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Bioconversion of chitin-containing wastes for the production of enzymes and bioactive materials

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ABSTRACT

The exploitation of chitinous materials seems to be an infinite treasure. The oligosaccharides of chitin/chitosan, prepared by hydrolyzing chitin/chitosan with chitinase/chitosanase, have various potential applications in the fields of food, agricultural, and pharmaceutical industries. Almost all of the chitinase/chitosanases-producing strains use chitin/chitosan (or colloidal chitin/chitosan) as a major carbon source. However, the preparation of chitin involves demineralization and deproteinization of shellfish waste with the use of strong acids or bases. The utilization of shellfish waste as the sole carbon/nitrogen source not only solves environmental problems but also decreases the production costs of microbial chitinases. To further enhance the utilization of chitin-containing seafood processing wastes, we have recently investigated the bioconversion of shellfish chitin wastes (shrimp shell, crab shell, and squid pen) for the production of enzymes and bioactive materials. Rather than attempting to review the literature, this paper summarized our recently published works in the field of microbial reclamation of these unutilized marine bioresources.

1. Introduction

Chitin is one of the most widespread bio-polymers in nature. Among the natural chitinous resources, fishery wastes (shrimp and crab shells) especially have the highest content. In addition to edible parts, wastes amount to as high as 60–80% of the whole shrimp and crabs. Except some are processed to make cheap feeds of shrimp and crab shells powder, most wastes are discarded at will (Wang, Hsu, & Liang, 2010; Wang & Li, 1994; Wang, Li, & Chong, 1997). These not recovered and unused wastes could become potential precious bioresources, if they are processed/converted by modern biotech to make highly value-added products (Jayakumar, Prabaharan, Nair, & Tamura, 2010; Muzzarelli et al., 2007; Wang, Li, Liang, Hsieh, & Tseng, 2010; Zhao, Park, & Muzzarelli, 2010).

Not well utilized aquatic resources (the so-called "aquatic processing wastes"), which contain some special natural ingredients, have already been extracted and utilized in industrial large-scales. Regarding the better use of these aquatic waste resources, the special emphasis of today's R & D is placed on how to utilize the residues (not-used biological resources) derived from fishery processing industry to produce high value-added materials. The examples include (1) the recovery of human health beneficial polyunsaturated fatty acids DHA and EPA from tuna, and bonito fish-eyes; (2) the extraction of pituitary gland from salmon heads; (3) the extraction of coenzyme, organic iron, vitamin and blood increasing agent from the blood of co-migratory fish meat; (4) the use of fish scales as a medicinal toothpaste abrasive; (5) the use of squid ink as a food coloring and anti-cancer agents; and (6) the Nordic developed enzyme extraction from fish waste to be applied to fish peeling (Kim & Mendis, 2006). The major representative examples were listed in Table 1. Among them, the utilization of chitin, chitosan, cholesterol, DHA and EPA, in particular, attracted much attention in recent years.

Most researches regarding recycling of shrimp and crab processing wastes mainly focused on the preparation of chitin and chitosan. In addition, researches also include the production of enzymes (Liang, Lin, Yen, Wang, & Wang, 2006; Oh, Shih, Tzeng, & Wang, 2000; Wang, Chang, & Lu, 1995; Wang & Chang, 1997; Wang & Chio, 1998; Wang, Shih, Liang, & Wang, 2002; Wang, Hsiao, & Chang, 2002; Wang et al., 2006a; Wang, Lin, Yen, Liao, & Chen, 2006; Wang & Yeh, 2006; Wang & Yeh, 2008; Wang, Yang, Liang, & Yen, 2008; Wang, Hsu, Yen, & Wang, 2008; Wang et al., 2008c; Wang, Wang, & Huang, 2008; Wang, Chen, & Wang, 2008; Wang, Pen, Liang, & Liu, 2008; Wang, Chao, Liang, & Chen, 2009; Wang, Chen, & Liang, 2009; Wang, Chen, Liang, & Lin, 2009; Wang, Lin, Liang, Liu, & Kuo, 2009; Wang, Liou, Liang, & Liu, 2009; Wang, Wu, & Liang, 2009; Wang, Chang, & Liang, 2010; Yang, Shih, Tzeng,

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Table 1
The recovery of high value-added products from aquatic products processing waste.

Recovered products	Wastes	Main use
Chitin/chitosan	Shrimp shell, crab shell, squid pen	Coagulant Cosmetic materials Medical materials
Astaxanthin	Shrimp shell, shrimp head	Food additives Feed additives
Cholesterol	Fish oil	Liquid crystal Cosmetic materials
DHA, EPA	Fish oil	Health foods
Taurine	Cooking liquid waste of fish and shellfish	Health foods Pharmaceuticals
Complexed polysaccharide	Squid ink	Pharmaceuticals
Edible black pigment	Squid ink	Food additives
Pituitary	Fish head	Pharmaceuticals
Coenzyme Q	Fish meat	Health foods
Organic iron	Fish meat	Health foods
RNA	Sardine or salmon's juvenile	Health foods
protamine	Fish seminal	Food preservation agent
Squalene	Sharks-liver	Health foods Cosmetic materials
Apatite	Fish-bone	Biological ceramic material
Flavor components	Cooking liquid waste	Natural seasonings

& Wang, 2000), deodorants (Twu, Huang, Chang, & Wang, 2003; Yen et al., 2002), biofertilizers (Wang, Hsu, et al., 2010; Liang et al., 2006; Wang et al., 2008c), biofungicides (Wang, Yieh, & Shieh, 1999a, b; Wang, Yen, Hsiao, Chang, & Wang, 2002; Wang et al., 2002d; Wang, Yen, Tzeng, & Hsieh, 2005), hydrogen gas (Evvyernie et al., 2001), carotenoids (Babu, Chakrabarti, & Sambasivarao, 2008; Sachindra, Bhaskar, Siddegowda, Sathisha, & Suresh, 2007), and antioxidants (Wang, Lin, et al., 2009; Wang, Liou, et al., 2009; Wang, Wu, et al., 2009; Sachindra & Bhaskar, 2008; Wang, Yang, Liang, & Wang, 2009; Wang, Liu, Liang, Kuo, & Wang, 2010) from these chitin-containing biowastes by microbial fermentation. The effect of shrimp shell wastes in mangrove river sediment on the biodegradation and microbial community changes have also been studied (Chen, Tseng, Hsieh, Wang, & Wang, 2010).

In addition to outlining the preparation of chitin and chitosan from the shrimp shell, crab shell, squid pen, as well as the application of chitinous materials, this paper mainly focused on introducing our recently published works in the field of microbial reclamation of chitin-containing wastes (Fig. 1).

2. Materials and methods

2.1. Materials

The shrimp shells powder (SSP), crab shells powder (CSP), and squid pen powder (SPP) used in these experiments were all purchased from Shin-Ma Frozen Food Co. (I-Lan, Taiwan) and prepared as described previously (Wang et al., 2006a). The dried materials obtained were milled to powders for using as the carbon/nitrogen source for the production of enzymes and bioactive materials.

