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Effect of Chitin and Chitooligosaccharide on In vitro Growth of *Lactobacillus rhamnosus* GG and *Escherichia coli* TG

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Abstract

Background and Objective: Given the rising tendency of using insects as food, research regarding the food safety issues and health implications of edible insects are necessary. Insects have an external skeleton that is mainly composed of chitin- a nontoxic, fiber-like polysaccharide. Chitin and its derivative compounds can take part in maintaining healthy gut microbiota, by promoting or inhibiting the growth of several gut bacteria depending on the chitinous substrate. Healthy composition of gut microbiota can prevent intestinal disease states and food digestion problems. The aim of the study is to characterize the impact of chitin and chitooligosaccharides on the growth of two gut bacteria *Lactobacillus rhamnosus* GG and *Escherichia coli* TG, to provide further understanding on possible outcomes of consuming insects.

Materials and Methods: Micro plate wells were prepared with tryptone soy broth in 0.5 and 0.1% wv^{-1} chitin concentrations and in 0.5, 0.1, and 0.05% wv^{-1} chitooligosaccharide concentrations. Bacteria were added and the growth parameters of *Lactobacillus rhamnosus* GG and *Escherichia coli* TG were obtained by measurement of optical density at 600 nm in 37ºC.

Results and Conclusion: Chitooligosaccharides enhanced the growth of *Lactobacillus rhamnosus* GG and inhibited the growth of *Escherichia coli* TG in the lowest tested concentration of 0.05% wv^{-1}. Chitin completely inhibited the growth of both bacteria in the lowest tested concentration of 0.1% wv^{-1}. Chitooligosaccharides appear promising as potential prebiotic compounds associated with insect food products. Chitin has a strong antibacterial effect on tested bacteria. However, the In vitro results should be verified in well-designed human studies.

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1. Introduction

Demand for food is expected to rise in the coming decades due to population growth. A promising solution to the challenges this presents is the use of insects as food and as animal feed. Insect farming can contribute to the Sustainable Development Goals set by the United Nations; when used in accordance with the principles of a circular economy, farmed insects can provide an environmentally sustainable alternative to current sources of animal protein [1,2].

Protein levels in edible insects are comparable to those in conventional livestock, and they are rich in polyunsaturated fats and micronutrients [3,4]. Beyond their nutritional value, food insects may also provide unique functional benefits to human health. Particularly interesting is chitin, the fiber-like main compound of the external skeleton of insects that is naturally present in variable amounts in commonly consumed insect species. Mealworms, as an example of insect species with the potential for being farmed on a commercial scale, have been estimated to contain between 4% and 8% chitin on a dry weight basis [5].

Recent studies have modified the industrial processing of food insects, with the aim of developing food ingredients that are more palatable for consumers than visually identifiable insects [6,7]. The process known as fractionation, besides extracting protein and fats, can yield a fraction rich in chitin [6]. These chitin fractions have the potential to serve as functional food additives or as ingredients in variety of applications.

Chitin is a polysaccharide composed of β (1→4)-linked *N*-acetyl-2-amino-2-deoxy-glucose units (GlcNAc) [8]. Chitin is water insoluble, but humans have digestive enzymes in their gastrointestinal (GI) tract that are capable of degrading chitin to some extent [9]. Chitinolytic

enzymes breakdown the glycosidic bonds between GlcNAc units and degrade chitin into its derivatives chitosan, chitooligosaccharides (COS), and chitooligomers [10-12]. Lysozyme is known to catalyze the deacetylation of 2-acetamino groups and the parting of glycoside bonds between GlcNAc units of chitin, thus producing chitosan, a partially deacetylated byproduct of chitin degradation [13,14]. Acidic mammalian chitinase (AMCase) can digest chitin into *N*-acetyl-COS compounds, which are COS compounds that consist only from few GlcNAc units [9,15,16]. Thus, they have low molecular weight (M_W) and are easily absorbed into blood circulation from the GI tract.

