Abstract: Chitinase (Pa-Chi) and chitin oligosaccharide deacetylase (Pa-COD) are involved in the production of a heterodisaccharide, β-D-N-acetylglucosaminyl-(1,4)-D-glucosamine (GlcNAC-GlcN). These enzymes were recovered from the supernatant of Vibrio parahaemolyticus KN1699 cell culture and purified and characterized. For each enzyme, an ORF encoding gene and its signal sequence were cloned from genomic DNA of strain KN1699. In addition, the expression plasmid was constructed for each enzyme gene and inserted into Escherichia coli cells, and recombinant Pa-Chi and Pa-COD (Pa-rChi and Pa-rCOD) were secreted into the culture medium with the aid of signal peptides. Di-N-acetylchitobiose ([GlcNAC]_2) was produced in 60% (w/w) yield by cultivating the Pa-rChi-secreting E. coli cells in 2% (w/v) β-chitin-containing medium. Moreover, GlcNAC-GlcN was produced in high yield by treating (GlcNAC)_2, with the culture supernatant of Pa-rCOD-secreting E. coli cells.

Key words: Vibrio parahaemolyticus, chitinase, chitin oligosaccharide deacetylase, heterodisaccharide, recombinant enzyme

Oligosaccharides can exhibit physiologically useful functions. Many of these oligosaccharides are prepared by enzymatic degradation of biomass polysaccharides, or by enzymatic conversion of oligosaccharides produced by higher plants. New oligosaccharides with potential therapeutic activities are currently being developed; the physiological properties of oligosaccharides obtained by the hydrolysis of chitin, a β-(1,4) polymer of N-acetyl-D-glucosamine (GlcNAC), are of particular interest. Studies have reported that hexa-N-acetylglucosaminohexaose, (GlcNAC)_6, exhibits antitumor and antimicrobial activity in mice by enhancing the immunological defense system. These findings have focused attention on GlcNAC and (GlcNAC)_n, as potential agents for arthritis therapy and immunotherapy, respectively, and have raised the possibility that chitin oligosaccharides could have physiologically useful functions. Generally, GlcNAC and chitin oligosaccharides are obtained by hydrolysis of chitin using strong acid. Chitin, one of the most abundant of all biomass polysaccharides, is the major component of the shells of crustaceans such as crab and shrimp, the exoskeletons of insects, and the cell walls of fungi. The hydrolysis of this biomass polysaccharide by enzymes possessing high product specificity would be more efficient than acid hydrolysis for the production of specific oligosaccharides. Various enzymes involved in chitin hydrolysis [e.g., chitinase (EC 3.2.1.14), β-N-acetylhexosaminidase (EC 3.2.1.52), chitin deacetylase (EC 3.5.1.41), and chitin oligosaccharide deacetylase (EC 3.5.1.→)] have been identified. Chitinase catalyzes the degradation of water-insoluble chitin into water-soluble chitin oligosaccharides through hydrolysis. A number of chitinases have been isolated from bacteria and their properties investigated. In addition, genes encoding a variety of chitinases have been cloned. Based on their amino acid sequences, these chitinases are classified as belonging to either glycoside hydrolase (GH) family 18 or 19 (http://afmb.cnrs-mrs.fr/CAZY/). β-N-Acetylhexosaminidase catalyzes hydrolysis of chitin oligosaccharides to release the monosaccharide GlcNAC. Enzymes from various sources have been classified in three GH families: 3, 20 (the main family), and 84. Microbial β-N-acetylhexosaminidases have been reviewed. Chitin deacetylase and chitin oligosaccharide deacetylase are involved in hydrolysis of the acetamide group of the GlcNAC residue of chitin and chitin oligosaccharides. Both enzymes, isolated from various sources, are classified as belonging to carboxylate esterase (CE) family 4 (http://afmb.cnrs-mrs.fr/CAZY/). Several microbial chitin deacetylases and chitin oligosaccharide deacetylases have been isolated and characterized. Previously, high-yield production of di-N-acetylchitobiose, (GlcNAC)_2, had been accomplished by cultivating the exo-type chitinase-producing marine bacterium, Vibrio anguillarum E-383a, in a medium containing colloidal chitin.

