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Preparation of Hetero-Chitooligosaccharides and Their Antimicrobial Activity on *Vibrio parahaemolyticus*

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Abstract This study was performed to investigate the antimicrobial effects of hetero-chitosans and their oligosaccharides on the halophilic bacterium, *Vibrio parahaemolyticus*. Nine classes of hetero-chitosan oligosaccharides were prepared based on their molecular weights, using an ultrafiltration membrane reactor system with chitosanase and celluase, from partially different deacetylated chitosans, 90%, 75%, and 50% deacetylated chitosan, respectively. Thirty-two strains of *V. parahaemolyticus* were isolated from various marine organisms such as shellfish, shrimps, octopus, and seabirds. Seventy-five percent deacetylated chitosan showed the highest antimicrobial acitivity. The minimal inhibitory concentration (MIC) was 0.5 mg/ml on 14 strains of *V. parahaemolyticus*, and MIC of the rest strains (18 strains) was 1.0 mg/ml. In addition, MIC of most hetero-chitosan oligosaccharides was 8.0 mg/ml. The results revealed that the antimicrobial effects of hetero-chitosans and their oligosaccharides against *V. parahaemolyticus* depend on the degree of deacetylation, their molecular weights, and strains tested.

Key words: Hetero-chitosan, chitooligosaccharide, antimicrobial activity, *Vibrio parahaemolyticus*

Chitosan is a natural biopolymer of N-acetylglucosamine (β-1,4 linked 2-acetamido-D-glucose), which is obtained after alkaline deacetylation of chitin derived from the cell walls of lower plants and the skeletal tissues of lower animals, including arthropods and mollusks [9]. It has received considerable attention for its commercial applications in the biomedical, food, and chemical industries. In recent studies, chitosan has attracted interest, because it converts into oligosaccharides that are not only water-soluble but also possess versatile functional properties such as antimicrobial activities [11, 6, 8, 20, 25], and it is also associated with antitumor activities [5, 24]. Antimicrobial activity of chitosan and its oligosaccharides has revealed that chitosan is more effective in inhibiting the growth of bacteria than chitosan oligosaccharides [8, 25]. Furthermore, the antimicrobial activity of chitosan depends on the molecular weight of chitosan oligosaccharides [8, 25]. However, most studies were performed with only one or a few chitosan or its oligosaccharides with different molecular weights [25, 28]. Therefore, there is little information available on the antimicrobial activity of hetero-chitosans and their oligosaccharides with different molecular weights.

Vibrio parahaemolyticus, a Gram-negative halophilic bacterium, is a food-borne pathogen prevalent in many Asian countries where marine foods are frequently consumed [10]. These strains cause disease not only in human but also in shellfish, shrimp, fish, and other invertebrates [2, 16]. Therefore, *V. parahaemolyticus* is an important pathogen in the aquaculture industry.

In the present study, hetero-chitooligosaccharides were prepared from three kinds of partially deacetylated chitosans with two enzymes; chitosanase and cellulase. The antimicrobial activity of hetero-chitosans and their oligosaccharides with different molecular weights was investigated against *V. parahaemolyticus* isolated from various marine sources.

Materials and Methods

Materials

Chitin prepared from crab shells was donated by Kitto Life Co. (Seoul, Korea). The chitosanase (35,000 units/g protein) derived from *Bacillus* sp. was purchased from Amicogen Co. (Jinju, Korea), and cellulase was donated by Pacific Chemical Co. Ltd. (Seoul, Korea). An ultrafiltration membrane (UF) reactor system for the preparation and the fractionation of hetero-chitosan oligosaccharides, based on molecular weight, was supplied by Millipore Co. (Bedford,

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MA, U.S.A.). All other reagents were of the highest grade available commercially.

