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# Cholesterol Assimilation of Two Probiotic Strains of *Lactobacillus casei* used as Dairy Starter Cultures

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## Abstract

**Background and Objective:** Consumption of milks fermented with lactic acid bacteria has been shown to improve lipid profiles; however, the mechanisms underlying this improvement are not clear. Using *in vitro* analyses, the aim of this study was to investigate how *Lactobacillus casei* strains AP and AG assimilate cholesterol.

**Material and Methods:** Bacterial growth in ox gall-supplemented media, quantity of assimilated cholesterol and activity of bile salt hydrolase were assessed in *Lactobacillus casei* strains AP and AG. Furthermore, cholesterol attachment to cell walls was assessed using scanning electron microscopy.

**Results and Conclusion:** *Lactobacillus casei* AG showed a higher cholesterol assimilation (13.05 mg $dl^{-1}$ ±0.48) than *Lactobacillus casei* AP (8.05 mg $dl^{-1}$ ±0.48) as well as a faster growth rate of the former strain than that of the latter one. Growth inhibition of *Lactobacillus casei* AP was associated with increased activity of bile salt hydrolase (halo size of 1.62 mm ±0.20), compared to that of *Lactobacillus casei* AG (1.37 mm ±0.07) and upregulation of the *bsh* gene. High cholesterol assimilations by *Lactobacillus casei* AG seem to attribute to membrane attachment via resistance to bile acids.

**Conflict of interest:** The authors declare no conflict of interest.

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## 1. Introduction

Cholesterol is an integral component of cellular membranes that participates in various biological processes; however, excessive quantities of this biochemical have been linked to atherosclerosis, cardiovascular diseases and hypertension [1]. Traditional approaches relying on drugs to decrease cholesterol levels can include unwanted side effects; hence, more practitioners are opting for combinatorial treatments involving medical and dietary therapies [2]. Of these novel treatments, consumption of fermented milks is especially promising [3]. Milk fermentation is commonly carried out using lactic acid bacteria (LAB) to break down lactose into lactate [4,5]. These bacteria can be isolated from raw milks, breast milks, infant feces and fermented foods. However, careful considerations must be included in the bacterial sources due to their potential effects on safety and nutritional quality of the final products [6,7]. Pioneering studies have demonstrated that consumption of milk fermented with lactobacilli as LAB decreases serum cholesterol in men [8]. A further contemporary investigation has discovered that dairy products fermented with certain strains of LAB include moderate cholesterol-lowering activities [9]. At present, the exact mechanisms underlying this phenomenon are still unclear. However, it is hypothesized that binding of cholesterol to bacterial cell walls and generation of cell-surface structures such as exopolysaccharide (EPS), as well as the deconjugation of bile acids via bile salt hydrolase (BSH), may be involved [3,10,11]. Regarding the former mechanism, findings from

*in vitro* studies have shown that viable and non-viable LAB can prohibit cholesterol to form a layer surrounding the cells [3]. In addition, EPS is capable of entering the upper gastrointestinal tract (GIT) with no degradations; thus, serving as an energy source for the gut microbes [12] and decreasing cholesterol uptake by the intestinal cells [13,14,15]. Significantly, this leads to shifts in bacterial populations (particularly *Bifidobacterium* spp.) and production of short-chain fatty acids, which may cause changes in serum cholesterol in response to dietary fiber intakes [16,17].

Regarding deconjugation by BSH, bile acids are conjugated with amino acids in the gallbladder to form bile salts after it is synthesized from cholesterol in the liver. Then, BSH hydrolyzes the amide bonds to release bile acids, which are largely excreted. To preserve adequate concentrations of bile acids by the body, serum cholesterol is channeled to the liver for use as a substrate [18], resulting in overall decreases of cholesterol levels in the blood [19]. Widodo et al. have previously found that isolated *Lactobacillus* (*L.*) *casei* strains AP and AG, belonging to LAB and being widely used in dairy industries, can act as probiotics [20] and hence lead to favorable improvements in triglycerides, low-density lipoproteins (LDL), high-density lipoproteins (HDL) and total cholesterol levels when consumed through fermented milk products [21]. However, mechanisms involved in hypocholesterolemic effects exerted by these strains have not been identified. Therefore, the aim of the present study was to address these mechanisms by investigating how *L. casei* strains AP and AG assimilate cholesterol using *in vitro* analyses.

## 2. Materials and Methods

### 2.1. Bacterial strains and culture conditions

*Lactobacillus casei* strains AP and AG were provided by the Faculty of Animal Science, Gadjah Mada University, Indonesia [20]. The bacterial strains were plated onto De Man-Rogosa-Sharpe (MRS) agar (Merck, Jakarta, Indonesia) and incubated at 37 °C for 24 h under microaerobic conditions by adding 0.5 g $l^{-1}$ L-cysteine (Sigma Aldrich, Singapore).

