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The Effect of the Hot Water Extracts of the *Paecilomyces hepiali* and *Cordyceps militaris* Mycelia on the Growth of Gastrointestinal Bacteria

Sanath Gamage[1], Jiro Nakayama[2], Yusuke Fuyuno[2], Shoji Ohga[1]

[1]Department of Agro-Environmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan
[2]Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan
Email: ohga@forest.kyushu-u.ac.jp, gamage.wgsb@gmail.com

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Abstract

The gastrointestinal health is very important aspect concerning human health. It refers to nutrient and vitamin absorption, food digestion and various infectious diseases. The *Paecilomyces hepiali* and *Cordyceps militaris* are highly enriched with cordycepin and ergosterol which are considered as anti bacterial substances. Present study finds out comparative effect of hot water extract of particular fungal material on growth of six species of gastrointestinal bacteria that belong to both aerobic and anaerobic and, consist with harmful and commensal categories. The appropriate concentration level of hot water extract of both strains was identified. The individual specific bacterial growing media were prepared and calculated; amounts of bacteria cultures were inoculated by using micro pipettes. The optical density and number of bacterial colonies were measured after 24 hours. The pure mycelial extract of *P. hepiali* with 2×10^{-3} g/ml of concentration has significant effect on depleting the growth of *E. coli*, *E. faecalis*, *S. aureus*, *L. gasseri* and *B. ovatus* bacteria. *B. longum* has no significant effect by particular extract. Same type of extract of *C. militaris* has significantly reduced the growth of every bacteria used in this study. Hot water extract of *C. militaris* cultivated on soy bean has significant growth retardation toward *E. coli*, *E. faecalis*, *S. aureus* and *L. gasseri*. It has stimulated the growth of *B. ovatus* and *B. longum* which are considered as beneficial bacteria for human gut. This study shows that extracts of both mycelia include antimicrobial substances like cordycepin and ergosterol which can be used as food supplements to enhance human gut health.

Keywords

Ophiocordyceps sinensis, *Cordyceps militaris*, Antibacterial Activity, Human

Gut Bacteria

1. Introduction

The wild *Ophiocordyceps sinensis* and *Cordyceps militaris* are important kind of medicinal fungi belonging to the phylum Ascomycota. Those are an abundant resource in nature with various biological activities and have been used extensively as a tonic and health supplement [1]. Chioza *et al.* reported that *Paecilomyces hepiali* and *Hirsutella sinensis* do coexist in *O. sinensis*. They found DNA of these fungi in both the caterpillar and fruiting bodies of natural *O. sinensis*. However, those isolates commonly exist in natural *O. sinensis* as a result of being endoparasites or epiphytes of the host insect [2]. In this study, *P. hepiali* and *C. militaris* have been used to prepare mycelial hot water extract.

O. sinensis is a macro fungus of biomedical importance, contains a number of bioactive components. Many of them are biological response modifiers which activate our immune systems for a multitude of defensive functions. The immunomodulating effects are associated with its antitumor activity [1] and lower fasting plasma levels of glucose and insulin improve oral glucose tolerance, and increase glucose-insulin index [3]. Extract of *O. sinensis* has inhibited hepatic fibrogenesis [4] in rats with CCl_4-induced liver fibrosis and reduced the weight loss, polydipsia, and hyperglycemia in streptozotocin-induced diabetic rats [5]. The water extract of *C. militaris* has reduced fasting serum glucose level and enhanced glucose utilization in skeletal muscles and improved insulin secretion in rats [6]. The bioactive constituents of particular fungus have been extracted such as cordycepin, polysaccharides, ergosterol, mannitol, and adenosine [7] [8]. Meanwhile, various pharmacological actions of these chemical constituents have been reported, including antitumor effect, antioxidant, nephroprotective, antiapoptotic, antibacterial properties and inflammatory effects [1] which are the most proverbial effect of *O. sinensis* and *C. militaris*.

The effect of *O. sinensis* and *C. militaris* may be caused by a single active ingredient or by the combined action of many active agents that existed in the extractions. Research is necessary to get an overview about the genus *Ophiocordyceps* and *Cordyceps* because of the increasing interest both for medicine and mycology [9] [10]. This study has been observed pharmacological actions of the *P. hepiali* and *C. militaris* for its significant role in the development of new drugs and therapeutics for various bacterial diseases.

