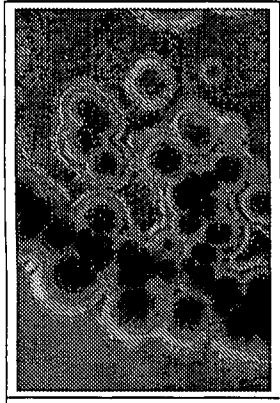
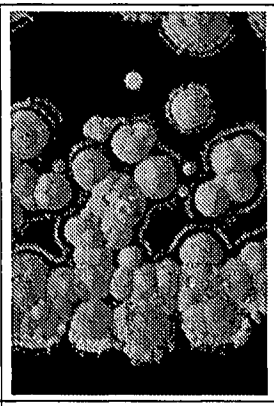
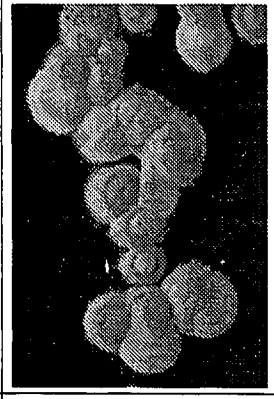
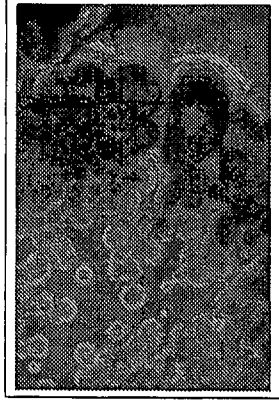
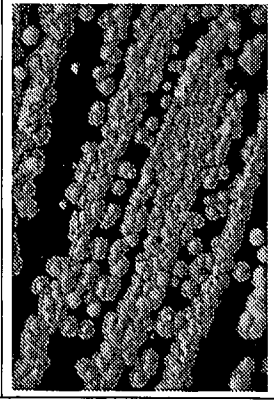
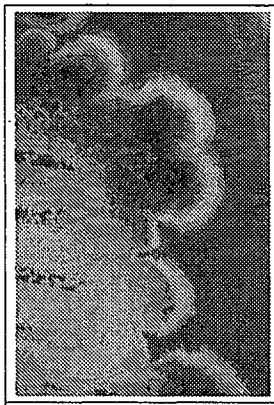
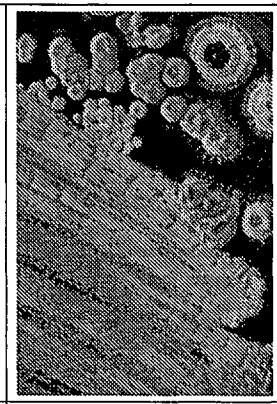
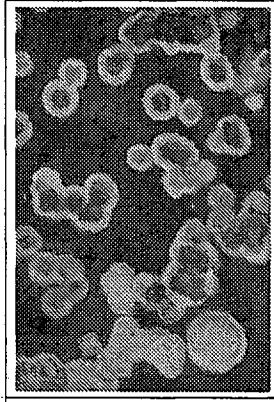
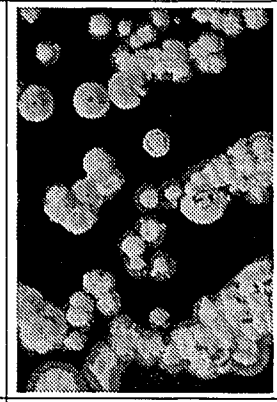
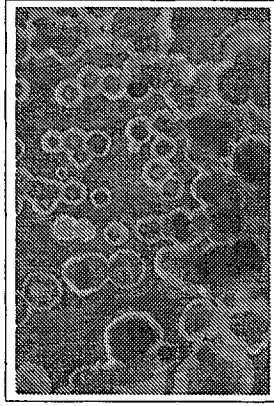
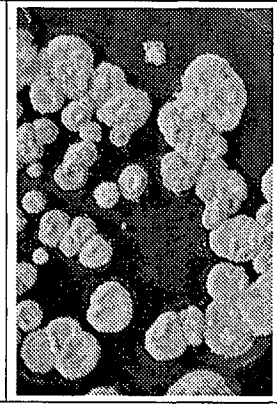
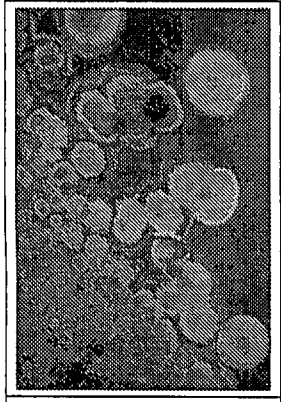
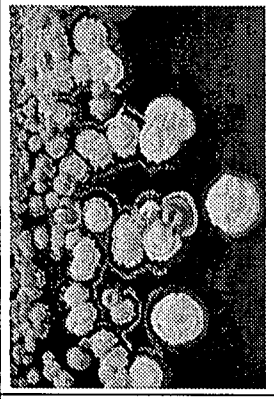
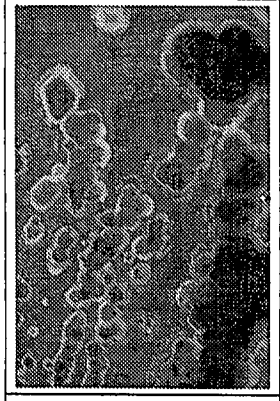
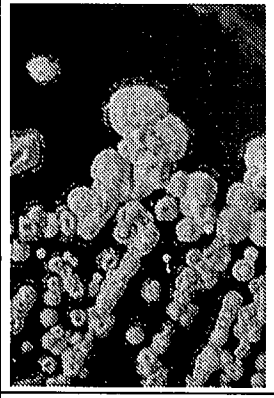
		409	2007.6.8
		410	2007.6.8
		411	2007.6.8

		416	2007.6.8
		422b	2007.6.9
		423	2007.6.8

		424	2007.6.8
		426	2007.6.8
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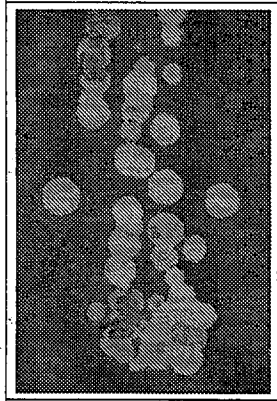
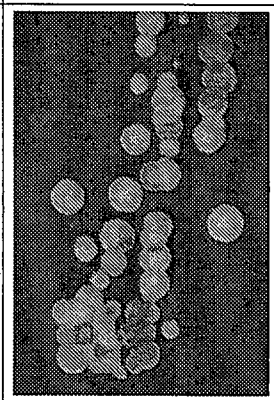
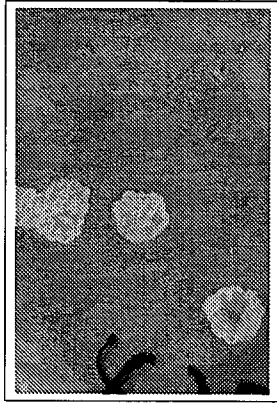
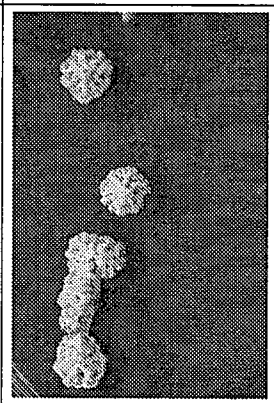
		436	2007.6.9
		438	2007.6.8

