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# MICROBIAL CHITINASES: ADVANCES IN RESEARCH AND POTENTIAL APPLICATIONS IN AGRICULTURE

**MOHAMMED KUDDUS**

Department of Biochemistry, University of Hail, PO Box 2440, Hail, Kingdom of Saudi Arabia

***Corresponding Author: Email: mkuddus@gmail.com; kuddus_biotech@yahoo.com;**

**Phone: +966504984419; Fax: 966-6-535-8250**

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## ABSTRACT

The chitinase enzymes, present in various organisms, including bacteria, fungi, insects and crustaceans, hydrolize β-1-4-linkage in the N-acetyl-D-glucosamine polymer of chitin which is a major component of fungal cell walls and arthropod exoskeletons. Therefore, they assist in fungal cell wall degradation and play major role in the plant defense by attacking fungal pathogens. The agricultural pests and pathogens are well known candidate that causes severe problems worldwide in the cultivation of economically important plants. Due to the harmful effect of chemical pesticides on the environment and development of resistance, it is needed to develop ecofriendly biopesticides to control agricultural pests and pathogens. Chitinases from microorganisms have wide range of biotechnological applications, especially in agriculture for biocontrol of phytopathogenic fungi and harmful insects. The present article includes recent development in microbial chitinase production and its applications in food and agriculture sectors.

**Keywords**: **Chitinase, chitinolytic microorganism, antifungal, biopesticides, chito-oligosaccharides**

## INTRODUCTION

In recent years, a significant amount of research on microbial chitinases has been carried out due to their important biophysiological functions and applications. Chitinases (EC 3.2.1.14) can be classified as endochitinases or exochitinases based on their mode of action on chitin or chitioligomers. Endochitinases

cleave chitin at internal sites to generate multimers of N-acetyl-D-glucosamine (NAG or GlcNAc). However, exochitinases catalyze the hydrolysis of chitin progressively to produce GlcNAc, chitobiose or chitotriose. Chitinases belong to two major families of carbohydrate enzymes, family 18 and family 19, depending on their sequence similarities, structure and mechanism of action. Family 18 includes chitinases found in bacteria, fungi, viruses, and animals, and class III or V of plant chitinases. Family 19 includes class I, II and IV chitinases of plant origin only, with exception of chitinase C from *Streptomyces griseus* [1] and chitinases F and G from *Streptomyces coelicolor* [2]. Chitinolytic enzymes have wide range of applications such as preparation of pharmaceutically important chitooligosaccharides and NAG, preparation of single-cell protein, isolation of protoplasts from fungi and yeast, control of pathogenic fungi, treatment of chitinous waste, and control of malaria transmission [3]. The production of inexpensive chitinolytic enzymes is important in the use of chitin containing waste particularly in the sea food industry, which not only can solve environmental problem but also do with added value in certain cases [4]. Chito-oligomers produced by enzymatic hydrolysis of chitin is useful for applications in various fields like in medical, agricultural and industrial applications, such as antibacterial, antifungal, hypocholesterolemic, antihypertensive activity, and as a food quality enhancer [5]. In recent year, efforts have been going on throughout the world to enhance the production of chitinase and isolation of gene(s) encoding for the chitinase enzyme to be utilized as canditate gene(s) to combat with fungal pathogens [6-7].

More than 100,000 kinds of fungi exist, of which about 8,000 fungi can cause disease in plants, and a relatively small number of them cause disease in humans and livestock [8]. Almost all the agricultural and horticultural crop species suffer severe yield losses due to fungal diseases. To minimize losses due to field crop diseases, one should identify the diseases and conditions that favor disease development and prepare management plan that are effective, practical, safe, and economical. Pesticides and organic compounds are widely used to control plant pathogens in many countries. However, the non-degradable components of their compounds have accumulated over the years and entered the food chain causing higher toxicity in animals and environment [9]. It also led to buildup of resistance of the pathogens to these fungicides. Hence, to

tackle this global problem, it is compelling to look for alternative and effective method for disease management practices, which include the use of antagonistic microbes as bicontrol agents and their metabolic products such as enzymes. The development of enzymatic and/or microbiological approaches for the control of plant pathogenic fungi is extremely urged as they offer a safe, cheap and ecofriendly method. The antagonistic activity against fungal pathogens is usually related to the production of antifungal compounds [10] and extracellular hydrolytic enzymes [11]. Chitinase and β-1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls, as for example, cell walls of *Fusarium oxysporum* and *Sclerotinia minor* [12]. Microbial degradation of chitin offers best solution for the problem leading to recycling of nutrients in the environment along with generating of useful products *viz.* chitinase, chito-oligosaccharides, N-Acetyl-D-glucosamine and single cell protein [13]. Most of the chitin is used as raw material for the production of the monosaccharide, which is the number one dietary supplement in the USA, used for pain relief of osteoarthritis [14]. Potential uses of naturally occurring bacteria, actinomycetes and fungi replacement or supplements for chemical pesticides have been addressed in many studies [15].