When consumed in insect-derived food or obtained through chitin degradation, chitin and its derivatives COS and chitosan are functional fibers that can lower LDL cholesterol levels in blood [17-19]. Chitosan and COS particles are non-allergenic bioactive nutrients and are reported to have immune enhancing, antioxidative, anti-inflammatory and antitumor capabilities [20-25]. If chitin is degraded into chitosan and COS particles with low enough M_W, they can be absorbed into the bloodstream and transferred to all organs and tissues, where they can then have the aforementioned beneficial effects [26,27].

Chitin and its derivatives may also help to maintain a balanced and healthy gut microbiota. The composition of the human gut microbiota has an effect on many intestinal disease states and digestion disorders [28,29]. A balanced composition keeps the amounts of potentially harmful bacteria low and reduces the risk of intestinal diseases [30,31]. Generally, the health-promoting effects of beneficial gut bacteria are attributed to their competitive exclusion of non-beneficial bacteria.

Chitin and its derivatives have been found to reduce the growth of some of the more harmful bacteria, such as *Escherichia* (*E.*) *coli, Vibrio (V.) cholerae*, *Shigella (S.) dysenteriae*, and *Salmonella (S.) typhimurium* [32-34]. By inhibiting the growth of non-beneficial bacteria, chitin and its derivatives enable the proliferation of beneficial gut bacteria species of the Lactobacillus and Bifidobacteria genera [35]. COSs have also been found to have direct prebiotic effects on Bifidobacteria and Lactobacillus species [12,36]. These effects are dependent on the M_W and on the degree of acetylation of the COS [36-38]. COS molecules can act as decoy molecules, preventing the adhesion of *E. coli*, or other more harmful bacteria, to the epithelium of the GI tract [39,40].

Although chitin and chitosan have been studied in depth for purposes of extraction, bioengineering, and healthcare applications, few studies have focused on the actual consumption of chitin and its nutrient effects in the context of edible insects. In the present study, we hypothesize that insect foods form a novel means can be used to create and maintain a healthy gut microbiota. We further hypothesize that COS obtained from insect-derived food through chitin degradation functions as a new prebiotic by reducing the growth of harmful bacteria and promoting the growth of probiotic bacteria, thus making consumption of insects beneficial for human health. Therefore, we examined the In vitro effects of chitin and chitosan on the growth of *Lactobacillus* (*L.*) *rhamnosus* GG, a well-known probiotic microorganism, and on *E. coli*, a bacterium that is not harmful in small numbers but is potentially an opportunistic pathogen especially when abundant in the gut.

2. Materials and Methods

2.1 Bacterial cultivations

L. rhamnosus GG (LGG) (ATCC 53103) and *E. coli* TG were chosen for the present study because *E. coli* is normally present in the GI tract and LGG, also normally present in the GI tract, is widely used in Finland as a food probiotic. The bacteria were cultivated in tryptone-soy-agar (TSA) plates using Tryptone Soy Broth (TSB) (CM0129, Oxoid Microbiology Products, Thermo Fischer Scientific Inc. Massachusetts, USA) and agar (MC006, Lab M, A Neogen Company, Lancashire, UK) at 37ºC. The *E. coli* grew colonies suitable for extraction in 22 to 26 hours, whereas for the LGG the same process took 4 to 6 days. Cultivation was continued throughout the experiment by extracting colonies to new TSA plates.

2.2 Preparation of chitin and COS solutions and bacterial stock solution

COS stock solution was prepared by mixing COS powder with a degree of deacetylation (DD)≥90% and M_W ≤1.5 kDa (237589, Bonding Chemical, Texas, USA) into *di*H_2O to a concentration of 50 g l^{-1}. As chitin is insoluble in water, a stock solution was prepared by dissolving chitin powder (C7170-100G, Sigma-Aldrich Corporation, Missouri, USA) into a 50% wv^{-1} NaOH solution (30620 1KG R, Sigma-Aldrich, Missouri USA) at a concentration of 50 g l^{-1}. Experiments on NaOH's chitin deacetylating effects were not included in this study, but knowing that NaOH might affect in such way, dilutions were prepared and used immediately after preparation of chitin's stock solutions.

The chitin and COS stock solutions were further diluted to concentrations of 5 g l^{-1} and 1 g l^{-1} for the chitin, and to 5 g l^{-1}, 1 g l^{-1} and 0.5 g l^{-1} for the COS. Corresponding control solutions for the chitin and COS solutions were prepared. A negative blank control of TSB broth without chitin or COS solutions and bacteria was also employed. A positive control with bacteria and TSB but without chitin or COS solutions was also employed (Table 1).

