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Chitinolytic Bacteria Producing Enzyme Against Insect and Fungal Plant Pathogens

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ABSTRACT

Chitinases have the ability to enzymatically break down chitin to its monomers or oligomers (which consist of a few monomeric units). Chitinases have been discovered in a several kinds of organisms like bacteria, plants, fungi, insects, and crustaceans. Since there is no chitin in vertebrates, it has been proposed to use chitinases as a therapy for fungal infections as well as some parasites. The use of microorganisms as biological agents to fight plant disease has also been described. This strategy is a good alternative for less environmentally friendly methods of controlling agricultural phytopathogens. The Production of cheap chitinolytic enzymes is a critical condition in the utilization of shell wastes. This would not only resolve the current environmental problem with polluting the sea but would also contribute to the economy of marine products.

KEYWORDS Chitinase, biopesticides, Chitin, Biosynthesis

Bacterial biopesticides

Bacterial biopesticides are the commonly applied and cost effective alternative to microbial pesticides. Bacteria in the genus ***Bacillus*** are commonly used for insect control (Ongena and Jacques, 2008).

Chitin degradation

Chitinases

Chitinases are hydrolytic enzymes, which break down chitin to its mono or oligomers though chitin. There are no large deposits of chitin observed in the environment. This indicates that a natural mechanism for its degradation, which involves chitinolytic enzymes, exists. Chitinases are the glycosyl hydrolase with the ability to hydrolyze the 1→4 β-glycoside bond of N-acetyl d-glucosamine, except chitinase (3.2.1.14), which has an ability to randomly break glycosidic bonds in chitin, a β-N-acetylglucosaminidase (3.2.1.30) plays an important role in the hydrolysis process of chitin (Sahai and Manocha, 1993).

Chitin

Chitin is a common natural polymer, which due to its nature is found in structures, which provide protection to organisms. Except chitin fibers, natural chitin contains proteins and glucans. Those molecules are responsible for a process called sclerotization, where molecules are linking each other with covalent bonds strengthening the overall structure. Chitin found in crustaceans, arthropods and fungal cell walls are characteristically packed, antiparallel arranged fibres of 18 to 25 chains, linked with proteins to form so-called Bouligand-type structure. This arrangement is called α-chitin (Gardner and Blackwell, 1975).

The structure of chitin makes it extremely resistant and less chemically active. Squid pens are form of β-chitin which less common in nature than α-chitin. The fibres in this type of chitin are parallel. The final type of chitin is γ-chitin, where for two parallel fibres there is one orientated antiparallel Chitin does not dissolve in water or any of the typical organic solvents (Einbu ***et al.,*** 2004).

Biosynthesis of chitinases

Chitinases are found in multiple chitin-utilizing species and their induction in not unified. Expression of induced hydrolases is controlled by hydrolysis product. This process requires the presence of inducer in the medium; otherwise chitinases are produced in very small quantities. Introduction of the inducer allows increasing synthesis of the desired enzyme. This was demonstrated by modifying medium with chito oligomers what increased the production of chitinase A (ChiA) by ***Bacillus cereus*** (Sato and Araki, 2008).

Chitinases in combination with β-1,3-glucanase are also utilized by higher plants in defensive mechanism against the invasion of pathogenic microorganisms where they are induced by infection or elicitor treatment (Ebrahim and Singh, 2011).

In arthropods and fungi, which contain chitin, chitinases have more structural rather than energy source or defensive role. Insects and crustaceans synthesize chitinases during ecdysis in the molting process to hydrolyze chitin in the cuticle. Since those organisms only produce chitinases at very specific moment in their life cycle, they are not suitable for commercial applications.

Production of chitinase

Bacteria and fungi are the most attractive organisms for the large-scale enzyme production in biotechnological processes. The production of chitinase is dependent on the presence of enzymes inductors but chitin flakes and powders do not show satisfactory induce capability. On the laboratory scale, hydrolysis of chitin can be performed in solution of hydrochloric acid. This technique is not preferable for the industrial scale due to a large environmental impact. Industrial scale chitin hydrolysis has been also achieved utilizing lactic acid fermentation (Cira ***et al.,*** 2002). This technique is far superior when it comes to environmental impact. In addition, lactic acid acted as a better chitinase inducer than colloidal chitin (Green ***et al.,*** 2005). The employment of ultra sonication and steam ex-

plosion further improves accessibility of chitin and has potential to be applied in large scale manufacturing of chitinase (Villa-Lerma ***et al.,*** 2013).