### 2.2. Cholesterol assimilation at various temperatures and concentrations of ox bile (ox gall)

Cholesterol powder (1.3% w $v^{-1}$) (Sigma-Aldrich, Singapore) was diluted using isopropanol and passed through 0.45-µm syringe filters. Briefly, bacterial cultures (1% w $v^{-1}$) in logarithmic growth phase were inoculated into MRS media supplemented with cholesterol solution (10% w $v^{-1}$) and ox gall at sub-lethal concentrations (0.4% w $v^{-1}$) and incubated at 37 °C for 24 h. After incubation, cholesterol levels were assessed. For the effect assessment of growth temperatures on cholesterol assimilation, bacteria were cultured on MRS media combined with the cholesterol solution (10% w $v^{-1}$) and ox gall at 0.4% (w $v^{-1}$) and then incubated at 15, 37 and 45 °C for 24 h. For the assessment of ox gall concentrations, bacteria were cultured at 37 °C for 24 h on MRS media with various ox gall concentrations (0.1, 0.3 and 0.4% w $v^{-1}$). Plain MRS media (with no ox gall supplementations) served as the control. Cholesterol levels and bacterial growth rates were assessed every 3 h for 24 h and bacterial growth was assessed at an optical density of 620 nm ($A_{620nm}$). All assays were carried out in triplicate.

### 2.3. Cholesterol assimilation assay

Cholesterol assimilation was assessed using modified version of a protocol by Widodo et al. [22]:

$$\frac{\text{Cholestrol quantity in control media} - \text{cholestrol quantity in media after incubation}}{\text{Cholestrol quantity in control media}} x\ 100\%$$

After incubation, cultures were centrifuged at 3000× g for 5 min at 4 °C. Concentration of cholesterol in the supernatant was assessed using cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) kit (Sigma-Aldrich, Singapore) [23]. Briefly, a 10-µL sample was added to 1 ml of CHOD-PAP reagent and vortexed. Then, samples were incubated at 37 °C for 10 min and the absorbance was measured at 500 nm using spectrophotometer (Thermo Fisher Scientific, USA). Absorbance values were converted into mg $dl^{-1}$ for comparison with cholesterol standards. Decreases in cholesterol concentration following bacterial assimilation were calculated by dividing the total quantity of cholesterol measured in the media after incubation by the total quantity of cholesterol in control media.

### 2.4. Bile salt hydrolase activity assay

The BSH activity was assessed based on a method by Allain et al. [24] with modifications. Briefly, bacterial cells (10 µl) at a concentration of $10^8$ CFU $ml^{-1}$ were spotted onto MRS agar supplemented with $CaCl_2$ (0.37 g $l^{-1}$) and various concentrations (0.1, 0.3 or 0.4% w $v^{-1}$) of ox gall. Plates were incubated at 37 °C for 72 h under microaerobic conditions and then the diameters of the halos were measured.

### 2.5. Scanning electron microscopy analysis

Scanning electron microscopy analysis was carried out based on a method by Allain et al. [24] with modifications. Briefly, bacterial strains were cultured in MRS media supplemented with cholesterol solution (10% w $v^{-1}$) and ox gall (0.4% w $v^{-1}$) and then incubated at 37 °C for 24 h. The supernatant was removed and bacterial cells were fixed onto glass coverslips with 15 ml of glutaraldehyde (2.5%) at 4 °C for 2 h. The coverslips were washed with 0.1 M of phosphate buffer for 15 min and cells were dehydrated using graded ethanol series (50, 60, 70 and 100% w $v^{-1}$). After drying with liquid $CO_2$, coverslips were coated with platinum (30 mV) using auto fine coater for 90 s and then samples were studied by SEM (JSM-6510LA; JEOL, Tokyo, Japan).

### 2.6. Quantitative polymerase chain reaction analysis of *bsh* expression

Expression of *bsh* was investigated using qPCR and specific *bsh*F and *bsh*R primers designed based on the *bsh* sequence for *L. casei* strain Zhang (accession no. EU5-99213.1). Quantitative polymerase chain reaction (qPCR) reaction mixture consisted of 5 µl of SsoFast EvaGreen Supermix, 1 µl of the forward primer, 1 µl of the reverse primer, 1 µl of cDNA and 2 µl of DNase-free water. The qPCR program was set as follows: preliminary denaturation at 95 °C for 30 s, followed by 39 cycles of denaturation at 95 °C for 5 s, annealing at 59 °C for 30 s and amplification at 65 °C for 5 s. Then, a cycle of melting was carried out at 95 °C for 5 s. All samples were analyzed in duplicate. Expression of *bsh* was assessed using $2^{-\Delta\Delta CT}$ method. Moreover, glyceraldehyde 3-phosphate dehydrogenase was used as the standard. Significant differences were identified using independent *t*-test.

