

CHOLESTEROL-LOWERING PROPERTY OF *BACILLUS CEREUS* STRAIN KAVK4 ISOLATED FROM BUTTER

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ABSTRACT

Objectives: The aim of the present study was to check the cholesterol-lowering property of *Bacillus cereus* strain KAVK4 at different concentration of cholesterol, different pH, different temperature, and different incubation time.

Methods: *B. cereus* strain KAVK4 was isolated from butter by spread plate method. The cholesterol-lowering property was studied by adding different concentration of cholesterol in nutrient agar medium. Further, the cholesterol-lowering ability was optimized at various parameters such as different concentration of cholesterol, different pH, different temperature, and different incubation time.

Results: *B. cereus* strain KAVK4 was utilized cholesterol and grown well in nutrient medium containing cholesterol at 1 mg/ml concentration. The cholesterol utilization was optimized at 1 mg/ml concentration, the optimal pH was at 8, the temperature at 30°C, and the incubation period was at 24 hrs.

Conclusion: *B. cereus* strain KAVK4 was able to utilize cholesterol from the culture medium. Hence, *B. cereus* strain KAVK4 has been reported as cholesterol lowering agent.

Keywords: Cholesterol lowering, *Bacillus cereus*, pH, Temperature, Time.

INTRODUCTION

Cholesterol is a steroidal compound found abundantly in animal and human tissues and in plasma lipoproteins either as free cholesterol or combined with long-chain fatty acids as cholesterol esters. Excess of cholesterol deposited in the body leads to the risk of cardiovascular disease [1]. Microorganisms such as bacteria have ability to degrade cholesterol that includes in the genera such as *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, *Mycobacterium*, *Streptomyces*, *Rhodococcus*, *Pseudomonas*, *Burkholderia*, *Chromobacterium*, and *Schizophyllum* [2-6]. According to Kim *et al.*, [7] cholesterol is degraded by *Bacillus subtilis* SFF34 during fermentation of flatfish.

Recently, many researchers have focused on the potential beneficial microorganisms for the treatment of obesity and hyperlipidemic activity. In concern to this, *Bifidobacterium longum* showed a significant effect in lowering serum total cholesterol both in rats and humans [8] and *Bifidobacterium* spp. act as an antiobesity agent on high fat diet-induced obese rats [9]. *Lactobacillus plantarum* produced trans-10, cis-12-conjugated linoleic acid was investigated for antiobesity effect in diet-induced obese mice [10]. Another study investigated on consumption of *Lactobacillus gasseri* SBT2055 fermented milk for 12 weeks was able to reduce adiposity and body weight in obese adults [11]. In the present study, *Bacillus cereus* strain KAVK4 was isolated from butter and to evaluate its action on cholesterol-lowering effect at various optimized parameters.

METHODS

Isolation and identification of *B. cereus* strain KAVK4

The butter sample was collected from the North Chennai, Tamil Nadu, India, and stored at 4°C. It was spread plated using nutrient agar medium and incubate at 37°C for 24 hrs. The culture was isolated and identification based on physical and biochemical characteristics [12]. The isolated bacterial culture was molecularly identified by sequencing of 16S rRNA gene region and submitted to National Center for Biotechnology Information.

Growth of the *B. cereus* strain KAVK4 in the presence of cholesterol

Nutrient medium containing cholesterol at various concentrations such as 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml were prepared, and *B. cereus* strain KAVK4 was inoculated and incubated at 37°C for 24 hrs. Nutrient medium without cholesterol was used as control plate.

Optimization for the utilization of cholesterol at various parameters

Cholesterol-lowering activity at different concentration of cholesterol

The cholesterol at different concentrations such as 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml in 10 ml nutrient broth were prepared, and *B. cereus* strain KAVK4 was inoculated and incubated at 37°C for 24 hrs. The cholesterol lowering ability was observed by colorimetric reading, optical density at 650 nm and compared with positive control.

Cholesterol-lowering activity at different pH

The cholesterol-lowering activity was checked at different pH such as 2, 4, 5, 7, and 8 were used in 10 ml of nutrient broth containing cholesterol (1 mg/ml). The *B. cereus* strain KAVK4 was inoculated and incubated at 37°C for 24 hrs. The cholesterol lowering ability was measured by colorimetric reading, optical density at 650 nm and compared with positive control.

Cholesterol-lowering activity at different temperature

The cholesterol-lowering activity at different temperatures such as 20°C, 25°C, 30°C, 35°C, and 45°C were maintained in 10 ml nutrient broth containing cholesterol (1 mg/ml). The *B. cereus* strain KAVK4 was inoculated and incubated for 24 hrs. The cholesterol lowering ability at different temperature were recorded, optical density at 650 nm and compared with positive control.