Preparation of Hetero-Chitosan and Their Oligosaccharides

Three kinds of partially deacetylated chitosans, designated 90% deacetylated chitosan, 75% deacetylated chitosan, and 50% deacetylated chitosan, were prepared from crab chitin according to our method previously described [21]. One-percent solution of hetero-chitosans was prepared by dispersing 100 g of hetero-chitosans in 1 l of distilled water, dissolving it and stirring while adding 550 ml of 1.0 M lactic acid, and making it (1 l) to 10 l by adding distilled water. The pH was adjusted to 5.5 with saturated sodium carbonate solution. The ultrafiltration membrane reactor system (Millipore Minitan™, Millipore Co., U.S.A.) was used for preparation of hetero-chitosan oligosaccharides in accordance with the previous method [6] with slight modifications. Ninety-percent deacetylated chitosan was hydrolyzed with an endo-type chitosanase (35,000 U/g protein) with a substrate-to-enzyme ratio of 1 to 1.5 units for 36 h in a batch reactor, and then heated at 98°C for 10 min to inactivate the enzyme. Thereafter, the hydrolysates were separated using an UF membrane reactor system. The UF membranes used in the system were molecular weight cut-off (MWCO) 10 kDa, 5 kDa, and 1 kDa, respectively. Seventy-five percent and 50% deacetylated chitosan were hydrolyzed with substrate-to-enzyme ratio of 1 to 5 units and of 1 to 10 units by cellulase (CMC 100,000 U/g protein) for 2 h at 43±2°C in a batch reactor. Subsequently, chitosanase was added in the reactor, hydrolyzed for 36 h at the same temperature, and separated by an UF membrane reactor system. The hetero-chitosan oligosaccharides were fractionated, depending on their molecular weights, using three different pore sizes of UF membranes, MWCO 10, 5, and 1 kDa, respectively. The degree of hydrolysis was expressed as the amount of reducing sugar (D-glucosamine) released from chitosan using Blix's method [1].

Table 1. *Vibrio parahaemolyticus* used in this study.

Strain No.	Source	Area	Year	References
1	ATCC 17802			
2	*Crangon affinis*	Nakdong River, Busan	1996	12
3	*Soletellina olivacea*	Dadaepo sandbank, Busan	1997	15
4	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14
5	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14
6	*Crangon affinis*	Nakdong River, Busan	1996	12
7	*Sterna albifrons*	Nakdong delta, Busan	1997	
8	*Octopus variabilis*	Kwangan sea product market, Busan	1997	14
9	*Ruditapes philippinarum*	Masan shellfish culture bed, Masan	1997	13
10	*Octopus variabilis*	Kwangan sea product market, Busan	1997	14
11	*Ruditapes philippinarum*	Masan shellfish culture bed, Masan	1995	13
12	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
13	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14
14	*Ruditapes philippinarum*	Kwangan sea product market, Busan	1997	13
15	*Crangon affinis*	Kwangan sea product market, Busan	1996	12
16	*Crangon affinis*	Kwangan sea product market, Busan	1996	12
17	*Octopas variabilis*	Kwangan sea product market, Busan	1996	14
18	*Crangon affinis*	Kwangan sea product market, Busan	1996	12
19	*Batillus cornutus*	Kwangan sea product market, Busan	1995	13
20	*Ruditapes philippinarum*	Kwangan sea product market, Busan	1997	13
21	*Sterna albifrons*	Kwangan sea product market, Busan	1997	
22	*Sterna albifrons*	Kwangan sea product market, Busan	1997	
23	*Scapharca broughtonii*	Kwangan sea product market, Busan	1995	
24	*Octopas variabilis*	Dadaepo Sea, Busan	1995	14
25	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
26	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
27	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
28	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
29	*Crangon affinis*	Dadaepo Sea, Busan	1996	12
30	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14
31	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14
32	*Octopas variabilis*	Kwangan sea product market, Busan	1995	14

