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Bioproduction of Chitin Hydrolysate Containing N-Acetylglucosamine by *Serratia marcescens* PT6 Crude Chitinase and Its Effects on Bacterial Growth Inhibition in Various Temperature

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Abstract. N-acetylglucosamine (GlcNAc), a chitin monomer, can be used as a natural preservative to ensure food quality and safety. Combining natural preservatives with low storage temperature offers physical hurdles to bacterial growth in food. This study aimed to produce chitin hydrolysate containing GlcNAc using *Serratia marcescens* PT6 crude chitinase and investigate its effect on bacterial growth rate as a function of temperature. Crude chitinase from partial purification was used to hydrolyze 1.3% colloidal chitin. The optimal enzymatic conditions were pH 6 and 45˚C for 120 min, at an enzyme:substrate ratio of 1:1, yielding a 65.6 μg/mL GlcNAc. Inhibitory activity of hydrolysate containing 2.5-7.5 ppm GlcNAc on *Escherichia coli*, *Staphylococcus aureus, Bacillus cereus*, and *Vibrio parahaemolyticus* was measured at 4, 15, and 30ºC in nutrient broth. Bacterial growth was measured using of optical density for each combination of GlcNAc concentration and temperature. Growth curves fitted by the Baranyi and Roberts model were developed using DMFit software. The growth rate was converted to the square root and then modeled as a function of temperature using the Ratkowsky square root model. Incubation temperature exerted a pronounced effect on the inhibition of all bacterial species ($P<0.0001$), with the greatest effect observed for *E. coli* at 30°C ($P<0.0001$), and the least effect for *V. parahaemolyticus* ($P=0.0878$). The inhibitory effect of GlcNAc in chitin hydrolysate was only significant for *E. coli* ($P<0.0001$) and *S. aureus* ($P=0.0041$). This study revealed that the effect of temperature in growth inhibition was more significant than GlcNAc addition. However, a reduction in bacterial growth with the addition of GlcNAc at 30°C was observed, which may be effective for food encountered thermal abuse conditions. Further investigation of the effect of GlcNAc on bacteria structure and metabolism is required to elucidate the mechanism of GlcNAc as a food preservative.

Keywords: Antibacterial, Chitinase, Growth rate, N-acetylglucosamine, *Serratia marcescens*

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INTRODUCTION

N-acetylglucosamine (GlcNAc), a monomer of chitin hydrolysate, is well known as a value-added product derived from the utilization of shellfish waste. Compared to its polymeric form, oligomer, dimer and monomer forms of chitin have greater solubility, which facilitates their utilization (Taokaew & Kriangkrai, 2023). Attractive characteristics of chitin and chitin hydrolysate products include biocompatibility, biodegradability, and non-toxicity, which promote use as a raw material over a wide range of applications.

The production of GlcNAc is achieved by hydrolyzing chitin using several chemical or biological processes. Chemical methods usually have high efficiency, easy operation, and low cost. However, the main concern of this method is the consumption of hazardous chemicals in the process, such as strong acids, which lead to considerable environmental pollution and equipment corrosion. Therefore, an environmental-friendly biological production of GlcNAc has gained much interest (Cao et al. 2022). An enzyme, chitinase, catalyse this process. Microbial chitinase is a promising source of this enzyme compared to animal or plant sources, offering easier and simpler handling in upstream and downstream processes of chitinase production.

Previously, we isolated *Serratia marcescens* PT6, a Gram-negative chitinolytic bacterium, from shrimp pond sediment, which showed good chitin hydrolysis activity (Triwijayani et al. 2018). Chitinolytic activity of *S. marcescens* has been reported in other studies (Tao et al. 2022; Li et al. 2020; Costa et al. 2019). Furthermore, Liu et al. (2023) investigated the synergistic hydrolysis of *S. marcescens* chitinases that degraded chitin efficiently and the effect of CMBs (Carbohydrate-binding molecules) on its chitinolytic system.