2.2. Measurement of enzyme activity

2.2.1. Chitinase activity

Colloidal chitin (1.3% in 50 mM phosphate buffer) was used as the substrate for the measurement of chitinase activity. The mixture of enzyme solution (0.5 mL) and substrate (1 mL) was incubated at 37 °C for 30 min. After centrifugation, the amount of reducing sugar produced in the supernatant was determined by the method of Imoto and Yagishita (1971) with *N*-acetylglucosamine as a reference compound. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of reducing sugars per min (Wang, Lin, et al., 2009).

2.2.2. Lysozyme activity

Lysozyme activity was determined spectrophotometrically by measuring the decrease in optical density at 660 nm. The reaction mixture contained 1.5 mL of an *M. lysodeikticus* cell suspension (optical density of 1.7) in 50 mM phosphate buffer (pH 7) and 1.5 mL of the enzyme solution. The mixture was incubated at 37 °C for 30 min, and the optical density at 660 nm was measured. The control sample contained 1.5 mL of the buffer instead of the enzyme. The turbidimetric assay for bacterial cell-lytic enzyme was performed according to the same method described above. Lysozyme activity was also measured as an increase in reducing power resulting from hydrolysis of ethylene glycol chitin (EGC) (in 50 mM phosphate buffer, pH 7) at 30 °C for 30 min (Wang, Shin, et al., 2002).

2.2.3. Protease activity

For measuring protease activity, a diluted enzyme solution (0.2 mL) was mixed with 1.25 mL of 1.25% casein in pH 7 phosphate buffer and incubated for 30 min at 37 °C. The reaction was terminated by adding 5 mL of 0.19 M trichloroacetic acid (TCA). The reaction mixture was centrifuged and the soluble peptide in the supernatant fraction was measured by the method of Todd with tyrosine as the reference compound (Todd, 1949). One unit of protease activity was defined as the amount of enzyme required to release 1 μmol of tyrosine per min (Wang, Lin, et al., 2009).

2.2.4. Nattokinase activity

Nattokinase activity was measured with a fibrin degradation assay developed by JBSL. First, 0.4 mL of 0.72% fibrinogen was placed in a test tube with 0.1 mL of 245 mM phosphate buffer (pH 7) and incubated at 37 °C for 5 min. Then, 0.1 mL of a 20 U/mL thrombin solution was added. The solution was incubated at 37 °C for 10 min, 0.1 mL of diluted enzyme solution was added, and incubation continued at 37 °C. This solution was again mixed after 20 and 40 min. At 60 min, 0.7 mL of 0.2 M trichloroacetic acid (TCA) was added, and mixed. The reaction mixture was centrifuged at 15,000 *g* for 10 min. Then, 1 mL of the supernatant was collected and the absorbance at 275 nm was measured. In this assay, 1 unit (fibrin degradation unit, FU) of enzyme activity is defined as a 0.01-per-minute increase in absorbance at 275 nm of the reaction solution (Wang, Chen, et al., 2009).

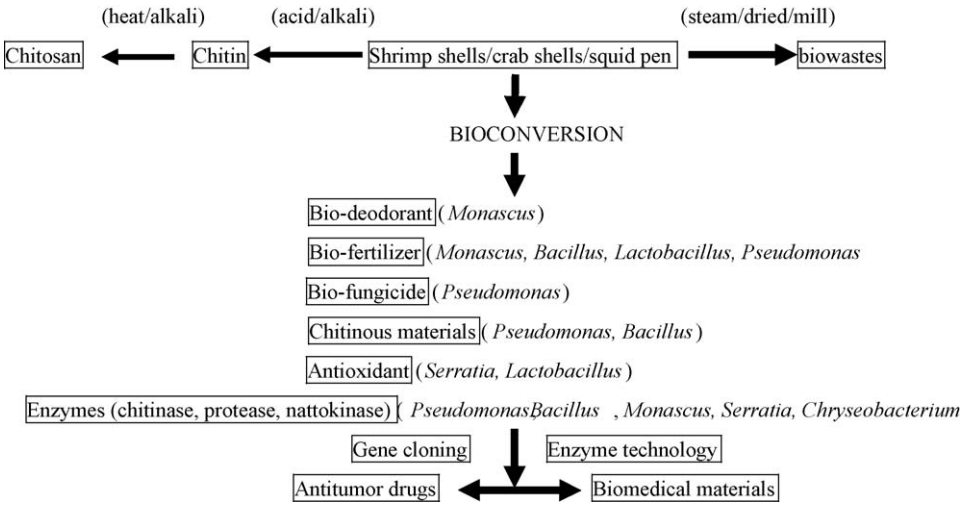

Fig. 1. Bioconversion of shrimp and crab shells.

2.3. In vitro antifungal activity tests

The antifungal activities of the culture supernatants were estimated using a growth inhibition assay described earlier (Wang, Shin, et al., 2002). Fungal spores were grown on potato dextrose agar (PDA) in Petri plates. After 10 days of incubation at 25 °C, the fungal spores were removed with sterile water containing 0.1% (v/v) Tween 80. The resulting suspension was filtered aseptically through a sterilized gauze. The concentrations of the spore suspensions were determined in a hemacytometer and adjusted to 1×10^6 spores/mL. The spore suspensions were stored at 4 °C before use. To test the antifungal inhibitory effect of the culture supernatants, Petri plates were filled with 5 mL of molten PDA precooled to 45 °C and divided into two groups (triplicate for each). To each plate in the experimental group (E), the culture supernatant (5 mL) was added. The ratio (v/v) of the tested solution and PDA added in the Petri plates was 1:1. To those of the control group (C), an equal amount of sterile water instead of tested solution was added. After the plates had been cooled, the fungal spores (20 μL) were placed onto the agar surface. Both groups were incubated for 72 h at 25 °C. The diameters of the largest and smallest fungal colonies were recorded and their averages were calculated. The inhibition ratios were calculated with the formula:

$$\text{inhibition ratio}\,(\%)\frac{C-E}{C} \times 100$$

where C is the average diameter of the largest and smallest colonies of the control groups and E is the average diameter of the largest and smallest colonies of the experimental groups. Generally if the inhibitory ratio was >20%, the tested fungus would be considered to be inhibited. To express the inhibitory activity of the culture supernatants, one unit of antifungal activity was defined as the amount of culture supernatants required to obtain a 50% inhibition under the above assay conditions.

2.4. Preparation of the chitooligosaccharides

The culture supernatant was concentrated to about 1/5 of the original volume with a rotary evaporator under diminished pressure. The pH of the sample was adjusted to pH 9 with 10% NaOH and followed by adding ethanol. Yellow agglomerates were formed in the solution. The agglomerates were concentrated with a rotary evaporator under diminished pressure and were collected after drying in vacuum. The supernatant was concentrated to about 1/10 of the original volume with a rotary evaporator under diminished pressure. Then, it was precipitated by adding acetone. The precipitates were collected after drying in vacuum (Wang, Hsu, et al., 2010).