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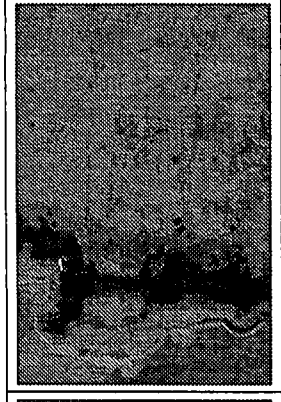
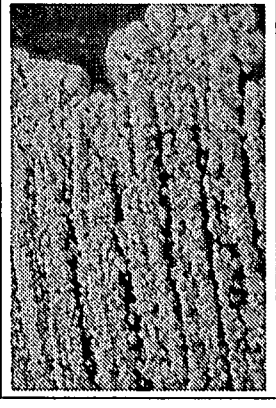
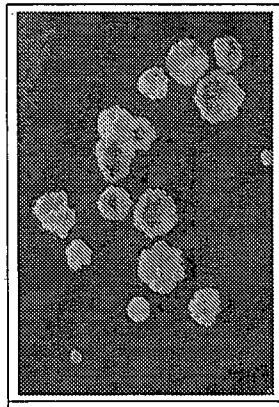
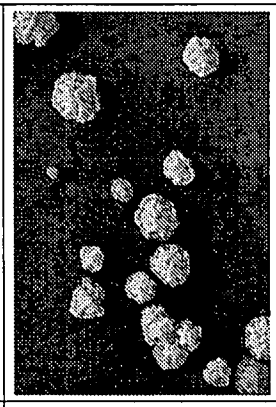
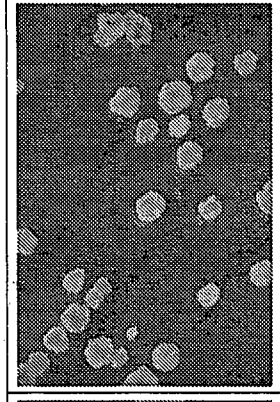
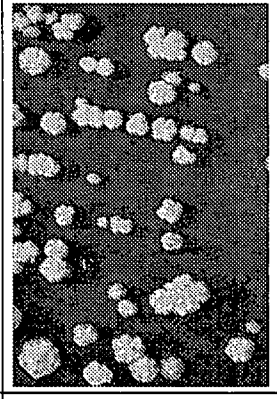
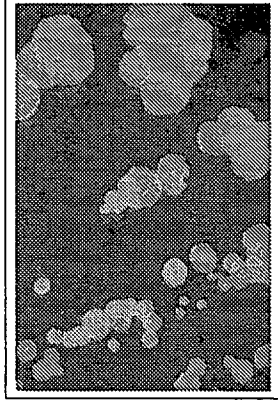
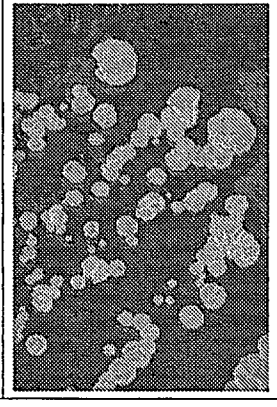
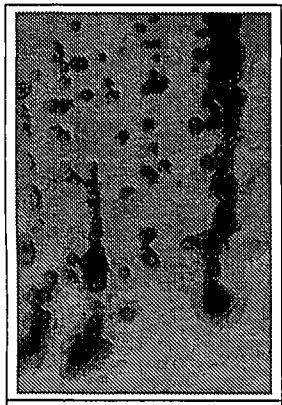
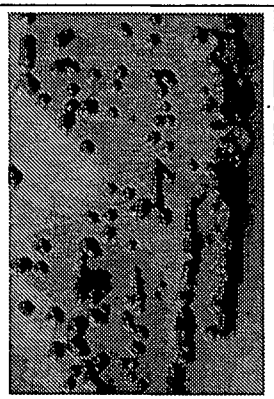
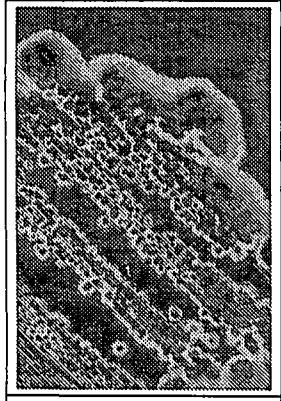
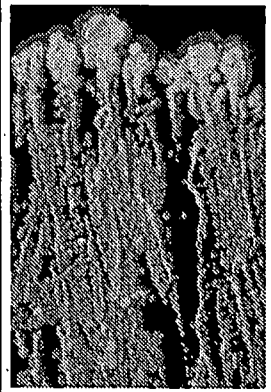
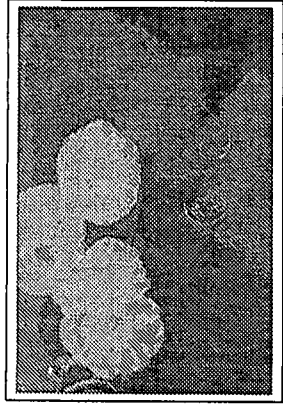
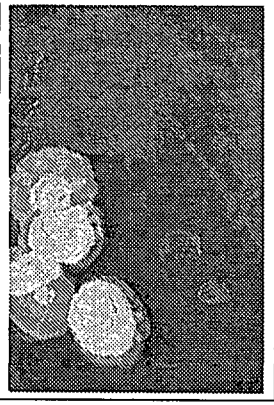
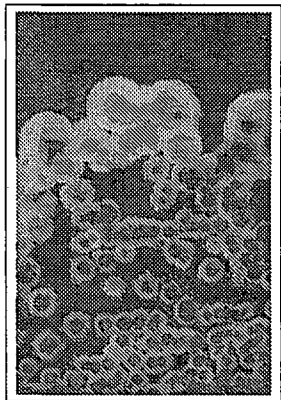
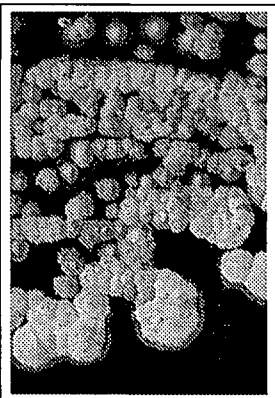
		441	2007.6.9
		447	2007.6.8