To obtain the enzymes that produce specific oligosaccharides from chitin effectively, we screened chitin-degrading bacteria isolated from dry beach soil and from the contents of marine fish intestines. Enzymes were screened by observing the formation of clear zones around
colonies on colloidal chitin-agar plates. A number of bacterial strains having chitin-degrading activity were isolated from Yatsu dry beach (Narashino, Chiba Prefecture, Japan). We chose one type of bacterium (KN1699) from those isolated, through tests involving both chitin decomposition and oligosaccharide production. The bacterium isolated was identified as *V. parahaemolyticus* from its morphological and physiological characteristics and nucleotide sequence encoding its 16S rDNA. Here, we describe the results of oligosaccharide production by extracellular chitin-degrading enzymes of strain KN1699, as well as the purification and characterization of the enzymes, cloning of enzyme genes, preparation of recombinant enzyme–secreting *Escherichia coli* cells, and oligosaccharide production using transformed *E. coli* cells and the recombinant enzyme.

**Oligosaccharide production by extracellular chitin-degrading enzymes of *V. parahaemolyticus* KN1699.**

The oligosaccharide produced by the action of crude enzyme (prepared from the supernatant of *V. parahaemolyticus* KN1699 cultures) on β-chitin was analyzed using silica gel thin layer chromatography (TLC). When phosphomolybdic acid reagent was used to visualize the oligosaccharide, TLC of the reaction mixture indicated a single product (Fig. 1a). Ninhydrin reagent also visualized this product, suggesting the product is an oligosaccharide possessing a free amino group. Although the mobility of this compound corresponded to that of chitobiose [GlcN]2, its structure needed confirmation. Therefore, the compound was purified and its structure analyzed by ESI-MS and ¹H NMR. The data indicated that the compound produced from β-chitin by the action of crude enzyme, prepared from the supernatant of *V. parahaemolyticus* KN1699 cultures, was β-D-N-acetylglucosaminyl(1,4)-D-glucosamine (GlcNAc-GlcN) (Fig. 1b).

This heterodisaccharide appeared to be produced by the reactions of both chitinase and chitin oligosaccharide deacetylase, which were secreted into the culture medium by *V. parahaemolyticus* KN1699. To confirm this supposition, the enzymes were purified and their reactions examined.

**Table 1.** Purification of chitinolytic enzymes from culture supernatant of *V. parahaemolyticus* KN1699.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total activity (U)</th>
<th>Specific activity (U/mg of protein)</th>
<th>Yield (%)</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pa</em>-Chi³ (NH₄)₂SO₄ precipitation</td>
<td>34.4</td>
<td>0.24</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>DEAE-Toyopearl 650M (1st)</td>
<td>18.2</td>
<td>1.61</td>
<td>52.9</td>
<td>6.71</td>
</tr>
<tr>
<td>DEAE-Toyopearl 650M (2nd)</td>
<td>14.9</td>
<td>2.13</td>
<td>43.2</td>
<td>8.88</td>
</tr>
<tr>
<td>Toyopearl HW-55F</td>
<td>10.7</td>
<td>2.32</td>
<td>31.2</td>
<td>9.67</td>
</tr>
<tr>
<td><em>Pa</em>-COD⁹ (NH₄)₂SO₄ precipitation</td>
<td>1.95</td>
<td>0.01</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>DEAE-Sepharose FF</td>
<td>1.04</td>
<td>3.05</td>
<td>53.3</td>
<td>305</td>
</tr>
<tr>
<td>DEAE-Toyopearl 650M</td>
<td>0.76</td>
<td>9.50</td>
<td>39.0</td>
<td>950</td>
</tr>
<tr>
<td>Phenyl Sepharose HP</td>
<td>0.62</td>
<td>31.0</td>
<td>31.8</td>
<td>3100</td>
</tr>
</tbody>
</table>

Strain KN1699 was grown at 28°C for 16 h with shaking in half-strength artificial seawater containing 1% (w/v) peptone, 0.1% (w/v) yeast extract, and 0.5% (w/v) powdered α-chitin. The enzyme assay was performed according to a method described previously. One unit of chitinase activity was defined as the amount of enzyme required to liberate reducing sugar equivalent to 1 μmol of GlcNAc per min under the assay conditions. One unit of chitin oligosaccharide deacetylase activity was defined as the amount of enzyme required to produce 1 μmol of GlcN residues per min under the assay conditions. *Pa*-Chi was purified from 1 L of culture supernatant. *Pa*-COD was purified from 2 L of culture supernatant.

