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Review

Oligochitosan: A plant diseases vaccine—A review

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ABSTRACT

Chitosan is one of the most abundant carbohydrate biopolymers in the world. Oligochitosan prepared from chitosan is a potent plant immunity regulator. The efficacy of oligochitosan on plant disease control is presented in this review. This paper summarizes recent progress made on oligochitosan activated plant innate immunity, including: signal perception; signal transduction; oligochitosan response genes and proteins; oligochitosan induced defense-related secondary metabolites accumulation. Based on published papers and our former results, we deduce that the mode of oligochitosan act on plant is similar with general vaccines act on human and animals. So we conclude that oligochitosan is a plant disease vaccine.

Contents

1. Introduction

Chitosan buildup with β-1,4 glucosamines (Fig. 1) is one of the most abundant marine-based biopolymers. Chitosan has been used in agricultural, textile, medicinal and environmental fields due to its unique properties as a polymer and its biodegradable, non-toxic characteristics (Kurita, 2006; Rinaudo, 2006). However, chitosan is insoluble in either organic solvents or water, which greatly limits its application. This problem can be solved by hydrolysis of chitosan to water-soluble oligochitosan perfectly (Kim & Rajapakse, 2005; Yin, Du, & Zhang, 2009). So, substantial efforts have been made worldwide in developing oligochitosan and many impressive accomplishments were achieved from 1980s.

Oligochitosan has shown a wide range of biological applications, including health food, plant growth stimulator, feed additive, antimicrobial agent, etc. In addition, oligochitosan is effective at eliciting plant innate immunity against plant diseases in lots of plants such as tobacco, rapeseed, rice, grapevine, etc. (Agrawal et al., 2002; Cabrera, Messiaen, Cambier, & Van Cutsem, 2006; Chen et al., 2009; Eikemo, Stensvand, & Tronsmo, 2003; Hadwiger & Beckman, 1980; Hadwiger, Ogawa, & Kuyama, 1994; Howe, Lightner, Browse, & Ryan, 1996; Kendra, Christian, & Hadwiger, 1989; Orozco-Cardenas & Ryan, 1999; Rakwal, Tamogami, Agrawal,

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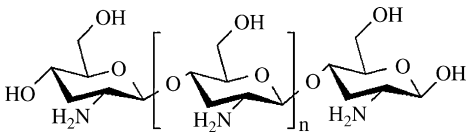

Fig. 1. Chemical structure of chitosan.

& Iwahashi, 2002). Oligochitosan, which is produced in our lab by enzyme hydrolysis and membrane separation coupling technique, is used as bio-pesticide on crop protection in several provinces in China. It has a potent protective effect on several kinds of plants such as crops, fruits, vegetables and trees (Yin, Li, Zhao, Du, & Ma, 2006; Zhao, She, Du, & Liang, 2007). From our former results and other scientific reports, we discover that oligochitosan acts on plants like common vaccines act on human and animals, so we supposed oligochitosan as a plant disease vaccine in this paper. This review aims to present an overview of the efficacy and mechanism of oligochitosan on plant immunity regulation, the latest progress and recent papers will be included.

2. Effect of oligochitosan on plant immunity

Unlike animals, plants are sessile and therefore they have developed sophisticated mechanisms to adapt to various biotic (fungi, bacteria, and insects) and abiotic (wounding, salinity, drought, salt, and cold) stresses. To resist these stresses, plants have evolved the ability to initiate various defense reactions such as hypersensitive responses, production of phytoalexins, and reinforcement of cell walls etc. The above resistant mechanism is named innate immunity (Chisholm, Coaker, Day, & Staskawicz, 2006; Jones & Dangl, 2006; Nurnberger, Brunner, Kemmerling, & Piater, 2004). Plant innate immune system comprises the mechanisms that defend plants from infection by other organisms and abiotic stresses, in a non-specific manner. In this paper, we focus on the effect of oligochitosan on plant immunity. The immunity stimulating activity of oligochitosan has been well documented in many different plant systems. Some important results will be introduced according to plant classification in the following text.

2.1. Food crops

Prof. Hadwiger in Washington State University first reported that oligochitosan can induce plant immunity in soybean plants in 1980 (Hadwiger & Beckman, 1980). When oligochitosan was applied to pea with or prior to *Fusarium solani*, the pea was protected from pathogen infection. Oligochitosan at concentration as low as 0.9 mg/ml and 3 mg/ml elicited phytoalexin induction and inhibited germination of *F. solani*, respectively. This founding leads to an increasing trend on oligochitosan and plant protection research. From then on, much excellent work has been done in this field.