Gastrointestinal Bacteria

Colonization of the gastrointestinal tract of newborn infants starts immediately after birth and occurs within a few days. Pioneer bacteria can modulate expression of genes in host epithelial cells [11] thus creating a favorable habitat for themselves, and can prevent growth of other bacteria introduced later in the ecosystem. The composition of the gut bacteria community in the stomach and

colon is distinctive, which is mainly due to different physicochemical conditions, such as intestinal motility, pH value, redox condition, nutrients and host secretions. Additionally, they can be influenced by many factors, such as the use of antibiotics, illness, stress, aging, bad dietary habits and lifestyle [12] [13].

Generally around 400 species of bacteria have evolved and adapted to live and grow in the human intestine. The large intestine contains a complex and dynamic microbial ecosystem with high densities of living bacteria, which achieve concentrations of up to 10^{11} or 10^{12} cells/g of luminal contents [14]. The genera *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus* are predominant in human beings, whereas aerobes (facultative anaerobes) such as *Escherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Proteus*, etc. are among the subdominant genera [15]. Bacteria can supply essential nutrients, synthesize vitamin K, aid in the digestion of cellulose, and promote angiogenesis and enteric nerve function. They produce a wide spectrum of enzymes that, being reductive and hydrolytic in nature, are actively involved in many processes in the colon, such as carbohydrate and protein fermentation, bile acid and steroid transformation, metabolism of xenobiotic substances, as well as the activation and destruction of potential mutagenic metabolites [12] [16]. Nitroreductase, azoreductase, N-oxide and sulfoxide reductase are the most extensively investigated reductive enzymes, while glucosidase and glucronidase are the most extensively studied hydrolytic enzymes [14].

Some of these bacteria are potential pathogens and can be a source of infection and sepsis under some circumstances for instance when the integrity of the bowel barrier is physically or functionally breached. However, the constant interaction between the host and its microbial guests can infer important health benefits to the human host. Other hand, management of microbiota in the gut is crucial specially reducing pathogenic bacteria [17].

This study has been focused on utilization of fungal material of *O. sinensis* and *C. militaris* as food supplements and evaluation of the antibacterial activities of six species of bacteria which are categorized as commensal and pathogenic have been done. The fungal extract or synthesized drug from particular fungi can be used as prebiotic for target bacteria or symbiotic with ameliorated bacteria strains and also used as a selective inhibitor towards harmful bacteria.

2. Materials and Methods

2.1. Fungal Strain

The *P. hepiali* strain which used in this study was originally brought from Jilin Agricultural University, China and assigned to accession number KUMB1081 and *C. militaris* assigned to accession number KUMB1061 in the mushroom culture bank at the Laboratory of Forest Production Control, Kyushu University.

2.2. Bacterial Strain

The strains which have been used this experiment and the places where bacterial

strains were originally brought have been shown in Table 1.

2.3. Culture Condition and Media Preparation

The *E. faecalis*, *S. aureus* and *E. coli* were grown in LB broth (Difco) at 37˚C. Culture media of the *E. coli* was shaken to provide optimum growing condition. Other strains have been incubated at 37˚C. Colony counting was done by using agar powder (Waco) ameliorated LB broth. The *L. gasseri*, *B. longum* and *B. ovatus* have been cultured in GAM broth (Nissui) at 37˚C. Those strains have been provided anaerobiosis conditions by using anaeropack (Mitsubishi Gas Chemical Co., Inc.). The cell enumerations have been carried out on modified GAM media which agar powder (Waco) was ameliorated to the media (24 hours at 37˚C).

Two different types of hot water extracts of both *P. hepiali* and *C. militaris* were prepared. Two flaks with 500 ml of potato dextrose broth (Difco) were prepared according to company instruction. Meanwhile, oil removed soy bean, wheat brand and distilled water were mixed at a ratio of 4:1:2 and put in to polypropylene bags. Both flaks and both bags were autoclaved at 121˚C for 30 minutes. After overnight cooling, a 5 mm diameter agar plugs with actively growing mycelium of both fungal strains were inoculated in to each potato dextrose broth and soy bean media. Those media were incubated under 25˚C for 20 days. Potato dextrose broth was used for obtain pure mycelia of both fungal strains. Day 20, both types of cultures have reached appropriate amount of mycelia. Liquid media were filtered (Advantec, 0.45 μm) and mycelial mats were collected. Mycelia were dried for 12 hours at 50˚C. From each strain, 5 g of pure mycelia were collected by following same method for few flasks and mycelia chopped by using electrical blender. When they broken in to small pieces, 500 ml of water was added and continue more few minutes. This mixture was boiled 3 hours and residual were filtered by using Advantec vacuum filter unit with 0.45 μm filter papers at first. Then 0.2 μm filter papers were used for obtaining more purified extract. Rotary evaporator was used to evaporate excess water from extract for concentrate the solution up to 2×10^{-3} g/ml. Pycnometer (specific gravity bottle) method was used measuring the concentrations of the extracts. Then several

Table 1. Information of bacterial strains (accession number and origin).

