *S. boulardii* grown with cholesterol, (F) cholesterol assimilation by *S. boulardii* spent broth and resting cells. SEM = scanning electron microscopy; YMB = Yeast and Mold Broth.

Table 3 – Effect of probiotic isolates on serum lipids level (mg/dL) and body weight (g)

	ND	HFD	HFD + statin	HFD + ARDMC1	HFD + Sb	*p*
21 days						
HDL-C	12.16 ± 2.14	16.50 ± 2.03	13.00 ± 3.52	12.80 ± 2.17	14.25 ± 2.87	0.6644
TG	74.27 ± 6.41^{a}	108.12 ± 11.44^{b}	87.87 ± 6.94^{c}	80.36 ± 6.35^{a}	85.63 ± 4.19^{a}	< 0.0001
TC	59.60 ± 5.37^{a}	98.88 ± 9.55^{b}	75.06 ± 7.73^{c}	81.42 ± 7.78^{c}	74.70 ± 3.76^{c}	< 0.0001
LDL-C	32.58 ± 5.84^{a}	62.86 ± 11.27^{b}	44.49 ± 9.15^{ac}	52.55 ± 7.84^{ac}	43.33 ± 7.19^{bc}	0.0001
VLDL-C	14.85 ± 1.28^{a}	21.62 ± 2.29^{b}	17.57 ± 1.39^{c}	16.07 ± 1.27^{ac}	17.13 ± 0.84^{ac}	< 0.0001
AI	4.02 ± 0.92	6.11 ± 1.48	5.35 ± 2.64	5.52 ± 1.29	4.43 ± 1.27	0.2905
LDL/HDL	2.78 ± 0.78	4.52 ± 1.13	3.88 ± 2.07	4.24 ± 1.11	3.20 ± 1.10	0.2296
Body weight	234.16 ± 7.36^{ab}	245 ± 8.94^{b}	229.16 ± 8.01^{a}	240.0 ± 6.12^{ab}	234.16 ± 3.76^{ab}	0.0088
42 d						
HDL-C	14.33 ± 2.08	16.33 ± 1.26	13.33 ± 4.16	15.50 ± 3.54	16.33 ± 1.53	0.4881
TG	93.37 ± 6.70^{a}	260.20 ± 14.32^{b}	119.43 ± 3.00^{a}	122.70 ± 10.04^{a}	108.73 ± 10.24^{a}	< 0.0001
TC	59.13 ± 1.45^{a}	173.97 ± 8.86^{b}	66.50 ± 0.44^{a}	62.75 ± 1.34^{a}	65.63 ± 3.30^{a}	< 0.0001
LDL-C	26.13 ± 2.04^{a}	110.37 ± 3.82^{b}	29.28 ± 3.43^{a}	21.71 ± 0.18^{a}	27.55 ± 6.24^{a}	< 0.0001
VLDL-C	18.67 ± 1.34^{a}	52.04 ± 2.82^{b}	23.89 ± 0.60^{a}	24.54 ± 2.01^{a}	21.75 ± 2.05^{a}	< 0.0001
AI	3.18 ± 0.57^{a}	9.67 ± 0.33^{b}	4.29 ± 1.49^{a}	2.88 ± 0.75^{a}	3.05 ± 0.48^{a}	< 0.0001
LDL/HDL	1.87 ± 0.44^{a}	6.82 ± 0.64^{b}	2.38 ± 0.91^{a}	1.35 ± 0.30^{a}	1.64 ± 0.48^{a}	< 0.0001
Body weight	268.33 ± 5.16^{a}	314.16 ± 5.84^{b}	295.83 ± 9.70^{c}	306.0 ± 6.51^{bc}	300.83 ± 7.35^{c}	< 0.0001

Values are represented as mean ± standard deviation, $n = 6$. Means with different letters in the same row are significantly different ($p < 0.05$), checked by Tukey's multiple comparison test, GraphPad Prism, version 5.0. AI = atherogenic index; ARDMC1 = *Saccharomyces cerevisiae* ARDMC1; HDL = high-density lipoprotein; HFD: high-fat diet; LDL = low-density lipoprotein; ND = normal diet; Sb = *Saccharomyces boulardii*; TC = total cholesterol; TG = triglyceride; VLDL = very low-density lipoprotein.

Low HDL-C and elevated levels of LDL-C, VLDL-C, TC, and TG are associated with the inception of CVD [40] and it is important to keep them at the threshold level. Cholesterol lowering by yeasts in *in vitro* conditions is due to the uptake of cholesterol as in growing yeast cells [11]. Yeasts also contain β-glucans that are reported to bind to bile acids in the intestine, resulting in a decrease in bile acid pool and enhanced cholesterol breakdown. Moreover, yeasts also enhance the production of short-chain fatty acids, which in turn reduce the synthesis of hepatic cholesterol [41]. In our study, the strain ARDMC1 that showed promising *in vitro* cholesterol assimilation activity also showed significant lowering ($p < 0.05$) of "bad cholesterols" LDL-C, VLDL-C, TC, and TG compared to the HFD group, without affecting the concentration of "good cholesterol" HDL-C in serum. Rats fed with yeast cells or yeast-supplemented functional food showed similar results in previous studies. Both AI and LDL-C/HDL-C ratio, which are two important risk factors for atherosclerosis and other CVDs [42], were found to be decreased in the probiotic-treated groups. The body weight increase of the rats with probiotic feed supplements may be attributed to the growth-promoting effects of yeast [9].

5. Conclusion

Our isolate *S. cerevisiae* ARDMC1 showed potential *in vitro* probiotic attributes and hypocholesterolemic activity. Supplementation of ARDMC1 to rats fed a HFD resulted in a significant decrease in serum cholesterol levels in rats. Therefore, it can be concluded that *S. cerevisiae* ARDMC1 has the capability to play an important role in the preparation of functional foods with health-promoting effects.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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