Rice is the most important staple food in the world. Agrawal et al. (2002) reported that oligochitosan has the ability to stimulate defense responses in leaves of rice. They found that reactive oxygen species (ROS) accumulated in rice after treatment with 0.1% oligochitosan. Additionally, the production of phenolic secondary metabolites also was upregulated after oligochitosan treatment. In another study, enhanced resistance against rice blast pathogen *Magnaporthe grisea* was observed in *H7S* rice seedlings treated with oligochitosan. In their experiment, the ability of different concentration of oligochitosan was analyzed; 5 μg/ml oligochitosan solution showed the best result and the disease control rate was more than 50% (Lin, Hu, Zhang, Rogers, & Cai, 2005).

Wheat is the primary food in cold area, so scientists in cold country such as Russian pay much attention on it. Experiments conducted on wheat indicated that oligochitosan with a molecular weight of 5–10 kDa and 65% degree of acetylation had good efficacy on *Bipolaris sorokiniana* control (Burkhanova, Yarullina, & Maksimov, 2007; Khairullin, Yarullina, Troshina, & Akhmetova, 2001). In our lab, the ability of oligochitosan to promote wheat resistance to pathogenic toxin was validated in greenhouse experiments (Liu, Du, & Bai, 2001).

2.2. Economic crops

Tobacco is an important economic crop and model plant. Many papers reveal that oligochitosan can induce tobacco resistance to tobacco mosaic virus (TMV), tobacco necrosis virus (TNV) and *Phytophthora parasitica nicotianae*. For example, the effect of different molecular weight and deacetylation degree of oligochitosan for tobacco protection against *P. parasitica* were studied by Falcon et al. (2008). The results showed that, different kinds of oligochitosan had distinct influence on *P. parasitica* control: less acetylated oligochitosan were better for inhibition of *P. parasitica* growth but partially acetylated oligochitosan were more effective to protect tobacco against this pathogen by systemic induction of plant immunity. The effect of oligochitosan on TMV control was studied in our lab from 1990s. Oligochitosan was sprayed on tobacco leaves to inhibit TMV infection and the best disease control effect was observed when TMV inoculation was conducted 24 h after 50 μg/ml oligochitosan application (Fig. 2, Zhao, She, Du, et al., 2007).

Oligochitosan with a molecular weight of 2–6 kDa and 85% degree of deacetylation has the best immunomodulate activity on potato to resist the late blight disease (Ozeretskovskaya, Vasyukova, Panina, & Chalenko, 2006). In another experiment, potato was infected with potato virus X after oligochitosan pretreatment. It was found that, the oligochitosan treatment significantly decreased the number of systemically infected plants compared to control plants and the treated leaves also accumulated less amount of virus than the control leaves (Chirkov et al., 2001).

Rapeseed is widely applied on human food, animal feed, chemical industry and bioenergy. *Sclerotinia* rot is the most serious disease in rapeseed production. The induced resistance of oligochitosan to *Sclerotinia sclerotiorum* in rapeseed was investigated in our lab. Whereas oligochitosan did not inhibit radial growth of *S. sclerotiorum* colonies *in vitro*, it reduced the size and frequency of rot compared to untreated plants when applied to rapeseed before inoculation with *S. sclerotiorum*. The best pretreatment time was 72 h before *S. sclerotiorum* inoculation and the optimum concentration of oligochitosan was 50 μg/ml (Yin, Bai, & Du, 2008). Furthermore, Based on its physical character, oligochitosan could be modulated into steady colloid solution and used as seed coating agent. In a seed coating experiment conducted on rapeseed, oligochitosan did not influence seed sprouting and emerge, but can obviously suppress the occurrence of *S. sclerotiorum* infection, the control rate was around 40% (Lu, Qian, Peng, & Ma, 2003).

2.3. Vegetable crops

Vasiukova et al. (2001) reported that oligochitosan displayed plant immunomodulate activity by inducing local and systemic resistance of tomato to nematodes and *Phytophthora infestans*. Other studies indicated that oligochitosan had the ability to protect tomato plants against *Colletotrichum* sp. and *Fusarium oxysporum* (Zhang & Chen, 2009). For example, oligochitosan inhibited the radial growth of *Colletotrichum* sp., with marked effect when the concentration exceeds 1.5%. Furthermore, oligochitosan significantly reduced the lesion size of tomato fruits, when tomato was pretreated with 1.0% and 2.5% (w/v) oligochitosan solution 10 days before inoculated with *Colletotrichum* sp. (Munoz, Moret, & Garces, 2009).

