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Research Article

CHITINASE AS THE MOST IMPORTANT SECONDARY METABOLITES OF STREPTOMYCES BACTERIS

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ABSTRACT

Fungal phytopathogens pose serious problems worldwide in the cultivation of economi cally important plants.

Chemical fungicides are extensively used in current agriculture.However, excessive use of chemical fungicides in agriculture has led to deteriorating human health , environmental pollution, damaged to ecosystem and development of pathogen resistance to fungicide.

Because of the worsening problems in fungal disease control , a serious search is needed to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. Microbial antagonists are widely used for the biocontrol of fungal plant diseases. Many species of actinomycates, particulary those belonging to the genus sterptomyces, are well known as antifungal biocontrol agents that inhibit several plant pathogenic fungi.

Another way biological control has been developed as an alternative of chemicals to tock with plant pathogenic fungi. Considering high presence of chitin in fungal cell wall, chitinase enzyme is camped as an effective biocontrol agent against phytopathogenic fungi. Streptomyces bacteria are able to produce various

chitinase enzymes, chitinases produced by streptomyces belong to the families 18 and 19 glycosyl hydrolases. The antifungal activity is mostly shown by fomily 19 Chitinases. In comparison with bacterial family 18 chitinases, the specific hydrolyzing activity of chitinase 19 against soluble and in soluble chitinous substrates has been markedly higher. Considering the importance of family to investigate antifungal potential of streptomyces bacteria isolated from east Azarbijan region soils based on molecular identification of family 19 chitinase. encoding gene in these bacteria.

To aim the purpose 110 soil samples were collected from East Azarbaijan and 310 strepomyces isolates were selected using macroscopic and microscopic observations. DNA genomic of all of the isolates were extracted and PCR reactions was done using chitinase 19 designed primers as marker.

Totally isolates were selected with molecular selection and antagonistic test were done. One of the isolates exhibit the most strong antifungal activity.

The strain was identified using 16srDNA gene, and the chitinase encoding gene were amplified partially to prove the PCR selection. Finally the bacterium were introduced as potentially biological fertilizer.

Keywords: streptomyces, family 19 chitinase, antifungal activity,16srDNA gene, biological control.

INTRODUCTION

Today, with the widespread use of chemicals in gardens and fields leading to the eradication of pathogens, there is a risk of insects and beneficial microorganism's destruction. Reducing environmental pollution caused by chemical pesticides led to new insights into the environmental control system and its upgrade. Practical use of biological control agents by reducing the survival of the business processes or the use of biological agents in the field is limited. So, it is important to screen for beneficial microorganisms. Streptomyces species are G^+ bacteria, standstill and filamentous bacteria which are located among environmental control factors. Streptomyces colonies are recognizable with chalky appearance and their smell like the soil. They are widespread in the nature and specially live in the soil and are very important parser. Streptomyces species are metabolically capable of metabolizing many different compounds including sugars, alcohols, amino acids and cyclic compounds by hydrolytic enzymes. Also a species of Streptomyces in soil salinity tolerant plants to stress and is involved in the control of fungal diseases (7, 8). Fertilization and plant disease control is the power of Streptomyces bacteria that enter the soil to increase tolerance of plants against pests and diseases, and it can be replaced chemical fertilizers, and pesticides. The bacteria can also inhibit soil-borne fungal disease causing decisive effect on crop production has increased (1,8).

One of the ways to control diseases caused by pathogenic fungi that have no adverse effects is the use of biological control methods. Unlike synthetic materials, the microbiological substances that are less toxic species have been effective, readily biodegradable and have low allergenic. In addition, this material does not

accumulate in food products and are also inexpensive and suitable for use on an industrial scale. Actinomycetes, particularly Streptomyces species are G^+ bacteria, many of which are soil-borne, broad-spectrum biological control agents such as antibiotics, hydrolytic enzymes such as chitinase and enzyme inhibitors against fungal pathogens, plants produce and secrete (14). Most of the other Streptomyces species in the temperature range 15 to 37 will grow. Furthermore, Streptomyces performance including different mechanisms, such as inhibition of pathogens by producing antibiotics, competition for iron through siderophore production, chitinase, glucanase and other sodium compounds such as phenyl acetic and phenyl acetic is suitable. The useful features of Streptomyces have attracted many researches for separation and recovery of this bacterium in biological control programs (2,7).