### 2.7. Statistical analysis

Data were expressed as the mean ±SD (standard deviation) of the mean. Cholesterol assimilation and bile salt hydrolase activity data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to assess the significance of comparisons. The $p < 0.05$ was considered significant and $p < 0.01$ was considered highly significant. All analyses were carried out using SPSS Software v.16.0 (IBM, New York, NY, USA).

## 3. Results and Discussion

The present study assessed cholesterol assimilation in two *L. casei* strains AP and AG. The growth media included ox gall at sub-lethal concentrations (0.4% w $v^{-1}$) to simulate environment of the human GIT. As Table 1 shows, the two bacterial strains decreased concentration of cholesterol in the supernatant after 24 h; however, *L. casei* AG was more effective than *L. casei* AP (57 vs. 35% decreasing rates). These findings and other relative studies have reported that *Pediococcus pentosaecesus* LAB6 and *P. pentosaceus* KID7 are capable of decreasing cholesterol levels in media supplemented with 0.3% (w $v^{-1}$) of ox gall by 58 and 33%, respectively [25]. Growth rates of *L. casei* strains AP and AG, as well as their corresponding cholesterol assimilation patterns over 24 h, are shown in Figs. 1 and 2. The two strains exhibited various growth phases as *L. casei* AG grew rapidly (reaching the logarithmic phase within 3-6 h), while *L. casei* AP included a long adaptive phase (6 h) before reaching the logarithmic phase at the end of a 24-h incubation period. The two strains needed growth adaptation before cholesterol assimilation began as *L. casei* AP needed 3 h to adapt, while adaptation by *L. casei* AG needed 6 h. Overall, decreases of cholesterol in the media were associated with increased bacterial growth, particularly during the logarithmic growth phase, suggesting that cholesterol removal was correlated with the bacterial growth. In an earlier study on 11 strains of lactobacilli by Liong and Shah [11], cholesterol assimilation by growing cells was significantly higher than that of resting or dead cells. Furthermore, no differences were seen in levels of cholesterol removal between the resting and the dead cells. Thus, increased removal of cholesterol by growing cells might indicate that the binding of cholesterol was dependent on cellular growth.

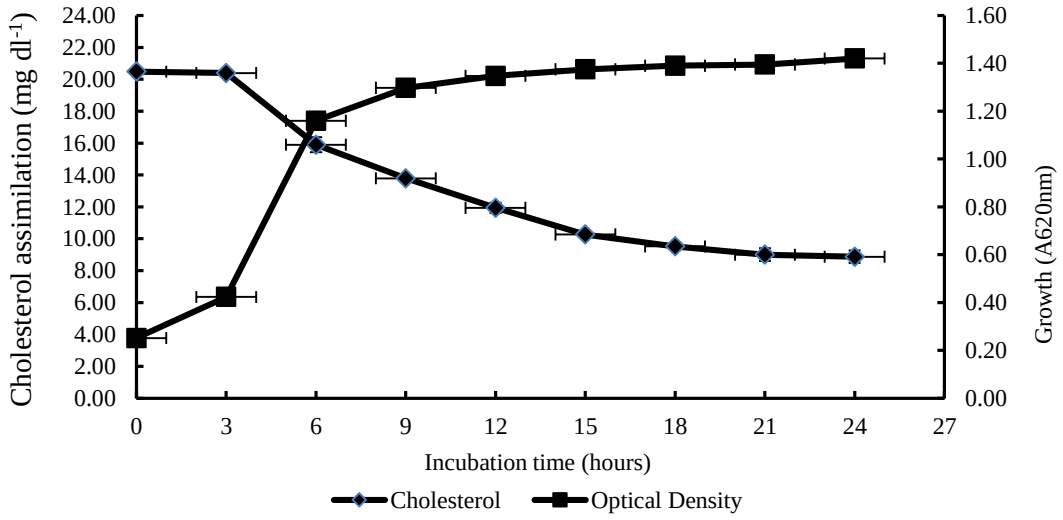


**Figure 1**. Cholesterol assimilation (black diamonds) and bacterial growth (black squares) of *Lactobacillus casei* strain AG in cholesterol-containing media with 0.4% ox gall
(w $v^{-1}$) at 37 °C. Error bars represent standard deviations

**Table 1**. Cholesterol assimilation by *Lactobacillus casei* strain AP and *Lactobacillus casei* strain AG in media with 0.4% ox gall (w $v^{-1}$) after 24 h of growth at 37 °C

| Culture | Cholesterol concentration before incubation (mg $dl^{-1}$) | Cholesterol concentration after incubation (mg $dl^{-1}$) | Cholesterol assimilation (mg $dl^{-1}$) | Cholesterol reduction (%) |
|---|---|---|---|---|
| Control | 22.94 ± 0.78 | 22.94 ± 0.78 | 0 | 0 |
| *L. casei* strain AP | 23.02 ± 1.12 | 14.96 ± 0.73 | 8.06 ± 0.48[a] | 35 |
| *L. casei* strain AG | 22.90 ± 0.69 | 9.85 ± 0.30 | 13.06 ± 0.48[b] | 57 |

