

Effect of hydrolyzed *Cordyceps militaris* on probiotic growth

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Abstract:

Galactomannan is polysaccharide that found in *Cordyceps militaris* which useful for manooligosaccharide production. This research aimed to study the effect of hydrolyzed *C. militaris* on probiotic growth. *C. militaris* were hydrolyzed by *Bacillus subtilis* KS1, at 37 °C, 150 rpm for 7 days and reducing sugar was determined. The supernatant of *C. militaris* hydrolysis was added into de Man, Rogosa and Sharpe (MRS) medium containing each probiotic (*Lactobacillus plantarum* TISTR 543 or *Lactobacillus casei* TISTR 390 or *Lactobacillus acidophilus* TISTR 1338) in 96-well culture plate and hydrolyzed copra meal was used as positive control in triplicate experiments. Culture plates were incubated for 48 hr. The optical density (600 nm) of probiotic was measured at before and after incubation. The result demonstrated that hydrolyzed *C. militaris* can be promoted all probiotics but the maximum promoting was observed in *L. acidophilus* TISTR 1338.

Keywords: *Lactobacillus acidophilus* TISTR 1338, *Cordyceps militaris*, probiotic growth

Introduction

Cordyceps militaris is medical fungus that belonging to Ascomycota, Sordariomycetidae, Hypocreales and Cordycipitaceae and well known for their pharmacological activities including anti-liver fibrosis, immunomodulatory, anti-inflammatory, lowering blood glucose, antitumor, and antibacterial activities and other favorable effects [1]. It contains biologically active substance such as nucleosides (cordycepin; 3'-deoxyadenosine, and adenosine), polysaccharides (galactomannan) and ergosterol [2, 3].

Galactomannan is mainly polysaccharide that found in *C. militaris* which is a non-starch polysaccharide that consists of a mannose in main chain with galactose side chains [4]. Mannooligosaccharides (MOS) are prebiotics that obtained from galactomannan hydrolysis using mannanase. MOS were applied as a feed additive to promote the growth of probiotics, prevent pathogen colonization and modulate the immune system of host animals [5].

Probiotics are live microorganisms that confer a health benefit on the host [6]. Many functional roles of probiotics such as restoration of the gut microbiome, reduction of the concentration of cancer-promoting in the gut, pathogen exclusion, epithelial barrier, bacteriocin production, prevention of gut inflammation and other intestinal or systemic disease phenotypes [7,8]. From previous research [3] found *C. militaris* polysaccharide composed of mannose and galactose. [3] The propose of this research was to study the effect of hydrolyzed *C. militaris* on probiotic growth for new alternative way of food additive as prebiotics.

Materials and methods

***C. militaris* culture**

C. militaris inoculum broth was purchased from kasetbuddy farm, Saraburi. *C. militaris* inoculum was inoculated into bottle containing rice medium. The bottles were incubated in dark at 18 °C for 15 days before continued culturing under light 18 °C for 45 days. *C. militaris* was collected and dried at 60 °C for 18 hrs. Dried *C. militaris* were grinded to fine powder and stored for later use.

Mannanase producing-bacteria

Bacillus subtilis KS1 was obtained from previous research [9]. *B. subtilis* KS1 was cultured using sterilized Luria-Bertani (LB) and incubated at 37 °C, 150 rpm for 48 hrs prior hydrolysis.

***C. militaris* hydrolysis**

One milliliter of *Bacillus subtilis* KS1 cultured broth were added into 100 ml LB broth containing 1% *C. militaris* incubated at 37°C, 150 rpm for 7 days. Copra meal hydrolysis was used as positive control with the same culture condition. One milliliter of cultured broth was collected every 24 hrs for sugar determination using dinitrosalicylic acid (DNS) method.

Effect of hydrolyzed C. militaris on probiotic growth

Cultured broth was sterile by filtration and used as crude mannoooligosaccharide (MOS). Probiotics, *Lactobacillus plantarum* TISTR 543, *Lactobacillus casei* TISTR 390 and *Lactobacillus acidophilus* TISTR 1338 were cultured using de Man, Rogosa and Sharpe (MRS) medium for 24 hrs and adjusted the concentration at optical density 600 = 0.1. Crude MOS was added into MRS medium containing each probiotic in 96-well culture plate and hydrolyzed copra meal was used as positive control in triplicate experiments. The optical density (600 nm) of each well were measured at before and after incubation at 37 °C for 48 hrs.

Results and discussion

***C. militaris* culture**

After incubated in dark at 18 °C for 15 days and continued culturing under light at 18 °C for 45. *C. militaris* was grown (Fig. 1) and collected before dried at 60 °C. Dried *C. militaris* were grinded to fine powder (Fig. 2) and used as substrate for mannanase hydrolysis using *Bacillus subtilis* KS1. The culture condition is not similar to Kang et al. (2014) which used at 25 °C for liquid state culture.



Figure 1. *Cordyceps militaris* growth under culture condition.



Figure 2. *Cordyceps militaris* powder.

***C. militaris* hydrolysis**

C. militaris was hydrolyzed using *Bacillus subtilis* KS1 for 7 days. Reducing sugar on each day was determined using DNS method. The results indicated that at day 7 demonstrated highest amount of sugar concentration at 0.936 mg/ml for *C. militaris* and 0.137 mg/ml for copra meal (Table 1.).