Cholesterol-lowering activity at different incubation time

Different incubation time such as 1 hr, 6 hrs, 12 hrs, 24 hrs, and 48 hrs were used to check the cholesterol-lowering ability in 10 ml nutrient

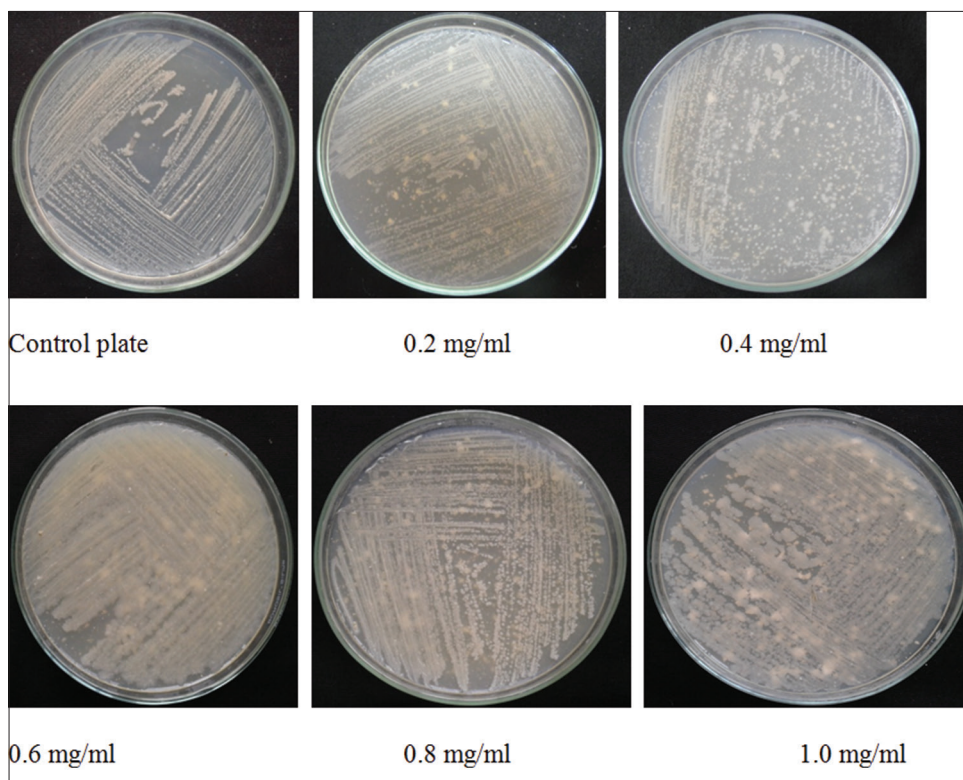


Fig. 1: Growth of the *Bacillus cereus* strain KAVK4 on the nutrient medium containing cholesterol with and without the presence (control) of cholesterol

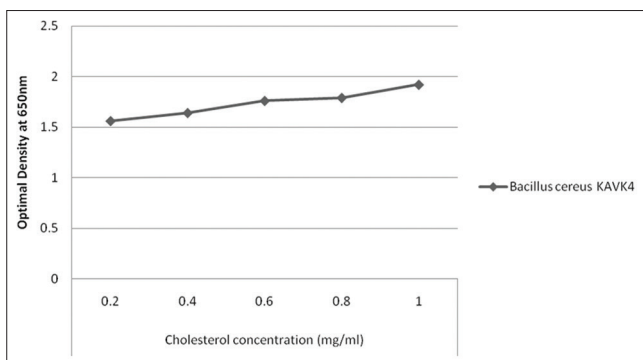


Fig.2: Cholesterol-lowering activity at different concentration of cholesterol

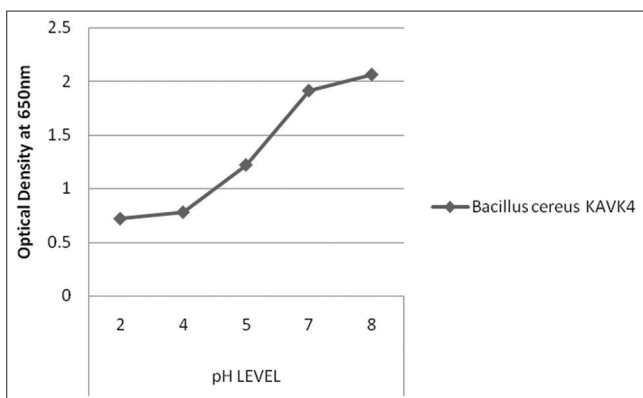


Fig.3: Cholesterol-lowering activity at different pH level

broth containing cholesterol (1 mg/ml). The *B. cereus* strain KAVK4 was inoculated and incubated at 37°C for 24 hrs. Colorimetric reading,

optical density at 650 nm were measured and compared with positive control.