Determination of Molecular Weight of Hetero-Chitooligosaccharides

The molecular weight distributions of the hetero-chitooligosaccharides were analyzed by gel permeation chromatography using Bio-Gel P-30 gel and TSKgel Amide-80 columns. The chitooligosaccharides with molecular weight ranged from 1 kDa to 10 kDa, i.e., COS I and COS II, were chromatographed through Bio-Gel P-30 gel permeation column (3×85 cm) with 0.1 N acetate buffer (pH 5.5) at a flow rate of 0.4 ml/min, and the fractionated peaks were detected by a ninhydrin method [26]. The standard P-82 series (pullulan: MW 47, 22.8, 11.8, and 5.8 kDa) and mannopentose-di-(*N*-acetyl-D-glucosamine) (MW 1.235 kDa) were used as standard markers. The measurement of chitooligosaccharides with a molecular weight below 1 kDa, COS III, was carried out by high performance liquid chromatography (HPLC) of TSKgel Amide-80 column (4.6×250 mm). HPLC operation was carried out with acetonitrile and 250 mM phosphoric acid (60% to 40%) as a mobile phase at a flow rate of 0.5 ml/min. The separated peaks were detected using a RI detector (integration time 30 min, peak width 0.20 min and peak sensitivity 0.5%).

Strains of *V. parahaemolyticus*

V. parahaemolyticus (ATCC 17802) tested for antimicrobial activity were isolated from shellfish, shrimp, octopus and birds during 1995 to 1997 in our laboratory (Table 1), or purchased from ATCC (Americal Type Culture Collection).

Assay for Antimicrobial Activity

Minimum inhibitory concentration (MIC) was tested by two-fold serial broth dilutions. Inoculation of bacteria/plate was performed with a Steer's-type multiple inoculator [23] on Mueller-Hinton agar (MHA, Difco Lab., Detroit, MI, U.S.A.) and the plates were incubated at 37°C for 17 h. The concentrations of chitosan and its oligosaccharides used for antimicrobial test were between 0.25 mg/ml to 16.0 mg/ml.

RESULTS AND DISSCUSSION

Preparation of Hetero-Chitosan Oligomers and Determination of Their Molecular Weights

Nine different kinds of hetero-chitooligosaccharides (COS) were prepared by an ultrafiltration membrane reactor system. The deacetylated chitosans of 90%, 75%, and 50% were hydrolyzed by an enzymatic reaction in an UF membrane reactor system, and fractionated by passing them through three ultrafiltration membranes of MWCO 10, 5, and 1 kDa, respectively. The hetero-chitooligosaccharides were named as 90-COS I, 75-COS I, and 50-COS I, which are 90%, 75%, and 50% deacetylated chitosan oligosaccharides passed out through MWCO 10 kDa membrane, but not through 5 kDa membrane, 90-COS II, 75-COS II, and 50-COS II, which are 90%, 75%, and 50% deacetylated chitosan oligosaccharides passed through 5 kDa membrane, but not through 1 kDa membrane, 90-COS III, 75-COS III, and 50-COS III, which are 90%, 75%, and 50% deacetylated chitosan oligosaccharides passed out 1 kDa membrane, respectively. The molecular weight measurements of hetero-COSs were carried out by both gel permeation chromatography and HPLC. The distribution of molecular weights of 90-COSs was different from that of the membranes used. Chromatograms using Bio-Gel P-30 gel column for 90-COS I and 90-COS II are shown in Fig. 1a. In the case of 90-COS I, five major peaks (21.0, 11.0, 10.0, 9.5, and 7.5 kDa) were distributed, and two peaks were greater than the pore size of the membrane (MWCO 10 kDa) used. These results indicate that the fractions having molecular weights greater than the pore size of the membrane used were passed though the membrane. In 90-COS II, major peaks are 7.0, 6.5, 5.0, 3.5, 3.0, and 2.0 kDa. The molecular weight distribution of 90-COS III, which was separated using TSK gel Amide 80 column chromatography on HPLC, is shown in Fig. 1b. The HPLC patterns indicated two large peaks with trimer and tetramer residues. In general, the RI detector used in HPLC analysis of chitooligosaccharides was not sensitive to higher molecular weight components. The chromatograms of 75-COS I and COS II are shown in Fig. 1c. 75-COS I had a size distribution ranged from 11.0 to 5.5 kDa. The molecular weight distribution of 75-COS II was in the range of 5.0 to 1.2 kDa, except for peaks of 7.3 and 6.0 kDa, and the major peaks were 4.2, 3.0, 2.0, and 1.2 kDa, respectively. The chromatogram of 75-COS III showed five major peaks of dimer, trimer, tetramer, pentamer, and hexamer, respectively (Fig. 1d). The 50-COS I had a size distribution which ranged from 10.0 to 5.5 kDa, except in the presence of an 18 kDa peak. The pattern of molecular weight distribution of 50-COS II was in the range of 6.0 to 1.0 kDa, and major peaks were at 4.5, 3.2, and 2.0 kDa, respectively (Fig. 1e). The 50-COS III showed four major peaks of dimer, trimer, tetramer, and pentamer, respectively (Fig. 1f). The molecular weight profiles of each oligosaccharide showed a distinct decrease in molecular weights according to the pore sizes of the membrane used. The yields of 90-COS I, 90-COS II, and 90-COS III were 18.1%, 46.2%, and 35.7%, respectively, those of 75-COS I, 75-COS II, and 75-COS III were 26.2%, 32.2%, and 41.6%, respectively, and of those 50-COS I, 50-COS II, and 50-COS III were 22.0%, 32.8%, and 45.2%, respectively (Table 2). In addition, total reducing sugars provided the information on the hydrolytic level of the oligosaccharides. The contents of low molecular weights (90-COS III, 75-COS III, and 50-COS III) were much higher than COS Is and COS IIs.