2.5. HPLC analysis of chitooligosaccharides

HPLC analysis of chitooligosaccharides was performed on a Hitachi L-7000 apparatus (column, Nucleosil 5 NH_2 4.6 mm × 250 mm; mobile phase, acetonitrile/water = 70/30, v/v; flow rate = 1.0 mL/min; detection, RI). After fermentation and filtration, the sample was analyzed to measure the amount of (GlcNAc)n, n = 1–6 in the culture supernatant by HPLC. The amounts of (GlcNAc)n, n = 1–6 were estimated with the calibration curve of standard 3.75 mg/mL, (GlcNAc)n, n = 1–6 (Wang, Hsu, et al., 2010). The yield of (GlcNAc)n, n = 1–6 was calculated by the following equation:

$$\text{The concentration of sample}\,(\text{mg/mL}) = \frac{3.75 \times \text{the area of sample}}{\text{the area of standard}}$$

2.6. Measurement of DPPH radical scavenging activity

The diluted culture supernatant (150 μL) was mixed with 37.5 μL of methanolic solution containing 0.75 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm against a blank (Wang, Liu, et al., 2010). The scavenging ability was calculated as follows: scavenging activity (%) = [(A_{517} of control − A_{517} of sample)/A_{517} of control] × 100.

3. Results and discussion

3.1. Preparation of chitin and chitosan

The chemical compositions of fisheries waste vary depending on the varieties of raw materials and the parts of the bodies. For example, in terms of the types of raw materials, shrimp shells contain higher chitin than crab shells (with a higher ratio of mineral salts). Regarding the different parts of crabs, the feet shells contain the highest chitin, ventral contain the highest proportion of protein, while dorsal shell and claws clamp contain a higher proportion of mineral salts. Containing 25–35% chitin, 20–30% protein, and 40–50% calcium carbonate-based mineral salts, crab shell is

often used as a raw material for the production of chitin and chitosan (Abdou, Nagu, & Elsabee, 2008; Brine & Austin, 1981). The shrimp and crab shell waste must be demineralized and deproteinized, in order to obtain chitin and chitosan (Chen, Li, Ji, Zhong, & Li, 2008; Gagne & Simpson, 1993; Jo, Jung, Kuk, Oh, Kim, & Park, 2008; Jung, Jo, Kuk, Kim, Oh, & Park, 2007; Oh, Kim, Nguyen, Jung, & Park, 2007; Sini, Santhosh, & Mathew, 2007; Zhao & Xia, 2009). The related research on chitin and chitosan in recent years has not only limited to basic analysis. The studies of the utilization value and industrial potential are also actively engaged in. The major areas of application include water treatment (Bhatnagar & Sillanpää, 2009; Kartal & Imamura, 2005; Prado, Torres, Faria, & Dias, 2004), food ingredients, biomedical applications (Jayakumar et al., 2010; Khor & Lim, 2003; Murakami et al., 2010; Muzzarelli et al., 2007; Okamoto, Yano, Miyatake, Tomohiro, Shigemasa, & Minami, 2003; Senel & McClure, 2004), and personal-care products (Krajewska, 2004; Perugini, Genta, Pavanetto, Conti, Scalia, & Baruffini, 2000; Rinaudo, 2006; Twu, Huang, Chang, & Wang, 2003; Yen et al., 2002).

3.1.1. Chitin

Both chemical and biological methods can be used for the preparation of chitin and chitosan from shrimp and crab shell. Conventionally, preparation of chitin from marine waste material involves demineralization and deproteinization with the use of strong acids or bases (Fig. 2). However, the use of these chemicals may cause a partial deacetylation of chitin and hydrolysis of the polymer, resulting in final inconsistent physiological properties. The chemical treatments also create waste disposal problems, because neutralization and detoxification of the discharged wastewater are necessary. Furthermore, the value of the deproteinized liquid is diminished because of the presence of sodium hydroxide (Brine & Austin, 1981; Gagne & Simpson, 1993; Oh et al., 2000; Wang, Hsu, et al., 2010; Yang, Shih, Tzeng, & Wang, 2000).

Regarding biological methods, enzymatic treatment by the use of protease (Oh et al., 2000; Wang & Chio, 1998), and microbial fermentation by the use of protease-producing bacteria (Jo, Jung, Kuk, Oh, Kim, & Park, 2008; Jung, Jo, Kuk, Kim, Oh, & Park, 2007; Oh et al., 2000; Oh, Kim, Nguyen, Jung, & Park, 2007; Sini, Santhosh, & Mathew, 2007; Wang, Hsu, et al., 2010; Wang et al., 2008c; Wang, Wang, et al., 2008; Yang, Shih, Tzeng, & Wang, 2000) are commonly used. The use of microbial proteolytic enzymes for deproteinization of marine crustacean wastes is a current trend in the conversion of wastes into useful biomass. It is a simple and inexpensive alternative to chemical methods employed in the preparation of chitin (Oh et al., 2000; Wang & Chio, 1998; Wang, Hsu, et al., 2010). The enzymatic hydrolysates or culture broth, derived from deproteinization of chitin wastes of shrimp, crab and squid pen, are rich in protein hydrolysates (amino acids and peptides) and could be used as medium for the incubation of other useful microorganisms. Consequently, the cost of wastewater treatment could be reduced. Due to these niches, both the enzymatic methods by the use of proteolytic enzymes and the microbial methods by the use of protease-producing microbes become more and more noteworthy (Table 2).

Recovery of an enzyme after its application is a major concern in process economics. Immobilization is considered favorable in saving enzymes for reuse. It also has the advantage of automation and continuous processing. Immobilizing enzymes into a reversible soluble-insoluble carrier, in contrast to conventional water-insoluble support, has been suggested as a means to solve the diffusion problems encountered in a heterogenous reaction system and to aid in product separation (Chen & Chang, 1994). We have demonstrated that the crude protease produced by *P. aeruginosa* K-187 can be covalently immobilized on a reversibly soluble polymeric support (AS-L). The utilization of the immobilized protease for the deproteinization of shrimp and crab shell powder (SCSP) has resulted in a 67% protein removal, which was a little lower than 72% of free enzymes (Oh et al., 2000).

Table 2
Biological methods for the preparation of chitin from biowastes of shrimp and crab.

Microbes/enzymes	Biowastes of shrimp/crab
Microbial fermentation	
Bacillus subtilis	Shrimp and crab shell
B. subtilis	Shrimp and crab shell
Bacillus sp.	Squid pen
B. subtilis	Shrimp shell
Chryseobacterium taeanense	Shrimp shell
C. indologenes	Shrimp shell
Euarotium repens	Shrimp and crab shell
L. paracasei	Squid pen
Monascus purpureus	Shrimp and crab shell
Pseudomonas aeruginosa	Shrimp and crab shell
P. aeruginosa	Shrimp and crab shell
P. aeruginosa	Squid pen
P. aeruginosa	Crab shell
Serratia marcescens	Squid pen
S. marcescens	Crab shell
S. ureilytica	Squid pen
L. paracasei/*S. marcescens*	Crab shell
Aspergillus niger	Shrimp shell
Enzymatic treatment	
Papain	Shrimp and crab shell
Papain	Demineralized shrimp shell
Bromelain	Shrimp and crab shell
Chymotrypsin	Demineralized shrimp shell
P. aeruginosa protease	Shrimp and crab shell
P. aeruginosa protease	Shrimp and crab shell

3.1.2. Chitosan

Chitosan can be prepared from chitin by deacetylization. Deacetylation methods include (1) heat-alkali method; (2) enzymatic method (enzyme hydrolysis by the use of chitin deacetylase); (3) microbiological method (fermentation by the use of chitin deacetylase-producing microbes). The heat-alkali method produces chitosan with higher degree of deacetylation (DD) and therefore is more frequently used (Rinaudo, 2006). However, the disadvantage of this method is that the DD and the degree of depolymerization (DP) of the chitosan produced are affected by the alkali concentration, amount ratio, reaction time and temperature. As for the enzymatic method and the microbiological method, although without the problems of waste alkali treatment and degradation of chitosan molecular chain, they require a long reaction time and the resultant deacetylation degree (DD) is not high (Kim, Zhao, Oh, Nguyen, & Park, 2008).