GlcNAc has been studied for its use in medicine, such as treating osteoarthritis, restoring and maintaining cartilage, reducing inflammation, and protecting the liver (Ahuja et al. 2021). In food processing, the application of GlcNAc is still limited, although several applications have been reported, including supplementation of GlcNAc in food products, as a growth promotor for probiotics in fermented milk (Chen et al. 2004) and as a sweetener in food and beverages (Wassenaar, 2004). Recently, Doi et al. (2022) reported that caramelized GlcNAc has a great potential to be an antioxidant agent to prevent oxidative deterioration in food.

Another potential bioactivity of GlcNAc suitable for application in food is antimicrobial activity. The antimicrobial activity of chitin hydrolysate is due to the size of the small particles which is to infiltrate the bacterial cell wall, reaching the cell membrane and disturbing its permeability (Benhabiles et al. 2012). GlcNAc has stronger antibacterial activity than chitin against *Staphylococcus aureus* and *Escherichia coli* (Raut et al. 2016). Antibacterial properties were also shown with the oligomeric form of GlcNAc, in which the 0.1% N-acetyl chitooligosaccharide exhibited bactericidal activities against *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Vibrio cholerae*, *Shigella dysenteriae*, *Bacteroides fragilis*, *Prevotella melaninogenica*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*; these antibacterial activities were more intense compared to chitin and chitosan (Benhabiles et al. 2012).

Due to its biocompatibility, GlcNAc can

be applied as a natural preservative in food to enhance shelf-life and assure food quality and safety. The use of chitin/chitosan derivatives and hydrolysates for food preservation has also been reported, such as in fresh papaya(Dotto et al. 2015), ice-stored Pacific white shrimp (Yuan et al. 2016), ready-to-cook meat product (Kanatt et al. 2013), and chilled fish (Reesha et al. 2015).

Hurdle technology prolongs product shelf-life and is increasingly being applied in the food industry since combining several factors in controlling microbial growth can be more effective in preserving food. Temperature is a main hurdle in fish preservation, water activity, pH, gas composition in packaging, and preservatives (antibacterial compounds) (Tsironi et al. 2020). We found that most reports evaluating the application of chitin derivatives and hydrolysates as antibacterial compounds have focused on molecule concentration (Benhabiles et al. 2012; Reesha et al. 2015), molecular weight (Lee et al. 2022; Garcia et al. 2018), degree of polymerization (Rahman et al. 2015; Kulikov et al. 2015), and degree of deacetylation (Liu et al. 2012; Byun et al. 2013). However, the effectiveness of chitin derivatives and hydrolysates against food-associated bacteria has not been reported previously, including the effect of incubation temperature. Applying chitin hydrolysate as a natural preservative, combined with low storage temperature, can produce cost-effective physical hurdles to bacterial growth in food.

This is the first report of the antibacterial activity of chitin hydrolysate containing GlcNAc and its effect on bacterial growth rate as a function of temperature. Optimal conditions for GlcNAc production using crude chitinase from *S. marcescens* PT6 are also reported. The results of this research provide insights into applying a natural preservative at different temperatures on inhibiting microbial growth and present early information about a potential hurdle technology approach for minimally processed foods.

MATERIALS AND METHODS

Inoculum and Medium Preparation

Serratia marcescens PT6 was obtained from of the Laboratory of Fish Product Quality and Safety collection, Universitas Gadjah Mada; the strain was previously isolated from shrimp pond sediment in Yogyakarta (Triwijayani et al. 2018). One loop-full of bacterial suspension from a glycerol stock was transferred into 7 mL colloidal chitin broth (CCB) (0.03% KH_2PO_4, 0.07% K_2HPO_4, 0.05% $MgSO_4.5H_2O$, 0.0001% $ZnSO_4$, 0.0001% $MnCl_2$, 2% colloidal chitin; w/v) and incubated at 37°C for 72 h. After that, the suspension was streaked on colloidal chitin agar (CCB plus 2% agar; w/v) and incubated at 37°C for 72 h. An inoculum was prepared by transferring one colony from agar into 7 mL CCB, with additional incubation at 37°C for 72 h.