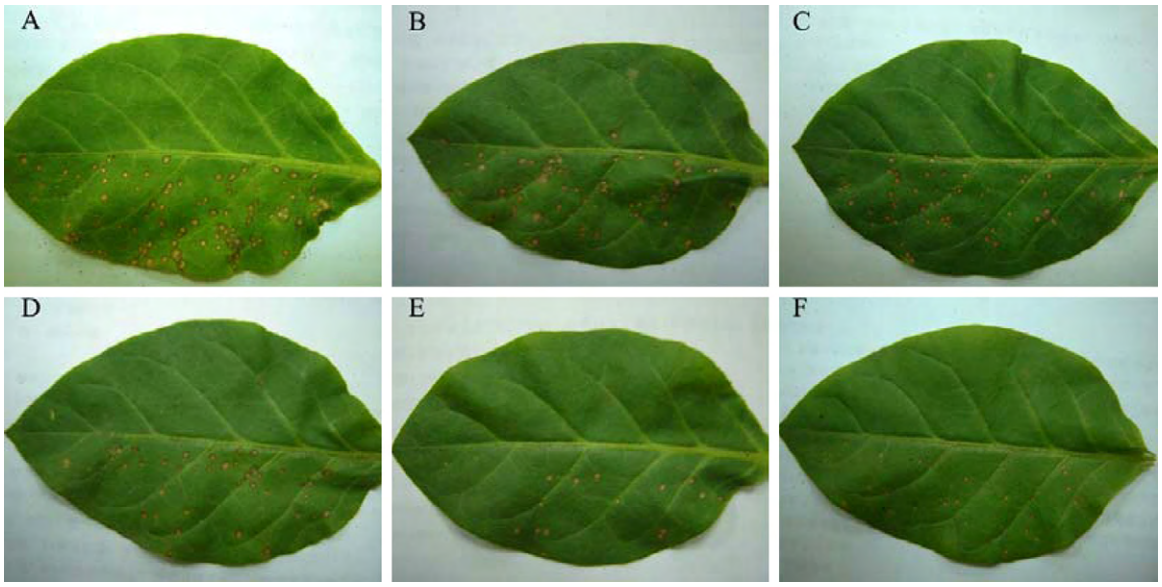

Fig. 2. The effect of oligochitosan on TMV control. (A) control; (B)–(F): 1 ppm, 10 ppm, 25 ppm, 50 ppm, 100 ppm oligochitosan treatment, respectively.

The control efficacy of oligochitosan on gray mould caused by *Botrytis cinerea* in cucumber plants was evaluated by Ben-Shalom, Ardi, Pinto, Aki, and Fallik (2003). Complete inhibition of *Botrytis conidia* germination was found at 50 μg/ml oligochitosan solution *in vitro*. Besides this fungicidal effect, oligochitosan pretreatment 1, 4 or 24 h before inoculation with *B. conidia* decreased gray mould on cucumber by 65%, 82% and 87%, respectively. However, oligochitosan spraying on the leaves 1 h after fungus inoculation decreased gray mould incidence only by 52%. These results suggest that pretreatment is crucial in oligochitosan mediated plant defense, which is similar with common vaccines act on animal. *Pythium aphanidermatum* (*Edson*) *Fitzp* is another plant pathogen which causes root and crown rot in cucumber. Recently, an interesting study showed that oligochitosan couldnot induce defense on cucumber to this pathogen when used alone. However, application of oligochitosan in combination with *Lysobacter enzymogenes 3.1T8* (a biocontrol bacteria) reduced the rate of diseased plants by 50–100% (Postma, Stevens, Wiegers, Davelaar, & Nijhuis, 2009). This result suggests that addition of oligochitosan can enhance the protective efficacy of other biocontrol agents.