Table 1. Compositions of the prepared solutions

Solution type	Volume (ml)	Chitin or chito-oligosaccharide stock solution (ml)	*di*H_2O (ml)	Tryptone soy broth powder (g)	Bacteria stock solution (ml)
Negative control	10	-	9.7	0.3	-
Positive control	10	-	9.504	0.3	0.1
Chitin 0.5% wv^{-1}	10	1	8.64	0.3	0.1
Chitin 0.1% wv^{-1}	10	0.2	9.408	0.3	0.1
Chitin control 0.5% wv^{-1}	10	1	8.7	0.3	-
Chitin control 0.1% wv^{-1}	10	0.2	9.5	0.3	-
Chitooligosaccharide 0.5% wv^{-1}	10	1	8.64	0.3	0.1
Chitooligosaccharide 0.1% wv^{-1}	10	0.2	9.408	0.3	0.1
Chitooligosaccharide 0.05% wv^{-1}	10	0.1	9.504	0.3	0.1
Chitooligosaccharide control 0.5% wv^{-1}	10	1	8.7	0.3	-
Chitooligosaccharide control 0.1% wv^{-1}	10	0.2	9.5	0.3	-
Chitooligosaccharide control 0.05% wv^{-1}	10	0.1	9.6	0.3	-

TSB powder (0.3 g) was added to all the chitin and COS solutions and their controls. All the solutions and controls were sterilized in an autoclave and stored overnight at 4ºC.

For each run of 96-well plates, a bacterial stock solution was prepared containing the same amounts of *E. coli* or LGG. Bacterial stock solution was prepared by extracting colonies from the TSA plates to cuvettes containing 1 ml of phosphate-buffered saline (PBS) (NaCl 8.5 g l^{-1}, K_2HPO_4 1.21 g l^{-1}, KH_2PO_4 0.34 g l^{-1}, with *di*H_2O added to a volume of 1 l, pH=7.2). The bacterial growth density was adjusted to match the Mac Farland standard, 3×10^8 bacteria per ml at optical density A=0.250 by measuring wavelength at 600 nm with a spectrophotometer (UV/VIS UV1601 Spectrophotometer, Shimadzu Corporation, Kyoto, Japan) and then adding bacteria to match the desired optical density. A tenfold dilution was made into PBS for the bacterial stock solution and 100 ml of the desired bacterial stock solution was added to the positive controls and to the chitin and COS solutions.

2.3 Measuring the optical density of cultivations

The 96-well plates were prepared by pipetting 300 ml of the chitin and COS solutions and their respective controls into their appointed wells. Each plate contained a total of 12 different types of well and eight repeats of each type. Three replicates were made for the *E. coli* plates and four for the LGG plates.

Bacterial growth was measured as an increase in optical density (OD) in the wells of a 96-well plate in a plate reader (Synergy H1, Hybrid Multi-Mode Reader, BioTek Instruments Inc, Vermont, USA). The plates were maintained at a constant temperature of 37ºC, and measurements of OD were taken every 30 minutes for 20 to 26 hours at a wavelength of 600 nm. A similar method for measuring the turbidity of cultures was used by Benhabiles et al. [33].

Gen5 data analysis software (Gen5 Software, software version 3.0, BioTek Instruments Inc, Vermont, USA) was used to record the results. Data values from the control wells were substituted from the corresponding values of the chitin and COS wells for purposes of background correction.

2.4 Statistical analysis

IBM SPSS software (IBM SPSS statistics 24.0 software, IBM Corp., Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test was applied to check the data for normal distribution. Differences were considered significant at $p\leq0.05$. One-way ANOVA was employed for comparison of normally distributed data. Because of unequally distributed variances, the Games-Howell nonparametric post-hoc test was applied to compare the differences between the groups. For the comparison of non-normally distributed data, the Kruskal-Wallis and Mann-Whitney U tests were employed.