RESULTS AND DISCUSSION

The *B. cereus* strain KAVK4 isolated from butter was assessed for cholesterol lowering property. Cholesterol-lowering activity of the *B. cereus* strain KAVK4 was carried out in the nutrient agar medium containing cholesterol plates at different concentration (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml) were prepared, and *B. cereus* strain KAVK4 was inoculated and incubated. The cholesterol at 1 mg/ml in nutrient agar medium utilized cholesterol and grown well in the medium (Fig. 1). According to Jayachitra *et al.* [13], *B. cereus* was isolated from soil of agriculture waste by an enriched cholesterol Tween-80 medium at 37°C and pH at 7.0. Hence, *B. cereus* strain KAVK4 used in this study was confirmed to degrade cholesterol. The mechanism involved for the degradation of cholesterol by bacteria may be as follows due to the fermentation products of lactic acid bacteria because it inhibits the cholesterol synthesis enzymes and that reduce cholesterol production, elimination of cholesterol in feces, bacteria binds with cholesterol, and thus inhibit the absorption of cholesterol back into the body, recycling of bile salt and facilitate its elimination that raises the demand for bile salt results in body cholesterol consumption and finally assimilation of lactic acid [14-19]. In our preliminary study, the cholesterol-lowering potential of *B. cereus* strain KAVK4 was optimized for the utilization of cholesterol at various parameters. *B. cereus* strain KAVK4 grown maximum and utilized cholesterol at 1 mg/ml concentration as shown in Fig. 2. At pH 8, maximum absorbance of cholesterol was observed in *B. cereus* strain KAVK4 as shown in Fig. 3. Temperature at 30°C and the incubation period was at 24 hrs were showed the maximum absorbance of cholesterol in *B. cereus* strain KAVK4 (Figs. 4 and 5). The result of the study proved that *B. cereus* strain KAVK4 was utilized cholesterol from the culture medium and grown well.

CONCLUSION

B. cereus strain KAVK4 used in this study was able to utilize cholesterol from the culture medium at various optimized parameters. Hence, we

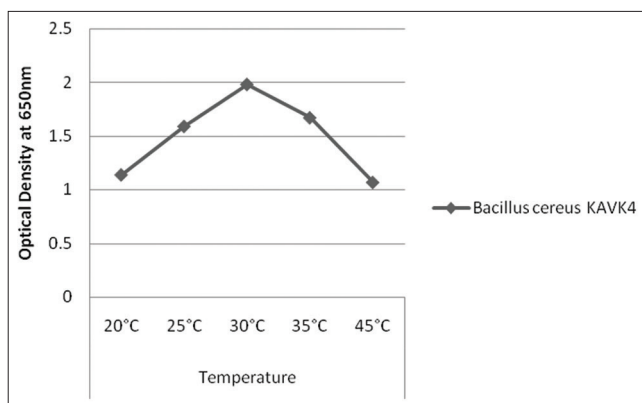


Fig.4: Cholesterol-lowering activity at different temperatures

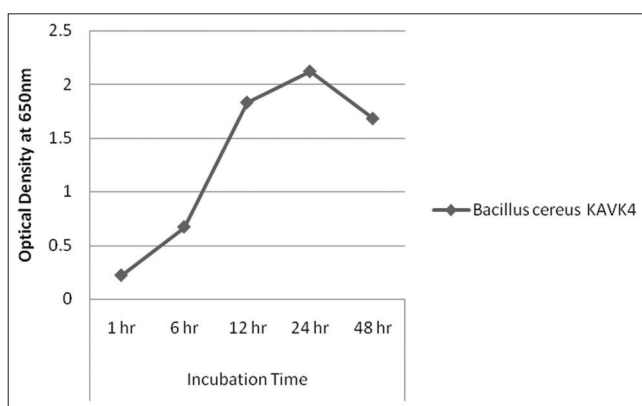


Fig.5: Cholesterol lowering activity at different incubation time

suggest that *B. cereus* strain KAVK4 should have beneficial effect on lowering of cholesterol.

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