The crude enzyme, which was prepared from supernatant of *V. parahaemolyticus* KN1699 culture, was added to 20 mM sodium phosphate buffer (pH 7.0) containing 0.5% (w/v) powdered β-chitin, then the mixture was incubated at 37°C with stirring. The reaction products of the various incubation times were subjected to silica gel thin layer chromatography (TLC) (Merck). After developing the TLC plates using 5:4:3 (v/v/v) n-butanol/methanol/16% aqueous ammonia as the mobile phase, the products were visualized using phosphomolybdic acid reagent. The product was isolated from the reaction mixture by column chromatography using charcoal (Wako Pure Chemical) and Toyopearl HW-40F resin (Tosoh) columns, followed by structural analysis. a, TLC results; Lane S, N-acetylchitooligosaccharide standards; b, structure of reaction product.
cific activity of the purified enzyme toward powdered β-chitin was 2.32 U/mg protein. We named this chitinase Pa-Chi.

Chitin oligosaccharide deacetylase was purified in four steps from 2 L of culture fluid (Table 1). Each column chromatographic step produced a single peak possessing deacetylase activity. The purified enzyme gave a single band on both SDS-PAGE and native-PAGE (data not shown), indicating highly purified enzyme. Chitin oligosaccharide deacetylase was purified 3100-fold with 31.8% recovery of initial total activity. The specific activity of the purified enzyme toward (GlcNAc)2 was 31 U/mg protein. Although the amount of deacetylase protein in the culture supernatant was very small, its specific activity was significantly higher than that of Pa-Chi. We named this chitin oligosaccharide deacetylase Pa-COD. The molecular masses of Pa-Chi and Pa-COD were estimated by SDS-PAGE to be 92 and 46 kDa, respectively, based on molecular mass standards. The N-terminal amino acid sequences of Pa-Chi and Pa-COD were APTAPSVDMY GSNNLQFSKIELAMET and QTDTKGIYLTFFDGIPI NASIDVINV, respectively. Pa-Chi and Pa-COD were most active at pH 8.0 and 8.5–9.0, respectively, and the optimum reaction temperature was 50–55°C for Pa-Chi and 45°C for Pa-COD.

The TLC mobility results indicate that (GlcNAc)2 is the only oligosaccharide produced by the hydrolytic action of Pa-Chi on β-chitin (Fig. 2a-1). The product did not react with ninhydrin (Fig. 2a-2), indicating that Pa-Chi hydrolyzed β-chitin to (GlcNAc). Reaction of Pa-Chi with (GlcNAc)2 also gave only (GlcNAc)2 as the final product (data not shown). The TLC results with Pa-COD indicated that this enzyme converts (GlcNAc)2 to a saccharide that is more polar than (GlcNAc)2 (Fig. 2b-1) and possesses a free amino group (Fig. 2b-2). The 1H NMR analysis confirmed the structure of this product as GlcNAc-GlcN. Pa-COD was confirmed to possess the greatest substrate specificity for (GlcNAc)2 out of the oligosaccharides examined. These results indicate that Pa-Chi is an exo-N,N-diacytethylchitobiodyrholase-like enzyme and Pa-COD catalyzes the hydrolysis of the acetamide group of the reducing end GlcNAc residue of (GlcNAc). The findings demonstrate that GlcNAc-GlcN is produced from chitin by the cooperative hydrolytic reactions of Pa-Chi and Pa-COD, both of which are present in the supernatant of V. paraahaemolyticus KN1699 cultures.

Cloning of enzyme genes and expression of recombinant enzymes in the culture medium of Escherichia coli cells.16,17)

The N-terminal amino acid sequences (26 residues) of both Pa-Chi and Pa-COD showed 100% identity with those estimated from the nucleotide sequences of the V. paraahaemolyticus RIMD 2210633 putative genes of corresponding enzymes (GeneBank accession no.: chitinase; BA000032-55, chitin oligosaccharide deacetylase; BA 000031-2638). This suggests similarity in the nucleotide sequences in the vicinity of both the chitinase and chitin oligosaccharide deacetylase genes in V. paraahaemolyticus KN1699 and RIMD 2210633 genomic DNA. Therefore, PCR forward and reverse primers for cloning Pa-Chi and Pa-COD genes were designed from upstream and downstream nucleotide sequences of putative chitinase and chitin oligosaccharide deacetylase genes in genomic DNA of strain RIMD2210633. After sub-cloning of the PCR products into the vector, their nucleotide sequences were analyzed. The ORF of Pa-Chi (GeneBank accession no.: AB 299855) consisting of 2544 bp and encoding 848 amino acid residues was found in the PCR product. The N-terminal sequence analysis of Pa-rCh showed that the signal sequence corresponds to the 21 N-terminal amino acids. The recombinant enzyme consisted of 827 amino acids, with a molecular weight of 88,001 Da. This molecular weight is consistent with that of purified Pa-Chi.35 As expected, two motifs conserved in the catalytic regions of GH family 18 chitinases, S-x-G-G (amino acid no. 271–274) and x-D-x-x-D-x-D-x-E (amino acid no. 307–315) (E is a catalytic amino acid residue),36 also were pre-

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**Fig. 2.** TLC analysis of oligosaccharides produced by the reactions of purified enzymes.