		455a	2007.6.8
		455b	2007.6.8
		464	2007.6.8

		465	2007.6.9
		472	2007.6.8
		484	2007.6.8

		487	2007.6.8
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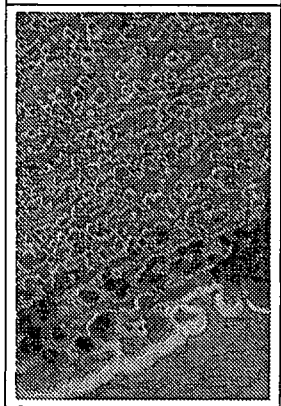
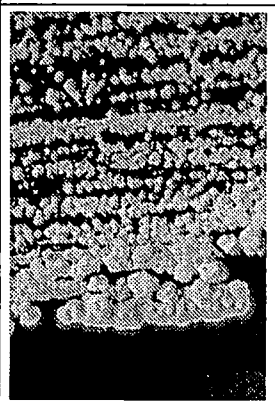
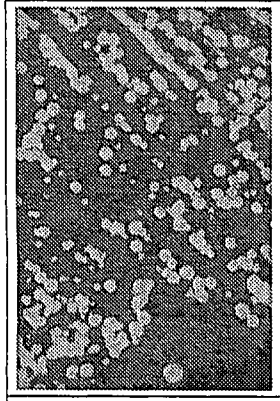
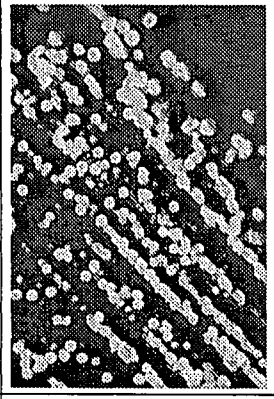
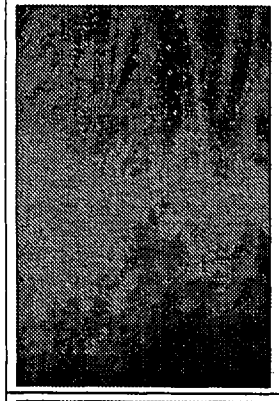
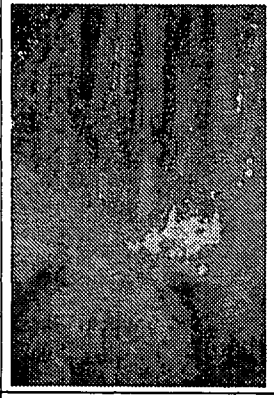
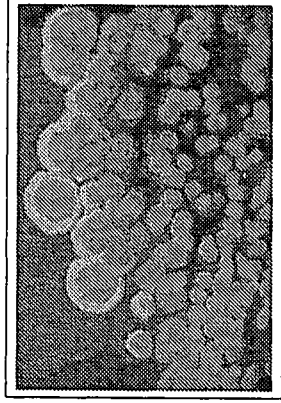
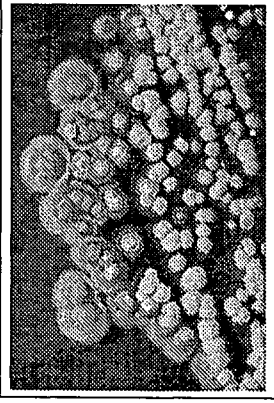
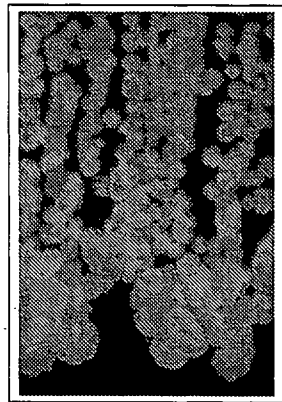
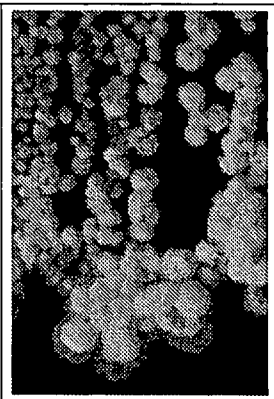
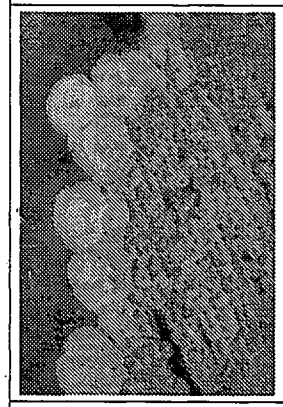
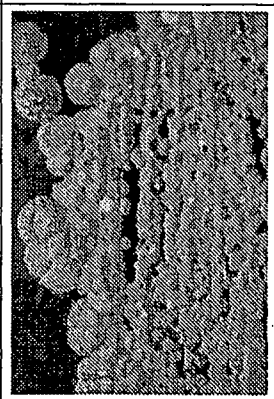
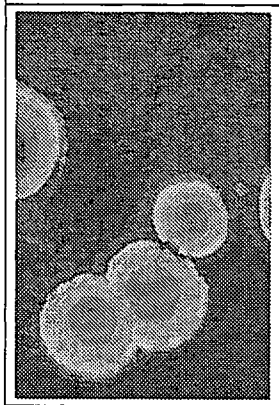
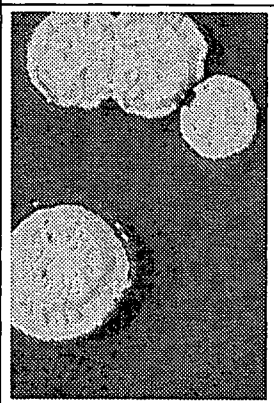
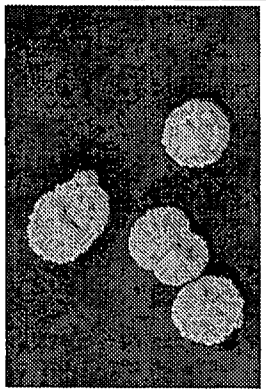
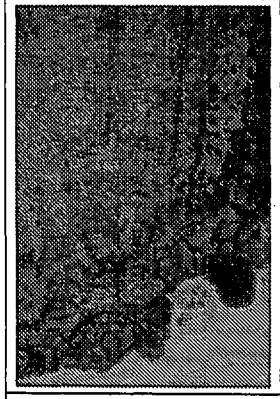
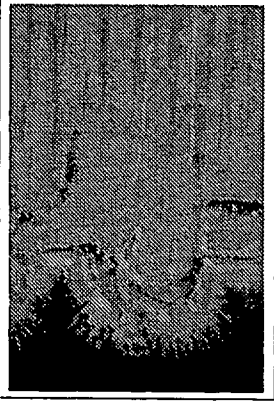
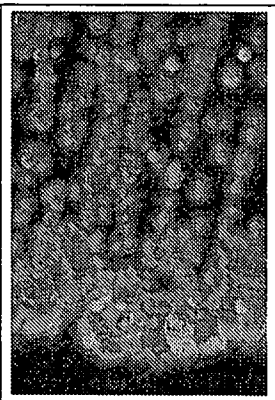
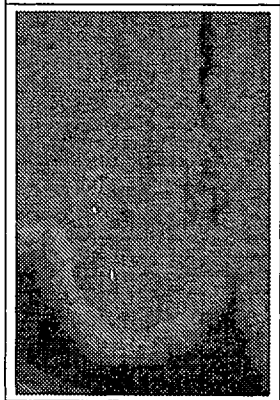
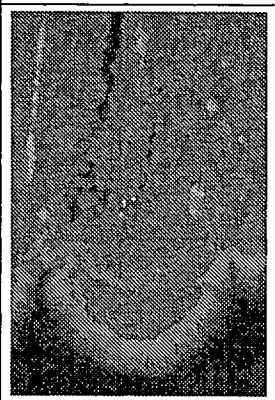
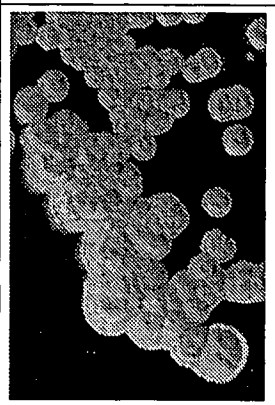
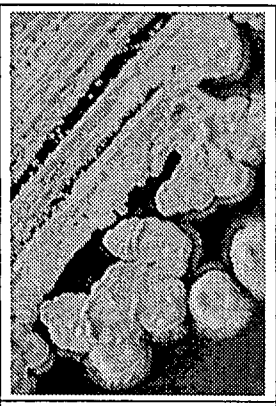
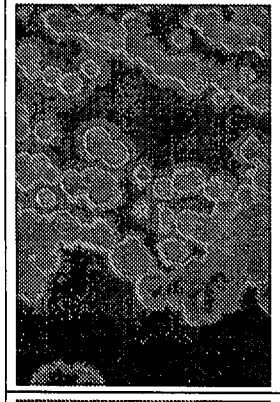
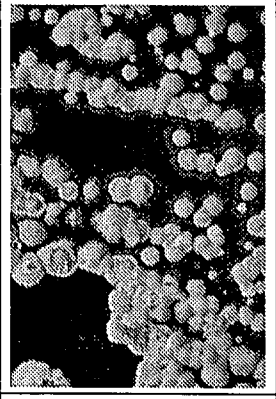
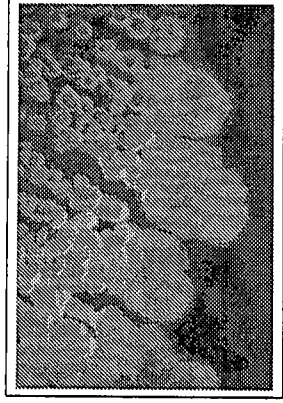
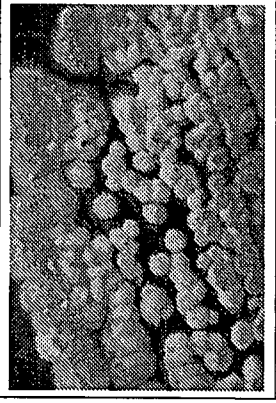
		493	2007.6.8