3. Results and Discussion

3.1 The effects of chitin and COS on the growth of *E. coli* TG

As shown in Figure 1, COS reduced the growth of *E. coli*, whereas chitin inhibited its growth. In all COS concentrations the total growth and the growth rate of *E. coli* were reduced compared to the untreated control. There were no significant differences between the 0.5% wv^{-1} and 0.1% wv^{-1} concentrations of chitin in respect of their ability to prevent *E. coli* growth. No growth was observed in *E. coli* in the presence of chitin.

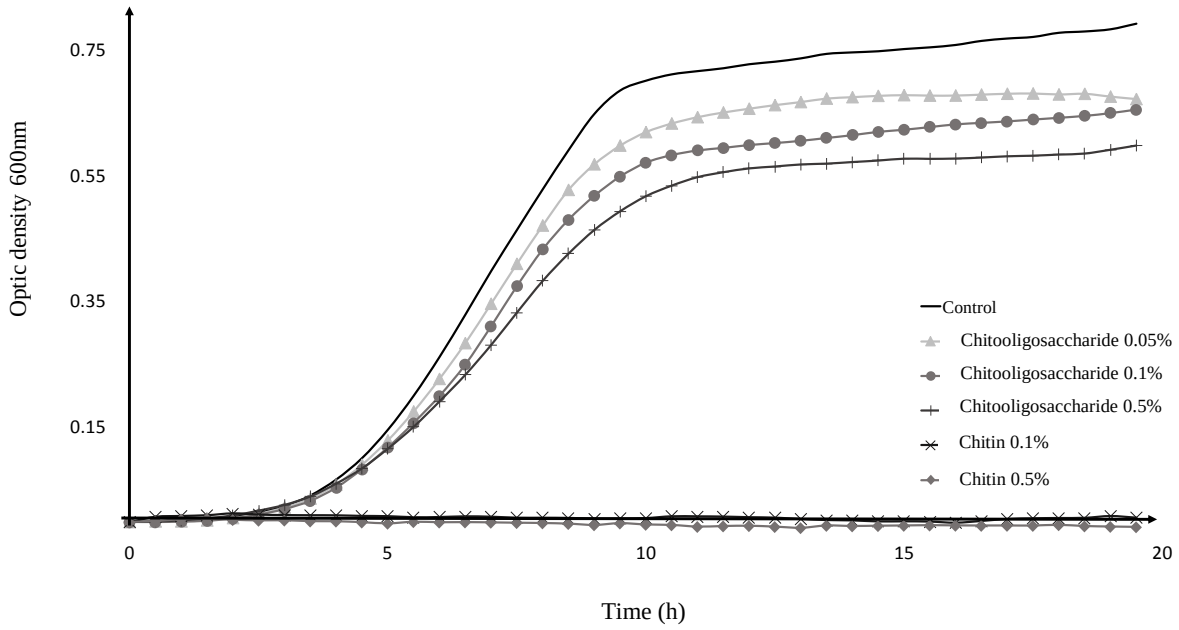

Figure 1. The effects of chitin and chitooligosaccharide on the growth of *Escherichia coli* TG

Rising values in y-axis's optical density signify for bacteria population's growth. The figure illustrates the inhibitory effects of chitin and chitooligosaccharide concentrations (% wv^{-1}) on the growth of *Escherichia coli*. Control is an untreated group without chitin and chitooligosaccharide.

The inhibitory effect of COS was more efficient at higher concentrations (Table 2). The growth rate was fastest in the untreated *E. coli* control. *E. coli*'s growth rate was slowed with rising COS concentrations. At COS concentrations of 0.5% wv^{-1} the growth of *E. coli* was approximately 63% slower than in the untreated control group. The total population growth of *E. coli* was also lower in higher COS concentrations.

Statistically significant differences were found between the untreated control and the COS concentrations in respect of maximum growth rate and total population growth (Table 2). Statistically significant differences were also found between COS 0.5% wv^{-1} and COS 0.05% wv^{-1} in respect to inhibiting the growth rate of *E. coli*: COS 0.5% wv^{-1} was more efficient than COS 0.05% wv^{-1}.