Purified Pa-Chi (30 mU) was added to 1 mL of 20 mM sodium phosphate buffer (pH 7.0) containing 0.5% (v/v) of powdered β-chitin, then the mixture was incubated at 37°C with stirring. Purified Pa-COD (3.12 mU) was added to 80 µL of 20 mM sodium phosphate buffer (pH 7.0) buffer containing 0.5% of (GlcNAc), then the mixture was incubated at 37°C. After developing the TLC plates containing the reaction products of various incubation times using 5:4:3 (v/v/v) n-butanol/methanol/16% aqueous ammonia as mobile phase, the products were visualized. TLC results of the reaction mixture with Pa-Chi are shown in a. TLC results of the reaction mixture with Pa-COD are shown in b. Reaction products on the TLC plates were visualized using the following reagents: a-1 and b-1, phosphomolybdic acid reagent; a-2 and b-2, ninhydrin reagent. Lane S1, N-acetylchitosan standards; lane S2, GlcNAc-GlcN.
sent in Pa-rChi. The carbohydrate binding domain classified in carbohydrate binding module (CBM) family 5 (http://afmb.cnrs-mrs.fr/CAZY/) was observed in the C-terminal region (amino acid no. 797–839) of Pa-rChi. The motif of Pa-rChi is illustrated in Fig. 3a. The amino acid sequence of this recombinant enzyme was highly homologous to several GH family 18 chitinases from Vibrionaceae bacteria: 100% identity with V. alginolyticus H-8 ChiB\(^{20}\) (GeneBank accession no. AJ292004), 99.8% identity with V. parahaemolyticus RIMD 2210633 Chi, 97% identity with V. alginolyticus 12G01 Chi (GeneBank accession no. AAPS01000021), and 93.1% identity with V. carchariae ChiA (GeneBank accession no. AF323180).\(^{20}\) The ORF of Pa-COD (GeneBank accession no. AB 275387) consisting of 1281 bp was found in the PCR product, and encoded 427 amino acid residues. The N-terminal sequence analysis of Pa-rCOD showed that the signal sequence corresponds to the 22 N-terminal amino acids. The recombinant enzyme consisted of 405 amino acids, with a molecular weight of 44,715 Da. This value is in good agreement with that obtained by SDS-PAGE analysis of Pa-COD.\(^{15}\) The carbohydrate-binding domain classified in CBM family 12 (http://afmb.cnrs-mrs.fr/CAZY/) was observed in the C-terminal region (amino acid no. 332–366, 381–415) of Pa-rCOD. The motif of this recombinant enzyme is illustrated in Fig. 3b. The amino acid sequence of Pa-rCOD showed high homology with those of CE family 4 chitin oligosaccharide deacetylases from Vibrionaceae bacteria. Three amino acids (290, 394 and 396) were different between Pa-rCOD and the putative deacetylase (405 amino acids) from V. parahaemolyticus RIMD2210633. Four amino acids (173, 290, 394 and 396) were different between Pa-rCOD and the deacetylase from V. alginolyticus H-8 (DA1, 405 amino acids; GeneBank accession number AJ292005).\(^{20}\) Moreover, Pa-rCOD showed 83% identity with the putative deacetylase from V. vulnificus CMC-6 and V. vulnificus YJ016, and 82% identity with the putative deacetylase from V. cholerae E1 Tor N16961. The ORF containing the ribosome-binding sequence, the signal sequence, and each enzyme gene was amplified by PCR and inserted into the pET-21(+) vector to make expression plasmid (Pa-rChi expression plasmid, pVP-Chi; Pa-rCOD expression plasmid, pVP-COD2) (Figs. 4a and 4b), and then E. coli BL21(DE3) cells were transformed with each plasmid. The E. coli cells harboring the plasmid were cultivated and production of recombinant enzyme was induced by addition of IPTG. Figure 5a shows the time course of chitinase activity in both lysates and the culture medium of E. coli cells harboring pVP-Chi. Time courses of deacetylase activity in both lysates and the culture medium of E. coli cells harboring pVP-COD2 are shown in Fig. 5c. Addition of IPTG initiated production of recombinant enzyme in E. coli cells, while its secretion into the culture medium increased gradually. Chitinase activity in the medium exceeded that in the E. coli lysates after 7 h of cultivation. After 12 h of cultivation, total deacetylase activity in the culture medium peaked at the same level as that in E. coli lysates. Figures