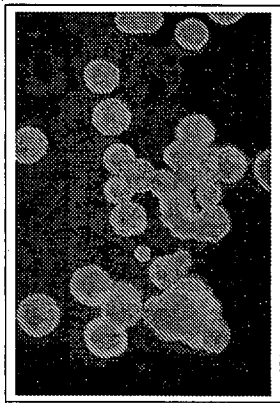
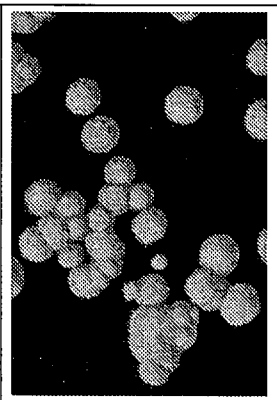
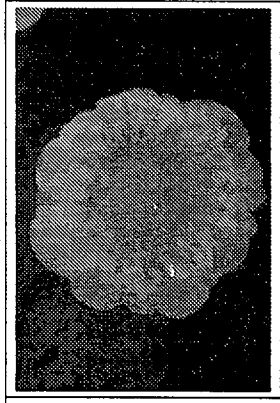
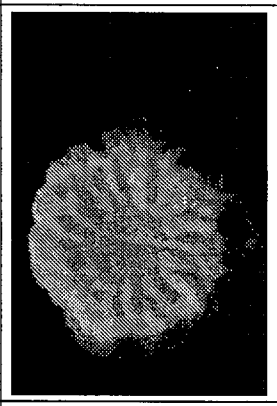
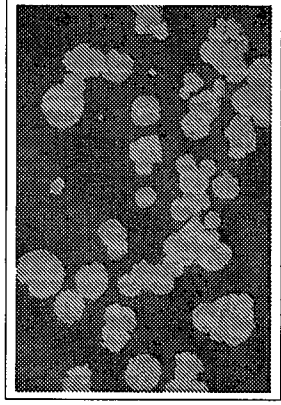
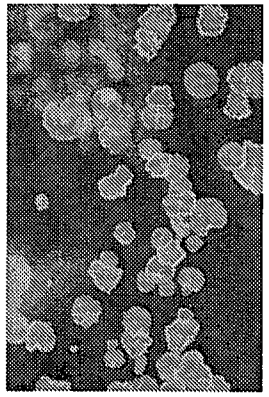
		498	2007.6.8
		501	2007.6.9
		505	2007.6.8

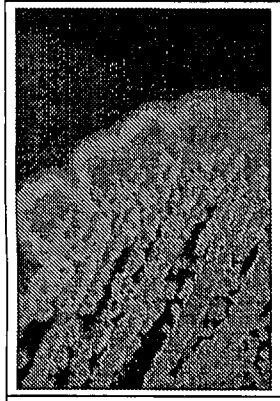
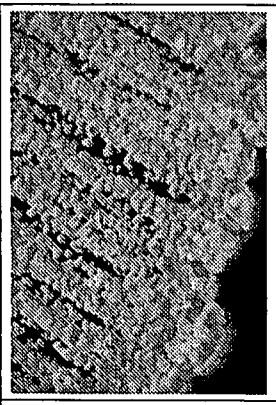
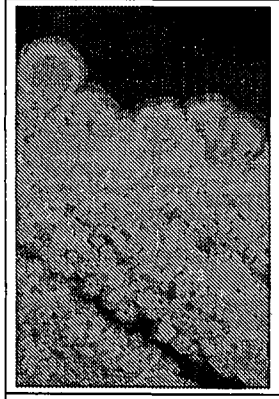
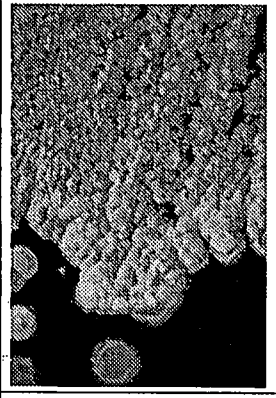
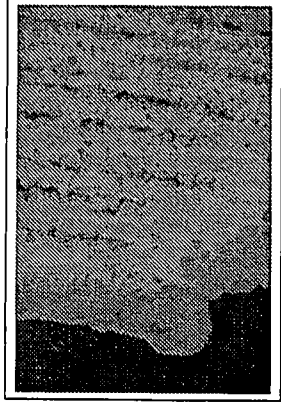
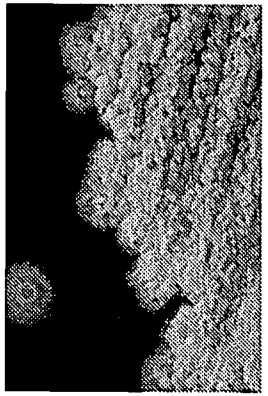
		507	2007.6.9
		508	2007.6.8
		512a	2007.6.8