Different superscripts indicate significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation, *L. casei*= *Lactobacillus casei*

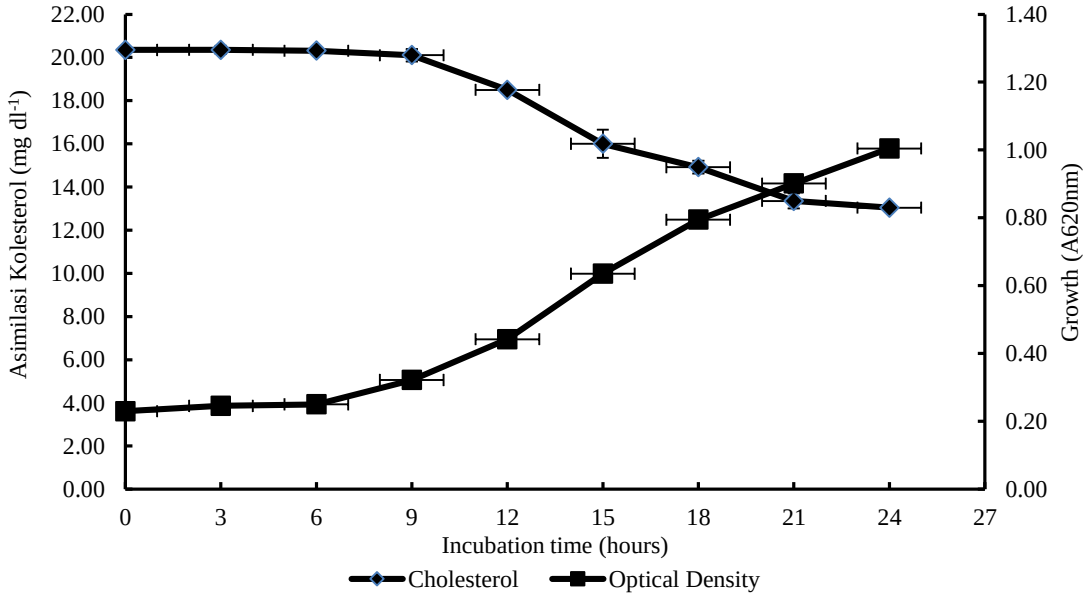


**Figure 2**. Cholesterol assimilation (black diamonds) and bacterial growth (black squares) of *Lactobacillus casei* strain AP in cholesterol-containing media with 0.4% ox gall (w $v^{-1}$) at 37 °C. Error bars represent standard deviations

The present findings revealed differences in cholesterol assimilation between the bacterial strains. An earlier investigation reported that *L. fermentum* and *L. acidophilus* isolated from Dangke (a fermented food native to Sulawesi, Indonesia) assimilated cholesterol in quantities of 3.2 and 5.1 mg $dl^{-1}$, respectively. Another study showed that a combination of *L. plantarum* and *L. acidophilus* increased cholesterol assimilation by 5.6 mg $dl^{-1}$ [26]. The current authors previously detected that *L. acidophilus* strains FNCC 101, FNCC 108 and FNCC 120 assimilated cholesterol in quantities of 0.45, 0.47 and 0.52 µg $ml^{-1}$, respectively [22]. In contrast to *L. casei* AP, ability of *L. casei* AG to assimilate cholesterol was not affected by the ox gall concentration (Fig. 3) as the biochemical quantities were nearly identical for the bacteria cultured on media supplemented with 0.1, 0.3 and 0.4% of ox gall. Although strains were able to assimilate cholesterol in presence of up to 0.5% (w $v^{-1}$) of ox gall, this concentration was unlikely to translate to levels present in the human body because concentration of the bile acids in the small intestine is normally below 0.5% (w $v^{-1}$). When 0.3% of ox gall was added to the growth media, cholesterol assimilation was shown to increase in strains of *L. casei* ASCC 1520, *L. casei* ATCC 15820 and *L. casei* CSCC 2607 and to decrease in strains of *L. casei* ASCC 279, *L. casei* ASCC 290 and *L. acidophilus* ATCC 4357 [11]. Cholesterol assimilation by the aforementioned strains ranged 1.203-3.225 mg $dl^{-1}$, which was lower than that by *L. casei* strains AP and AG (range of 2.77-11.54 mg $dl^{-1}$) in the present study. Tahri et al. [27] reported that *B. breve* ATCC 15700 assimilated up to 51% of the provided cholesterol when cultures were grown in media with taurocholic acid. Moreover, a study by Pereira and Gibson [28] demonstrated that *L. brevis* NR1C1684, *L. casei* Shirota and *Enterococcus faecalis* assimilated more than 5 mg $g^{-1}$ cholesterol in presence of 0.2% (w $v^{-1}$) ox gall. In the current study, *L. casei* strains AP and AG responded variously to the presence of bile acids in the media, suggesting that cholesterol assimilation was strain dependent.