Bacterial strain name	Accession number	Origin
Enterococcus faecalis	JH2-2	Laboratory of Microbial Technology, Kyushu University
Escherichia coli	JCM5491^T	Japan Collection of Microorganisms (JCM)
Lactobacillus gasseri	JCM1131^T	
Bifidobacterium longum	JCM1217^T	
Bacteroides ovatus	JCM5824^T	
Stapylococcus aureus	ATCC12600^T	American Type Culture Collection (ATCC)

levels of concentrations were prepared by adding required amount of water. Both strains which were fully colonized on soy bean media were dried as above method and 50 g were collected. Compared to pure mycelia, soy bean media includes many substances which are removed at filtration process. That's why 50 g of soy bean media was taken and they were broken in to small parts by using blender. Then 500 ml of water were added in to blender and continue more few minutes. Same procedure was continued for making concentrated hot water extract.

2.4. Experiment Set up

The inhibition of the growth of bacterial strains in the presence of hot water extract of both *P. hepiali* and *C. militaris* has to be determined according to the method of [18] with some modifications. As mentioned, soy bean extract and pure mycelial extract were concentrated until 2×10^{-3} g/ml. References to that four different concentration levels were created. The different responses of four different concentration levels were identified by using pure mycelial extract of *P. hepiali* at first. Selected concentration level was used for further identification of anti bacterial activities on six types of gut bacteria. Activation of bacteria which were obtained from storage freezer as Glycerol stock was done. Agar ameliorated LB and GAM media were used to prepare per cultures of individual bacteria. Anaerobic environment was provided by using anaeropack pack for anaerobic strains which were grown on agar ameliorated GAM media. All strains were incubated at 37˚C. After 24 hours single colony from pre culture of each bacteria were inoculated in to respectable growing media and incubated at 37˚C for 24 hours to obtain bacteria culture. Each strain has been inoculated (2% v/v) into LB broth or GAM broth with hot water extracts. Cultures were incubated at 37˚C and, after 24 hours, optical density of culture has been measured and compared to the control culture. Same time small volume (10 µl) of culture was subjected to serial dilution and using micro pipettes, 5 µl from each steps were inoculated in to petri dishes which were prepared by using agar ameliorated LB and GAM media. After 24 hours numbers of colony were counted and compared with control.

2.5. Statistical Analysis

The analyses were done using Minitab 18 statistical software (Minitab Inc.) and Microsoft Excel. Identification of statistical differences within treatments was done by Analysis of Variance (ANOVA) followed by Tukey's post hoc test. All the analyses were done with 0.05 significance levels. All graphs are presented with standard error bars.

3. Results and Discussion

The identification of antibacterial effect expressed by hot water extracts of both *P. hepiali* and *C. militaris* was done. The limited spectrum of antibacterial activ-

ity of the aqueous extracts has been compared with the control. It was hard to justify since all the extracts contained the metabolites, though not in the same proportions. As shown in Table 2, effective concentration of extract was evaluated for further experiment by using both aerobic and anaerobic bacterial strains. Nutritional requirements of higher fungi play critical role in production of mycelia in submerged cultures. It has been found that medium constituents strongly affect chemical composition, structure and productivity [19] [20]. Mycelial biomass is one of the useful product containing various bioactive compounds, production yield of mycelial biomass should be simultaneously considered in the processes of submerged culture of *P. hepiali* and *C. militaris*.

3.1. Effect of Concentration Levels on Bacterial Growth

The clue was given by the result shown in Table 2 that extract of *P. hepiali* has anti bacterial effect. The bacterial growth of *E. coli* and *L. gasseri* in every concentration level show less growth compared with its own controls in terms of optical density of 600 nm [13] [21]. Suggestion can be made as the extracts have one or more antibacterial compound that has been dissolved. It has been reported the active components of *Cordyceps* and *O. sinensis* are peptides, a glycoprotein, DNase [3], adenosine, adenine, hypoxanthine, cordycepin [22].

The inner cellular material of the both mycelium of *P. hepiali* and *C. militaris* are important for inhibit bacterial growth. As expected, high concentration has high effect on bacterial growth inhibition. Both aerobic and anaerobic bacterial strains were responded with growth retardations. It has been reported that high concentration of extract provided significant effect on bacterial inhibition [23]. The concentration of 2×10^{-3} g/ml has shown significantly lower bacterial growth compared with other low concentration levels in terms of OD 600 nm. In this study, 2×10^{-3} g/ml of concentration has been used for further treatments.