Production of Crude Chitinase

A production medium (CCB plus 1.5% starch and 0.15% yeast extract; w/v) was prepared, adjusted to pH 7, and inoculated with 0.5% bacterial suspension. Fermentation was carried out in a shaking incubator (100 rpm) at 30°C for 3 days. The culture supernatant was collected by centrifugation (Eppendorf 5810-R) at 20,124 x *g* and 4°C for 10 min. Proteins were precipitated from the supernatant using ammonium sulphate (60% saturation) in a stirrer for 1 h at 4°C. Pellets were collected by centrifugation at 2,465 x *g* and 4°C for 15 min. Then they were dissolved

in 5 mL phosphate buffer (50 mM, pH 7) and dialyzed overnight in 50 mM phosphate buffer (pH 7) using a 13.2 MWCO dialysis membrane (Membra-Cel). The dialysate (crude enzyme) was collected, centrifuged (2,465 x *g* and 4°C for 15 min) to remove particles, and then kept at 4°C before use.

Enzymatic Reaction Conditions

To determine the effect of pH on chitinase activity, 0.5 mL crude enzyme was added to 1 mL of 1.3% colloidal chitin prepared in citrate buffer at pH 4, 5, and 6; phosphate buffer at pH 7; and borate buffer at pH 8 and 9; the preparations were incubated in a shaking water bath at 37°C for 30 min. The mixture was then boiled for 3 min to stop the reaction. After cooling, the mixture was centrifuged at 10,621 x g for 5 min. Chitinase activity was then measured in the supernatant. The highest chitinase activity obtained from the pH treatments was used for temperature optimization. The 0.5 mL crude enzyme was added to 1 mL of 1.3% colloidal chitin (adjusted to optimum pH) and incubated at 30, 35, 40, 45, and 50°C for 30 min. After boiling for 3 min, the mixture was centrifuged at 10,000 rpm for 5 min. The supernatant was measured for its chitinase activity.

Measurement of Chitinase Activity

The amount of GlcNAc released from the enzymatic reaction was measured following Reissig et al. (1955). The reaction solutions (0.25 mL) from the previous step were mixed with 0.05 mL potassium tetraborate (pH 9.1). The mixture was boiled for 3 min and then cooled. Next, 1.25 mL p-dimethylamino-benzaldehyde (10% (w/v) in 7:1 of glacial acetic acid and 10 N HCl) was added to the mixture and incubated at 37°C for 20 min for color development. The color intensity was detected by UV-Vis spectrophotometer at 584 nm. Standard GlcNAc solutions (0, 0.5, 1, 1.5, 2, and 2.5 µg/mL) were used for calibration. One unit of chitinase activity was defined as the amount of GlcNAc (µmol) the enzyme produces per minute.

Production of Chitin Hydrolysate in Various Incubation Times and Enzyme/Substrate Ratios

The results of optimizing pH and temperature from the previous steps were used to evaluate the effect of incubation time and enzyme/substrate ratio on the production of chitin hydrolysate. The enzymatic hydrolysis was carried out by mixing 1 mL of 1.3% colloidal chitin and 0.5 mL crude enzyme with a total activity of 0.0008 U. The incubation times for the reaction were 30, 60, 90, and 120 min. The incubation time that resulted in the highest hydrolysate concentration was used to define the optimum ratio of the enzyme (E) and substrate (S). The variation in E:S ratios were 1:1, 1:2, and 2:1 (in a 1.5 mL reaction mixture). Measurement of GlcNAc was performed as above.

Confirmation of Hydrolysate Products

TLC performed hydrolysis product confirmation after Patil and Jadhav (2014). A 10 µL of hydrolysate obtained from the enzymatic reactions was spotted on Silica gel 60 F254 plates (Merck) with a GlcNAc standard (50 µg/mL). After drying, the plates were inserted into a chamber containing the mobile phase (isopropyl alcohol: ethyl acetate: aquadest: pyridine, 3:3:1:0.4 (v/v)). Hydrolysis products on the plates were visualized using aniline diphenylamine. The samples' retention factor (Rf) was compared to the GlcNAc standard.