2.4. Fruits

Grape is a familiar nourishing fruit; it can be eaten raw or used for making jam, juice, wine, etc. However, grape production and quality is severely harmed by many pathogens such as *B. cinerea*. Oligochitosan is used as biopesticide in many grape producing countries. Aziz first reported that oligochitosan with 1500 Da molecular weight and 80% deacetylation degree dramatically reduced the infection of grapevine leaves by *Plasmopara viticola* and *B. cinerea* at 200 μg/ml treatment. Dose-response experiments showed that the greatest control effect of *B. cinerea* was achieved with 75–150 μg/ml oligochitosan. However, higher concentrations of oligochitosan did not present greater efficiency but oligochitosan and bluestone co-treatment had good effect (Aziz et al., 2006; Trotel-Aziz, Couderchet, Vernet, & Aziz, 2006). Similarly, oligochitosan can induce plant immunity on apple and watermelon. The experiments conducted in our lab recently showed that oligochitosan pretreatment could elicit apple to resist canker and alternaria leaf diseases. Both the control rate of these two diseases is higher than 90% (Zhao & Du, unpublished paper).

Besides the plants and diseases we mentioned hereinbefore, it has also been reported that oligochitosan can activate plant defense to other diseases on several other plants such as barley (Faoro, Maffi, Cantu, & Iriti, 2008), pearl millet (Manjunatha, Roopa, Prashanth, & Shetty, 2008), carrot (Molloy, Cheah, & Koolaard, 2004), sunflower (Nandeeshkumar et al., 2008), coconut (Lizama-Uc et al., 2007), araucaria (Dos Santos, El Gueddari, Trombotto, & Moerschbacher, 2008), etc. In the general survey in more than 20 provinces in China, we found that oligochitosan had plant immunity regulation activity in 24 plants (Table 1). All these results validated that oligochitosan is a potent plant immunomodulator.

3. Mechanism of oligochitosan on plant immunity regulation

Though many application experiments were conducted, the mechanisms of oligochitosan on plant immunity regulation remain to be elucidated. This aspect will be described detailed in the following paragraphs based on our research results and other literatures.

3.1. Signal perception

Signal perception is the first challenge for plant immune system startup (Akira, Uematsu, & Takeuchi, 2006). Perception of elicitors by an array of pattern recognition receptors comprises the basis for communication between plants and elicitors in a number of studied plant systems (Zipfel, 2008).

The receptors of oligochitin (acetylated product of oligochitosan) were found on several plants. Shibuya, Kaku, Kuchitsu, and Maliarik (1993) first reported that there were high-affinity binding site of octa-N-acetylchitopentaose at the surface of rice cells as well as in microsomal membranes prepared from these cells. From then on, high-affinity binding proteins of oligochitin were identified from rice, soybean, wheat, barley and carrot by photo-affinity labeling and affinity cross-linking of carbohydrate and protein techniques (Day et al., 2001; Okada, Matsumura, Ito, & Shibuya, 2002; Shibuya et al., 1996; Yamaguchi, Ito, & Shibuya, 2000).

Further results were acquired in recent years. Kaku et al. (2006) purified an oligochitin-binding protein (CEBiP) from plasma membrane of rice cells and cloned its full length gene. Structural analysis indicated that CEBiP did not contain any intracellular domains, which suggests that another component is required for oligochitin

Table 1
The effect of oligochitosan on plant diseases control (survey in China).

Plants	Plant diseases	Control rates (%)
Tobacco	Tobacco mosaic virus, Potato virus	76.49–87.5
Panax notoginseng	Virus disease	>90
Tulip	Peronospora	84.24–88.6
Piper nigrum L.	Mosaic virus	73.54–81.3
Tomato	Solani, infestans, virus disease, bacterial wilt	84.47–88–24
Cucumber	Peronospora	78.96–82.65
Pepper	Virus diseases, blight, anthracnose, Pepper phytophthora blight	78.58–90
Egg plant	Virus diseases	93.18–100
Chinese cabbage	*Erwinia carotovora* subsp	78.62–85
Asparagusplettuce	Peronospora	45.8–62.3
Wax gourd	Blight	84.81–95
Cauliflower	Black rot	63.6–64.2
Geen cucumber	Blight	45.5–57.6
Cowpea	Virus diseases	31.13–58.8
Papaya	Mosaic virus	70–96
Watermelon	Black rot, Didym ella bryoniae, light, virus diseases	81.71–85.40
Muskmelon	Powdery mildew	71.34–86.26
Banana	Bunchy top	83.7–94.6
Apple	Mosaic virus, Venturia inaequalis	76.68–93.85
Soybean	Virus diseases	75.1–100
Cotton plant	Cotton yellow wilt	85.5–87.2
Maize	Sphacelotheca Reiliana, corn northern leaf blight, corn southern leaf blight	23.9–45. 35
Rice	Rice blast	71.41–92.0
Peanut	Virus diseases	23.9–26.5

signal transduction through the rice plasma membrane into the cytoplasm. According to this suggestion, two research groups found a receptor-like kinase designated CERK1 or LysM RLK1 which was cooperate with CEBiP for oligochitin elicitor signaling in *Arabidopsis* (Miya et al., 2007; Wan et al., 2008).