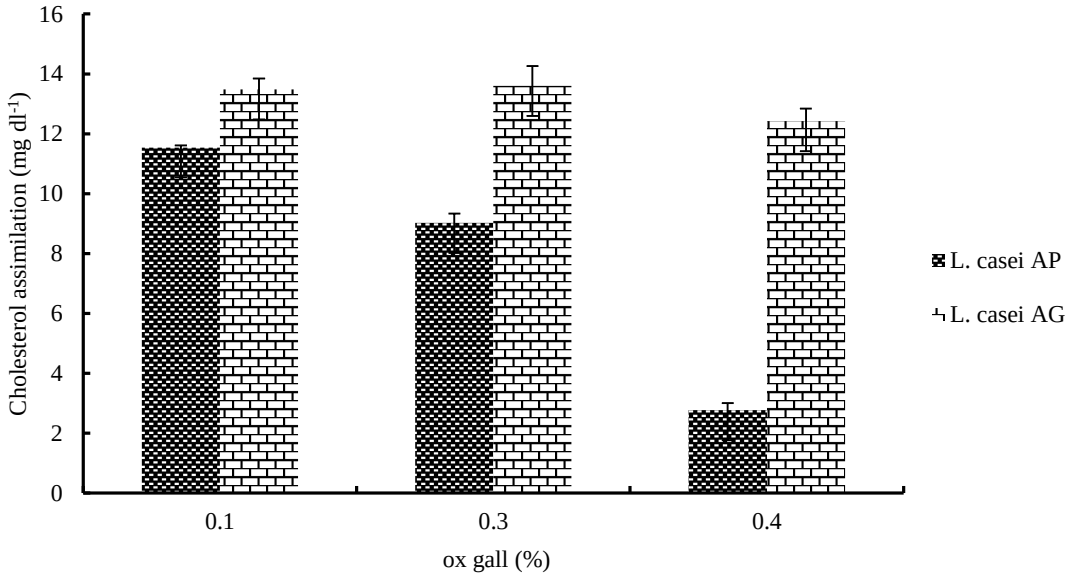


**Figure 3**. Cholesterol assimilation of *Lactobacillus casei* strain AP and AG cultivated at 37 °C in cholesterol-containing media with various ox gall concentrations (0.1, 0.3 and 0.4% w $v^{-1}$). Error bars represent standard deviations

Use of culture media with various quantities of ox gall demonstrated that *L. casei* AP was more sensitive to shifts in ox gall concentration than that the *L. casei* AG was (Fig. 3). Cholesterol assimilation by *L. casei* AP was 11.52 mg $dl^{-1}$ ±0.04 in media with 0.1% of ox gall and decreased to 8.75 mg $dl^{-1}$ ±0.66 and 2.99 mg $dl^{-1}$ ±0.14 in media with 0.3 and 0.4% of ox gall, respectively. Concentration of bile salts in the media was detected to include dose-dependent effects on decrease of cholesterol by *L. casei* AP, with higher concentrations decreasing cell numbers and growth rates. Therefore, the authors postulate that the number of viable cells is an important factor affecting cholesterol decrease *in vitro*. The modest cholesterol assimilation by *L. casei* AP following increases in bile salt concentration could possibly be attributed to high BSH activity, since this protein is produced by the bacterial cells (Table 2). High BSH activity in *L. casei* AP was associated with upregulation of *bsh* expression when the bacterial cells were cultured for 3 h in media supplemented with 0.1% of ox gall (Fig. 4). Previously, Tannock [29] demonstrated that elevated production of BSH in strains of lactobacilli slowed the bacterial growth and caused lysis after 24 h of incubation with bile salts. Moreover, he showed that the growth rate decelerated further when the deconjugated bile acid concentration reached 1 mM. Deconjugated bile acids are known to inhibit cell growth and lead to cell lysis if pH of the media reaches 7.0 [30]. Although it has been suggested that cells deconjugate bile salts to prevent membrane disruption, accumulation of deconjugated bile acids inhibits growth of lactobacilli. Therefore, it is unlikely that accumulation of deconjugated bile acids occurs *in vivo*. This finding might not be representative of processes that occur in the human body.