Measurement of Bacterial Inhibitory Activity

Bacterial inhibition tests were performed using *Escherichia coli* FNCC 0091, *Vibrio parahaemolyticus* JCM 2147, *Staphylococcus aureus* FNCC 0047, and *Bacillus cereus* (collection of the Laboratory for Fish Product Quality and Safety, UGM). These species were cultured overnight in nutrient broth (NB) at 37°C, diluted in NB, and then 100 µL of the overnight cell suspension (10^6 log CFU/mL) was added to 800 µL NB media and 100 µL of different dilutions of chitin hydrolysate, and then mildly vortexed. The final concentration of GlcNAc in the treatment culture was 2.5, 5, and 7.5 µg/mL. A 100 µL aliquot of buffered phosphate (the solution to dilute chitin hydrolysate) was added as a negative control. Mixtures were incubated for 12 h at 30°C, 10 d at 15°C and 10 d at 4°C. Bacterial growth was measured each hour for the 30°C incubation and every 24 h for 4°C and 15°C by turbidity OD_{600nm} measurement. An OD value higher than 0.2 was chosen to indicate of the apparent growth of cells in the culture.

Data Analysis

ANOVA analysis was performed to observe the effect of pH, temperature, and enzyme/substrate ratio on chitinase. Duncan's multiple-range test (DMRT) was used to detect differences among the mean values of all parameters. DMFit, available from ComBase (http://www.combase.cc), was used to fit the Baranyi and Roberts model (Baranyi & Roberts, 1994) to bacterial optical density growth curves for each combination of chitin concentration and temperature. The growth rate was converted to the square root and then modelled as a function of temperature using the Ratkowsky square root model (Ratkowsky et al. 1982), and as a function of GlcNAc concentration using the Excel linear regression function.

RESULTS AND DISCUSSION

ANOVA analysis shows that the activity of the crude chitinase from *S. marcescens* PT6 was affected by pH ($P<0.05$). DMRT analysis implies no significance in chitinase activity among pH 5 to 8 (the same letter of c in Figure 1A). This indicated that chitinase activity remained stable at pH values ranging from 5 to 8, with the highest chitinase activity recorded at pH 6 (0.0042 U/mL). A significant reduction in chitinase activity was observed at pH 4 (71% reduction) and pH 9 (33% reduction) compared to the activity at pH 6. The activity of the crude chitinase from *S. marcescens* PT6 was also affected by temperature ($P<0.05$). DMRT analysis shows that chitinase activity at 45 and 50°C significantly different from other temperatures (Figure 1B). Enzyme activity increased with increasing temperature until an optimum was reached at 45°C, at 0.0048 U/mL, then activity declined. Similar results for the optimum pH for chitinase from *S. marcescens* were reported by other researchers.

Emruzi et al. (2020) reported that crude chitinase from *S. marcescens* B4A was stable over the pH of 5-8 and optimum at pH 7. Furthermore, Li et al. (2020) found that the hydrolyzing activity of recombinant chitinase rCHI-2 from *S. marcescens* remained stable at the pH range of 6 to 9, with the optimum activity at pH 6. A significant reduction in chitinase activity was observed at pH 5.5 and 10.

A wider range of optimum temperatures for the *Serratia marcescens* chitinase reaction was reported previously. A slightly higher optimum temperature than our result is reported by Emruzi et al. (2020), who showed

that the activity of crude chitinase from *S. marcescens* B4A was optimum at 50°C. A higher temperature for optimum chitinase activity was reported for *S. marcescens* rCHI-2 (Li et al. 2020), where optimum activity was observed at 55°C. A reduction of 85% activity was reported after 1 h incubation at 45°C.