3.2. Production of chitinases with novel properties

Chitinase producing strains, from soils and marine muds, have been used by many researchers to produce chitinase by using chitin, colloidal chitin or other chitinous materials as a major carbon source. However, isolation of chitinase producing strain by using shrimp shells or squid pens as the sole C/N source has rarely been reported. In the industrial production, chitin or chitosan is extracted from shrimp or crab shells with the use of strong acids and alkalines. There exists an enormous difference between the prices of shrimp or crab shells raw materials and chitin or chitosan products. In order to lower the cost of enzyme production, the authors screened for chitinolytic enzymes (chitinase, chitosanase) and protease producing microorganisms by utilizing cheap shrimp shell powder or squid pen powder, which contains chitin and protein, as the sole C/N sources. Many novel chitinase/chitosanase/protease producing bacteria have been screened out from the soil in Taiwan (Table 3).

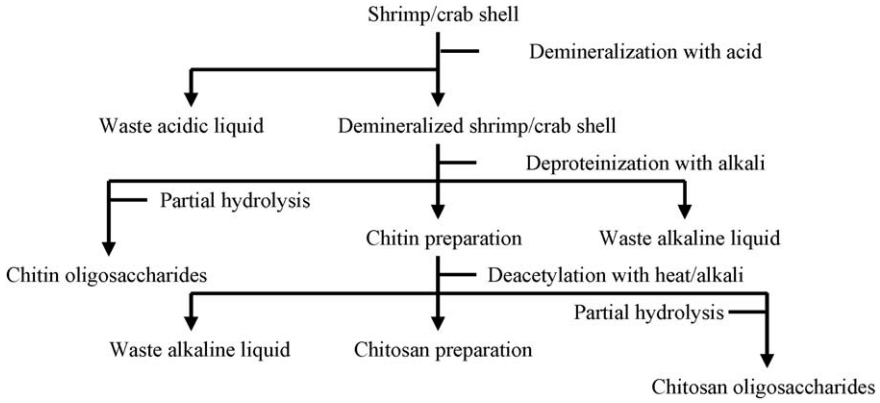

Fig. 2. Chemical methods for the preparation of chitin, chitosan, and their related derivatives.

Table 3
The chitinolytic and proteolytic enzymes-producing bacteria isolated from Taiwan soil using shrimp/crab shell or squid pen as the sole carbon/nitrogen source.

Strains	Enzyme	C/N source
Bacillus sp. TKU004	Metalloprotease	2% SPP
	Chitosanase	2% SPP
B. cereus	TKU006 metalloprotease	2% SSP
	Chitinase	2% SSP
B. cereus YQ308	Antifungal chitinase	2% SCSP
B. subtilis TKU007	Serineprotease/nattokinase	1.5% SSP
B. subtilis Y-108	Metalloprotease	3% SCSP
B. subtilis W-118	Antifungal chitinase	3% SCSP
B. amyloliquefaciens V656	Antifungal chitinase	2% SCSP
B. cereus TKU018	Chitosanase	1% SSP
B. subtilis TKU007	Chitosanase	1.5% SSP
Chryseobacterium taeanense TKU001	Metalloprotease	1% SSP
C. indologenes TKU014	Metalloprotease	0.5% SSP
Lactbacillus paracasei TKU010	Metalloprotease	1% SPP
L. paracasei TKU012	Metalloprotease	1% SPP
P. aeruginosa K-187	Chitinase	3% SCSP
protease	3% SCSP	
P. aeruginosa M-1001	Antifungal protease	1% SCSP
Pseudomonas sp. TKU015	Chitinase	0.5% SSP
	chiTosanase	0.5% SSP
	Nattokinase	1% SSP
Serratia marcescens TKU011	Chitosanase	2% SPP
	Metalloprotease	1% SPP
S. ureilytica TKU013	Chitinase	1.5% SPP
	Protease	1.5%SPP
Serratia sp. TKU020		
Serratia sp. TKU017	Chitinase	2% SSP
	Protease	2% SSP
Serratia sp. TKU016	Chitosanase	1% SSP
protease	1% SSP	1% SSP
Vibrio fluvialis TKU005	Alkaline protease	3% SSP

SPP, squid pen powder; SSP, shrimp shell powder; SCSP, shrimp and crab shell powder.

3.2.1. *Bifunctional chitinase/lysozymes*

Chitin is one of the most abundant renewable natural resources second only to cellulose. Chitin, cellulose, and peptidoglycan exist in shells of crustaceans (animal), cell wall of plant, and cell wall of bacteria (microorganism), respectively. The structures of these three carbohydrate polymers are very similar. The monomers construct chitin and cellulose are *N*-acetylglucosamine and glucose, respectively. On the other hand, peptidoglycan is builded up of two different monomers, *N*-acetylglucosamine and *N*-acetylmuramic acid. The only difference between these two monomers is the functional group bound to the third carbon. Because of the similarity of the structures, some enzymes were found to possess bifunctional activity of hydrolyzing chitin and peptidoglycan (Wang & Chang, 1997). These bifunctional chitinase/lysozymes include hen egg-white lysozyme and fig chitinase *etc*.

Some animal and higher plant chitinases also have lysozyme activity (chitinase/lysozyme), while bifunctional chitinases have not been isolated from microorganisms. We have isolated two kinds of bifunctional chitinases/lysozymes from cell-free culture broth of *Pseudomonas aeruginosa* K-187, an alkali-tolerant bacterium isolated from the soil using SCSP as the sole C/N source (Wang et al., 1995; Wang & Chang, 1997). Both chitinases showed lysozyme activity with enzyme specificity similar to plant chitinases. The purified enzymes had antibacterial and cell lysis activities with many kinds of bacteria. This was the first report of a bifunctional chitinase/lysozyme from a prokaryote (Wang & Chang, 1997).

3.2.2. *Antifungal chitinases*

When it was grown in a medium containing SCSP of marine waste, *Bacillus amyloliquefaciens* V656 produced antifungal chitinases. In contrast to other known bacterial chitinases, the unique characteristics of *B. amyloliquefaciens* V656 chitinases include

Table 4
Productivity of chitinolytic enzymes by the isolated strains.