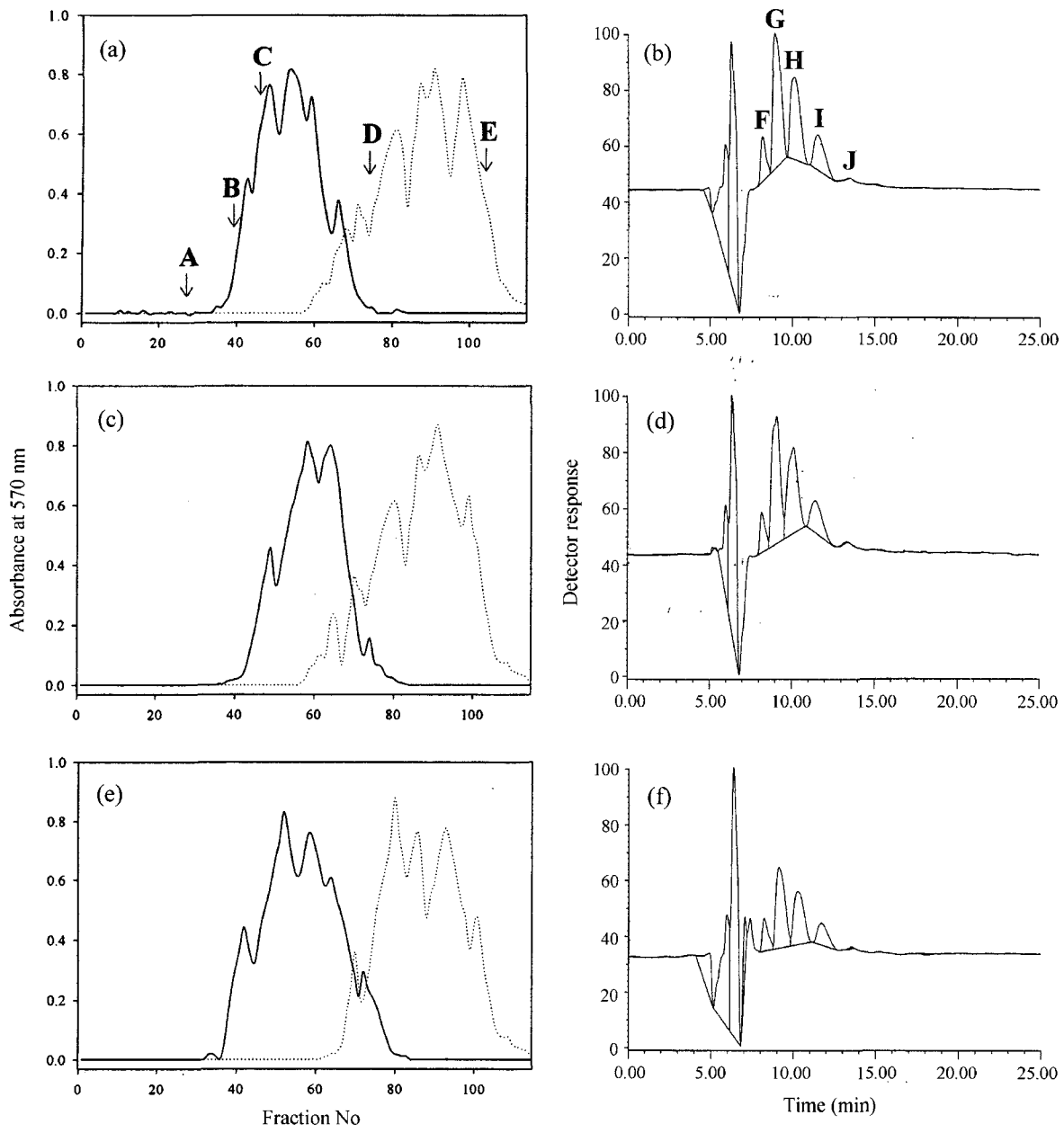


Fig. 1. Molecular weight distribution profiles of COS IS, COS IIS, and COS IIIS, which are the oligosaccharides fractionated with membranes of molecular weight cut-offs (MWCO) of 10, 5, and 1 kDa, respectively, in the ultrafiltration membrane reactor system. Samples loaded were chromatographed through Bio-Gel P-30 gel permeation with 0.1 N acetate buffer (pH 5.5) at a rate of 0.5 ml/min and the peaks separated were detected by ninhydrin colorimetric analysis (a, 90-COS I and II; c, 75-COS I and II; e, 50-COS I and II; COS Is, a solid line; COS IIs, a dotted line). Chromatogram was performed through HPLC on TSK gel NH_2-60 column (b, 90-COS III; d, 75-COS III; f, 50-COS III). HPLC was carried out with 60% acetonitrile as mobile phase at a flow rate of 0.8 ml/min using RI detector. Standard markers for gel permeation chromatography were Shodex standard P-82 Pullulan series (A, MW 47.0 kDa; B, MW 22.8 kDa; C, MW 11.8 kDa; D, MW 5.8 kDa) and mannopentaose-di-(*N*-acetyl-D-glucosamine) (E, MW 1.235 kDa). The standard markers for HPLC analysis of chitosan oligosaccharides were ranging from monomer, to heptamer. F, dimer; G, trimer; H, tetramer; I, pentamer; J, hexamer.

Ilyina *et al.* [4] reported that low molecular weight chitosans with molecular weights ranging from 4 to 8 kDa were prepared from commercial crab chitosan by means of chitinolytic complex from *Streptomyces kurssanovii*, and the yields were 70–80%. Zhang *et al.* [30] reported that 24% acetylated chitosan was depolymerized by a mixture of cellulase, α-amylase, and proteinase to give oligosaccharides, and that the removal of products by membrane separation yielded maximum amount of products having a polymerization degree in the range of 3–10. In

Table 2. Characterizations of hetero-chitosan oligosaccharides fractionated using an ultrafiltration membrane reactor system.

Hetero-chitosan oligosaccharides[1]	Yield (%)	TRS[2] (mg/g chitosan)
90-COS I	18.1	53.2±4.1
90-COS II	46.2	82.1±7.4
90-COS III	35.7	312.8±13.5
75-COS I	26.2	51.6±3.6
75-COS II	32.2	81.6±7.3
75-COS III	41.6	238.5±10.2
50-COS I	22.0	49.9±3.7
50-COS II	32.8	76.2±7.7
50-COS III	45.2	212.9±9.8