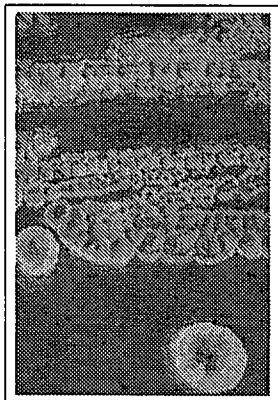
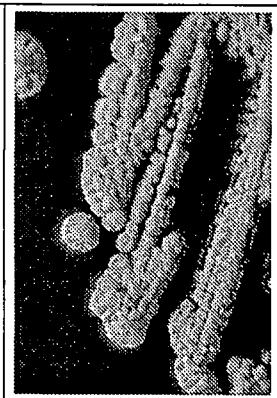
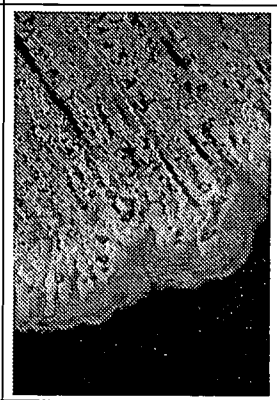
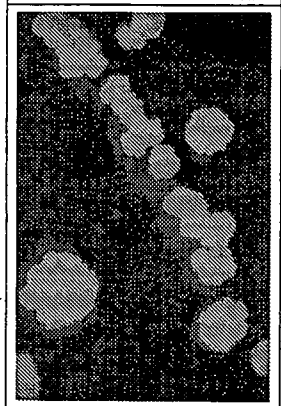
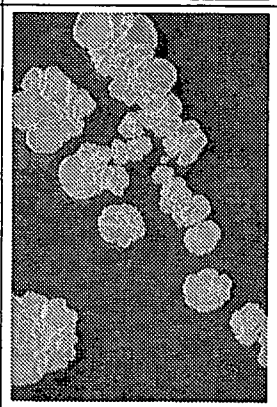
		513	2007.6.9
		519	2007.6.9
		525	2007.6.9

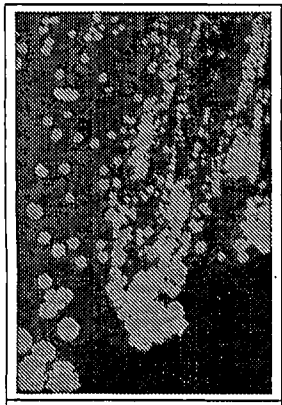
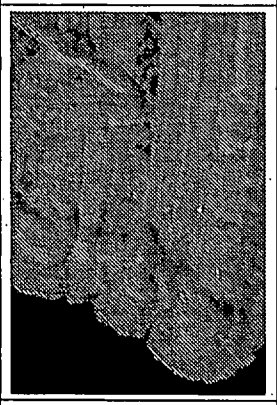
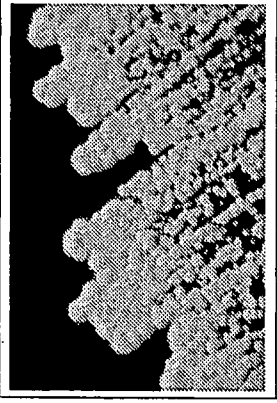
		536a	2007.6.9
		536b	2007.6.9
		537b	2007.6.9

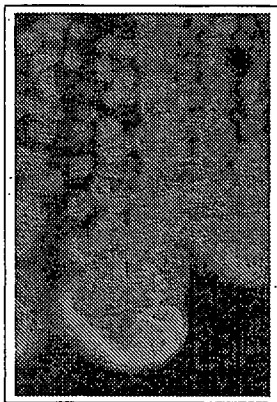
		537c	2007.6.8
		538b	2007.6.8
		546	2007.6.8

		550	2007.6.9
		551	2007.6.9
		553	2007.6.9

		558	2007.6.8
		561	2007.6.8
		562	2007.6.8

		563	2007.6.8
		565	2007.6.8
		568b	2007.6.8

		569	2007.6.9
		573	2007.6.9
		575	2007.6.8

		577b	2007.6.9
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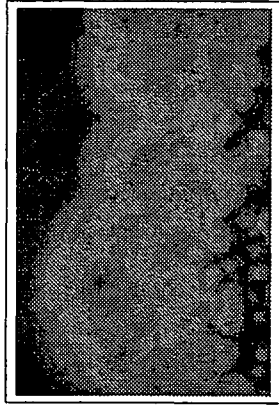	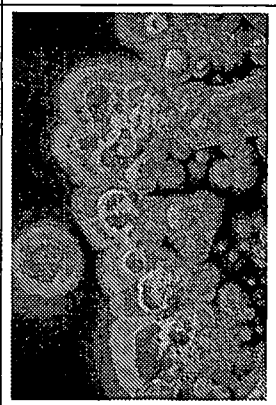	583	2007.6.8

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2007.6.8	2007.6.8	2007.6.8

605	607	608
2007.6.8	2007.6.8	2007.6.9

別紙４

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
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雑誌

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COMPUTATIONAL STUDY ON CONFORMATION OF OLIGOPEPTIDES CONTAINING CHIRAL CYCLIC α,α-DISUBSTITUTED

Masaaki Kurihara[1], Yukiko Sato[1], Wataru Hakamata[1], Haruhiro Okuda[1], Yosuke Demizu[2], Masanobu Nagano[2], Naomi Kawabe[2], Mitsunobu Doi[3], Masakazu Tanaka[2] and Hiroshi Suemune[2]

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Introduction

Prediction of the conformation of peptides using computational simulation is an interesting challenge for the design of functionalized and bioactive peptides. We have shown the Monte Carlo conformational search using MacroModel is useful for conformational study of oligopeptides prepared from α,α-disubstituted α-amino acids. Moreover, we have studied conformational analysis of oligopeptides containing chiral α,α-disubstituted α-amino acids to predict the helical screw sense of helical structures (α-helix, 3_{10}-helix). Here we report computational study on conformation of oligopeptides containing cyclic α,α-disubstituted α-amino acids with side-chain chiral centers.