Applications of chitinase

Several reports have been published where N-acetyl β-dglucosamine and chitin oligomers from chitin (α and β forms) were employed Setthakaset ***et al***., 2008). This indicates that large scale chitin hydrolysis by chitinase is possible. Just like celluloses, chitins are often a waste product of industrial processes. Developing a commercial scale hydrolysis of the chitin could resolve the problem of utilisation of these byproducts. It has been demonstrated that Mucor circinelloides NBRC 6746 was successfully utilised to produce biodiesel in the presence of N-acetyl β-dglucosamine (Inokuma ***et al.,*** 2013). Even though M. circinelloides produces chitinase in the absence of added inducer, the application of chitin monomers improved yield of ethanol from 6.00 g/L, 0.46 g/L after 16 and 12 days when colloidal and powder chitin were used respectively to 18.6 g/L after 72 h when N-acetyl β-d-glucosamine was used. This equals to 0.75 g/L/h of ethanol made. The application of chitinase significantly improves fermentation, which can be implemented in many biotechnological processes.

The use of chitinase in medicine does not diminish studies into their use as subsidiary reagents in chitinous materials but rather expands their applications. An example of using chitinase from ***S. marcescens*** and ***S. griseus*** to induce lysis of MCF-7 cells in culture and human breast cancer xenograft B11–2 in severe combined immunodeficiency mice have been reported (Pan ***et al.,*** 2005).

Insects are currently the biggest group of pests. Regardless from progress in the development of new generations, more potent pesticides, insects are causing significant loss to the economy (Oerke ***et al.,*** 2006). Just like crustaceans, insects are protected by chitinous cuticle. This natural defence allows them to survive in various, sometimes-harsh environments. Several crops including cotton, tomatoes, sunflower, beans, maize, and several cucurbitous and citruses are targeted by cotton bollworm ***Helicoverpa armigera***. Research shows that larval weight and feeding rates can be reduced and the number of successful pupation events is decreased as a result of treatment with cultural fluid from ***T. harzianum***, which has strong chitinase activity. Larval and pupal mortality has also been significantly increased (Binod ***et al.,*** 2007).

Several strains of bacteria and fungi producing chitinolytic enzymes are active against fungal plant pathogens. Genus ***Trichoderma*** applies several mechanisms to increase pathogen resistance of crops. Those mechanisms include colonization of the soil and/or parts of the plant, occupying a physical space, and avoiding the multiplication of pathogens; producing cell wall degrading enzymes against the pathogens; as well as antibiotics that can kill the pathogens, promote plant development, and induce defensive mechanisms of the plant (Saba ***et al.,*** 2012). Tabaco and potato plants are either tolerant or resistant against the foliar pathogens ***Alternaria alternata, Alternaria solani***, and ***Botrytis cinerea*** and to the soil-borne pathogen ***Rhizoctonia solani*** as a result of expression of genes of the chitinase Chit42 form ***T. harzianum*** (Howell, 2003). Some chitinolytic bacteria have strong activity against plant pathogens. It has been reported that ***Paenibacillus*** sp. and Streptomyces sp. can be used to control ***Fusarium*** wilt of cucumber (Cucumis sativus) caused by ***Fusarium oxysporum f. sp. B. cereus*** 28–9 also demonstrated strong activity and control against botrytis leaf blight in lilies. ***B. cereus*** 28–9 produced at least two chitinases (Huang ***et al.,*** 2005). Fungal protoplasts have gained substantial importance in mycological research in recent years. The culture fluid of ***Streptomyces cyaneus*** SP-27, with the addition of α-1,3-glucanase from ***Bacillus circulans*** KA-304, form ***Schizophyllum*** commune protoplasts. The N-terminal amino acid sequence of chitinase A (MW 29000) has a sequential similarity to those of several ***Streptomycete*** family 19 chitinase (Yano ***et al***., 2008).

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