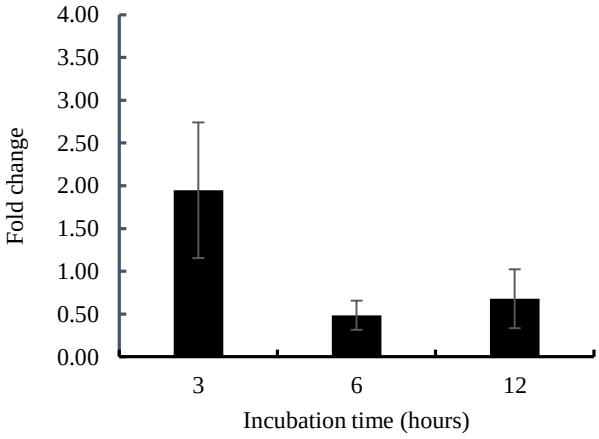


**Figure 4**. Bile salt hydrolase (*bsh*) expression in *Lactobacillus casei* strain AP cultivated in media supplemented with 0.1% ox gall (w $v^{-1}$) at various incubation times. Data are expressed as means; error bars represent standard deviations

Assimilation of cholesterol by *L. casei* AG seemed to minimally be affected by the concentration of bile salts (Fig. 3), suggesting that *L. casei* AG was a bile-tolerant strain. Considering that BSH activity was lower in *L. casei* AG than in *L. casei* AP (Table 2) and *bsh* expression was not upregulated (data not shown), cholesterol assimilation in *L. casei* AG could be attributed to properties in the cell wall that enable cholesterol binding (Figs. 5 and 6). Figure 5a and 6a show no cholesterol attachment when *L. casei* strains AP and AG were cultured in media without cholesterol. In contrast, Figures 5b and 6b show attachment of cholesterol on the surface of *L. casei* strains AP and AG when they were cultured in media containing cholesterol (1.5% w $v^{-1}$). This attachment caused cholesterol removal from the media and decreased cholesterol levels in the supernatant. This finding suggests that cholesterol removal in the growth media of *L.*

*casei* strains AP and AG was associated with cholesterol attachment to the cell membranes. This has been reported as the primary mechanism underlying cholesterol assimilation in *B. longum* and *L. acidophilus* [10,11,31]. Noh et al. [32] reported that LAB removed cholesterol from the media through the attachment of the biochemical to the surface of the bacterial cell wall. This caused dissolving of cholesterol in the media and its decreases and consequently changes in the bacterial cell structure. The correlation between cholesterol removal and modification of cell structure has been reported by Taranto et al. [33].

**Table 2**. Bile salt hydrolase activity as indicated by the diameter of halos of *Lactobacillus casei* strain AP and *Lactobacillus casei* strain AG cultivated at 37 °C on agar with various concentrations of ox gall

| Strain | Oxgall concentration (%) | Precipitation halo (mm) |
|---|---|---|
| *Lactobacillus casei* strain AP | 0.1 | 1.62 ± 0.20$^{c}$ |
| | 0.3 | 1.58 ± 0.18$^{c}$ |
| | 0.4 | 0.84 ± 0.48$^{a}$ |
| *Lactobacillus casei* strain AG | 0.1 | 1.37 ± 0.07$^{bc}$ |
| | 0.3 | 1.15 ± 0.06$^{b}$ |
| | 0.4 | 0.77 ± 0.44$^{a}$ |

Different superscripts indicate significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation

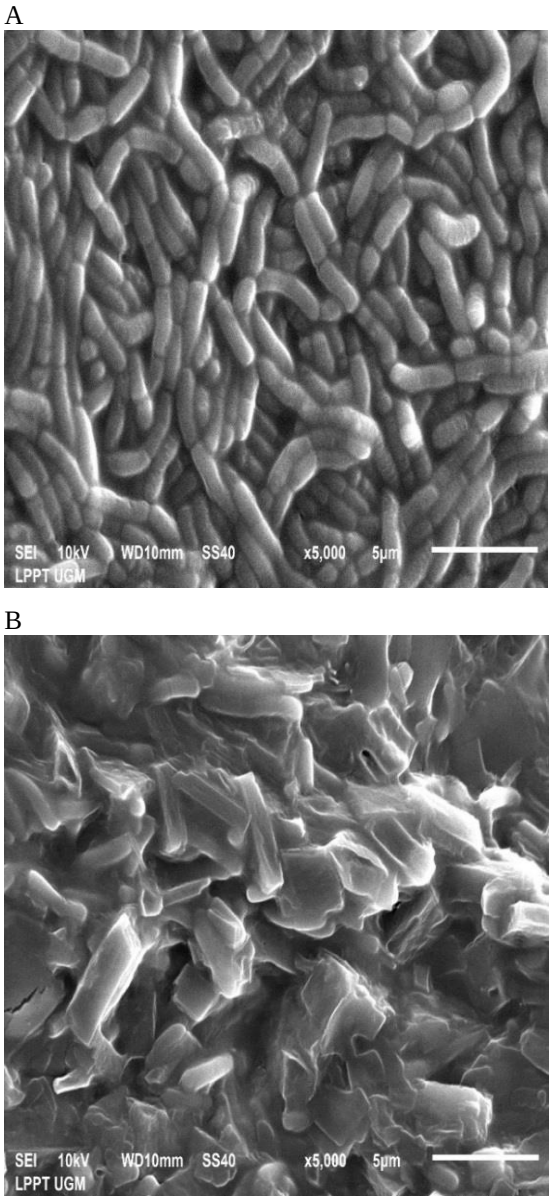


**Figure 5**. Representative scanning electron micrographs of *Lactobacillus casei* strain AG cultivated (A) without and (B) with cholesterol (1.5% w v$^{-1}$). Scale bars represent 5 µm