Strains	Type	MW	Opt. pH	Opt. T.	Productivity	C/N source
P. aeruginosa K-187	Chitinase	30 kDa	8	50 °C		3% SCSP
	Chitinase	32 kDa	7	40 °C		
Pseudomonas sp. TKU015	Chitinase	68 kDa	6	50 °C	0.011 U/mL	0.5% SSP
	Chitosanase	30 kDa	4	50 °C	0.026 U/mL	
B. amyloliquefaciens V656	Chitinase	14 kDa	7	40 °C	0.017 U/mL	2% SCSP
	Chitinase	17 kDa	6	40 °C		
B. cereus TKU006	Chitinase	35 kDa	5	40 °C	0.06 U/mL	2% SSP
B. cereus TKU018	Chitosanase	44 kDa	5	60 °C	0.02 U/mL	1% SSP
	Chitosanase	22 kDa	7	50 °C		
B. subtilis TKU007	Chitosanase	25 kDa	7	37 °C	0.03 U/mL	1.5% SSP
S. marcescens TKU011	Chitosanase	21 kDa	5	50 °C	0.03 U/mL	2% SPP
S. ureilytica TKU013	Chitinase	60 kDa	6	50 °C	0.05 U/mL	1.5% SPP

SPP, squid pen powder; SSP, shrimp shell powder; SCSP, shrimp and crab shell powder.

Table 5
Protein/chitin/mineral salts compositions of the three used C/N sources.

C/N source	Composition (%)		
	Protein	Chitin	Mineral salt
Squid pen	61	38	1
Shrimp shell	48	38	14
Shrimp and crab shell	29	31	40

extremely low molecular weights and nearly neutral optimum pH values (Table 4) (Wang, Shih, et al., 2002). SCSP was also used as a substrate for the isolation of an antifungal chitinase-producing bacterium, *B. cereus* YQ308 (Chang, Chen, & Wang, 2003). The purified YQ308 chitinase inhibited the hyphal extension of the phytopathogenic fungi *F. oxysporum* and *Pythium ultimum*.

We have reported that SCSP of marine wastes is an effective inducer for the production of antimicrobial chitinases by *P. aeruginosa* K-187 (Wang & Chang, 1997), *B. amyloliquefaciens* V656 (Wang, Shih, et al., 2002), *B. cereus* YQ308 (Chang et al., 2003), *B. subtilis* W-118 (Wang, Lin, et al., 2006), and *M. purpureus* CCRC31499 (Wang, Hsiao, et al., 2002).

3.3. Production of proteases with novel properties

3.3.1. Extracellular proteases from Lactobacillus

Bioconversion of squid pen has been proposed as a waste treatment alternative to the disposal of chitin containing seafood processing wastes. Squid pen contains abundant protein and chitin, while its mineral salts are much lower than those of shrimp or crab shell (Table 5). Therefore, we have investigated microbial reclamation of squid pen for the production of lactobacilli protease, and published the first report on the production and purification of extracellular proteases from lactobacilli (Wang, Wang, et al., 2008). Two protease-producing bacteria, strain TKU010 (Wang et al., 2008c) and TKU012 (Wang, Wang, et al., 2008), which were isolated from infant vomited milk, were identified as *Lactobacillus paracasei*. Different to that of TKU012, the 4th day culture supernatant of TKU010 showed maximal activity of about 5-fold growth enhancing effect on lettuce weight (Fig. 3). This effect was not shown with *L. paracasei* TKU012.

The result of identification of TKU010 protease showed that nine tryptic peptides were identical to *Serratia* protease (serralysin) with 35% sequence coverage. In comparison with the tryptic peptides of TKU012 protease, TKU010 protease possessed two additional peptides with sequences of AATTGYDAVDDLLHYHER and QTFTHEIGHALGLSHPGDYNAGEGNPTYR (Wang et al., 2008c). These structural differences between TKU010 protease and TKU012 protease might be the reason for the different properties of vegetable growth enhancing effect.

Some other specific proteases have also been purified from *Chryseobacterium taeanense* TKU001 (Wang, Yang, et al., 2008), *C. indologenes* TKU014 (Wang, Hsu, et al., 2008), *Bacillus* sp. TKU004 (Wang et al., 2006a), *B. cereus* TKU006 (Wang, Chao, et al., 2009), and *B. subtilis* TKU007 (Wang & Yeh, 2006) by using shrimp shells as the sole C/N source (Table 6).

It is also observed in Table 6, generally speaking, squid pen is more suitable as C/N source for fermentation production of protease by *Lactobacillus* species (Wang et al., 2008c; Wang, Wang, et al., 2008) and *Serratia* species (Wang, Yang, et al., 2009; Wang, Lin, et al., 2009), while shrimp shell is more suitable as C/N source for fermentation production of protease by *Chryseobacterium* species (Wang, Yang, et al., 2008; Wang, Hsu, et al., 2008), *Pseudomonas* species (Wang, Chen, et al., 2009), and *Bacillus* species (Wang, Chao, et al., 2009; Wang & Yeh, 2006). Besides, only protease but not chitinolytic enzyme activity was detected in the culture supernatant of chitin/protein-containing seafood processing wastes fermentated by *Lactobacillus* species and *Chryseobacterium* species. This is different from those of *Pseudomonas* species, *Bacillus* species, and *Serratia* species, in which both activities of protease and chitinolytic activity were detected.

3.3.2. Nattokinase from Pseudomonas

Nattokinase is a potent fibrinolytic enzyme, which is mostly obtained from the fermentation of soybean by *Bacillus natto*. Reports about the production of nattokinase by bacteria other than *Bacillus* species seems to be scarce. It is speculated that squid pen could be used as an inducer for fermentation production of proteolytic enzyme since squid pen is also rich in protein. Consequently, the authors used squid pen as the sole C/N source to isolate TKU015, a new species strain of *Pseudomonas*, from soil on the campus of Tamkang University (Taipei, Taiwan) (Wang, Chen, et al., 2009). *Pseudomonas* sp. TKU015 fermented squid pen and produced chitinase and chitosanases (Wang, Chen, et al., 2008) besides nattokinase (Wang, Chen, et al., 2009). The nattokinase produced by *Pseudomonas* sp. TKU015 does not have protease activity (using casein as substrate). This is different from *B. subtilis* TKU007, a chitosanase and nattokinase-producing strain (Wang & Yeh, 2008). For example, *B. subtilis* TKU007 nattokinase possess protease activity as well. Besides, different from that *B. subtilis* TKU007 use shrimp shell as the sole C/N source, *Pseudomonas* sp. TKU015 produced nattokinase only when it used squid pen as the C/N source. If shrimp shell is used, nattokinase activity can not been detected.

3.4. Production of antifungal materials

Synthetic chemical fungicides have long served as agents for reducing the incidence of plant disease. However, they are costly, can cause environmental pollution, and may induce pathogen resistance. Considering the limitations of chemical fungicides, it seems appropriate to search for a supplemental control strategy. Biological control, the use of a microorganism or its secretion to prevent disease, offers an attractive alternative or supplement to pesticides and genetic resistance for the management of plant disease, without the negative effects of chemical control. Therefore, it becomes an important aspect of suitable agriculture.