Whereas in the present study COS with 1.5 kDa inhibited *E. coli*'s growth in 0.05% wv^{-1} the lowest concentration tested, Fernandes et al. reported that COS with M_W lower than 5 kDa and 3 kDa inhibited *E. coli*'s growth in a concentration of 0.25% wv^{-1} [32]. Jeon et al. observed inhibitory effects for *E. coli* in COS concentrations between 0.06% wv^{-1} and 0.12% wv^{-1}, which is the lowest reported minimum inhibitory concentration with confirmed M_W's of COS particles ranging from 24 kDa to 7 kDa [41]. Though Benhabiles et al. reported lower minimum inhibitory concentration of COS on *E. coli* to be 0.003% wv^{-1}, they did not describe the actual M_W of COS used. Based on their reporting the M_W was presumably <12 kDa, but it cannot be confirmed [33].

Table 2. The effects of chitooligosaccharide on the growth of *Escherichia coli* TG

Escherichia coli TG	Latency (h)	Max growth rate (OD h^{-1})	Total growth (OD)
Control	4.935 (4.70-5.29)	0.203 (0.20-0.21)^{a}	1.032 (0.96-1.06)^{a}
Chitooligosaccharide 0.05% wv^{-1}	4.915 (4.81-5.01)	0.178 (0.16-0.19)^{b}	0.869 (0.83-0.95)^{b}
Chitooligosaccharide 0.1% wv^{-1}	4.789 (4.72-5.04)	0.161 (0.14-0.17)^{b,c}	0.907 (0.66-0.92)^{b}
Chitooligosaccharide 0.5% wv^{-1}	4.639 (4.51-4.80)	0.127 (0.12-0.15)^{c}	0.820 (0.66-0.86)^{b}

Results are reported in median values with range in parenthesis from min to max. Latency is reported in hours and the total amount of population's growth is reported in optical density (OD) (i.e. Absorbance at 600 nm). The maximum rate of growth is reported as OD versus the culture time (h). Different letters in the same columns indicate the statistically significant difference in a confidence level of $p \leq 0.05$.

In the current study chitin inhibited the growth of *E. coli* growth completely in 0.1% wv^{-1} concentration in contrast to the results of Raut et al. who found that 0.1% wv^{-1} chitin concentration only decreased *E. coli*'s growth by 18% [34]. Benhabiles et al. reported minimum inhibitory concentrations of chitin for *E. coli* at 0.01% wv^{-1}. These variations may be attributed to using chitins of different M_W and DD or different bacterial strains used [33]. Chitin's effects on bacteria should be also studied in conditions that don't require it to be soluble, thus not including NaOH for solvent. In this experiment NaOH effect on bacteria was expected to be minimal since it was diluted in ratios of 1:10 and 1:50 for the desired chitin concentrations.

3.2 The effects of chitin and COS on the growth of *Lactobacillus rhamnosus* GG

As shown in Figure 2, COS promoted the growth of LGG, whereas chitin prevented its growth entirely. For each of the COS concentrations, the total growth and the growth rate of the LGG exceeded that of the untreated controls. There were no significant differences between the 0.5% wv^{-1} and 0.1% wv^{-1} concentrations of chitin in respect to their ability to prevent LGG growth. No growth was observed in LGG in the presence of chitin.

Each of the COS concentrations showed a statistically significant difference in the maximum growth rate compared to the control group (Table 3). The growth promoting effect of COS was found to be greater at higher COS concentrations. The growth rate was slowest in the control and almost twice as fast in the highest COS concentration. Furthermore, the amount of total population growth was greater at higher COS concentrations. Although there were no statistically significant differences among the COS and the controls in respect of latency or total amount of growth, the latency time for COS 0.05% wv^{-1} was lower than for the control group.