The bacterial growths of selected six types of bacteria in the presence of pure mycelial hot water extract of *P. hepiali* and *C. militaris* have been shown in Table 3. Control plots of each bacterial strain have been represented its growth without adding any extract.

Table 2. This table represents four different concentration levels of pure mycelial extract of *P. hepiali* effect on two different bacterial strains. The values in last four columns represent every mean ± SD of OD 600 nm values measured at 24 hours after inoculations. Values in the same column and its own control column with different letters differ significantly according to Tukey's test ($p < 0.05$).

Concentration of extract	Optical density 600 nm			
	E. coli	*E. coli control*	*L. gasseri*	*L. gasseri* Control
2×10^{-3}	0.322 ± 0.058d	0.932 ± 0.107ab	0.459 ± 0.074fd	0.852 ± 0.067abc
1×10^{-3}	0.61 ± 0.087c	0.971 ± 0.056ab	0.668 ± 0.197cd	1.14 ± 0.038a
5×10^{-4}	0.75 ± 0.046bc	1.106 ± 0.125a	0.648 ± 0.072cd	0.94 ± 0.119abc
2.5×10^{-4}	0.653 ± 0.114c	1.088 ± 0.103a	0.77 ± 0.139bc	1.072 ± 0.046ab

Table 3. This table represents the effect of pure mycelial hot water extracts of both *P. hepiali* and *C. militaris* on six different bacterial strains. The values in each column represent every mean ± SD of OD 600 nm values measured at 24 hours after inoculations. Values in the same row with different letters differ significantly according to Tukey's test ($p < 0.05$).

Bacterial strains	Optical density 600 nm		
	Extract of *P. hepialid*	Extract of *C. militaris*	Control
Aerobic			
E. coli	0.866 ± 0.074[b]	0.819 ± 0.094[b]	1.029 ± 0.098[a]
E. faecalis	0.756 ± 0.076[b]	0.591 ± 0.072[c]	0.994 ± 0.123[a]
S. aureus	0.58 ± 0.1[b]	0.652 ± 0.069[b]	1.118 ± 0.162[a]
Anaerobic			
L. gasseri	0.696 ± 0.062[b]	0.675 ± 0.03[b]	0.811 ± 0.078[a]
B. longum	0.856 ± 0.066[a]	0.635 ± 0.066[b]	0.913 ± 0.082[a]
B. ovatus	0.77 ± 0.078[b]	0.792 ± 0.046[b]	0.924 ± 0.072[a]

It has been reported that cordycepin mainly dissolved in submerged cultures [24], referring to extract preparation of this study extracellular secretions in submerged culture has been filtered and completely wash away. The mycelia with remaining intracellular substances were used for extract preparation. It has been suggested that antibacterial substances can be found inner cellular part of mycelia as well. That might be cordycepin or ergosterol. As for the extracellular polysaccharides from mycelial cultures of *Cordyceps*, different constituents have been demonstrated according to species and culture conditions [24] [25].

3.2. Effect on Aerobic Bacterial Growth

Even though inside of gut consist with lack of oxygen environment, there are so many type of facultative anaerobes bacteria. The effects of pure mycelial hot water extracts of both *P. hepiali* and *C. militaris* on aerobic bacteria have been shown in **Figure 1**.

The inhibition effect done by extract of both *P. hepiali* and *C. militaris* was not a selective effect on specific bacterial strain but the strength of inhibition was different among bacteria strain and between two extracts. Pure mycelial hot water extract of both *P. hepiali* and *C. militaris* have significant effect of bacterial growth inhibition towards *E. coli*, *E. faecalis* and *S. aureus*.

3.3. Effect on Anaerobic Bacterial Growth

The human gut accommodates hundred of anaerobic bacteria as well. Out of them, growth of three selected bacterial strain at the presence of hot water extracts of pure mycelia has been presented in **Figure 2**.

The inhibition effect done by pure mycelial extracts of *P. hepiali* was represented a selective effect on specific bacteria. Particular extract has no any