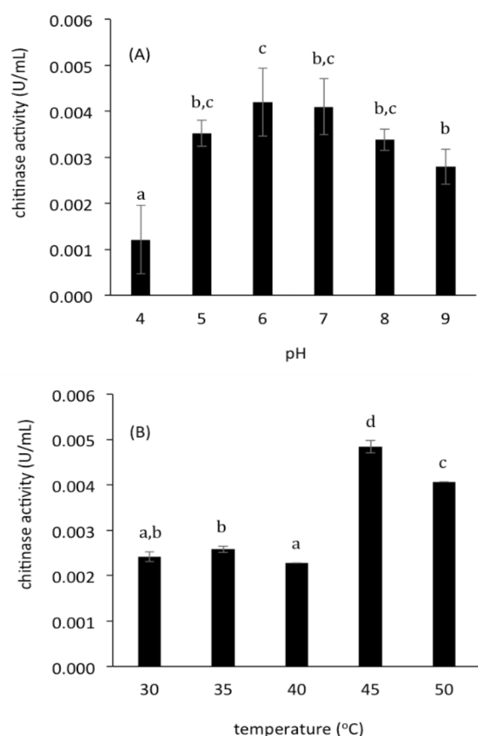

Fig. 1: Crude chitinase (U/mL) activity of *Serratia marcescens* PT6 at (A) different pH (incubated at 37°C), and (B) different incubation temperature (at pH 6). a-d: Different letters in the same graph indicate a significant difference ($P<0.05$) using DMRT analysis. The error bars represent standard deviation.

The highest catalytic activity of the enzyme in a given reaction is achieved at an optimum range of pH and temperature. The reduction of enzyme activity below or above the optimum pH was caused by the alteration of ionic charge in the system, resulting in the loss of catalytic activity. Meanwhile, increasing temperature helps to increase the catalytic activity due to kinetic energy until the optimum activity is reached. Above the optimum point, the temperature may alter the enzyme three-dimensional structure, causing a decrease in catalytic activity (Phillips, 2019). Moreover, the temperature of the enzymatic reaction also affects the composition of chitin hydrolysate products (Kuk et al. 2005). This study revealed that the optimum condition for the enzymatic reaction of *S. marcescens* PT6 to hydrolyse chitin was at pH 6 and 45°C (Fig. 1).

Standard GlcNAc, a monomer of the chitin polymer, was used to measure the final hydrolysis product from the enzymatic reaction of colloidal chitin with crude chitinase. The amount of GlcNAc produced from the enzymatic reactions was affected by incubation time ($P<0.05$). Figure 2 shows that the concentration of GlcNAc increased with increasing incubation time. The highest concentration of GlcNAc was obtained after 120 min incubation. Further confirmation of hydrolysis using TLC showed that the main hydrolysis product was GlcNAc (data not shown).

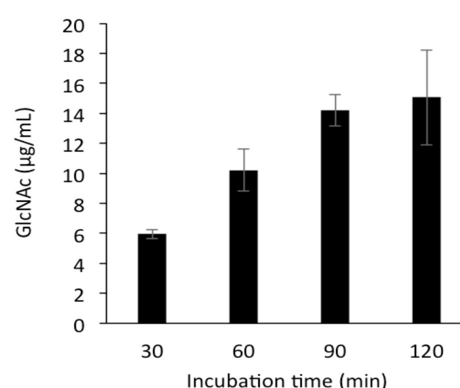

Fig. 2: GlcNAc (µg/mL) released from colloidal chitin following treatment with crude *Serratia marcescens* PT6 chitinase (produced at pH 6, 45°C) from 30 to 120 min; the error bars represent standard deviation.

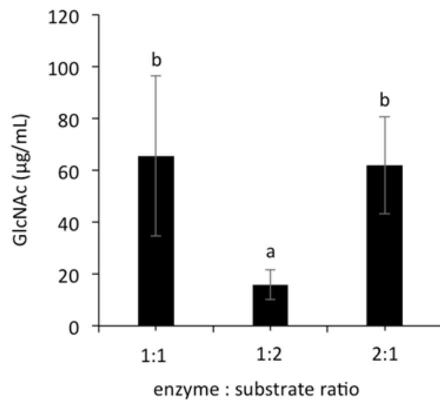

Fig. 3: GlcNAc (µg/mL) released from colloidal chitin following treatment with *Serratia marcescens* PT6 crude chitinase for 120 min at enzyme:substrate ratios of 1:1, 1:2, 2:1. Chitinase was produced at pH 6 and 45°C. a-b: Different letters indicate a significant difference (P<0.05) using DMRT analysis. The error bars represent the standard deviation.