In the past few years, numerous microorganisms with antifungal activities have been isolated. Their active factors have been identified, and the mechanisms by which antifungal factors inhibit growth of potentially pathogenic fungi have been demonstrated. To further enhance the utilization of chitin containing marine crustacean waste, we have recently investigated the bioconversion of shrimp and crab shell for biofungicide production.

3.4.1. Antifungal materials from Bacillus

Two strains of *B. subtilis*, W113 and W118, were isolated from soil (Wang et al., 2002). It was demonstrated that crude fungicides, obtained from the culture broth of these strains grown aerobically in a medium containing chitin, displayed antifungal activity on phytopathogenic *Fusarium oxysporum*. The crude fungicides were remarkably thermostable. The inhibitory activities were retained to some extent even after the crude fungicides were heated at 100 °C for 30 min. The inhibitory activities are not significantly affected by variation of pH, even at extreme pH. Furthermore, it was thought to have a high molecular weight because it precipitated upon ammonium sulfate treatment and non-dialyzable. Many known antifungal compounds produced by *Bacillus* were small molecules (Akihiro, Takashi, & Makoto, 1993; Silo-Suh, Lethbridge, Raffel, He, Clardy, & Handelsman, 1994). Many of them were found unstable at extreme pH and temperatures. In contrast, the fungicides of strains W113 and W118 were extremely thermostable and pH stable. These evidences suggest that these fungicides are different from the antifungal compounds previously reported.

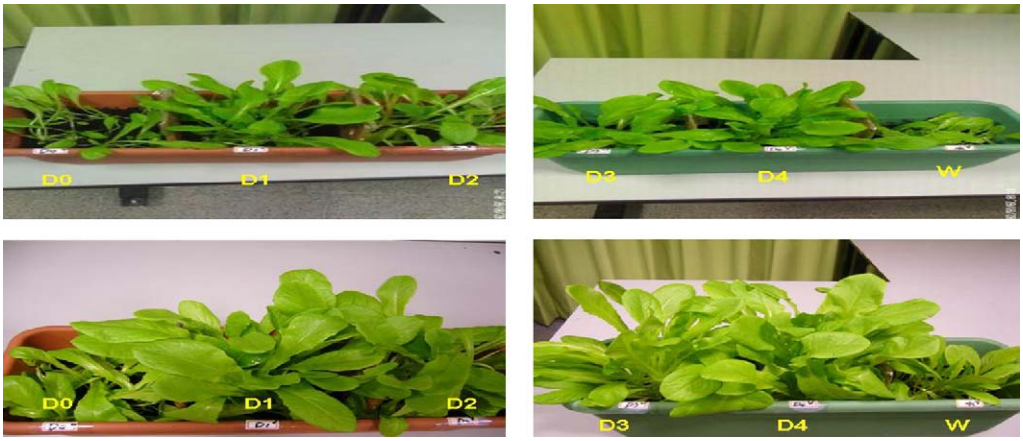

Fig. 3. Enhancing effect of culture supernatant of *L. paracasei* TKU010 on the growth of lettuce. The concentration of each resultant culture supernatant was adjusted with water (W) to 10%. The 4th day culture supernatant (D4) showed maximal activity of about 5-fold growth enhancing effect on lettuce weight. Up photo, the 3rd week; bottom photo, the 4th week.

3.4.2. Antifungal materials from Pseudomonas

P. aeruginosa K-187, which was isolated from soil in Taiwan, has been shown in our previous studies to produce chitinase in a SCSP medium (Wang et al., 1995; Wang & Chang, 1997; Wang & Chio, 1998). The purification and characterization of two bifunctional chitinases/lysozymes extracellularly produced by this microorganism in SCSP medium has also been demonstrated (Wang & Chang, 1997). However, the bifunctional chitinase/lysozyme thus produced showed no antifungal activity. The culture broth of *P. aeruginosa* K-187 grown aerobically in an SCSP medium displayed antifungal activity and inhibited growth of many phytopathogenic fungi (Wang et al., 1999a).

We carried out the isolation and characterization of an antifungal compound named pafungin from culture broth of *P. aeruginosa* K-187 (Wang, Yieh, & Shieh, 1999b). Pafungin was characterized to be a protrein–carbohydrate complex, which contains 17% of amino acids and 62% of carbohydrate, with a molecular weight of 66,000. Most of the antifungal proteins were shown to have chitinase activities. However, pafungin showed no activity of chitinase and/or lysozyme. Therefore, these results suggested pafungin was not the bifunctional chitinase/lysozyme produced by this microorganism in SCSP (Wang & Chang, 1997). The role of purified pafungin in the suppression of *F. oxysporum*, which causes damping-off disease, was studied. Suppression of swelling and lysis of the hyphae were observed in the presence of pafungin.

We also isolated *P. fluorescens* K-188, which exibited antifungal activities in the presence of SCSP. The acid or alkali liquid waste from SCSP treatment in the chitin production process also could be a feedstock for antifungal material production by strain K-188. The antifungal activity could be originated from K-188 cells, the protease activity, and an antifungal protein with molecular weight of 11,000 (Wang, Yen, Tzeng, & Hsieh, 2005).

3.4.3. Antifungal materials from Monascus

The culture supernatants from five strains of *Monascus* species, *M. purpureus* CCRC31499, *M. purpureus* CCRC32966, *M. purpureus* CCRC31530, *M. rubber* CCRC31535, and *M. pilosus* CCRC31527 were tested for their antifungal activity against *F. oxysporum*. Among them, *Monascus* species was selected because it is a safe and widely used traditional food fungi. The addition of SCSP resulted in enhancement of the yield of antifungal activity on *F. oxysporum*. Among these five strains, *M. purpureus* CCRC31499 exibited maximal antifungal activity, when cultured in media supplemented with 1% SCSP (Wang, Yen, et al., 2002). In addition to antifungal (Wang, Yen, et al., 2002), chitinolytic (Wang, Hsiao, et al., 2002) and proteolytic (Liang et al., 2006) activities, the culture broth of *M. purpureus* CCRC31499 also exhibited activity of enhancing rape growth (Liang et al., 2006).

SCSP was prepared by treating shrimp and crab processing wastes with boiling and crashing. Why the strains showed higher production of antifungal activity with SCSP than with chitin is not clear. The shell of shrimp and crab is composed mainly of chitin impregnated with proteins that has been variously modified with lipid and mineral salts. The impregnated mineral salts are primarily calcium carbonate with minor amounts of magnesium, phosphate, silica, and sulfur (Lavall, Assis, & Campana-Filho, 2007; Wang et al.,

Table 6
Productivity of proteases by the isolated strains.