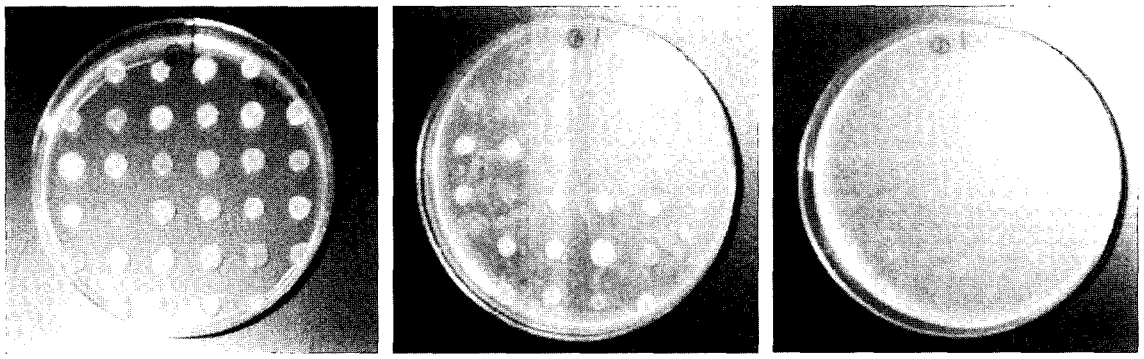
[1] 90-COS I, 75-COS I and 50-COS I, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out through MWCO 10 kDa membrane but not passed out 5 kDa membrane; 90-COS II, 75-COS II and 50-COS II, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out through MWCO 5 kDa membrane but not passed out 1 kDa membrane; 90-COS III, 75-COS III and 50-COS III, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out 1 kDa membrane.
[2] TRS data are expressed as mean standard deviation of three determinations.

addition, chitosan could also be degraded by nonspecific enzymes such as cellulase, lipase, and bromelain [17, 18, 27]. Jeon and Kim [7] reported continuous production of chitooligosaccharides from chitosan with a dual reactor system, which was composed of an ultrafiltration membrane reactor and a column reactor packed with an immobilized enzyme.

Antimicrobial Activity of Hetero-Chitosans and Their Oligosaccharides

As shown in Fig. 2 and Table 3, hetero-chitosans and their oligosaccharides inhibited the growth of *V. parahaemolyticus*. The inhibitory activity differed depending on the degree of deacetylation and molecular weight of chitosan. Seventy-five percent deacetylated chitosan showed the highest antimicrobial activity, and chitooligosaccharides with higher molecular weights showed higher activity against *V. parahaemolyticus*. MICs of 75% chitosan were 1.0 mg/ml on 18 strains of *V. parahaemolyticus* except for 14 strains (0.5 mg/ml). In addition, the MIC of most hetero-chitosan oligosaccharides was 8.0 mg/ml.

Uchida *et al.* [25] has reported that chitosan hydrolysate, which is slightly hydrolyzed with chitosanase, was more effective as an antibacterial agent than native chitosan and chitosan oligomers, and the MIC of the chitosan hydrolysate for *E. coli* and *S. aureus* were 0.025% and 0.5%, respectively. Cho *et al.* [3] reported that the antibacterial activity of chitosan for *E. coli* and *Bacillus* sp. increased with decreasing viscosity from 1,000 to 10 cp. Generally, the degree of chitosan polymerization is known to affect its antimicrobial activity, and the effectiveness of different chain-length chitosan hydrolysates against microorganisms has been described. In the present study, the growth of the bacteria was reduced, as the molecular weight of the hetero-chitosan oligosaccharides decreased. The relationship between molecular weight and the antimicrobial activity of chitosan oligosaccharides has been reported: Jeon *et al.* [8] reported that the molecular weights of chitosan oligosaccharides were critical for microorganism inhibition and the efficacy increased with molecular weight. Sekiguchi *et al.* [22] investigated antibacterial activities of chitosan oligosaccharides (molecular weight ranging from 2,350 to 21,600 Da) for various bacteria, and found that the growth of *Bacillus cereus* on agar culture was suppressed by 0.2-0.3% chitosan oligosaccharide with a 11,000 Da molecular weight. Yalpani *et al.* [28] found that chitosan oligosaccharides with various degrees of polymerization reduced the viability of *E. coli* by 2.47-2.84 log cycle at a concentration of 0.1%. Yun *et al.* [29] found that the differences in MIC values were correlated with different molecular weights of chitosan, ranging from