α-helix 3_{10}-helix

Fig. 1. Helical structures of oligopeptides

Results and Discussion

Conformational search calculations of oligopeptides 1, 2, containing chiral cyclic α,α-disubstituted amino acids, have performed using the Monte Carlo method of MacroModel (ver. 8.1, Schrödinger, Inc.). When AMBER* force field was used, the global minimum energy conformation of peptide 1 was a left-handed α-helix, which was more stable than a left-handed 3_{10}-helix by 4.2 kcal/mol. The results were in agreement with its X-ray structure, which showed a left-handed α-helix [1]

The global minimum energy conformation of peptide 2 was a right-ha helix, which was more stable than a left-handed 3_{10}-helix by 1.6 kcal/m results were obtained by STO-3G level molecular orbital calculat difference of energies was small. There were both right- and left-handed 3 in the solid state [2, 3]. These results indicated computational simulat conformational search calculations could predict the helical screw oligopeptides containing chiral cyclic α,α-disubstituted amino acids.

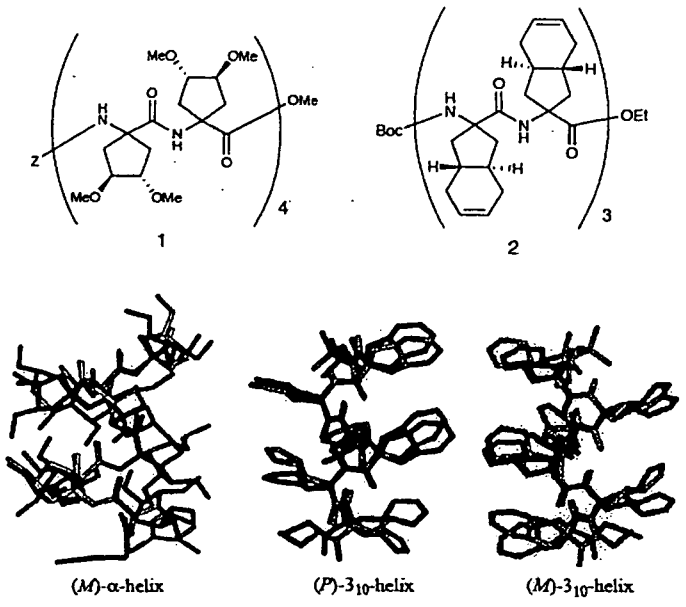

Fig. 2. Modeled structures (light) and X-ray structures (dark) of oligopeptides 1, 2.

Acknowledgements

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BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Purification and characterization of *Vibrio parahaemolyticus* extracellular chitinase and chitin oligosaccharide deacetylase involved in the production of heterodisaccharide from chitin

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Abstract A chitin-degrading bacterial strain, KN1699, isolated from Yatsu dry beach (Narashino, Chiba Prefecture, Japan), was identified as *Vibrio parahaemolyticus*. Treatment of powdered chitin with crude enzyme solution prepared from the supernatant of KN1699 cultures yielded a disaccharide, β-D-*N*-acetylglucosaminyl-(1,4)-D-glucosamine (GlcNAc-GlcN), as the primary chitin degradation product. The extracellular enzymes involved in the production of this heterodisaccharide, chitinase (*Pa*-Chi; molecular mass, 92 kDa) and chitin oligosaccharide deacetylase (*Pa*-COD; molecular mass, 46 kDa), were isolated from the crude enzyme solution, and their hydrolysis specificities were elucidated. These studies confirmed that (1) *Pa*-Chi hydrolyzes chitin to produce $(GlcNAc)_2$ and (2) *Pa*-COD hydrolyzes the acetamide group of reducing end GlcNAc residue of $(GlcNAc)_2$. These findings indicate that GlcNAc-GlcN is produced from chitin by the cooperative hydrolytic reactions of both *Pa*-Chi and *Pa*-COD.

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Introduction

Chitin, a β-(1,4) polymer of *N*-acetylglucosamine (GlcNAc), is one of the most abundant biomass polysaccharides, composing the shells of crustaceans such as crab and shrimp, the exoskeletons of insects, and the cell walls of fungi. Oligosaccharides obtained by the hydrolysis of chitin have shown physiological activities. For example, the antitumor (Suzuki et al. 1986; Tokoro et al. 1988; Tsukada et al. 1990) and antimicrobial actions (Tokoro et al. 1989; Kobayashi et al. 1990) of hexa-*N*-acetylchitohexaose [$(GlcNAc)_6$] in mice are known to be caused by enhancement of the immunological defense system. Various enzymes involved in chitin hydrolysis [i.e., 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)] are known. Chitinase catalyzes the degradation of water-insoluble chitin into water-soluble chitin oligosaccharides by its hydrolytic reaction. A number of chitinases have been isolated from bacteria, and their properties have been investigated (Dahiya et al. 2006). In addition, the genes encoding a variety of chitinases have been cloned. Based on their amino acid sequences, these chitinases are classified in either glycoside hydrolase (GH) family 18 or 19 (http://afmb.cnrs-mrs.fr/CAZY/). Most bacterial chitinases belong to GH family 18. β-*N*-Acetylhexosaminidase catalyzes the hydrolysis of chitin oligosaccharides to release the monosaccharide GlcNAc. The enzymes from various sources have been classified in GH families 3, 20 (the main family), and 84.

An excellent review of microbial β-*N*-acetylhexosaminidases has been published (Scigelova and Crout 1999). Chitin deacetylase and chitin oligosaccharide deacetylase are involved in the hydrolysis of the acetamide group of the GlcNAc residue of chitin and chitin oligosaccharides (Tsigos et al. 2000). Both enzymes, isolated from various sources, are classified in carbohydrate esterase (CE) family 4 (http://afmb.cnrs-mrs.fr/CAZY/). Several microbial chitin deacetylases and chitin oligosaccharide deacetylases have been isolated and their properties elucidated (Kafetzopoulos et al. 1993; Tsigos and Bouriotis 1995; Gao et al. 1995; Tokuyasu et al. 1996; Ohishi et al. 1997).

To obtain the enzymes that produce specific oligosaccharide from chitin effectively, we screened chitin-degrading bacteria isolated from dry beach soil and from the contents of marine fish intestines. The screen used was 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 kind of bacterium (strain KN1699), from these bacteria isolated, through the tests with both chitin decomposition and oligosaccharide production. In this paper, we report the identification of the strain KN1699, oligosaccharide production by extracellular enzyme of this strain, and purification and characterization of the enzyme involved in the oligosaccharide production.