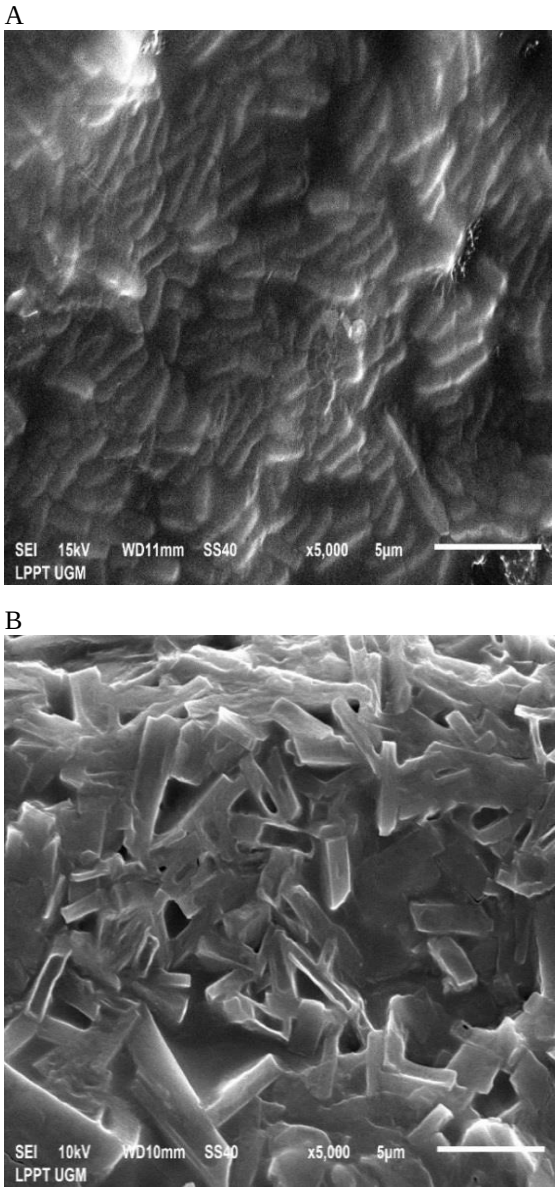


**Figure 6**. Representative scanning electron micrographs of *Lactobacillus casei* strain AP cultivated (A) without and (B) with cholesterol (1.5% w v$^{-1}$). Scale bars represent 5

Kimoto et al. [34] reported that heat-killed cells, which were not able to uptake cholesterol, could remove cholesterol from the media. This indicated that the mechanism involved cholesterol binding to the cells; however, the authors described that the quantity of cholesterol removed from the media by the live cells was higher than that by the heat-killed cells. The present data revealed that temperature affected cholesterol assimilation in the two strains. Figure 7 shows that the optimal cholesterol assimilation for *L. casei* strains AG and AP occurred at 37 °C with assimilated quantities of 12.86 mg $dl^{-1}$ ±0.34 and 7.11 mg $dl^{-1}$±0.30, respectively. At 45 °C, these quantities mildly decreased to 12.29 mg $dl^{-1}$ ±0.18 and 4.14 mg $dl^{-1}$ ±0.36. Furthermore, cholesterol assimilation was the lowest for the two strains when cultured at 15 °C. Collectively, these results and other results from a previous investigation by the current authors showed that *L. casei* strains AP and AG could grow at temperatures up to 45 °C but not below 10 °C [35]. The growth profiles and ability to assimilate cholesterol in presence of ox gall suggested that bile salts inhibited cholesterol assimilation in *L. casei* AP but not in *L. casei* AG. Importantly, bile salts reduced growth of *L. casei* AP, particularly in the logarithmic phase, with associated effects on cholesterol decreases. This finding was similar to the finding of a linked report that increasing concentrations of bile salts decreased the bacterial growth rate and cholesterol uptake [36]. These evidence suggested that *L. casei* AG was resistant to bile salts and free bile acids.

## 4. Conclusion

This study demonstrated that *L. casei* AG could assimilate more cholesterol than that the *L. casei* AP could, as evidenced by the bacterial ability to grow in media with high concentrations of bile salts. Mechanistically, this allowed cholesterol to attach to the cell walls of growing bacteria, leading to the removal of cholesterol from the media. In conclusion, milks fermented with *L. casei* AG can be recommended for the patients diagnosed with dyslipidemia.

## 5. Acknowledgements

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## 6. Conflict of Interest

The authors declare no conflict of interest.