Strains	Type	MW	Opt. pH	Opt. T.	Productivity	C/N source[a]
L. paracasei TKU010	Metallo-	49 kDa	10	50 °C	0.12 U/mL	1% SPP
L. paracasei TKU012	Metallo-	49 kDa	10	50 °C	0.14 U/mL	1% SPP
C. taeanense TKU001	Metallo-	41 kDa	8	60 °C	0.11 U/mL	1% SSP
	Metallo-	75 kDa	7	60 °C		
C. indologenes TKU014	Metallo-	56 kDa	10	30–50 °C	0.06 U/mL	0.05% SSP
	Metallo-	40 kDa	7-8	40 °C		
	Metallo-	40 kDa	8-9	40–50 °C		
Pseudomonas sp. TKU015	Serine-	21 kDa	7	50 °C	2.3 U/mL[b]	1% SSP
Bacillus sp. TKU004	Metallo-	27 kDa	7	60 °C	0.06 U/mL	2% SPP
B. cereus TKU006	Metallo-	39 kDa	9	50 °C	0.41 U/mL	2% SSP
B. subtilis TKU007	Serine-	28 kDa	7–8	50 °C	0.33 U/mL	1.5% SSP
S. marcescens TKU011	Metallo-	50 kDa	8	50 °C	0.4 U/mL	1% SPP
S. ureilytica TKU013		50 kDa	10	40 °C	0.2 U/mL	1.5%SPP
		50 kDa	10	40 °C		

[a] SPP, squid pen powder; SSP, shrimp shell powder; SCSP, shrimp and crab shell powder.
[b] Nattokinase activity.

1995). The reason why SCSP is more effective than chitin in antimicrobial chitinases production by these strains is the difference in composition of SCSP and chitin.

3.5. Production of antitumor chitooligomers

Recent studies on chitin and chitosan have attracted interest for converting them to oligosaccharides, because the oligomers not only are water soluble but also possess versatile functional properties such as antitumor, antimicrobial and antioxidant activities (Wang, Hsu, et al., 2010). It is known that high DP oligomers, such as chitopentaose, chitohexaose and chitoheptaose are more biologically active than the low DP oligomers. Therefore, in order to utilize the oligomers as functional food materials and pharmaceuticals, products containing a large fraction of higher oligosaccharide are desirable (Liang, Chen, Yen, & Wang, 2007; Wang et al., 2008g; Kuroiwa, Noguchi, Nakajima, Sato, Mukataka, & Ichikawa, 2008; Lin, Hsiao, & Chiang, 2009). Traditionally, these oligosaccharides are processed by chemical methods in industries. There are many problems existing in chemical process, such as a large amount of short-chain oligosaccharides produced and also environmental pollution caused (Zhao & Xia, 2009; Chen, Li, Ji, Zhong, & Li, 2008). Alternatively, with its advantages in environmental compatibility, biological methods have become more and more popular in recent years (Wang, Hsu, et al., 2010; Wang et al., 2008c; Wang, Wang, et al., 2008).

These oligomers can be obtained from the partial hydrolysis of chitin and chitosan. As the higher oligosaccharides such as pentamers and hexamers are intermediate products in the hydrolysis of chitin and chitosan, it is necessary to control the hydrolysis reaction to produce them at high yield. Besides selection of bacteria for the production of chitinolytic enzymes, there are other methods which may be used to increase the yield of high DP oligomers (Lin, Hsiao, & Chiang, 2009). We have studied the hydrolysis of chitin, colloidal chitin and water-soluble chitosan by crude enzyme solution produced by *B. amyloliquefaciens* V656 (Wang, Shih, et al., 2002) and *Pseudomonas aeruginosa* K-187 (Wang & Chang, 1997) using SCSP as the sole C/N source. These two strains were selected because the chitinases produced by strain V656 and K-187 possess novelty of being antifungal chitinase and bifunctional chitinase/lysozyme respectively. The results of investigation of hydrolysis conditions showed that regardless of V656 antifungal chitinase or K-187 chitinase/lysozyme was used for hydrolysis, the hydrolysates with 12 h of hydrolysis contained optimal $(GlcNAc)_6$ and showed higher antitumor activity. Among those chitinous materials, the most effective one was the hydrolysate of water-soluble chitosan. V656 antifungal chitinase and K-187 chitinase/lysozyme hydrolyzates inhibited the growth of the mice CT26 adenocarcinoma cells and reduced the survival rates to 34% and 80% in 1 day, respectively. V656 antifungal chitinase showed result obviously better than that of K-187 (Liang et al., 2007).

It was found that the hydrolysates of V656 antifungal chitinase inhibited the survival of CT26 cells in a concentration- and time-dependent manner (Fig. 4). The antitumor activity of pure $(GlcNAc)_6$ was far more lower than that of chitooligomer mixture (SC oligomers) in the same concentration. This phenomenon might be due to the synergistic effect of the oligomers (DP = 1–6) of GlcNAc in the hydrolysates. The hydrolysates induced characteristic DNA fragmentation of the CT26 cells. These results suggested that the hydrolysates from chitinous materials are potent apoptosis-inducing agents for CT26 cells (Liang et al., 2007).

3.6. Production of antioxidant

It has been reported that chitin, chitosan and peptide have antioxidative (Castagnino, Ottaviani, Cangiotti, Morelli, Casettari, & Muzzarelli, 2008; Wang, Chang, et al., 2010; Wang, Chen, et al., 2009; Wang, Li, et al., 2010; Wang, Lin, et al., 2009; Wang, Liou, et al., 2009; Wang, Liu, et al., 2010; Yen, Yang, & Mau, 2008) and anticarcinogenic properties (Liang et al., 2007). To increase the utilization of these chitin/protein-containing seafood processing wastes, we incubated *Bacillus subtilis* TKU006 (Wang, Chao, et al., 2009), *B. cereus* TKU018 (Wang, Wu, et al., 2009), *Serratia ureilytic* TKU013 (Wang, Lin, et al., 2009; Wang, Liu, et al., 2010), *S. marcescens* TKU011 (Wang, Yang, et al., 2009), *Serratia* sp. TKU016 (Wang, Chang, et al., 2010), and *Serratia* sp. TKU017 (Wang, Li, et al., 2010) under the optimal culture conditions and analyzed the antioxidant activity of the culture supernatant. The antioxidant activity assayed was the DPPH scavenging ability.

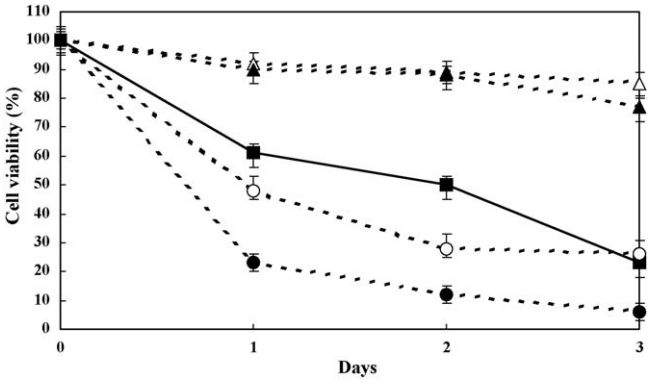

Fig. 4. Cell viability of CT26 after incubation with $(GlcNAc)_6$ (–△–, 500 μg/mL; –▲–, 1000 μg/mL), camptothecin (CAM) (—■—, 5 μM), and hydrolyzates of water-soluble chitosan (–○–, 500 μg/mL; –●–, 1000 μg/mL).