Materials and methods

Microorganism

Gram-stain and physiological characterization of strain KN1699 was accomplished using a Color Gram 2 kit (bioMerieux) and an API 20 E kit (bioMerieux), respectively, and the strain was classified according to Bergey's Manual of Systematic Bacteriology (Farmer et al. 2005). The genotype of strain KN1699 was investigated by comparing the nucleotide sequence of its 16S rDNA to the sequence database Basic Local Alignment Search Tool for Nucleotide (BLASTN; http://www.ncbi.nlm.nih.gov/blast/). Chromosomal DNA from KN1699 was isolated using a High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche). The 16S rDNA was amplified from the isolated DNA by PCR using a MicroSeq 16S rDNA Full Gene PCR kit (Applied Biosystems), and the resulting PCR product was purified using Quantum Prep PCR Kleen Spin Columns (Bio-Rad). DNA sequence analysis was performed by the dideoxynucleotide method (Sanger et al. 1977). The nucleotide sequence of the PCR product was determined using an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems).

Chemicals

Glucosamine (GlcN), GlcNAc, chitin oligosaccharides [$(GlcNAc)_{2-6}$], and β-chitin flake were purchased from Seikagaku Kogyo. Powdered α-chitin was obtained from Wako Pure Chemical Industries. β-Chitin flake was ground to a powder using a blender (Wonder Blender WB-1, Osaka Chemical). Colloidal chitin was prepared using powdered α-chitin according to the method of Shimahara and Takiguchi (1988). Artificial seawater was prepared using the Sealife (Marine Tech) salt mixture. All other chemicals were of analytical grade.

Preparation of the crude enzyme solution

Strain KN1699 was grown at 28°C for 16 h with shaking (135 rpm) in 10 ml of half-strength artificial seawater containing 1% (*w/v*) peptone, 0.1% (*w/v*) yeast extract, and 0.5% (*w/v*) powdered α-chitin. To produce sufficient chitinase for purification and characterization, 5 ml of the above culture was added to 1 l of fresh medium and cultivated for 60 h as described above. After centrifuging the cells (6,000×*g*, 15 min at 4°C), proteins in the supernatant were precipitated by adding $(NH_4)_2SO_4$ (80% saturation) and collected by centrifugation (6,000×*g*, 20 min at 4°C). The resulting precipitate was dissolved in 50 ml of 20 mM sodium phosphate buffer (pH 7.0), and the solution was dialyzed against the same buffer to afford crude enzyme solution.

Purification of enzyme

Purification of chitinase (*Pa*-Chi): The crude enzyme solution was loaded on a DEAE-Toyopearl 650M resin (Tosoh) column (φ2.5×20 cm), pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0), and the enzyme was eluted with a linear gradient of 0–0.4 M NaCl in the same buffer (total volume, 800 ml). The active fractions were collected, dialyzed against 20 mM sodium phosphate buffer (pH 7.0), then the enzyme solution was again loaded on the DEAE-Toyopearl 650M resin column and eluted with a linear gradient of 0–0.3 M NaCl in the same buffer (total volume, 800 ml). After the active fractions were collected and concentrated by diaflow filtration using an Amicon PM-10 membrane, *Pa*-Chi was further purified by gel filtration chromatography using a Toyopearl HW-55F resin (Tosoh) column (φ1.6×100 cm) pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0) containing 0.2 M NaCl.

Purification of chitin oligosaccharide deacetylase (*Pa*-COD): Ten milliliters of the KN1699 starter culture was diluted into 2 l of half-strength artificial seawater containing 1% (*w/v*) peptone and 0.1% (*w/v*) yeast extract, and the

culture was incubated at 28°C for 48 h with shaking (135 rpm). The cells were removed by centrifugation (6,000×*g*, 15 min at 4°C), and proteins in the supernatant were precipitated with $(NH_4)_2SO_4$ (80% saturation) and collected by centrifugation (6,000×*g*, 20 min at 4°C). The precipitate was dissolved in 20 mM sodium phosphate buffer (pH 7.0) and dialyzed against the same buffer. The resulting enzyme solution was loaded on a DEAE Sepharose Fast Flow resin (GE Healthcare Bio-science) column (ϕ3.2× 11 cm) pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0) and eluted with a linear gradient of 0–0.4 M NaCl in the same buffer (total volume, 800 ml). The active enzyme fractions were collected, dialyzed against 20 mM sodium phosphate buffer (pH 7.0), and loaded on a DEAE-Toyopearl 650M resin column (ϕ1.5×10 cm) pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0). Enzyme was eluted with a linear gradient of 0–0.4 M NaCl in the same buffer (total volume, 200 ml). The active fractions were collected, dialyzed as above, and loaded on a Phenyl Sepharose HP resin (GE Healthcare Bio-science) column (ϕ1.0×26 cm) pre-equilibrated with 20 mM sodium phosphate buffer (pH 7.0) containing 0.7 M $(NH_4)_2SO_4$. *Pa*-COD was eluted from the column with a linear gradient of 0.7–0 M $(NH_4)_2SO_4$ in the same buffer (total volume, 200 ml).

Protein analysis

The homogeneities of the enzyme preparations were confirmed by native- and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). In the SDS-PAGE, 2-mercaptoethanol was adopted as a reducing agent and was added to the sample buffer. Proteins in the polyacrylamide gels were stained using Coomassie Brilliant Blue R250 (Tokyo Kasei). Protein concentrations of the enzyme solutions were determined by the Lowry's method using bovine serum albumin (Sigma) as a standard. The *N*-terminal amino acid sequences of the enzymes were determined using a Perkin Elmer Biosystems model Procise 49X HT protein sequencer.

Assay of enzyme

Assay of chitinase activity: The assay mixture, consisting of 50 μl enzyme solution and 950 μl 0.5% (*w/v*) powdered β-chitin in 20 mM sodium phosphate buffer (pH 7.0), was incubated at 37°C for 30 min. The enzymatic reaction was terminated by heating at 100°C for 5 min in a hot dry bath, and then the reaction mixture was centrifuged at 10,000×*g* for 4 min. The amount of reducing sugar released was determined using a modification of the Schales methodology (Imoto and Yagashita 1971), using GlcNAc as a standard. One unit of chitinase activity was defined as the amount of enzyme required to liberate reducing sugar equivalent to 1 μmol of GlcNAc per minute under the assay conditions.

Assay of chitin oligosaccharide deacetylase activity: The assay mixture consisted of 75 μl enzyme solution and 425 μl 1-mM $(GlcNAc)_2$ in 20 mM sodium phosphate buffer (pH 7.0). The reaction mixture was incubated at 37°C for 30 min, then the reaction was stopped by heating at 100°C for 5 min in a hot dry bath. The amount of GlcN residues produced by the enzymatic reaction was determined according to the method of Dische and Borenfreund (1950) using GlcN as a standard. One unit of chitin oligosaccharide deacetylase activity was defined as the amount of enzyme required to produce 1 μmol of GlcN residues per minute under the assay conditions.