![Fig. 3. Motifs of recombinant enzymes. a, Pa-rChi; b, Pa-rCOD.](image)

![Fig. 4. Plasmids for expression of recombinant enzymes.](image)

An ORF containing the ribosome-binding sequence, signal sequence, and enzyme gene was amplified with different restriction enzyme sites by PCR. After the PCR product was isolated from agarose gel slice and digested with two corresponding restriction enzymes, the resulting DNA fragment was ligated into pET-21(+) (Novagen), which was digested by these restriction enzymes to yield the following expression plasmids: a, pVP-Chi for Pa-rChi production; b, pVP-COD2 for Pa-rCOD production.
5b-1 and -2 show SDS-PAGE results of the time course of the Pa-Chi production in both transformed E. coli cells and their culture medium. The SDS-PAGE results showing time courses of the production of Pa-rCOD in both transformed E. coli cells and their culture medium are shown in Figs. 5d-1 and -2. In both culture media, the proteins with molecular masses of approximately 90 kDa and 45 kDa were confirmed as the main products, while other proteins secreted by the transformed E. coli cells were present in very low concentrations. Chitinase activity was eightfold greater in the E. coli culture medium than in V. parahaemolyticus KN1699 culture medium. The amount of deacetylase activity in the E. coli culture medium was 150 times greater than in the culture medium of the strain KN1699. The N-terminal amino acid sequence of Pa-rChi and Pa-rCOD were same as the corresponding wild-type enzymes, indicating that the signal peptide regions of the expression products were removed as the proteins crossed the E. coli cell membrane. Substrate specificities and hydrolysis products of both recombinant enzymes were the same as those of corresponding wild-type enzymes.

Production of oligosaccharide from chitin by transformed E. coli cells and recombinant enzyme.

GlcNAC-GlcN, produced from chitin by cooperative reactions of both Pa-Chi and Pa-COD, is a unique oligosaccharide. Similar to other rare oligosaccharides, GlcNAC-GlcN is likely to possess physiologically useful activity. Gram-scale production of this heterodisaccharide was attempted to obtain material for function studies. However, the production of both Pa-Chi and Pa-COD from V. parahaemolyticus KN1699 was extremely low. Therefore, we decided to produce this heterodisaccharide using overproduced recombinant enzymes of Pa-Chi and Pa-COD (Pa-rChi and Pa-rCOD). To make GlcNAC-GlcN using Pa-rCOD, it is necessary to obtain (GlcNAC) through hydrolysis of chitin by Pa-rChi. As reported by Takiguchi and Shimahara, production of chitin oligosaccharides by fermentation using chitinase-secreting microorganisms is convenient because the enzyme does not need to be extracted from the culture supernatant, and the
hydrolytic reaction can be performed effectively due to continuous production and secretion of the enzyme. Therefore, for the production of (GlcNAc), from chitin, we performed fermentation using Pa-rChi-secreting E. coli cells. Evidence exists that addition of chitin to the culture medium is effective for recombinant chitinase production by transformed E. coli cells. Therefore, we investigated Pa-rChi production by E. coli cells harboring pVP-Chi in LB medium containing powdered chitin (Fig. 6a). Although the addition of IPTG into the culture medium accelerated production of recombinant enzyme from an early stage, remarkable production of the enzyme began after 2 days of cultivation in the medium containing powdered chitin. After cultivation for 4 days, the amount of chitinase activity in the culture medium containing powdered chitin was 4-fold greater than in the medium containing IPTG. SDS-PAGE results of Pa-rChi production in both culture supernatants corresponded to activity (Fig. 6b). The reason why addition of chitin into culture medium induces production of recombinant chitinase by transformed E. coli cells is not well understood. However, the phenomenon is useful for the production of (GlcNAc), from chitin by fermentation using E. coli cells harboring pVP-Chi. By cultivating transformed E. coli cells in LB medium containing 50 μg/mL ampicillin and 2% (w/v) powdered β-chitin, (GlcNAc): accumulation in the broth reached a maximum on the third day of culture, with a stoichiometric yield of 60% (w/w). Continued culture caused a rapid decrease in disaccharide concentration in the broth. In contrast, results of chitinase activity assays and SDS-PAGE analysis using culture supernatant at each culture time (Figs. 5a, b) confirmed that the cumulative amount of Pa-rChi increased throughout the culture period. Many strains of E. coli are known to uptake and metabolize (GlcNAc). This suggests that production and consumption of (GlcNAc), by Pa-rChi and E. coli cells occurs synchronically during the culture period. Almost all of the β-chitin added appears to be converted to (GlcNAc): by culturing for 3 days. A rapid decrease in (GlcNAc): after 3 days of culture may be due to both disappearance of substrate chitin and consumption of the disaccharide by E. coli cells. Fermentation using Pa-rChi-secreting E. coli cells and powdered β-chitin produced (GlcNAc): in high yield, similar to fermentation using V. anguillarum E-383 a and colloidal chitin. Addition of 25 units of Pa-rCOD, prepared from the culture supernatant of E. coli cells harboring pVP-COD, to 50 mL of 20 mM sodium phosphate buffer (pH 7.0) containing 0.5 g (GlcNAc): at 37°C produced GlcNAc-GlcN in 89% (mol/mol) yield after 2 days. The methods and results for production of this heterodisaccharide from chitin are illustrated in Fig. 7.