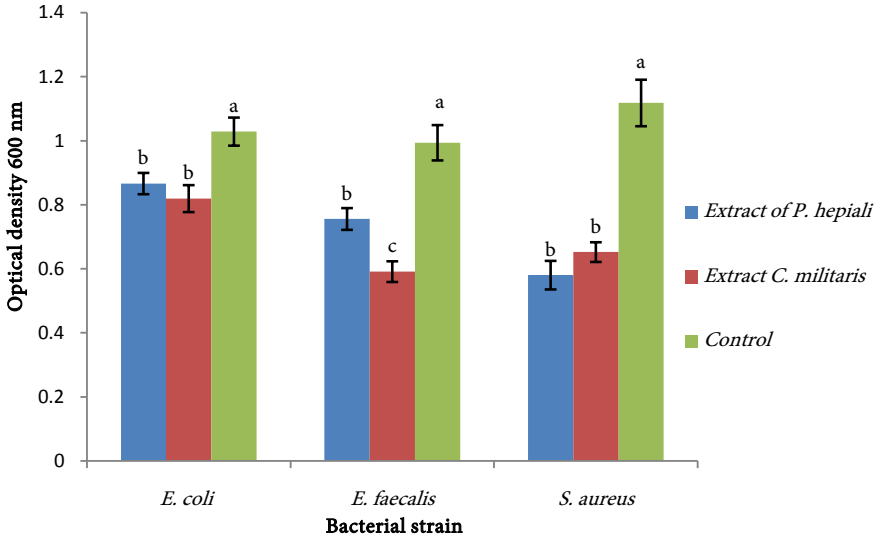

Figure 1. This graph represents the effect of pure mycelial hot water extracts of both *P. hepiali* and *C. militaris* on three different aerobic bacterial strains. The values in each column represent every mean ± SD of OD 600 nm values measured at 24 hours after inoculations. The error bars represent standard error. Means values in the same bacterial strain that do not share a letter are significantly different according to Tukey's test ($p < 0.05$).

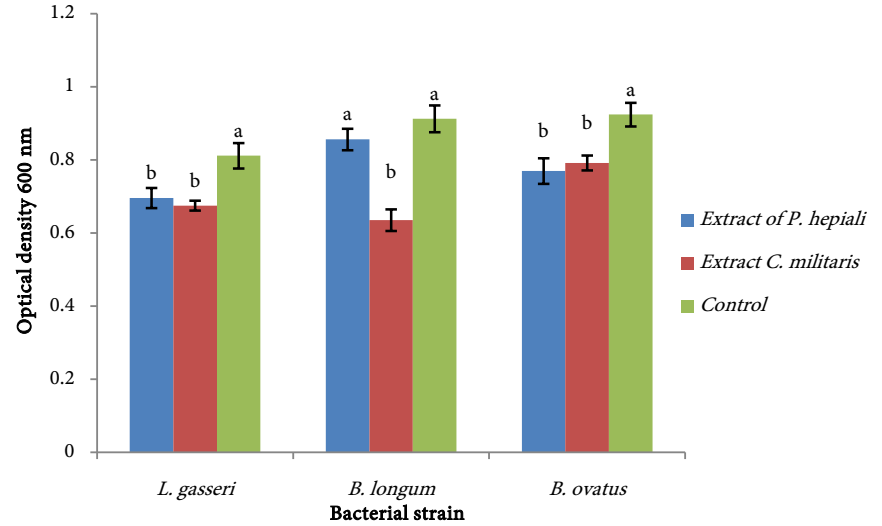

Figure 2. This graph represents the effect of pure mycelial hot water extracts of both *P. hepiali* and *C. militaris* on three different anaerobic bacterial strains. The values in each column represent every mean ± SD of OD 600 nm values measured at 24 hours after inoculations. The error bars represent standard error. Means values in the same bacterial strain that do not share a letter are significantly different according to Tukey's test ($p < 0.05$).

significant effect on growth inhibition of *B. lonum* strain. Meanwhile, extract of *C. militaris* has significnly inhibited bacterial growth of every selected strain in terms of OD 600 nm measurements.

Even though extracellular polymeric substances washed away from pure my-

celial hot water extract of both *P. hepiali* and *C. militaris*, when the mycelia submerged culture was filtered and cleaned, active compound has still been remained to inhibit bacterial growth. There were some extracellular polymeric substances which have strong biological activities as detected from fruiting bodies of *Cordyceps* [19] [20]. In this regard, much attention was given to prepare hot water extract with active compound from both extracellular and inner cellular parts and, soy bean solid growing media was selected.

3.4. Effect on Bacterial Growth of Hot Water Extract of *C. militaris* Grown on Soy Bean Media

It was generally accepted that mycelia of many different mushrooms and entomopathogenic fungi can be grown, to some extent, on a wide range of carbon sources [24] [26]. However, the nutriant source yielding maximum growth differs from species to species. *C. militaris* has been investigated in this study utilizing soy bean as the preferred major nutriant source. The omission of nitrogen in the medium greatly affects fungal growth and metabolite production. Nitrogen source may be supplied to media in the form of ammonia, nitrate, or as organic compounds, such as amino acids or proteins. In comparison with organic nitrogen sources, inorganic nitrogen sources often yield relatively lower mycelial biomass production than organic sources [27] [28].