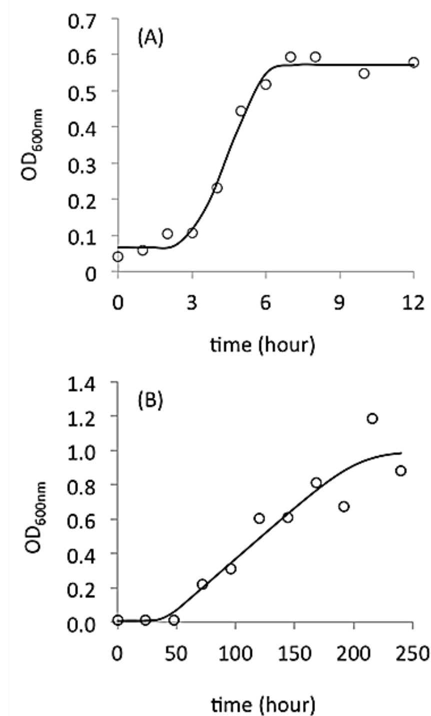

Fig 4. Representative growth profiles for *E. coli* at 30°C (A) and 15°C (B), in negative control medium containing buffered phosphate. The line is the Baranyi and Roberts model fit.

Although our result shows that the accumulation of GlcNAc seemed stable between 90-120 min, prolonged incubation might increase the amount of GlcNAc was obtained from the reaction. Purusotham et al. (2012) observed the time course hydrolysis of colloidal chitin by chitinases from *S. proteamaculans* 568 for 720 h. They showed that DP1 chitin (GlcNAc) accumulated at a lower rate at the beginning of incubation and increased at the later stage.

The type of chitinase and reaction time affects the chitin hydrolysate's composition (Gutiérrez-Román et al. 2014; Purushotham et al. 2012). Tuveng et al. (2017) confirmed that *S. marcescens* BJL200 secretes ChiA, B, C, and CBP21 in high amounts during growth in a chitin medium, yielding a dominant product of GlcNAc after 48 h incubation.

The concentration of GlcNAc released from the enzymatic reaction was also affected by the enzyme/substrate ratio, with the highest GlcNAc concentrations obtained at enzyme:substrate ratios of 1:1 and 2:1 (Fig. 3). Enzyme-substrate ratio affected the reaction rate and finally affected the amount of product in a given time. Aktuganov et al. (2018) found the nonlinear dynamic of colloidal chitin hydrolysis and the decreasing reaction rate by the end of incubation at the low enzyme–substrate ratio.

All tested bacteria grew at 15 and 30°C, but not at 4°C after 10 d incubation. Figure 4 shows examples of growth profiles for *E. coli* in the control medium. Lag time duration varied among strains and environmental conditions and displayed no consistent temperature and GlcNAc level trends. Lag time duration varied from 0 (not detected) to 105 h, 0 to 94 h, 0 to 109 h, and 0 to 107 h, for *B. cereus*, *E. coli*, *S. aureus*, and *V. parahaemolyticus*, respectively.

Growth rates ranged from 0.059 square

root log CFU/h for *E. coli* in 2.5 ppm GlcNAc at 15°C to 0.418 square root log CFU/h for *E. coli* in 0 ppm GlcNAc at 30°C (Table 1). For all species, temperature was highly significant (P<0.0001) for growth rate (data not shown). The effect of GlcNAc on growth rate varied by species and was significant for *E. coli* (P<0.0001) and *S. aureus* (P=0.0041), but only marginally for *B. cereus* (P=0.0258), and not significant for *V. parahaemolyticus* (P=0.0878). GlcNAc produced a lower growth rate (except for *E. coli* at 15°C) than the negative control, but with no apparent trend in effect above 2.5 ppm GlcNAc (Table 1).

The most prominent inhibitory effect was observed at 30°C, compared to other incubation temperatures for all four bacterial species (Fig. 5). The decline in growth rate was observed at 2.5 ppm at 30°C, but not at higher GlcNAc concentrations. The inhibitory effect of 2.5 ppm GlcNAc on growth rate was 42.9, 17.2, 16.9, and 8.4% for *E. coli*, *S. aureus*, *V. parahaemolyticus*, and *B. cereus*, respectively (Table 1). The corresponding Ratkowsky square root model parameters for growth rate as a function of temperature at different GlcNAc concentrations are shown in Table 2.