**PRODUCTION OF MICROBIAL CHITINASE**

Fermentation technology plays a very important role in the field of enzymology; not only for the production of various enzymes but also it reduces cost of production. For the production of chitinase enzymes, microorganisms are the preferred source, because they have shortest generation time, high yield of conversion of substrate into product, great versatility to environmental conditions, and simplicity in genetic manipulation and in cultivation conditions. Due to habitats multiplicity, microorganisms usually produce various types of chitinase, with distinct specificity regarding to substrate utilization and also to optimum pH and temperature range. Bacteria like *Serratia marcescens, Xanthomonas maltophilia, Stenotrophomonas maltophilia* and *Paenibacillus illinoisensis* have been proved as potent chitinolytic bacterial agents [16-17]. *Myrothecium verrucavia* [18] and *Trichoderma* sp. [19] were found as main source of chitinase among fungi. Submerged fermentation systems are attractive for cultivation of microorganism and production of biological products due to easy modification in cultivation conditions like pH, dissolved oxygen,

temperature, agitation, and nutrient concentration. A list of chitinase producing microorganisms and their production parameters are presented in table 1.

**BIOCONTROL OF FUNGAL PHYTOPATHOGEN**

The fungal plant pathogen causes severe problems worldwide in the cultivation of economically important plants, especially in the tropical and subtropical regions [39]. Chitinase enzymes are able to lyse the cell wall of numerous fungi. The microbes which produce these enzymes are capable of eradicating fungal diseases that are major problem for global agricultural production (Table 2). Normaly chemical fungicides are used to reduce fungal pathogens. Though, the extreme use of chemical fungicides led to problems related to contamination and degradation of the natural environment, along with induced pathogen resistance. These chemical compounds may be toxic to beneficial insects and microorganisms in the soil, and may also enter into the food chain [61]. Biological control of plant pathogens by soil bacteria is a well established phenomenon and chitinase production has been shown to play an important role in the suppression of various diseases [62]. Roberts and Selitrennikof [63] reported that plant and bacterial chitinases shows different antifungal activity, due to presence of two different classes of enzymes which shows different mechanisms of action. For example plant chitinases functioned as endochitinases in comparison to bacterial chitinases that work as exochitinases. Bolar et al. [64] studied synergistic activity of endochitinase and exochitinase from *Trichoderma atroviride* against the pathogenic fungus (*Venturia inaequalis*) in transgenic apple plants.

**CHITINASE GENE IN TRANSGENIC PLANTS**

To increase the antifungal potential of plants, transgenic plants are generated by transferring chitinase gene(s) from microorganisms. The inserted gene sequence is known as the transgene. There are numerious available literatures on molecular cloning and expression of chitinase gene; and the production of transgenic plants to get resistance against pathogens (Table 3). Most of the chitinases from chitinolytic bacteria, such as the *chiA* gene produced from *Serratia marcescens* and *Enterobacter agglomerans*, are potential agents for the biological control of plant diseases caused by various phytopathogenic fungi [78]. The chitinase genes expressed in *baculovirus* showed an increased killing rate of insect pathogens [79].

**Table 1: Fermentation conditions for chitinase production (published from 2005 onward)**

| Microorganisms | Incubation period (h) | Optimum Temp (°C) | Optimum pH | Conc./ Substrate | Reference |
|---|---|---|---|---|---|
| *Vibrio alginolyticus* | 72 h | 30 | 6.5 | 0.3% CC* | [20] |
| *Serratia marcescens* | 72 h | 30 | 7 | 1% CC | [21] |
| *Serratia marcescens* | 144 h | 30 | 6 | 2% CC | [22] |
| *Streptomyces* sp. | NM** | 30 | 7 | 0.4% chitin | [23] |
| *Serratia marcescens* | 32 h | 32 | 8 | 0.75% CC | [24] |
| *Bacillius subtilis* | 96 h | 35 | 7 | 0.3% CC | [25] |
| *Serratia marcescens* | 92 h | 30 | NM | 1.7% Chitin | [26] |
| *Bacillus cereus* | 36 h | 55 | 7 | NM | [27] |
| *Bacillus* sp. | 72 h | 55 | 7 | 0.5% CC | [28] |
| *Micrococcus* sp. | 42 h | 35 | 8 | NM | [29] |
| *Serratia marcescens* | NM | 30 | 7.9 | 1% Chitin | [30] |
| *Paenibacillius* sp. | NM | 30 | 7 | NM | [31] |
| *Streptomyces* sp. | 60 h | 35 | 6 | 1% Chitin | [32] |
| *Streptomyces* sp. | 72 h | 30-35 | 7 | 1.6% CC | [33] |
| *S. canus* | 120 h | 40-60 | 8 | 1.2% CC | [34] |
| *S. Pseudogriseolus* | 132 h | 40-50 | 8 | 1% CC | [34] |
| *Aeromonas* sp. | 48 h | 30 | 8 | 0.7% CC | [35] |
| *Bacillus laterosprous* | NM | 35 | 8 | 0.3% CC | [36] |
| *S. venezuelae* | 96 h | 30 | NM | 0.6% CC | [11] |
| *Bacillus* sp. | 72 h | 37 | 6.5 | 1% Chitin | [37] |
| *Enterobacter* sp. | 48 h | 30 | 7 | 5 % CC | [38] |
| *Zymomonas* sp. | 48 h | 30 | 6 | 15 % CC | [38] |