Table 3. The effects of chitooligosaccharide on the growth of *Lactobacillus rhamnosus* GG

Lactobacillus rhamnosus GG	Latency (h)	Max growth rate (OD h^{-1})	Total growth (OD)
Control	18.069 (16.18-19.15)	0.049 (0.03-0.06)[a]	0.470 (0.33-0.61)
Chitooligosaccharide 0.05% wv^{-1}	17.655 (16.25-20.01)	0.073 (0.06-0.09)[a,b]	0.599 (0.44-0.72)
Chitooligosaccharide 0.1% wv^{-1}	18.140 (16.23-18.59)	0.082 (0.07-0.09)[b]	0.667 (0.53-0.74)
Chitooligosaccharide 0.5% wv^{-1}	18.403 (16.14-19.66)	0.087 (0.08-0.10)[b]	0.633 (0.49-0.71)

Results are reported in median values with range in parenthesis from min to max. Latency is reported in hours and the total amount of population's growth is reported in optical density (OD) (i.e. absorbance at 600 nm). The maximum rate of growth is reported as OD versus the culture time (h). Different letters in the same columns indicate the statistically significant difference in a confidence level of $p \leq 0.05$.

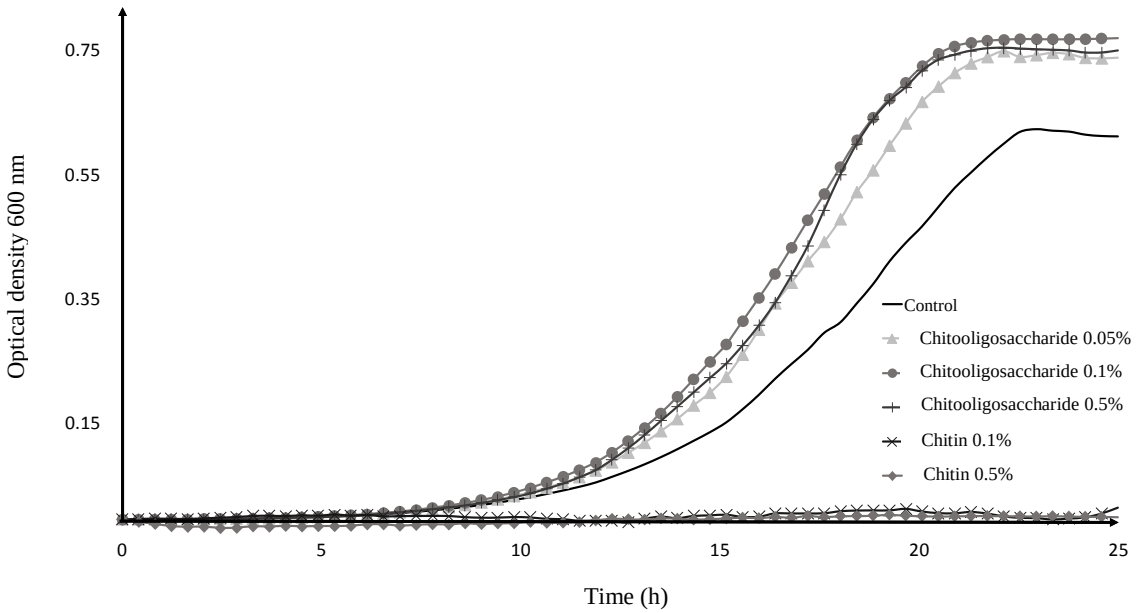

Figure 2. The effects of chitin and chitooligosaccharide on the growth of *Lactobacillus rhamnosus* GG

Rising values in y-axis's optical density signify for bacteria population's growth. The figure illustrates the inhibitory effects of chitin and the growth promoting effects of chitooligosaccharide concentrations (% wv^{-1}) on the growth of Lactobacillus. Control is an untreated group without chitin and chitooligosaccharide.

Contrary to results in the current study, Jeon et al. reported that COS to inhibited the growth of Lactobacilli in concentrations of 0.1% wv^{-1} and 0.03% wv^{-1} [41]. They also stated that COS was more efficient in inhibiting the growth of non-beneficial bacteria than the beneficial ones except for Lactobacilli. This contradiction may be accounted for different M_W's of the COS's used; 24 kDa and 7 kDa compared to 1.5 kDa used in the present experiment. Also, they used different lactobacilli, e.g. *L. casei*, *L. bulgaricus*, and *L. fermentum*. The effect on different strains of Lactobacillus should be verified on COSs with similar characteristics.