Table 1: Square root of bacterial growth rate at different temperatures and different chitin hydrolysate containing GlcNAc concentrations

Bacteria	GlcNAc (ppm)	Temperature	
		30°C	15°C
V. parahae-molyticus	0[a]	0.2441	0.0922
	2.5	0.21	0.0679
	5	0.2276	0.0658
	7.5	0.2306	0.0754
E. coli	0	0.4181	0.0774
	2.5	0.2517	0.1077
	5	0.1766	0.0856
	7.5	0.1896	0.0721
B. cereus	0	0.3455	0.1126
	2.5	0.2514	0.064
	5	0.2402	0.0843
	7.5	0.2522	0.0603
S. aureus	0	0.2914	0.1641
	2.5	0.1872	0.0705
	5	0.1926	0.0622
	7.5	0.1275	0.0609

[a]Note: Experiments were conducted in two trials; entries in the table are the averages of the results from the two trials.

Table 2: Ratkowsky square root model parameters for growth rate as a function of temperature at different chitin hydrolysate containing GlcNAc concentrations

Bacteria	GlcNAc (ppm)	b	T_{min}[a]	R^2
V. parahaemo-lyticus	0	0.0099	2.3636	0.9032
	2.5	0.0089	4.4607	0.9364
	5	0.0093	4.5054	0.9550
	7.5	0.0080	1.8250	0.9241
E. coli	0	0.0147	5.0204	0.9051
	2.5	0.0086	4.0814	0.9478
	5	0.0069	2.6667	0.9876
	7.5	0.0074	4.1486	0.9850
B. cereus	0	0.0116	4.0517	0.9639
	2.5	0.0095	-4.4737	0.9357
	5	0.0096	-4.6563	0.9614
	7.5	0.0093	-4.1828	0.9291
S. aureus	0	0.0103	0.0291	0.9912
	2.5	0.0075	4.0267	0.9745
	5	0.0089	5.0787	0.9122
	7.5	0.0075	4.4133	0.7660

[a]Note: T_{min} is the notional minimum temperature for growth, in °C.