The soybean steep powder and tryptone were more efficient for mycelial growth and also enhancements obtained from using the organic sources of nitrogen may indeed not only reflect the form that the nitrogen is in, but also the fact that other non nitrogen components could play a role in the improvements [29].

The bacterial strains which belong to aerobic and anaerobic categories were treated by hot water extract of *C. militaris* which has been cultivated on soy bean media. The growth inhibition effect was messured as OD 600 nm absorbtion and colony count method as shown in **Table 4**.

As graphically shown in **Figure 3**, hot water extract of *C. militaris* cultivated on soy bean significantly effect on growth retardation of *S. aureus*, *E. coli*, *E. faecalis and L. gasseri*. Mean while, *B. ovatus* and *B. longum* have grown in hot water extract treated media at a high bacteria density compared to its controls.

The colony count method was followed to obtain visual detection of the effect after optical density measurements. Colony count measurements of the bacterial cultures after 24 hours of inoculation have been presented in **Figure 4**. Serial dilution method was followed with 0.01 dilution factor. Hot water extract of *C. militaris* grown on soy bean has significant effect on growth retardation of *S. aureus* and *E. coli*. In terms of colony count there was no any significant growth retardation of bacterial strains of E. *faecalis*, *L. gasseri*, *B. ovatus* and *B. longum*.

It has been identified that the active compounds as cordycepin and ergosterol which are correspondent to the antibacterial effect [13]. The cordycepin was first

Table 4. This table represents the effect of hot water extracts of *C. militaris* grown on soy bean media towards six different bacterial strains. The values in second and third columns represent every mean ± SD of OD 600 nm values and fourth and fifth columns represent every mean ± SD of colony count done at 24 hours after inoculations. Values of OD 600 and colony count share different letters with their own control are significantly different according to Tukey's test ($p < 0.05$).

Bacterial strains	OD 600	Control	Colony count	Control
Aerobic				
E. coli	0.771 ± 0.022^{cde}	1.222 ± 0.129^{a}	16.75 ± 3.77^{b}	38.5 ± 4.95^{a}
E. faecalis	0.734 ± 0.072^{de}	1.129 ± 0.123^{ab}	35.3 ± 24.6^{a}	62 ± 49.5^{a}
S. aureus	0.704 ± 0.078^{e}	0.992 ± 0.042^{abcd}	20.75 ± 6.65^{b}	49 ± 5.66^{a}
Anerobic				
L. gasseri	0.861 ± 0.048^{bcde}	1.166 ± 0.035^{a}	83 ± 7.53^{a}	104.5 ± 23.3^{a}
B. longum	0.965 ± 0.158^{abc}	1.146 ± 0.066^{a}	74 ± 10.1^{a}	90 ± 8.49^{a}
B. ovatus	1.159 ± 0.055^{a}	1.114 ± 0.155^{ab}	116.25 ± 8.77^{a}	107 ± 15.6^{a}

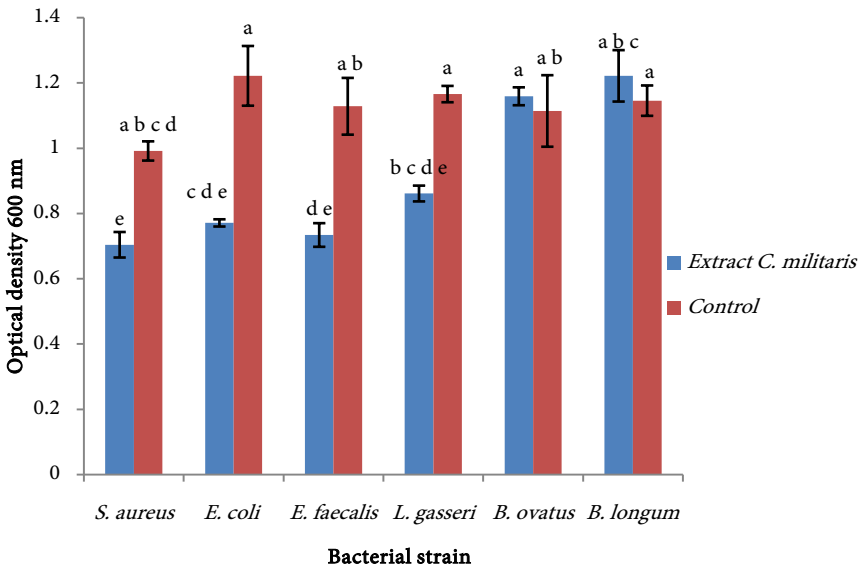

Figure 3. This graph represents the effect of hot water extracts of *C. militaris* grown on soy bean media towards six different bacterial strains. The values in each column represent every mean ± SD of OD 600 nm values measured at 24 hours after inoculations. The error bars represent standard error. Means values in the same column that do not share a letter are significantly different according to Tukey's test ($p < 0.05$).