To investigate the effects of pH on the activities and stabilities of the enzymes, they were incubated in following buffers: pH 3.0–6.0, sodium citrate buffer; pH 6.0–8.0, sodium phosphate buffer; pH 8.0–11.0, sodium borate buffer. Temperature studies on the activity and the stability of the enzymes were conducted in sodium phosphate buffer (pH 7.0).

Thin layer chromatography analysis

Oligosaccharides produced from β-chitin, $(GlcNAc)_2$, and $(GlcNAc)_3$ by the enzymatic reactions were analyzed by Silica Gel thin layer chromatography (TLC) using 5:4:3 (*v/v/v*) *n*-butanol/methanol/16% aqueous ammonia as the mobile phase. Silica Gel 60 TLC plates (0.25 mm) were obtained from E. Merck. After developing the TLC plates twice, compounds were visualized by spraying with an aqueous solution of 2.4% (*w/v*) phosphomolybdic acid, 5% (*v/v*) H_2SO_4, and 1.5% (*v/v*) H_3PO_4 (phosphomolybdic acid reagent) or ethanol containing 0.5% (*w/v*) ninhydrin (ninhidrin reagent), followed by heating.

Purification and identification of oligosaccharide

Aqueous solutions containing the oligosaccharide produced by incubation of powdered β-chitin with crude enzyme were filtered through no. 4 filter paper (Kiriyama), then the filtrate was loaded on a charcoal (Wako Pure Chemical) column (φ2.0×15 cm) pre-equilibrated with water. The oligosaccharide was eluted from the column with water and lyophilized. After the resulting white powder was dissolved in a small amount of water, the solution was loaded on a Toyopearl HW-40F resin (Tosoh) column (ϕ2.5×90 cm) pre-equilibrated with water. The oligosaccharide was eluted from the column with 20% (*v/v*) ethanol. The oligosaccharide-containing fractions were collected and dried then re-dissolved in a small amount of water and lyophilized to afford the oligosaccharide as a white powder.

The structure of the oligosaccharide was characterized by ^1H nuclear magnetic resonance (NMR) spectrometry using D_2O as a solvent and mass spectrometry.

The ^{1}H NMR spectra were recorded with a Varian Mercury 400 spectrometer at 20°C. Chemical shifts were expressed in ppm downfield shift from $(CH_3)_4Si$. Mass spectra were obtained with a Waters MicroMass ZQ instrument under positive or negative ion electron-spray ionization conditions.

Results

Identification of the strain

The bacterial isolate obtained from soil of Yatsu dry beach, strain KN1699, was identified from its morphological and physiological characteristics (Table 1) and from the nucleotide sequence encoding its 16S rDNA. According to Bergey's Manual of Systematic Bacteriology, the isolate was classified as a bacterium belonging to the genus *Vibrio*. A search for similarity with 16S rDNA in the BLASTN database confirmed that the isolate was most closely related to *Vibrio parahaemolyticus* O3:K6 (Table 2). These taxonomic studies allowed us to conclude that strain KN1699 was *V. parahaemolyticus*.

Analysis of the oligosaccharide produced by enzymes in the culture fluid

The oligosaccharide produced by the action of crude enzyme (prepared from the supernatant of *V. parahaemolyticus* KN1699 cultures) on β-chitin was analyzed using TLC. When phosphomolybdic acid reagent was used to visualize the oligosaccharide, TLC analysis of the reaction mixture showed a single product (Fig. 1a), which was also visualized by ninhydrin reagent (Fig. 1b). These results suggest that the product is an oligosaccharide possessing a free amino group. Although the mobility of this compound corresponded to that of chitobiose [$(GlcN)_2$], it is necessary to confirm its structure. Therefore, the compound was purified and its structure analyzed by electron-spray ionization mass spectrometry (ESIMS) and ^{1}H NMR. The ESIMS spectra of the product corresponded to $[M-H]^-$ and $[M+H]^+$ species at *m/z* of 381 and 383, respectively, indicating that this compound is a disaccharide consisting of GlcN and GlcNAc. The ^{1}H NMR signals obtained (400 MHz, D_2O) were assigned as follows; α-anomer (non-reducing end sugar moiety) δ 1.93 (s, 3H, $COCH_3$), 3.31–3.53 (m, 3H, H-3, H-4, and H-5), 3.61–3.64 (m, 2H, H-2, and H-6a), 3.80 (dd, 1H, $J_{5,6b}$ 1.2 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.43 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), (reducing end sugar moiety) δ 2.61 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.6 Hz, H-2), 3.31–3.53 (m, 1H, H-4), 3.55–3.59 (m, 1H, H-6a), 3.68 (dd, 1H, $J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 3.68 (dd, 1H, H-3), 3.73 (ddd, 1H, $J_{4,5}$ 10.0 Hz, H-5), 5.06 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1); β-anomer (non-reducing end sugar moiety) δ 1.93 (s, 3H, $COCH_3$), 3.31–3.53 (m, 3H, H-3, H-4, and H-5), 3.61–3.64 (m, 2H, H-2, and H-6a), 3.80 (dd, 1H, $J_{5,6b}$ 1.2 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.44 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1), (reducing end sugar moiety) δ 2.48 (t, 1H,

Table 1 Morphological and physiological characteristics of strain KN1699

Characteristics	Results
Form	Rods
Gram stain	Negative
Motility	Positive
Oxidase	Positive
Nitrate reduction	Positive
Indole production	Positive
Gas from D-glucose	Negative
Acetoin production	Negative
Hydrogen sulfide production	Positive
Gelatin hydrolysis	Negative
Urea hydrolysis	Negative
Utilization of citrate	Negative
Utilization of sucrose	Negative
Arginine dihydrolase	Negative
Lysine decarboxylase	Positive
Ornithine decarboxylase	Positive
β-Galactosidase	Negative
Growth in NaCl	0.5–7%
Growth on TCBS	Green

Table 2 BLASTN results of comparison of the 16S rDNA of strain KN1699 to the sequence database

Accesion number	Species	Strain number	Similarity (%)
BA000031	*Vibrio parahaemolyticus*	03:K6	100
BA000032	*Vibrio parahaemolyticus*	03:K6	99.9
AY345403	*Bacterium*	K2-74	99.9
AF319769	*Vibrio* sp.	Ex25	99.9
AY911391	*Vibrio parahaemolyticus*	MP-2	99.9
AF500207	*Vibrio* sp.	CJ11052	99.8
AJ874352	*Vibrio natriegens*	01/097	99.8
AY542526	*Vibrio* sp.	KYJ962	99.8
AY738129	*Vibrio campbelli*	90-69B3	99.7
AF388387	*Vibrio parahaemolyticus*	ATCC17802	99.5
AF246980	*Vibrio* sp.	98CJ11027	99.4
AY911396	*Vibrio harveyi*	SW-3	99.3
AF319770	*Vibrio* sp.	Ex97	99.3
AF064637	*Vibrio* sp.	NAP-4	99.3
AF388389	*Vibrio parahaemolyticus*	ATCC17802	99.3
AJ874353	*Vibrio natrieggens*	01/252	99.2