Fig. 6. Influence of powdered chitin on recombinant chitinase production by transformed E. coli.

IPTG and powdered β-chitin were added into different cultures of transformed E. coli at final concentrations of 1 mM and 2% (w/v), respectively, and the cultures were incubated with shaking for an additional 4 days. Aliquots of culture broth were withdrawn at the chosen time points and centrifuged to obtain culture supernatants. These culture supernatants were dialyzed against 20 mM sodium phosphate buffer (pH 7.0), a, Pa-rChi production; ○, total chitinase activity in the supernatant from culture using LB medium; ●, total chitinase activity in the supernatant from culture using LB medium containing IPTG; ■, total chitinase activity in the supernatant from culture using LB medium containing powdered β-chitin; b, SDS-PAGE results with Pa-rChi for each culture supernatant.

Further studies are underway using the prepared GlcNAc-GlcN to investigate its physiological functions.

This study was supported in part by a grant from Nihon University and by a “High-Tech Research Center Project” of the Ministry of Education, Science, Sports and Culture of Japan to promote advanced scientific research.

Fig. 7. Outline of the oligosaccharide production from powdered chitin.
REFERENCES


*Vibrio parahaemolyticus*が生産するキチン分解酵素の精製、諸性質解析、クローニングおよびリコンビナント酵素のオリゴ糖生産への利用

門倉一成，坂本裕希，六谷明子，池上孝紀，平野貴子，山本真，齋藤香織，畑野航，奈井史朗，杉田治明，奥 忠武，西尾俊幸

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ある。

【質問】
(甲)農研機構 食総研 市安
位置特異的に脱アセチル化する酵素は貴重。部分脱アセチル化物のリゾチームによる重合、あるいは逆反応による還元末端のみの選択的アセチル化などについて、可能性があると思うので、是非ご検討いただきたい。

【答】
貴重ご意見ありがとうございます。この部分脱アセチル化オリゴ糖およびPa-tCODを使用して検討を行っています。

【質問】
(甲)農研機構 矢追
1）IPTG に代わって β-キチンで発現誘導できたのはなぜか？
2）発酵法を選んだ理由は？

【答】
1）β-キチン添加によって起こるキチナーゼの発現誘導の理由については未解明なので不明です。おそらく β-キチンの分解によって生成した(GlcNAc)_2 による発現が誘導されたのではないかと考えています。興味深い現象ですので、今後詳細に検討を行って現象解明を進めていきたいと考えています。
2）発酵法は培養工程でフレッシュな酵素を連続的に供給できるため、キチンのような不溶性のフィルム基質に最適な生産法であると考え、この方法を採用しました。

【質問】
京大院・生命科学 山本
このアセチラーゼはキトビオースの還元末端側のアセチル基に特異的に作用するのですか、キチンには作用しないのですか、キチナーゼとアセチラーゼのそれぞれのリコンビナント酵素を分泌する大腸菌の菌体を用いた発酵法によってオリゴ糖生産等はできませんか。

【答】
本酵素はキトビオース以外にもキトトリオースにも作用することを確認しています。しかし、キチンには作用しないことを確認しています。それぞれのリコンビナント酵素を分泌させた発酵法での生産については、まだ未検討なので、今後検討を行っていきたいと考えています。