isolated from *C. militaris* and its structural formula was confirmed as 3'-deoxyadenosine [30]. Cordycepin is the most considerable adenosine analogue from some *Cordyceps* [31], which is a derivative of the nucleoside adenosine. It was later found to be present in small amounts in *O. sinensis*. The cordycepin is a category of compounds that exhibits significant therapeutic potential and has many intracellular targets, including nucleic acid, apoptosis, and cell cycle. It has been reported that the variety of molecular mechanisms that

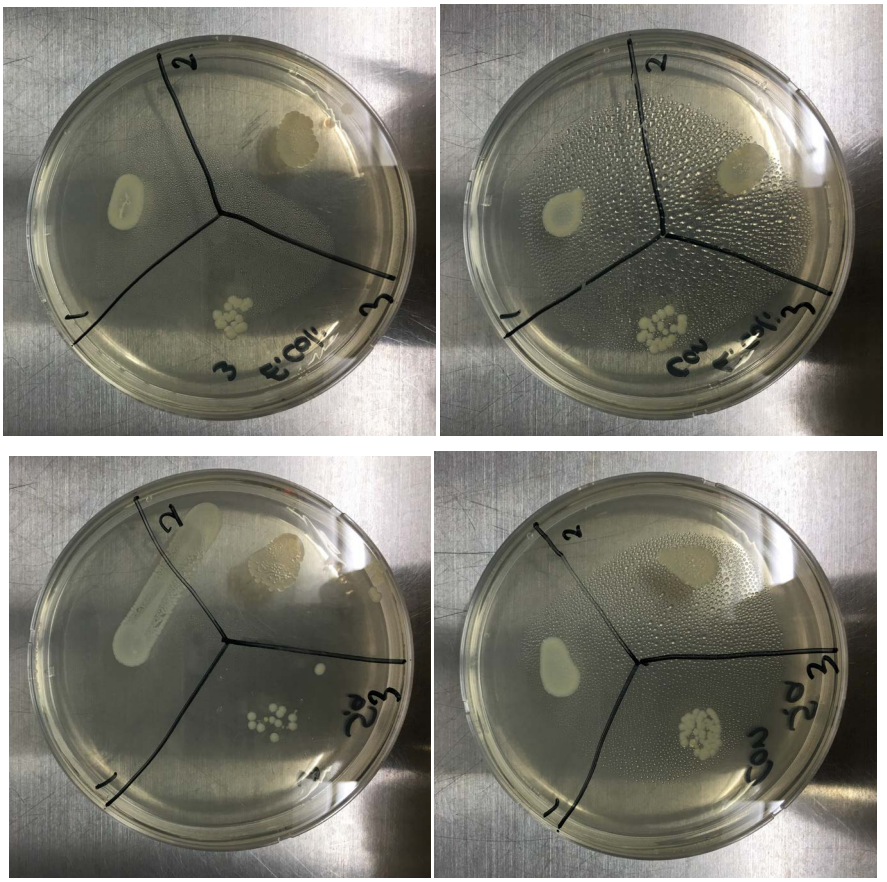

Figure 4. This figure represents the appearance and density of colonies of *S. aureus* and *E. coli* bacterial strains measured at 24 hours after inoculations. The effect of hot water extracts of *C. militaris* grown on soy bean media has been shown compared with colony growth of their own control. Dilution factor was 0.01 (10^{-2}). Numbers on the dishes represented level of dilution (1). 10^{-4} folds dilution (2). 10^{-6} folds dilution (3). 10^{-8} folds dilution.

mediate the pharmacological effects of cordycepin. Besides, they deem that cordycepin can participate in various molecular processes in cells because of its similarity with adenosine [8]. Cordycepin is a broad spectrum biocidal compound possessing not only antitumor activity but also antibacteria, antivirus, and insecticidal activities [9]. It was confirmed as a marker for *C. militaris* within the content profiles of nucleosides in *Cordyceps* product [32]. Cordycepin could be an attractive therapeutic candidate with oral activity against I/R-associated heart diseases such as myocardial infarction [1] and also it is a potent anti-inflammatory and analgesic medicine [33]. Cordycepin can intensively regulate the functions of human immune cells in vitro [34]. It has stimulated the release of some cytokines of resting PBMCs and influenced proliferation of PBMCs and transcription factors in THP-1 cell line.