Interestingly, research papers about the binding protein or receptor of oligochitosan is few. All the former identified oligochitin-binding proteins donot have specific binding site with oligochitosan. It is suggested that acetyl group play an important role in this carbohydrate–protein interaction. In published papers, only a lectin specific binding protein for oligochitosan has been purified by chitosan affinity chromatography from cultured cells of *Rubus* by Lienart, Gautier, and Domard, 1991. However, whether there are binding proteins for oligochitosan on other plants is still unknown. In our lab, we found there were high specific affinity binding of oligochitosan to strawberry, tobacco and rapeseed by using fluorescent labeled oligochitosan (Guo et al., 2009; Zhao, She, Yu, Liang, & Du, 2007). Time dependent manner was also observed in these experiments (Fig. 3). The identification of these binding proteins is in process.

Besides the signal reception via receptors, some literatures report another mode of oligochitosan signal perception. Oligochitosan has been shown to enter the plant nucleus and has the ability to act at the chromatin level directly (Choi, Klosterman, & Hadwiger, 2001; Hadwiger, 2008). A recent paper showed that oligochitosan released from *F. solani* has the ability to alter pea nucleosome organization. The highly positive charged oligochitosan possesses a high-affinity with the negative charged nuclear DNA. This character might lead oligochitosan to compete with histones and other nuclear proteins for binding sites on the plant DNA. The researchers suggested that the histone H2A/H2B release and the consequently chromatin conformational changes in the vicinity of the PR (pathogenesis related) genes probably will activate the PR genes without the requirement for specific transcription factors (Isaac, Hartney, Druffel, & Hadwiger, 2009).

3.2. Signal transduction

Once oligochitosan signal enter the cytoplasm, the oligochitosan signaling transmit quickly and develop a complicated signaling network. This network is so complex that it still remains unclear though much work has been done. According to published papers, Ca^{2+}, ROS, nitric oxide (NO), jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) all involved in oligochitosan activated signal pathway.

Kluesener et al. (2002) reported that oligochitosan elevated free cytosolic calcium concentration in guard cells of *Arabidopsis*. In another study carried out with cotton, Ca^{2+} channel blocker $LaCl_3$ or calmodulin antagonist trifluoroacetic acid co-treated with oligochitosan (DP = 3–7) depressed defense-related enzyme activities induced by oligochitosan (Guo, Bai, Du, & Li, 2004). These results revealed that Ca^{2+} is an important factor on oligochitosan signal transduction.

ROS and NO are also considered as primary defense signaling molecules (Bolwell, 1999; Delledonne, Zeier, Marocco, & Lamb, 2001; Zaninotto, La Camera, Polverari, & Delledonne, 2006). Among different ROS, hydrogen peroxide (H_2O_2) is one kind of the most important ROS; it can cross membranes and directly effect cell sig-

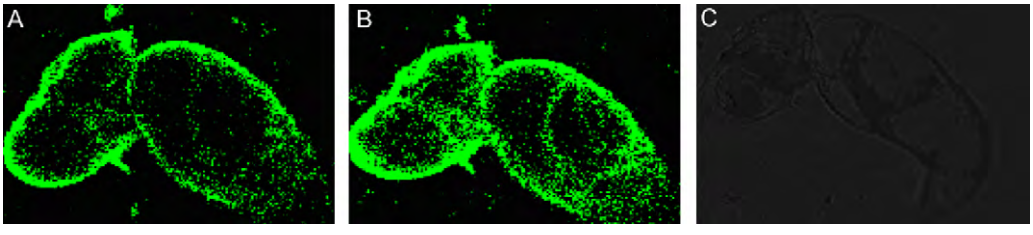

Fig. 3. The binding process of labeled oligochitosan to strawberry cells. (A) the binding of 2-AMAC-oligochitosan to strawberry cells in 2 min; (B) the binding of 2-AMAC-oligochitosan to strawberry cells in 10 min; (C) strawberry cells screened with visible light.