(A) (B) (C)

Fig. 2. Photograph of *V. parahaemolyticus* isolated from various sources and grown on culture medium (Mueller-Hinton agar) containing 75% deacetylated chitosan at concentration of 1.0 mg/ml and 0.5 mg/ml.
A, control; B, 0.5 mg/ml; C, 1.0 mg/ml.

Table 3. Growth inhibitory effect of hetero-chitosans and their oligosaccharides against various *Vibrio parahaemolyticus*.

Strain No.	Minimal Growth inhibitory Concentrations (mg/ml)											
	90% chitosan[1]	90-COS I[1]	90-COS II[1]	90-COS III[1]	75% chitosan[1]	75-COS I[1]	75-COS II[1]	75-COS III[1]	50% chitosan[1]	50-COS I[1]	50-COS II[1]	50-COS III[1]
1	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0
2	0.5	4.0	8.0	8.0	0.5	4.0	4.0	8.0	0.5	8.0	8.0	8.0
3	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
4	0.5	4.0	8.0	8.0	0.5	4.0	8.0	8.0	0.5	8.0	8.0	8.0
5	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0
6	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0
7	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
8	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
9	1.0	4.0	8.0	8.0	0.5	4.0	4.0	4.0	1.0	8.0	8.0	8.0
10	1.0	8.0	8.0	8.0	0.5	8.0	8.0	8.0	1.0	8.0	8.0	8.0
11	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
12	1.0	4.0	8.0	8.0	1.0	4.0	8.0	8.0	1.0	8.0	8.0	8.0
13	1.0	8.0	8.0	8.0	1.0	4.0	4.0	4.0	1.0	8.0	8.0	8.0
14	1.0	8.0	8.0	8.0	0.5	8.0	8.0	8.0	1.0	8.0	8.0	8.0
15	1.0	8.0	8.0	8.0	0.5	4.0	4.0	4.0	1.0	8.0	8.0	8.0
16	1.0	8.0	8.0	8.0	0.5	4.0	8.0	8.0	1.0	8.0	8.0	8.0
17	1.0	4.0	8.0	8.0	1.0	4.0	8.0	8.0	1.0	8.0	8.0	8.0
18	1.0	4.0	4.0	4.0	0.5	4.0	4.0	4.0	1.0	4.0	4.0	4.0
19	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
20	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
21	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
22	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
23	1.0	4.0	4.0	4.0	0.5	4.0	4.0	4.0	1.0	4.0	4.0	4.0
24	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
25	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
26	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
27	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
28	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0	0.5	4.0	4.0	4.0
29	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
30	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0
31	0.5	8.0	8.0	8.0	0.5	4.0	4.0	4.0	1.0	4.0	4.0	8.0
32	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0	1.0	8.0	8.0	8.0

[1]90%, 75% and 50% chitosan, the 90%, 75% and 50% deacetylated chitosan; 90-COS I, 75-COS I and 50-COS I, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out through MWCO 10 kDa membrane but not passed out 5 kDa membrane; 90-COS II, 75-COS II and 50-COS II, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out through MWCO 5 kDa membrane but not passed out 1 kDa membrane; 90-COS III, 75-COS III and 50-COS III, the 90%, 75% and 50% deacetylated chitosan oligosaccharides passed out 1 kDa membrane.

0.05% to >0.2% for *E. coli* and 0.04% to 0.1% for *S. aureus*.

In conclusion, hetero-chitosan oligosaccharides were prepared, using an UF membrane reactor system with chitosanase and cellulase, from differently deacetylated chitosans. The antimicrobial effects of hetero-chitosans and their oligosaccharides against *V. parahaemolyticus* were dependent on the degree of deacetylation, molecular weights, and tested strains.

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