Table 1. Reducing sugar of *C. militaris* and copra meal hydrolysis

Day	Reducing sugar concentration (mg/ml)	
	<i>C. militaris</i>	Copra meal
1	0.204	0.057
2	0.314	0.058
3	0.338	0.060
4	0.436	0.065
5	0.715	0.084
6	0.719	0.107
7	0.936	0.137

Effect of hydrolyzed C. militaris on probiotic growth

Hydrolyzed *C. militaris* was obtained from hydrolysis using *Bacillus subtilis* KS1 for 7 days and was filter sterile before added into 96-well culture plate containing each probiotic. Three strains of probiotic bacteria, *Lactobacillus plantarum* TISTR 543, *Lactobacillus casei* TISTR 390 and *Lactobacillus acidophilus* TISTR 1338 showed similar growth curves and

took approximately 18 hr and 36 hr to reach mid-exponential phase and stationary phase, respectively [10]. The results indicated that 60 μ l hydrolyzed *C. militaris* can be promoted all probiotics, but the maximum promoting was observed in *L. acidophilus* TISTR 1338 when supplemented with 10 μ l hydrolyzed *C. militaris* (Table 2-4). In addition, hydrolyzed copra meal can be promoted all selected probiotics but lower than hydrolyzed *C. militaris*.

Table 2. The average optical density at 600 nm of *Lactobacillus plantarum* TISTR 543.

Volume (μ l)	Hydrolyzed <i>C. militaris</i>		Hydrolyzed copra meal	
	Before	After ($\bar{x}\pm$ SD)	Before	After ($\bar{x}\pm$ SD)
10	0.11	0.67 \pm 0.02	0.11	0.14 \pm 0.02
20	0.11	0.67 \pm 0.04	0.11	0.14 \pm 0.03
30	0.11	0.70 \pm 0.03	0.11	0.16 \pm 0.03
40	0.11	0.71 \pm 0.02	0.11	0.16 \pm 0.03
50	0.11	0.72 \pm 0.03	0.11	0.17 \pm 0.02
60	0.11	0.89 \pm 0.01	0.11	0.17 \pm 0.04
70	0.11	0.75 \pm 0.02	0.11	0.19 \pm 0.02

Table 3. The average optical density at 600 nm of *Lactobacillus casei* TISTR 390.

Volume (μ l)	Hydrolyzed <i>C. militaris</i>		Hydrolyzed copra meal	
	Before	After ($\bar{x}\pm$ SD)	Before	After ($\bar{x}\pm$ SD)
10	0.11	1.27 \pm 0.03	0.11	0.73 \pm 0.03
20	0.11	1.34 \pm 0.02	0.11	0.65 \pm 0.02
30	0.11	1.38 \pm 0.03	0.11	0.48 \pm 0.04
40	0.11	1.39 \pm 0.02	0.11	0.42 \pm 0.04
50	0.11	1.50 \pm 0.03	0.11	0.38 \pm 0.03
60	0.11	1.56 \pm 0.03	0.11	0.30 \pm 0.04
70	0.11	1.43 \pm 0.02	0.11	0.21 \pm 0.03

Table 4. The average optical density at 600 nm of *Lactobacillus acidophilus* TISTR 1338.

Volume (μ l)	Hydrolyzed <i>C. militaris</i>		Hydrolyzed copra meal	
	Before	After ($\bar{x}\pm$ SD)	Before	After ($\bar{x}\pm$ SD)
10	0.11	1.24 \pm 0.02	0.11	0.13 \pm 0.03
20	0.11	1.96 \pm 0.03	0.11	0.16 \pm 0.02
30	0.11	1.58 \pm 0.03	0.11	0.17 \pm 0.03
40	0.11	1.50 \pm 0.02	0.11	0.17 \pm 0.04
50	0.11	1.47 \pm 0.03	0.11	0.19 \pm 0.02
60	0.11	1.34 \pm 0.01	0.11	0.24 \pm 0.03
70	0.11	1.30 \pm 0.02	0.11	0.27 \pm 0.02

Conclusions

C. militaris was cultured in bottle containing rice medium under dark condition for 15 days and continued culturing under light condition for 45 days at 18 °C. *C. militaris* was collected, dried and grinded to fine powder. *C. militaris* powder and copra meal were hydrolyzed for 7 days using *B. subtilis* KS1. Cultured broth was collected for sugar determination using DNS method. The results found that at day 7 showed highest amount of sugar concentration at 0.936 mg/ml for *C. militaris* and 0.137 mg/ml for copra meal. Hydrolyzed *C. militaris* was added into 96-well plate containing each probiotic and incubate for 48 hr. Results presented that hydrolyzed *C. militaris* can be enhanced all probiotics, but the maximum stimulating was observed in *L. acidophilus* TISTR 1338 when supplemented with 10 μ l hydrolyzed *C. militaris*. *C. militaris* is alternative way for prebiotics production and feed additive for human and livestock. Although the *C. militaris* is expensive but only small amount can be promoted the probiotic.

Acknowledgements

This work was supported by Roi Et Rajabhat University.

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