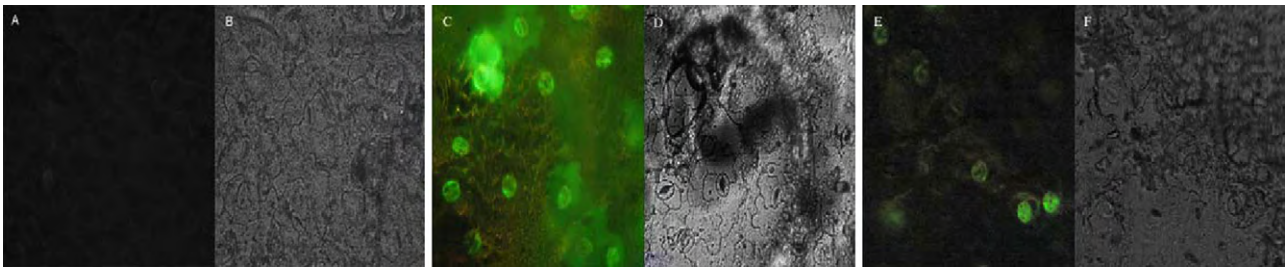

Fig. 4. Oligochitosan induced production of H_2O_2 and NO in epidermal cells of *Brassica napus* leaves: (A, C and E) fluorescent images; (B, D and F) bright field images; (A and B) control; (C and D) oligochitosan treated and H2DCF-DA labeled; (E and F): oligochitosan treated and DAF-2DA labeled.

naling. Production of H_2O_2 and NO in epidermal cells of rapeseed were observed shortly after oligochitosan treatment in our lab by using fluorescent probe H2DCF-DA and DAF-2DA (Fig. 4). And the interaction between H_2O_2 and NO had been investigated by using *L*-NAME (an inhibitor of NOS that also inhibits plant NO synthesis) and catalase (an H_2O_2 scavenger), respectively (Li et al., 2009).

Dose-response was observed during oligochitosan induced accumulation of H_2O_2 in many plants. For example, when oligochitosan was applied to rice cells, the generation of H_2O_2 could be detected when the concentration of oligochitosan was 15 mg/ml, reached maximum at 60 mg/ml and decreased at higher concentrations. A time-dependent response was also observed in the same experiment: the production of H_2O_2 reached the peak at near 1 h, and then decreased over the next 2 h (Lin et al., 2005). In another experiment, Srivastava found that chitosan (the polymer of oligochitosan) could induce NO and ROS production in abaxial epidermis of *Pisum sativum*. The calcium chelators, BAPTA-AM or BAPTA, have no marked effect on chitosan induced NO or ROS, indicated that Ca^{2+} didnot participate in this chitosan induced NO or ROS generation process in *P. sativum* (Srivastava, Gonugunta, Puli, & Raghavendra, 2009).

SA and JA are essential plant hormones required for signal transduction leading to plant resistance (Koornneef & Pieterse, 2008; Loake & Grant, 2007; Reymond & Farmer, 1998), SA mediates systemic acquired resistance (SAR) and JA mediates induced systemic resistance (ISR). Previous studies showed that oligochitosan could elicit plant hormones especially JA production in plants. Doares, Syrovets, Weiler, and Ryan, 1995 reported JA levels in leaves of tomato increased 3-fold within 2 h after oligochitosan (DP = 4) treatment. Further results were got in rice, 12-oxo-phytodieonic acid (OPDA, precursor of JA) and JA accumulation was detected within 3 min in oligochitosan treated rice leaves. OPDA peaked quickly around 5 min and returned to its basal level within 15 min, whereas JA induction upon oligochitosan treatment peaked at 1 h and decreased within 6 h (Rakwal et al., 2002). The concentrations of JA in oligochitosan treated tobacco leaves were determined by high performance liquid chromatography with a coulometric array detector in our lab. The concentration of JA reached the peak value after being treated for 6 h (Du, Li, & Guo, 2003). All these results suggested that oligochitosan activates plant immunity through the JA/ET pathway in some plant systems. However, other reports mentioned a contrary effect. For example, Obara, Hasegawa, and Kodama, 2002 found MeSA (methyl-salicylic acid) accumulated in rice leaves 7 h after oligochitosan treatment. MeSA is a derivative of SA which plays an important role in SA mediated SAR.