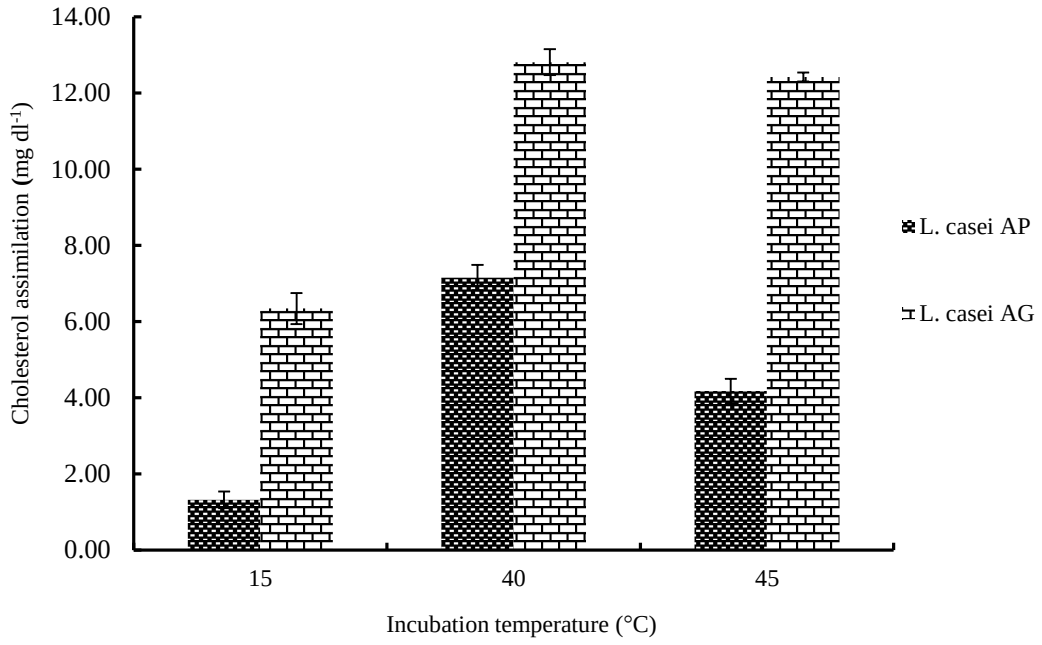


**Figure 7**. Cholesterol assimilation by *Lactobacillus casei* strain AP and *Lactobacillus casei* strain AG cultivated in media supplemented with 0.4% ox gall (w $v^{-1}$) at various temperatures. Data are expressed as means; error bars represent standard deviations

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# جذب کلسترول در دو سویه زیست‌یار لاکتوباسیلوس کازئی مورد استفاده به‌عنوان کشت‌های آغازگر لبنی

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- جذب کلسترول
- *لاکتوباسیلوس کازئی*
- زیست‌یارها

***نویسنده مسئول**

**وی دودو وی دودو**

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## چکیده

**سابقه و هدف:** نشان داده شده است که مصرف شیرهای تخمیرشده با باکترهای لاکتیک اسید پروفایل لیپیدی را بهبود می‌بخشد؛ اگرچه اساس سازوکار آن شفاف نمی‌باشد. هدف این مطالعه بررسی چگونگی جذب کلسترول توسط سویه‌های AP و AG *لاکتوباسیلوس کازئی* با استفاده از آزمون‌های برون تنی[۱] می‌باشد.

**مواد و روش ها:** رشد باکتریایی بر روی محیط کشت حاوی مکمل ox gall، میزان جذب کلسترول و فعالیت نمک صفراوی هیدرولاز در سویه‌های AP و AG *لاکتوباسیلوس کازئی* و نیز اتصال کلسترول به دیواره سلولی با استفاده از میکروسکوپ الکترونی روبشی[۲] مورد ارزیابی قرار گرفت.

**یافته‌ها و نتیجه‌گیری:** *لاکتوباسیلوس کازئی* AG میزان بیشتری کلسترل را جذب کرد (۰/۴۸ $mg\ dl^{-1}$ ± ۱۳/۰۵) نسبت به *لاکتوباسیلوس کازئی* AP (۰/۴۸ $mg\ dl^{-1}$ ± ۸/۰۵)، همچنین میزان رشد سریعتری را از خود نشان داد. مهار رشد *لاکتوباسیلوس کازئی* AP با افزایش فعالیت هیدرولاز نمک صفراوی (اندازه هاله ۰/۲۰ ± ۱/۶۲mm)، در مقایسه با *لاکتوباسیلوس کازئی* AG (اندازه هاله ۰/۰۷ ± ۱/۳۷mm) و نیز تنظیم بالادستی ژن *bsh* همراه بود. به‌نظر می‌رسد جذب بالای کلسترول توسط *لاکتوباسیلوس کازئی* AG با اتصال به غشا و مقاومت نسبت به اسیدهای صفراوی مرتبط باشد.

**تعارض منافع:** نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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۱ *in vitro*

۲ Scanning Electron Microscopy (SEM)