The researchers have shown that cordycepin is generally present in solid substrate grown *Cordyceps*, but not in liquid cultured *Cordyceps*. The presence or absence of cordycepin is dependent upon many factors, including the method of

mycelial culture [24]. In this study hot water extract of *C. militaris* grown on soy bean solid media has some sort of beneficial effect in terms of human gut health because compared with pure mycelial extract; soy bean extract has inhibited few harmful bacterial strains while stimulating beneficial bacterial growth.

The ergosterol content in *C. sinensis* has been determined with HPLC method and high yield has been obtained. The ergosterol is a characteristic of fungi sterol, an important source of vitamin D2 and existed in free and combined states [35]. It is an important raw material in the production of steroid hormone drugs and food, feed, and pharmaceutical raw material [36]. The cytotoxicity and antimicrobial activity of ergosterol have been proven. It possesses weak cytotoxicity against HL-60 and BEL-7402 cell lines and moderate antimicrobial activity against the bacteria *E. aerogenes* and *P. aeruginosa* and the fungus *C. albicans* [12]. Genetically modified strains which biosynthesis high yield of ergosterol can be produced [37].

Adenosine is a major nucleoside in *Cordyceps* and plays an important role in biochemical process in the organism [1]. The content of adenosine is much higher in cultured *C. sinensis* than in the natural one. Among them, cultured *C. sinensis* has a large number of adenosines, which are much higher than those in cultured *C. militaris* [38]. Nucleotide named AMP can be degraded to adenosine and the source of inosine in natural *C. sinensis* may be the oxidative deamination of adenosine.

The soy bean media which *C. militaris* has actively been grown or its hot water extract can be used as a prebiotic. Because it includes food ingredient that beneficially affects the host by selectively stimulating the growth, activity, or both of *B. ovatus* and *B. longum* bacterial species already resident in the colon. Moreover, fully colonized soy bean media of *C. militaris* can be used as method of synbiotics microflora management, in which probiotics and prebiotics are used in combination. *C. militaris* grown on soy bean media can be used for conjunction with *B. ovatus* and *B. longum* strains. This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation [39]. Among the various human intestinal microorganisms, bifidobacteria are often taken as useful indicators of human health under most environmental conditions, on the basis that they play important roles in metabolism such as amino acid and vitamin production, aid defense against infection, are associated with longevity, antitumor activity, pathogen inhibition, improvement of lactose tolerance of milk products, and immune potentiation.

The selective growth inhibitors play important role of understanding of the biochemical or molecular mechanisms of the bacterial infection and prevention of human diseases. It would be desirable to both inhibit the growth of potential pathogens and increase the numbers of bifidobacteria in the human gut. Selective growth promoters for bifidobacteria or inhibitors for harmful bacteria are especially important for human health, because intake of these extract may nor-

malize functions that result in the prevention of diseases caused by pathogens in the gastrointestinal tract [13]. However, human gastrointestinal tract is a complex and hostile environment, it appears unlikely that a few probiotic bacterial strain capable of influencing the microbial ecology of the host and of beneficially affecting lactose intolerance, the incidence of diarrhea, mucosal immune responses, blood cholesterol concentrations, and the induction of cancer.

4. Conclusion

The *P. hepiali* and *C. militaris* derived materials intake would be expected to alter the growth and composition of the intestinal flora and modulate the genesis of potentially harmful agents, thus maintaining optimal human health. On the basis of our data and earlier findings, inhibitory action of hot water extracts of *P. hepiali* and *C. militaris* toward the *S. aureus*, *E. coli*, *E. faecalis* and *L. gasseri* used without any adverse effect on bifidobacteria used may be an indication of at least one of the pharmacological actions of *P. hepiali* and *C. militaris*. It is noteworthy that *C. militaris* has a more potent activity than the much more expensive *C. sinensis*. The natural resources of the *P. hepiali* and *C. militaris* are diminishing, and it is feasible to artificially cultivation of the fruiting bodies. Thus, it would be important to find out if both *Cordyceps* species possess similar activities. The bioactive constituents with potential therapeutic value which belongs to both *P. hepiali* and *C. militaris* should be isolated. Additionally, cordycepin may also have potential in the preservation of food and selective media for the propagation of bifidobacteria. New methods and technologies need to be adopted to extract and analyze the components, requiring evaluation along the modern scientific line. Discoveries are needed about these special creatures to harvest its value for development of mankind. More researches have to be made on the herbal-medicinal and therapeutic values of *O. sinensis* and *C. militaris* species.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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