ABA is another important plant hormone that participates in plant diseases and stress resistance (Leung & Giraudat, 1998; Ton, Flors, & Mauch-Mani, 2009). The role of ABA in oligochitosan induced plant immunity was studied by Iriti recently (Iriti & Faoro, 2008; Iriti et al., 2009). Oligochitosan application induced ABA accumulation in tobacco leaf tissues 24 h after treatment with oligochitosan, and activated resistance against TNV. However, treatment with the nordihydro-guaiaretic acid (an ABA inhibitor) before oligochitosan application will reduce tobacco resistance to TNV. This experiment indicates that ABA also plays an important role in oligochitosan induced plant defense mechanism.

In general, these results suggest that oligochitosan defense signaling is mediated by several signal molecules. This phenomenon might roots in the variety of plant–disease interaction. It also proves that oligochitosan is a broad spectrum plant immunomodulator.

3.3. Oligochitosan response genes and proteins

Oligochitosan response genes is a research hotspot of oligochitosan induced plant immunity mechanism. Many significant defense/signal-related genes such as *MAPK* (Yin, Bai, Zhao, & Du, 2010), *SKP1* (Zhang et al., 2007), *OPR1* (Jang et al., 2009), *WRKY* (Hofmann, Sinha, Proels, & Roitsch, 2008) were found to be upregulated by oligochitosan in rice, tobacco, rapeseed, etc.

Besides single gene cloning, high-throughput techniques were used for oligochitosan response genes identification. In our lab, 96 tobacco genes which regulated by oligochitosan were identified by using mRNA differential display. Among these genes, a novel Ser/Thr protein kinase gene was isolated and designated as oligochitosan induced protein kinase (*oipk*) (Feng et al., 2006). A plant *oipk* antisense expression vector was constructed and transformed into tobacco by *Agrobacterium tumefaciens*. Decreased phenylalanine ammonia-lyase (PAL) activity and resistance to TMV were observed in this transgenic tobacco (Yafei et al., 2009). These results indicated that *oipk* was involved in the oligochitosan mediated signaling in tobacco.

Since its appearance in 1996, DNA microarray has been widely used for monitoring gene expression. A rapeseed cDNA microarray containing 8095 ESTs was used to analyze the gene transcript changes in *Brassica napus* elicited by oligochitosan. Transcript levels for 393 genes altered 2-fold or more in oligochitosan treated plants compared to control plants. Among these regulated genes, 136 were induced and 257 were repressed. These 393 genes were involved in different processes and had different functions including primary metabolism, transcription, defense, signal transduction and etc. An important JA synthase gene (OPR3); some genes that regulated the JA/ET pathway (such as *MPK4*, *EREBP*) and several JA-mediated genes were induced by oligochitosan, suggesting that oligochitosan activated the plant innate immunity in rapeseed through JA/ET signaling pathway (Yin et al., 2006).

Lots of literatures showed that oligochitosan can induce some defense-related proteins, enzymes and their activity such as glucanase, chitinase, PAL, etc. For example, bioassay in rice demonstrated that oligochitosan could induce higher levels of glucanase activity than in control seedlings (Rodriguez et al., 2007). Similar results were got in wheat (Burkhanova et al., 2007; Yusupova, Akhmetova, Khairullin, & Maksimov, 2005), tobacco (Falcon et al., 2008) and soybean (Khan, Prithiviraj, & Smith, 2003). In our lab, we found the activities of PAL, peroxidase, catalase, polyphenolox-

Table 2
Comparison of oligochitosan and general animal vaccines.

Content	Animal vaccines	Oligochitosan
Immunity inoculated effect	Yes	Yes
Specificity of resistance	Yes	No
Microbe-associated molecular patterns (MAMP)	Yes	Yes
Pattern recognition receptors	Yes	Yes
Signaling molecules	Nitric oxide (NO)	NO
	Reactive oxygen species (ROS)	ROS
	Salicylic acid (SA)	SA
Kinase mediated signaling	Yes	Yes
Resistant gene family	Immunoglobulin gene superfamily	PR gene family
Specialized antigen-presenting cells	Yes	Unknown
Autoantibodies	Yes	Unknown

idase and superoxide diamutase in rapeseed and tobacco leaves were upregulated after oligochitosan treatment (Yafei et al., 2009; Yin, Zhao, & Du, 2008).

Nowadays, proteomics has become one of the most powerful tools for identifying response proteins. In order to get more information about plasma membrane proteins involved in oligochitosan induced rice immunity, plasma membrane proteomic analysis of rice was conducted after oligochitosan treatment (Chen, Li, & He, 2007). A total of 14 up- or down- regulated proteins were observed at 12 h or 24 h after oligochitosan treatment. Mostly of these proteins were related to signal transduction. In addition, Ferri found that 50 mg/ml chitosan treatment changed the expression level of 73 proteins in treated samples compared with controls by using Two Dimensional Gel Electrophoresis. Among these 73 proteins, 11 proteins that belong to PR protein-10 family were strongly upregulated (Ferri et al., 2009).