As shown in Table 7, regarding *B. subtilis* TKU006 and *S. ureilytica* TKU013, the effects of these three chitin/protein-containing wastes as the only C/N source for antioxidant production were in order of squid pen, shrimp shell, and crab shell. Among them, antioxidant activity from *S. ureilytica* TKU013 was the highest (82%), while the lowest (22%) was from *S. marcescens* TKU011. The lowest antioxidant derived from *S. marcescens* TKU011 might be associated with that the highest activity of chitosanase and protease (the 5th day) emerged 1 day after the highest antioxidant activity (the 4th day).

As shown in Table 8, most other reported antioxidant producing strains belonged to fungi. Among them, antioxidant productivity from *S. ureilytica* TKU013 was higher (82%). Besides, using squid pen, which is a waste, as C/N source is obviously different from using red bean or soybean, which is foods, by other strains. In further investigation of other chitinous materials (shrimp shell, crab shell, chitin, and chitosan) on the production of antioxidant by *S. ureilytica* TKU013, no increase the antioxidant activity was found (Wang, Lin, et al., 2009).

It is judged that the antioxidant activity might partly come from the treatment of autoclave (121 °C, 15 min), which degrades (extracts) these chitinous materials and produces some of the antioxidants. Therefore, the antioxidant activity of the culture medium (day 0) was also analyzed. The results showed the antioxidant activities (<30%) were found in the supernatants of autoclaved medium containing squid pen, shrimp shell, and crab shell, but no antioxidant activity was found in those of chitin and chitosan. Namely, the antioxidant activity partly comes from materials extracted from autoclaved chitin/protein-containing seafood processing wastes.

In addition, it is judged the chitooligosaccharides, produced by hydrolysis of chitin-containing wastes by the chitinolytic enzymes in the culture supernatant, may also have antioxidant activity. And thus we used of the method of organic fractionation as described previously (Liang et al., 2007) to recover the chitooligosaccharides (DP < 8) in the culture supernatant of *S. ureilytica* TKU013.

Table 7
Summary of the optimal culture conditions for antioxidants production by the isolated strain grown on the three chitin-containing preparations.

C/N source	Antioxidant activity (%)			
	B. subtilis TKU006[a]	*B. cereus* TKU018[b]	*S. ureilytica* TKU013[c]	*S. marcescens* TKU011[d]
Squid pen	56 (1%, 4 day)	75% (1.5%, 4 day)	82 (1.5%, 4 day)	22% (1%, 4 day)
Shrimp shell	56 (2%, 3 day)		60 (1.5%, 1 day)	
Crab shell	39 (1%, 4 day)		50 (2%, 1 day)	

[a] Wang, Chao, et al. (2009).
[b] Wang, Chen, et al. (2009).
[c] Wang, Liu, et al. (2010).
[d] Wang, Pen, et al. (2008).

Table 8
Comparison of culture conditions for the production of antioxidant by *S. ureilytica* TKU013 and other microorganisms.

Origin	C/N source	Culture time	Antioxidant activity	Reference
S. ureilytica TKU013	Squid pen	4 days	82%	Wang, Lin,Liang, Liu, and Kuo (2009)
Bacillus subtilis	Red bean	2 days	<80%	Chung et al. (2002)
Aspergillus awamori	Soybean	8 days	50%	Dyah et al. (2009)
A. awamori	Sesame	10 days	<25%	Miyake et al. (2005)
A. awamori	Black bean	3 days	80%	Lee, Hung, and Chou (2007)
A. oryzae	Soybean	8 days	50%	Dyah et al. (2009)
Monascus pilosus	PDB[a]	8 days	<40%	Kuo, Hou, Wang, Chyau, and Chen (2009)

[a] Potato dextrose broth.

Table 9
Summary of the productivity of antioxidants and enzymes by the isolated strain grown on squid pen-containing medium.

Strain	Activity of			
	Antioxidant (%)	Chitinase (U/mL)	Chitosanase (U/mL)	Protease (U/mL)
B. subtilis TKU006[a]	56 (4 day)	0.089 (2 day)		0.057 (2 day)
B. cereus TKU018[b]	75 (4 day)		0.022 (3 day)	
S. ureilytica TKU013[c,d]	82 (4 day)	0.037 (2 day)		0.200 (2 day)
S. marcescens TKU011[e,f]	22 (4 day)		0.300 (5 day)	0.400 (5 day)

[a] Wang, Chao, et al. (2009).
[b] Wang, Chen, et al. (2009).
[c] Wang, Liu, et al. (2010).
[d] Wang, Lin, et al. (2009).
[e] Wang, Yang, et al. (2009).
[f] Wang, Pen, et al. (2008).

Results showed that the antioxidant activity of this TKU013 chitooligomer mixture reached up to 65%. In order to further confirm the relationship between antioxidant activity and chitooligomers, the antioxidant activityies of *N*-acetylglucosamine oligomers with degree of polymerization (DP) of 1–6 were investigated. It was found that the maximum antioxidant activities were 32%, 37%, 25%, 38%, 47%, 25%, respectively.

Compared with the optimal enzyme (chitinase, chitosanase, and protease) production conditions, except *S. marcescens* TKU011, the difference in culture time was that the optimal enzyme production was all 1–2 days later than that of antioxidant (Table 9). Since all the culture supernatants from squid pen fermented by other strains (*B. subtilis* TKU006, *B. cereus* TKU018, and *S. ureilytica* TKU013) had antioxidant and chitinolytic activity, the increase in antioxidant activity of culture supernatant in the culture time demonstrated that antioxidant chitooligosaccharides were present in the culture supernatant. To sum up, the mixture of chitooligomers had a high antioxidant activity than pure chitooligomer, this phenomenon is also found in the antitumor activity (Liang et al., 2007).

Literature survey found that the shrimp shell wastes are rich sources of phenolic compounds. Phenolic compounds play an important role in antioxidative properties, and phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory, and vasodilatory actions. Besides this, the shrimp shell powder-fermented supernatant may also be rich in some compounds with amino groups to enhance its antioxidant properties (Wang, Li, et al., 2010). It is expected that this bioactive material-rich liquor will have beneficial biological functions owing to the inherent protein and chitin hydrolysis and other bioactive materials produced during fermentation. With these methods, we have shown that chitin wastes of shrimp, crab, and squid can be recovered and utilized. The utilization is effective in the production of enzymes and antioxidants, and therefore facilitates their potential uses in industrial applications and functional foods.

4. Conclusion

A large amount of wastes are generated from fisheries. Heads, shells, and viscera of shrimp, crab and fish can amount to as much as 80% of the raw fish material. Currently, only part of these fish wastes are made into fish meals, shrimp meals, fish extract, and shrimp extract which are mainly sold as live stock feed at rather low market prices. The discarded wastes constitute a serious source environmental pollutant. As a consequence, proper management of fishery wastes must be carried out to meet environmental requirements as well as to recover economical resource. The results of this study was primarily discovering and using precious species in Taiwan for the microbial reclamation of marine bioresources. We studied and still keep studying the bioconversion of chitin-containing seafood processing waste (such as squid pen, shrimp shell and crab shell) into useful enzyme, biofertilizer, antioxidant, antimicrobial and antitumor materials. The exploitation of bioresources is an infinite treasure. In the future, with more in-depth studies, we believe there will be more useful enzymes or bioactive compounds beneficial to human health and the environment.

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