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Research Article

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Cholesterol esterase in *Streptococcus thermophillus*

Giang Vo Le Duy and Tu Nguyen Hoang Khue*

[1]*School of Biotechnology, Hochiminh City International University, Vietnam National University- Hochiminh city*
Quarter 6, Linh Trung ward, Thu Duc district, Hochiminh city, Vietnam

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ABSTRACT

Finding out the source reducing cholesterol is essential. In the study, Streptococcus thermophillus was cultured in MRS medium. After precipitation with 60% ammonium sulfate, the obtained precipitant was dissolved in distilled water and used to react with cholesterol oleate for 20 minutes at 37°C and then titrated with 1M NaOH and by using phenolphtalein as an indicator till the permanent red color appeared. The tested samples were reacted with cholesteryl oleate and then were run on thin layer chromatography (TLC) with solvent system and then were detected by spraying with 20% perchloric acid and then dried. Finally, the test was observed under ultraviolet (UV) at the wavelength of 230nm. The result reported that Streptococcus thermophillus could produce cholesteryl esterase.

Keywords: *Streptococcus thermophillus*, cholesterol esterase, titration

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INTRODUCTION

In the body, cholesterol works with high-density lipoproteins (HDL), often referred to as "good cholesterol". High-density lipoproteins carry cholesterol from the body's tissues to the liver for removal. When cholesterol is attached to a fatty acid, it is a cholesterol ester. Normally, the cholesterol esters are broken down by lysosomal acid lipase called cholesterol esterase into cholesterol and a fatty acid and then excreted or used by the body as nutrients. Cholesterol esterase is acid lipase, being an enzyme that catalyzes the hydrolytic cleavage of cholesterol and other sterol esters and triglycerides. This enzyme is found in the lysosome (compartments that digest and recycle materials in the cell) [4] or some bacteria as *Streptomyces lavendulae* [2], [3]. In enzymology, cholesterol esterase is known as a sterol esterase catalyzing the chemical reaction as below:

Cholesterol ester + H_2O ⟶ Cholesterol esterase Cholesterol + Fatty acid

In this study, we detected cholesterol esterase in *Streptococcus thermophilus* isolated from food. This strain is commonly used in the pharmaceutical and food products.

For healthcare, cholesterol causes the big problem in human circulation system. Cholesterol esterase is the main key to solve it. The medicine used in the cholesterol treatment is bile acid resins, ezetimibe (zetia), fibric acid, niacin, commonly. Up to now, there was no information about study of cholesterol esterase in *Streptococcus thermophilus* while this strain is used as biological products for human. This project was the first step to detect cholesterol esterase from *Streptococcus thermophilus* that will be useful for health care so far.

EXPERIMENTAL SECTION

Materials

Foods as yogurts suspected to contain *Streptococcus thermophilus* were selected for study isolated from yogurts and identified was used as a cholesterol esterase-producing microorganism. MRS agar and MRS broth were purchased from Merck. Kit API 50CHL and software were supplied by BioMérieux. Palmitic acid, oleic acid, vitaminD, ethanol, cholesteryl oleate, triton X-10, ammonium sulfate were in purified form.

Streptococcus isolation

The isolation on yogurts and fermented products was performed. The samples were incubated in MRS broth at 45^{o}C in 24-48 hours anaerobically, then spreaded onto MRS agar. The white colonies with the milky smell were picked up and transferred onto MRS agar. The incubation condition was at 37^{o}C in 24-48 hours aerobically. The desired colonies were stained and observed under microscope and then was identified by the API 50CHL kit (BioMérieux).

Cultivation for cholessterol esterase production

Streptococcus thermophilus was grown in MRS agar at 37^{o}C for 2 days in the aerobic condition. Then, they was transferred into MRS broth and incubated at 37^{o}C on the aerobic condition. Palmitic acid and vitamin D were added in the MRS broth. Palmitic acid added in the MRS broth to get the final concentration was 0.012% while the vitamin D amount was added in medium to get the final concentration of 7.5 % for optimizing the cholesterol esterase. All the samples were incubated 3 days at 37^{o}C aerobically.

Partially purification of cholesterol esterase

All samples were centrifuged at 12000 rpm for 15 min and were carried out at 4^{o}C with 60% ammonium sulfate. The precipitate was collected, dialyzed and dissolved in 100 ml distilled water by prepared for experiment.

Detection of free fatty acid by titration

Preparation sample for experiment: 1ml mixture of precipitate dissolved in distilled water was added with 0.5ul cholesteryl oleate and 0.5 % Triton X-100. Then, this mixture was incubated at 37^{o}C for 20min. The detection of free fatty acid by titration was performed according to Arti Nigam [1]. 1 to 2 drops of phenolphtalein solution were added to the sample and then titrated against 1M NaOH till a persistent faint pink color develops. The amount of 1M NaOH was recorded and calculated.

Thin layer chromatography

Thin layer chromatography was performed according to Arti Nigam [1]. The mobile phase was prepared by mixing chloroform: methanol: acetic acid: distilled water with the ratio 5:15:4:2. The standard oleic acid and cholesterol were prepared with 1% and 2%, respectively. For the spraying solution, the 20% percholric acid in aqueous base was used. 5 µl of each sample was applied. After running, the TLC plate was taken out, air dried, and detected percholric acid. The spots of the resolved lipid were located and compared with the spots of standard.

RESULTS AND DISCUSSION

Microorganism identification

3 isolated microorganisms were cocci, positive gram (Figure 1). After identification of them by using the API 50 CHL kit (BioMérieux) and analyzing with the API software, there was only one microorganism identified as *Streptococcus thermophilus* with Id: 100% and T: 0,5.