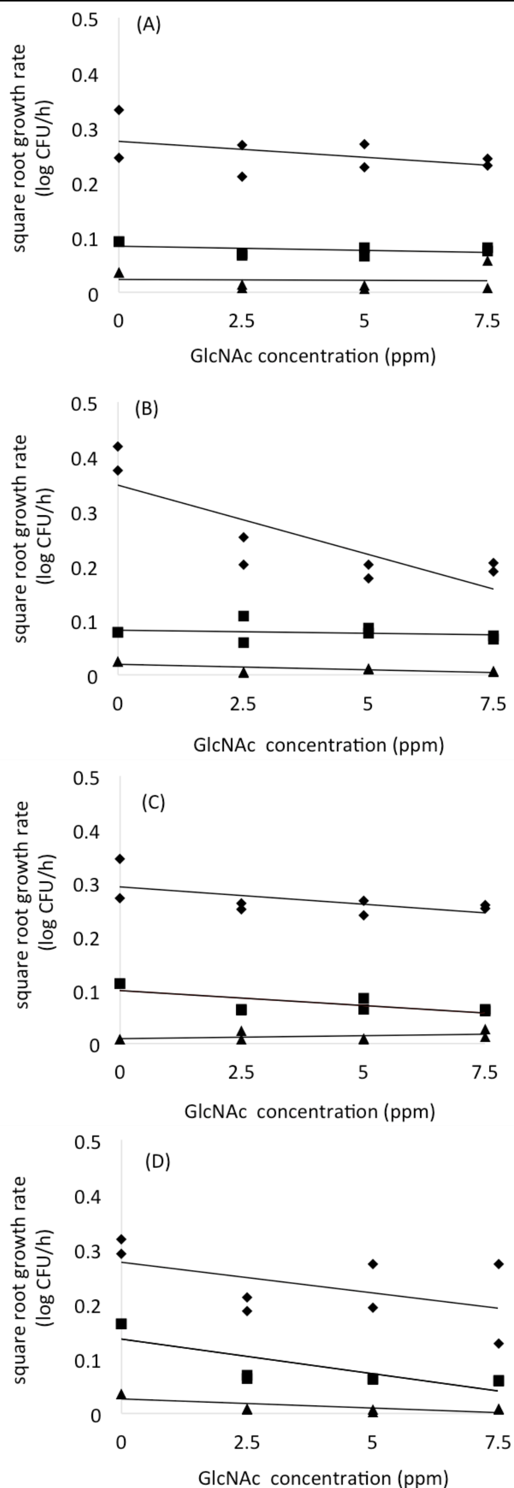

Fig. 5: Square root of growth rate for (A) *V. parahaemolyticus*; (B) *E. coli*; (C) *B. cereus* and (D) *S. aureus* as a function of GlcNAc concentration (0; 2.5; 5; 7.5 ppm) and at (▲) 4, (■) 15, and (♦) 30°C.

This study revealed that the effect of temperature on bacterial growth for all tested bacteria was noticeable. In contrast, inhibitory effect of GlcNAc was prominent only for certain bacteria (*E. coli* and *S. aureus*). Moreover, growth rate reduction by GlcNAc was observed at 2.5 ppm, and higher concentrations did not enhance the reduction (Table 1). The antibacterial mechanism of GlcNAc has not yet been elucidated., Still, the mechanism might be similar to the N-acetyl chito-oligosaccharides described by Benhabiles et al. (2012). It may be due to its ability to pass through bacterial cell walls and react with the plasma membrane, causing cell damage. Another possible antibacterial mechanism of GlcNAc may be associated with the antibacterial action of its dimer ($GlcNAc_2$) reported by Abidin et al. (2019) with two mechanism approaches. First, $GlcNAc_2$ binds to the bacterial cell wall and disrupts cell membrane permeability; or second, deposition of $GlcNAc_2$ on the cell wall causes the blockage of nutrient flow into the cell. However, the concentration of GlcNAc causing growth rate reduction in this study was lower than that reported in other studies. Raut et al. (2016) reported that 0.1% N-acetylglucosamine inhibits the growth of *Staphylococcus aureus* and *Escherichia coli*. The 0.1% N-acetyl chitooligosaccharide concentration showed bactericidal activities against *Salmonella typhimurium* (Benhabiles et al. 2012).

The inhibitory effect of GlcNAc on bacterial growth was notably observed at 30°C, and *E. coli* was the most affected bacterium (Fig. 5). Similarly, Tsai and Su (1999) reported that temperature had a strong influence on the antibacterial activity of chitosan. Higher temperatures (25 and 37°C) increased the bactericidal effect of 150 ppm chitosan on *E. coli*, compared to 4 and 15°C.

They suggested low temperature may have affected the reaction rate between chitosan and the cells. Moreover, the survival of bacteria under stress conditions is reportedly due to adaptation responses involving both physiological and genetic mechanisms through specific or general stress responses (Gottesman, 2019). Adaptive shock responses during low temperatures occurred in *E. coli*, such as the production of cold shock proteins (Zhang et al. 2018), increasing the accumulation of compatible solutes (trehalose and betain) that served as cryoprotectants (Li et al. 2018), and change in membrane fatty acid composition to maintain membrane fluidity (Li et al. 2018), which could interrupt the action of GlcNAc to inhibit bacterial growth during low temperature.

CONCLUSIONS

The enzymatic production of chitin hydrolysate using *Serratia marcescens* PT6 shows an optimum yield at the reaction condition pH 6, 45°C, 120 min, at an enzyme:substrate ratio of 1:1. The main compound found in the chitin hydrolysate was GlcNAc. The activity of GlcNAc as the antibacterial compound varied with bacterial species and incubation temperature. *E. coli* was most susceptible to 2.5 ppm GlcNAc at 30°C. Further investigation of the effect of GlcNAc on bacteria structure and metabolism is required. Although this study shows that the effect of temperature was more significant, GlcNAc reduced bacterial growth at 30°C, which may be encountered under thermal abuse conditions for food. The temperature in combination with GlcNAc requires further study as a food shelf-life extension method.

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