3.3 Impact of receiving chitin and COS from consuming insects

According to the results of the current study, In vitro COS with $M_W \leq 1.5$ kDa and DD $\geq 90\%$ promotes the growth of LGG and reduces the growth of *E. coli* in a dose-dependent manner, indicating that the COS tested here has the potential for prebiotic activity In vivo. More competent effects of COS' promotion of LGG's growth and inhibition of *E. coli* could be expected from insect foods that contain higher amounts of COS or from efficient chitin digestion by AMCase in the GI tract, which would result in higher yields of COS.

The fact that AMCase produces *N*-acetyl-COS from chitin In vivo after the consumption of insects is very promising in respect to obtaining beneficial effects for gut microbiota and for human health. According to Mateos-Aparicio, et al. COSs with many acetylated residues are more efficient in promoting the growth of beneficial Lactobacillus than deacetylated COSs [36]. However, regarding the inhibition of the growth of non-beneficial bacteria, Benhabiles et al. reported that the degree of acetylation of COS is inconsequential [33]. The N-acetyl-COS compound, a direct result of AMCase breakdown of chitin, is thus efficient in both promoting the beneficial bacteria and inhibiting the non-beneficial bacteria. Whether N-acetyl-COS would be further deacetylated, it would still promote the growth of LGG in accordance to the findings of this study.

The inhibitory effects on *E. coli* growth have been hypothesized by Zheng and Zhu and tested by Je and Kim to be resulting from chitin's and COS's ability to disrupt bacterial cell membranes [37,42]. By inhibiting the growth of *E. coli*, chitin reduces the levels of harmful bacteria, but it also prevented the growth of favorable LGG in this study. While chitin has an inhibitory effect to beneficial LGG growth, it may still contribute to gut health as functional fiber. Chitin's strong antibacterial effect could be utilized for commercial purposes, such as natural preservatives in the food industry to improve the shelf-life of foods [43-45].

Chitin and COS demonstrated similar outcomes on tested bacteria independent from each other. In future studies, simultaneous exposure to chitin and COS in different ratios should be carried out as both substances will be present in the GI tract following an insect meal. As no research on the inhibitory effects of chitin on Lactobacillus was found by the authors, these factors should be further elucidated.

From varying results in antimicrobial studies stated above, it can be concluded that the structure (M_W and DD) of chitin and its byproducts contribute to the antiproliferative or proliferative effects of the molecules. Reporting of the results of antimicrobial activities of chitin and its degradation products In vitro should always include exact M_W data. In vivo studies of effects of chitin from insect foods should focus on studying the functionality of chitin since its structure can vary greatly in insects themselves. Also, chitin will be degraded by digestive enzymes, making it less significant to measure exact characteristics of the chitin that is being consumed. The ratio of different chitin and its derivative chitosan and COS compounds present in the GI tract after eating insects is of importance when assessing the functional properties of insect foods on the gut microbiota and human health. These effects should be demonstrated In vivo.

The effects of chitin from insect consumption depend on how well chitin is being degraded by digestive enzymes, the amount of derivative compounds formed in the process, how long or short chained the derivative molecules are and what kind of bioactive side chains they contain. To obtain all the positive effects of chitin and its byproducts, both lysozyme and AMCase are needed for efficient chitin breakdown. Presumably the health benefits of the consumed insect foods are fewer in the people whose AMCase expression is insufficient for efficient chitin degradation, compared to those with abundant AMCase expression [9]. Understanding the function and efficiency of AMCase, lysozyme, gut bacteria and other possible factors in the chitin digestion is essential to gain knowledge on the amounts and types of COS and chitosan produce. To fully understand the comprehensive effects of chitin and its derivatives in humans, further research should be aimed at human intervention studies using insect foods or insects as a part of diets.

4. Conclusion

In the present study COS ameliorated the growth of LGG and inhibited the growth of *E. coli* TG In vitro, showing the potential of similar prebiotic-like effects to be expected In vivo. Chitin completely inhibited the growth of both tested bacteria but due to its strong antimicrobial effect, chitin could serve as a natural food preservative. When consumed in insect foods, chitin and its derivatives which cannot be absorbed may function as fiber. Chitin can add to the value of insects as food items with its functional fiber like characteristics and the gut microbiota enhancing effects of its digested state degradation products.