3.4. Defense-related secondary metabolites accumulation

The effect of oligochitosan on plant defense-related secondary metabolites production was studied from 1980s. In 1980, Hadwiger (Hadwiger & Beckman, 1980) found oligochitosan from *F. solani* cell walls could induce pisatin (a phytoalexin) in soybean pod in 24 h. Similar results were got in parsley (Conrath, Domard, & Kauss, 1989) and *Catharantus roseus* (Kauss, Jeblick, & Domard, 1989). The elicitation of coumarins accumulated in *Ruta graveolens* shoots after treatment of oligochitosan was studied by Orlita et al. (2008). 0.1% oligochitosan induced production of not only simple coumarins but also furanocoumarins and dihydrofuranocoumarins. In a recently published paper, the level and composition of volatile secondary metabolites in tomato leaves were analyzed after oligochitosan treatment (Zhang & Chen, 2009). Compared with the control, the amount of volatiles from old and adult leaves was dramatically increased by oligochitosan. Furthermore, the antifungal activity of these volatiles against spore germination and hyphal growth by *B. cinerea* and *F. oxysporum* was significantly enhanced.

Besides these phytoalexins, oligochitosan also can elicit the production of lignin (Bautista-Banos et al., 2006). Through deposition of lignin, the plant cell wall will strengthen. Bhaskara Reddy found that synthesis of phenolic acids was stimulated in primary wheat leaves following oligochitosan treatment, and levels of these phenolic acids increased significantly with increasing oligochitosan concentration. The synthesis of precursors of lignin such as ferulic, *p*-coumaric and sinapic acids having antimicrobial activity like benzoic, caffeic, protocatechuic, and chlorogenic were also stimulated by oligochitosan treatment (Bhaskara Reddy, Arul, Angers, & Couture, 1999). In their experiment, lignin content of primary leaves also showed a similar upregulated pattern. In another study, lignin deposition in the root cell wall of tomato increased 2.8, 5.1 and 6.8 times at 12, 24 and 36 h after oligochitosan elicitation, respectively (Mandal & Mitra, 2007). Vander, Varum, Domard, El Gueddari, and Moerschbacher, 1998 studied the effect of oligochitin and oligochitosan (several kinds of different molecular weight and deacetylation degree) on elicit lignin deposition in wheat leaves. They found that all oligochitosan but not oligochitin induced the deposition of lignin, suggesting that oligochitosan is more effective on inducing structural resistance in wheat.

The mechanism of oligochitosan induced plant immunity is still elusive though much work has been done. From existing publications, only the tip of the iceberg was investigated and many questions remain unanswered, such as the identification of oligochitosan receptors and the identification of integrated signaling pathway induced by oligochitosan in plants, etc. So, further research is required on the mechanisms of oligochitosan induced plant immunity.

4. Concluding remarks

In general, there are four characteristics of oligochitosan mediated plant immunity:

(1) Oligochitosan is effective on several plant–disease interactions, a survey in China suggests that oligochitosan can produce resistance to more than 30 diseases on 24 plants (Table 1).
(2) Aiming to different diseases, the most effective concentration, deacetylation degree and polymerization degree of oligochitosan are different. This phenomenon might originate from the distinct action mechanism of different plant–pathogen interactions.
(3) Oligochitosan can be used in cooperation with other pesticides and biocontrol agents.
(4) Pre-treat plants with oligochitosan before pathogen infection will get good control efficacy than oligochitosan treatment after pathogen infection. This is in accordance with the PRIME mechanism in induced systemic resistance of plants. PRIME mechanism is put forward during the past several years (Conrath et al., 2006; Jung, Tschaplinski, Wang, Glazebrook, & Greenberg, 2009; Pare et al., 2005). It has been demonstrated that pretreatment of plants with elicitor of systemic resistance will prime the plants for stronger elicitation when it suffers to plant disease sometime later. This characteristic is similar with general animal vaccines and the comparison of animal and plant immune systems can be found in Table 2.

Based on the comparability, we deduce oligochitosan as a potential plant disease vaccine. To our knowledge, this is the first paper to put forward the conception of plant disease vaccine. However, the details of this inference remain to be further studied.

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