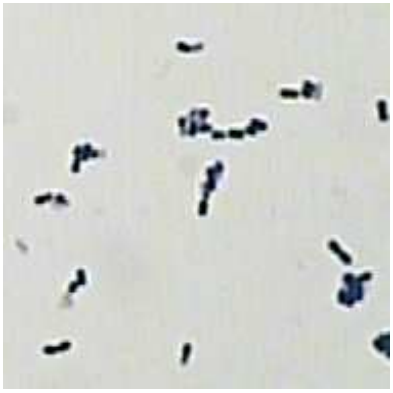

Figure 1: Morphology of *Streptococcus thermophilus* under microscope after Gram stain

Titration to detect of fatty acid

After precipitation with 60% ammonium sulfate, the obtained precipitant was dissolved in distilled water and used to react with cholesterol oleate for 20 minutes at 37^{o}C. The solutions were titrated with 1M NaOH and by using phenolphtalein as an indicator till the permanent red color appeared (Figure 2).

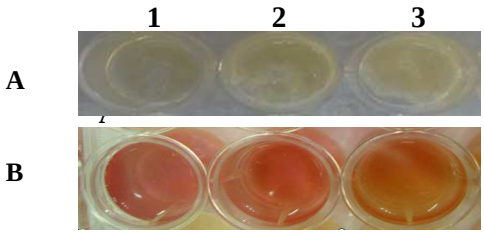

Figure 2: The solution before (A) and after titration (B) (From left to right of A and B: cultured MRS broth (1), added palmitic acid (2), added vitamin D (3))

The volume of 1M NaOH was recorded as in table 1. With the same amount of 1M NaOH, the color of sample under vitamin D condition was weaker than in MRS broth or under palmitic acid. With the same amount of biomass produced in MRS broth or under palmitic acid, the color was red-orange (Figure 2B-3). Therefore, vitamin D may interfere the cholesterol esterase production. The mechanism for cholesteryl esterase production should be studied so far. Remarkably, with this procedure of precipitation and dialysis, the palmitic acid and vitamin D were removed out of the samples.The obtained samples might contain any acidic products due to there were the change color (Figure 2B). After adding phenolphthalein as indicator and titration with 1M NaOH, all solutions had changed color that were summarized as in Table 1. The volume of NaOH made color change is less than 100 µl. It was demonstrated having the reaction between NaOH and acid. To make sure the products formed after hydrolyzation, thin layer chromatography was performed to detect which is fatty acid.

Table 1: The volume of 1M NaOH (µl)

Microorganism	Cultured MRS	Palmitic acid	Vitamin D
Streptococcus thermophilus	100	100	100
	Dark red	Dark red	Red-Orange

Thin layer chromatography

The tested samples were reacted with cholesteryl oleate and then were run on TLC with solvent system. Then the spots were detected by spraying with 20% perchloric acid and then dried. Finally, the test was observed under UV (Figure 3). The image showed the spots had equal Rfs with the oleic acid and cholesterol. So, it make be confirmed that have ester hydrolysis was acted. From which, in the precipitate, they have cholesteryl esterase. However, there were also the existence of spots in the lowest pattern (Figure 3) showing that more products were formed after hydrolysis. Similarily, the other fatty acids will be formed. These spots will be analyzed so far.

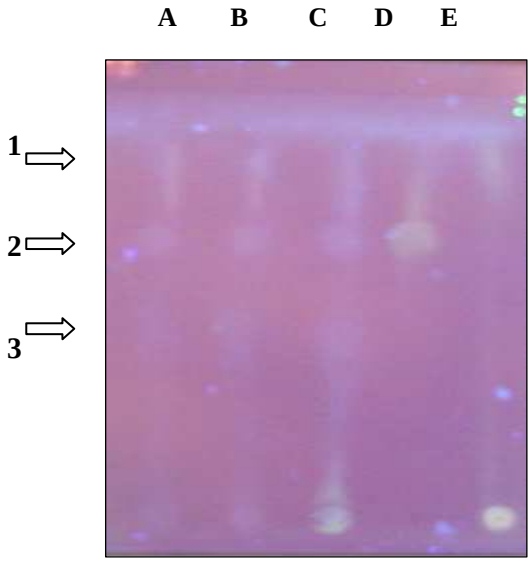

Figure 3: Thin layer chromatography for cholesterol detection in *Streptococcus thermophillus*.
From left to right: (A) sample from MRS, (B) sample from MRS with palmitic acid, (C) sample from MRS with vitamin D, (D) oleic acid, (E) cholesterol. From up to down: (1) cholesterol pattern, (2) oleic acid pattern, (3) others in tested samples.

The precipitation of cholesterol esterase with 60% ammonium sulfate was used to detect the activity. Thin layer chromatography was used to determine the free fatty acid that is the product of cholesterol ester hydroxylation. After culture the microorganism, the media were used to detect the cholesteryl esterase. The positive results showed that the cholesteryl esterase was produced extracellularly. The location of this enzyme in the *Streptococcus thermophillus* will be done in next step. In this research, *Streptococcus thermophillus* grew up in MRS without or with palmitic acid, vitamin D having the cholesterol esterase. This was the basic step for next research about the cholesterol esterase in lactic acid bacteria.

CONCLUSION

This was the primary detection of cholesterol esterase in lactic acid bacteria in foods. In order to understand and collect the purified enzyme for treatment and diagnosis, more purified steps and chemical reaction as well as structure analysis should be studied. With the purified cholesterol esterase, the genes encoding for this enzymes will be identified so far to understand more the mechanisms of cholesterol reduction of lactic acid bacteria when using in human health care.

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