Chitin's, chitosan's and COSs functionality and effects on gut microbiota and health are determined by their M_W and DD. Chitin's characteristics in the actual insect and thus in the insect food products can vary greatly. Because of this, the reporting of In vitro findings on antimicrobial effects should be standardized to include information on the M_W and DD.

For health benefits, chitin must be digested by AMCase and lysozyme into COS and chitosan in the GI tract. Otherwise, COS and chitosan must be available in sufficient amounts from the insect food itself. Enzymatic degradation of chitosan in GI tract results on a wide range of different chitin byproducts such as chitosans and COS's. Therefore, In vivo studies should focus on the complex matrices of chitin's and its byproducts and their functions and the ratios in GI tract following an insect meal.

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6. Conflict of Interest

The authors affirm that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interests in the subject matter or materials discussed in this manuscript.

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اثر کیتین و کیتواولیگوساکارید بر رشد *لاکتوباسیلوس رامنوس جی جی* و *اشرشیا کلی تی جی* در شرایط درون تنی

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چکیده

سابقه و هدف: با توجه به گرایش رو به رشد استفاده از حشرات به عنوان غذا، تحقیق درباره مسائل مربوط به ایمنی و پیامدهای سلامتی حشرات خوراکی ضروری می‌باشد. اسکلت خارجی حشرات عمدتاً از کیتین، پلی‌ساکاریدی فیبر مانند و غیرسمی تشکیل شده است. کیتین و ترکیبات مشتق شده از آن می‌توانند در حفظ سلامت ریزاندامگان‌های اختصاصی روده با افزایش یا مهار رشد برخی از باکتری‌های روده بسته به ماده اولیه کیتینی نقش داشته باشند. ترکیبات سالم ریزاندامگان‌های اختصاصی روده می‌توانند از بیماری روده و مشکلات هضم مواد غذایی پیشگیری کنند. هدف از مطالعه مشخص کردن اثر کیتین و کیتواولیگوساکاریدها بر رشد دو باکتری روده‌ای *لاکتوباسیلوس رامنوس جی جی* و *اشرشیا کلی تی جی* به منظور درک بیشتر در خصوص پیامد احتمالی مصرف حشرات می‌باشد.

مواد و روش‌ها: چاهک‌های میکروپلیت با تریپتون سوی براث حاوی کیتین در غلظت‌های ۰/۵ و ۰/۱ درصد وزنی-حجمی و کیتواولیگوساکارید در غلظت‌های ۰/۵، ۰/۱ و ۰/۰۵ درصد وزنی-حجمی آماده شدند. باکتری‌ها اضافه و رشد داده شدند. با اندازه‌گیری چگالی نوری در ۶۰۰ نانومتر در درجه حرارت ۳۷ درجه سلسیوس میزان *لاکتوباسیلوس رامنوس جی جی* و *اشرشیا کلای‌تی جی* به دست آمد.

یافته‌ها و نتیجه‌گیری: کیتواولیگوساکاریدها در کمترین غلظت (۰/۰۵ درصد وزنی-حجمی) رشد *لاکتوباسیلوس رامنوس جی جی* را افزایش دادند و رشد *اشرشیا کلی تی جی* را مهار کردند. کیتین در کمترین غلظت (۰/۱ درصد وزنی-حجمی) رشد هر دو باکتری را به طور کامل مهار کرد. به نظر می‌رسد کیتواولیگوساکاریدها به عنوان ترکیبات بالقوه کمک زیست‌یار[1] موجود در مواد غذایی حاوی حشرات آیندهای روشن داشته باشند. کیتین اثر ضدباکتریایی بر باکتری‌های مورد آزمون دارد. هرچند، نتایج به دست آمده در شرایط درون تنی نیز باید با مطالعات انسانی اصولی تایید شود.

تعارض منافع: نویسندگان اعلام می‌کنندکه هیچ تعارض منافعی وجود ندارد.

تاریخچه مقاله

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واژگان کلیدی

- کیتین
- کیتواولیگوساکارید
- *اشرشیا کلای*
- مواد غذایی تهیه شده از حشرات
- *لاکتوباسیلوس*

***نویسنده مسئول**

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[1] Prebiotic- غذاهای غیرقابل هضمی که باعث تحریک رشد ریزاندامگان‌های پس‌روده/ کولون می‌شوند