*CC= Colloidal chitin, **NM = Not mentioned

**Table 2: Bacterial agents for biocontrol of fungal plant pathogens (published from 2005 onward)**

| Microbes | Pathogen | Reference |
|---|---|---|
| *Penicillium ochrochloron* | *Fusarium oxysporum* | [40] |
| *Streptomyces* sp. | *Candida* sp. | [41] |
| *Aspergillus niger* | *Fusarium culmorum, Fusarium solani, Rhizoctonia solani* | [42] |
| *Trichoderma viride* | *Rhizoctonia solani, Fusarium oxysporum, Pythium ultimum* | [43] |
| *Streptomyces* sp. | *Fusarium oxysporum, Corynesopra cassiicola* | [44] |
| *Streptomyces hygroscopicus* | *Rhizoctonia solani, Fusarium oxysporum, Alternaria alternate, Aspergillus niger, Sclerotinia scleotiorum* | [45] |
| *Serratia marcescens* | *Rhizoctonia solani, Bipolaris* sp., *Alternaria raphani* | [46] |
| *Bacillus subtilis* | *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum.* | [25] |
| *Bacillus* strain | *E. coli, S. pyogenes, Klebsiella pneumonia, Lactococcus* sp., *A. niger, C. albicans, S. cerevisae* | [47] |
| *Pseudomonas* sp. | *Fusarium oxysporum* | [48] |
| *Bacillus amyloliquefaciens* | *Ralstonia solanacearum* | [49] |
| *Bacillus pumilus* | *Rhizoctonia solani, Verticillium* sp., *Nigrospora* sp., *Stemphyllium botryosum, Bipolaris* sp. | [50] |
| *Serratia marcescens* | *Fusarium solaini, Aspergillus flavus* | [51] |
| *Streptomyces griseus* | *Fusarium oxysporum, Alternari alternate, Rhizoctonia solani, Fusarium solani, Aspergillus flavus* | [52] |
| *Trichoderma* sp. | *Sclerotium rolfsii, Fusarium ciceri* | [53] |
| *Bacillus licheniformis* | *Gibberella saubinetii, Aspergillus niger* | [54] |
| *Streptomyces* sp. | *Aspergillus niger, Candida albicans* | [55] |
| *Streptomyces hygroscopicus* | *Sclerotium rolfsii, Colletotrichum gloeosporioides* | [56] |
| *Streptomyces venezuelae* | *Aspergillus niger, Alternaria alternate, Helminthosporium sativum* | [11] |
| *Enterobacter* sp. | *Fusarium moniliforme, Aspergillus niger, Mucor rouxi, Rhizopus nigricans* | [57] |
| *Bacillus amyloliquefaciens* | *Rhizoctonia solani* | [58] |
| *Trichoderma* sp. | Phytopathogenic fungi | [59] |
| *Bacillus* sp. | *Fusarium incarnatum* | [60] |
| *Stenotrophomonas maltophilia* | *Bipolaris sorokiniana* | [16] |

**Table 3: Biocontrol of fungus in transgenic plant**

| Gene/Agent | Target pathogen/Disease | Plant | Reference |
|---|---|---|---|
| EgCHI1, EgCHI2, EgCHI3 | *Ganoderma boninense, Trichoderma harzianum* | Oil palm leaves | [65] |
| *Chitinase* (chi ) | *Verticillium dahliae* | Cotton | [66] |
| Chitinase gene | *Fusarium graminearum* | Wheat | [67] |
| Endochitinase | *Alternaria* sp. | Broccoli | [68] |
| Class-1 chitinase | *R. solani* | Indica rice | [69] |
| Endochitinase (chit42) | *Alternaria alternata, A. solani, Botytis cinerea, R. solani, Sphaerotheca humuli* | Potato | [70] |
| Chitinase ($RCC_2$) | *Botrytis cinerea* | Cucumber | [71] |
| Chitinase gene | Black spot disease (*Diplocarpon rosae* wolf) | Rose | [72] |
| Bean chitinase promoter | *Botrytis cinerea, Rhizoctonia solani, Sclerotium rolfsii* | Tobacco | [73] |
| *LOC_Os11g47510* | *Rhizoctonia solani* | Rice | [74] |
| Endo-chitinase gene | *Alternaria solani* | Potato | [75] |
| Chitinase gene | *Spodoptera littoralis* | Maize | [76] |
| Chitinase *chi11* | *Rhizoctonia solani* Kuhn | Rice | [77] |

## CHITINASE AS A BIOPESTICIDE

In an agricultural system, pesticides are used to protect plants/crops from damage by insects and disease. However, the use of chemical pesticides on the crops has resulted in significant harmful effects to public health and the environment also. Therefore, the control of crop pests by the use of biological agents, such as enzymes and microbes, has a great potential as an alternative to the use of chemicals. Accordingly crude chitinase from microorganisms have been used to control crop pest population [80]. Chitinase produced by *Bacillus* sp. are potential for biocontrol of *Aphis gossypii* [81]. Koga [82] spray chitinase directly to the strawberry plants and observed that plants were free from insects and pathogenic fungi. Chitinase of *Bacillus subtilis* showed highest insecticidal activity against *Spodoptera litura* Fab [83]. The larval chitinases from tomato moth (*Lacanobia oleracea*), and tobacco hornworm (*Manduca sexta*) were shown to have insecticidal activity in transgenic plants [84]. Kabir et al. [85] isolated *Bombyx mori* chitinase and investigated that it serves as a biocontrol agent against the adult Japanese pine sawyer, *Monochamus alternatus*. Recently, Suganthi et al. [86] isolated a chitinase from *Pseudomonas fluorescens* that showed insecticidal activity against tea mosquito bug. These findings open up the possibility of using chitinase as a biopesticidal agent for future crop improvement programs and integrated pest management strategies.

## CHITINASE AS NEMATICIDAL AGENT

In many studies it was found that the chitinase has nematicidial effect on egg shell of nematodes. The egg-shell is the toughest part of nematode eggs and plays an important role in their resistance to chemical and biological nematicides [87].

The nematicidal effect of chitinase on plant-parasitic nematodes was first investigated by Mankau and Das [88-89]. They found that the chitin amendments controlled the citrus nematode *T. semipenetrans* and the root-knot nematode *M. incognita.* Later, chitin amendments were used to control *Tylenchorhynchus dubius* [90], *M. arenaria* [91], *M. javanica* [92], and *H. avenae* [93]. Chitinase killed *T. dubius* by producing structural changes in the nematode cuticle [94]. Purified chitinase inhibited egg hatch of *Globodera rostochiensis* up to 70% *in vitro*, and the chitinase-producing bacteria *Stenotrophomonas maltophilia* and *Chromobacterium* sp. reduced egg hatch of nematode both *in vitro* and in soil [95]. *Pseudomonas chitinolytica,* with strong chitinolytic activity, reduced *M. javanica* infection and improved growth of tomato, *Lycopersicon esculentum* [96]. The chitinolytic fungus, *Paecilomyces lilacinus,* destroyed nematode eggs and efficiently controlled *M. incognita* [97]. The chitinase produced by *Monacrosporium thaumasium* showed nematicidal activity and demonstrated significance reduction of *Panagrellus redivivus* larvae [98].

**PRODUCTION OF CHITOOLIGOSACCHARIDES AND PLANTS DEFENSE**

The enzymatic production of N-acetyl chitooligosaccharides from chitin is important to agriculture and biotechnology sectors. Higher chitooligomers such as chitohexaose and chitoheptaose elicit chitinase activity in the plants, thus triggering defense mechanisms against fungal pathogens [99]. Chitopentaose can be applied as plant growth promoters as well as biocontrol agents for plant pathogens. The enzymatic production of chitooligosaccharides with a high degree of polymerization was carried out using an ultrafiltration membrane reactor system [100]. The chitinase isolated from a novel thermophilic *Humicola grisea* effectively hydrolyzes colloidal chitin to produce chitooligosaccharides [101].

**CONCLUSION**

Application of biological agents, as an alternative of chemicals/pesticides, holds great promise to control plant pathogens. On tha basis of data summarized in this article we can conclude that the microbial chitinases may be used as an important factor for biological control of phytopathogens. One of the major applications of chitinases is development of transgenic plants and biocontrol of plant pathogens. More study on microbial chitinase by using genetic engineering tools will offer better understanding aboute chitinase genes and their biotechnological

applications in agriculture sector. By understanding biochemistry and protein engineering we can produce chitinases with specific functions that will make them more useful for various processes in near future.

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