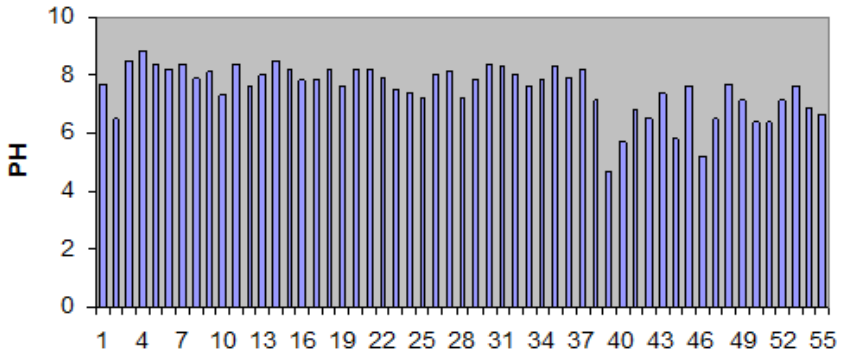
Streptomyces have direct antifungal activity, which has been previously reported. Reverse the osteolytic activity of these bacteria mainly is as a result of hydrolyses enzymes chitinase and glucanase (7). Chitin is a major component of fungal cell walls and is one of the most important features of the Streptomyces chitinase enzyme substrate capable of using chitin as a carbon source. Due to the high percentage of chitin in the cell walls of fungi, chitinase enzyme is considered as a biological control agent effective against fungi PhytoPathogen. The Streptomyces bacteria are able to produce various kinds of chitinase enzymes. Chitinase enzyme produced by Streptomyces belonging to two families 18 and 19 of qlycosil hydrolyses (9,12). Family 19 indicate anti fungi activities. Azerbaijan Shargi due to the cultivation of cucurbits and vegetable production is a major province in the country. So we should consider seriously the soli diseases *phytophthora* sp.44D, *Fusarium solani* in order to increasing function and have careful information to control the disease.

MATERIALS AND METHODS

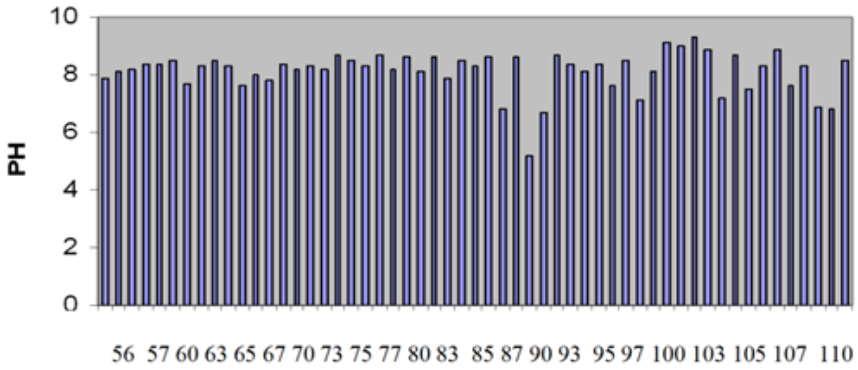
A total of 110 soil samples from a depth of 30-15 cm in spring and summer soil were collected from different areas of East Azarbaijan province. The soil samples were isolated from the acetone mists. Isolated from a soil sample dilutions were prepared according to $10^{-3}, 10^{-2}, 10^{-1}$. Investigation showed that the second was the best dilution to isolate the cause of the suitability of potential microbial concentration in the dilution. After planting soil diluted the sample isolate 310 was isolated microscopic and macroscopic observations. PH of soils in different areas of the conclusion was that 94 (85%) of the samples have a pH above 7.

PH	Name of the area of sampling	Number of sampling	Number of picked up samples	Row	PH	Name of the area of sampling	Number of sampling	Number of picked up samples	Row
7.9-8.4	Shahverdi	9	56-64	8	7.9-8.5	Tabriz	8	1-8	1
7.3-8.4	Mountain of Sahand	13	65-77	9	7.8-8.4	Jolfa	6	9-14	2
8.1-8.9	Shabestar	6	78-83	10	8.2-8.5	Kaleibar	7	15-21	3
7.1-8.5	Mountain of Misho	10	84-93	11	7.9-8.6	Ahar	8	22-29	4
7.8-8.3	Marand	4	94-97	12	9-9.3	Malekan	15	30-44	5
7.2-8.9	Bonab	5	98-102	13	8.9-9	Azarshahr	6	45-50	6
6.5-8.7	Heris	8	103-110	14	8.1-8.5	Khodaafarin	5	51-55	7

Table 1: The sampled areas of the soil.



Soil Samples (a)



Soil Samples(b)

Figure 1: East Azarbaijan region soils pH, a) number 1 to 55, b) number 56 to 110

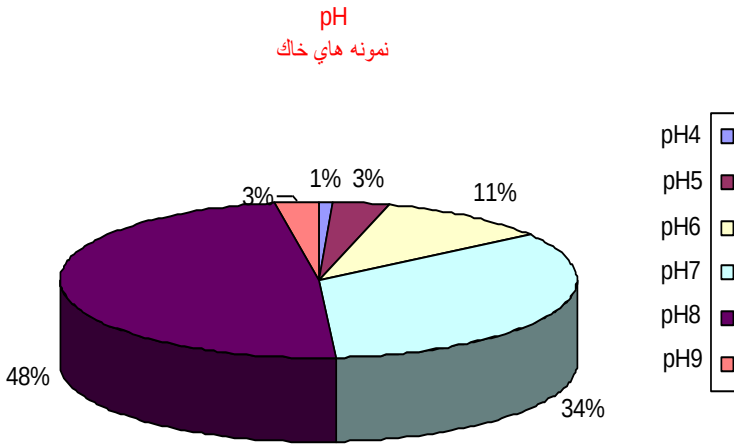


Figure 2: pH percent of evaluated soil samples.

Seven days after the second dilution culture plate colonies were counted.

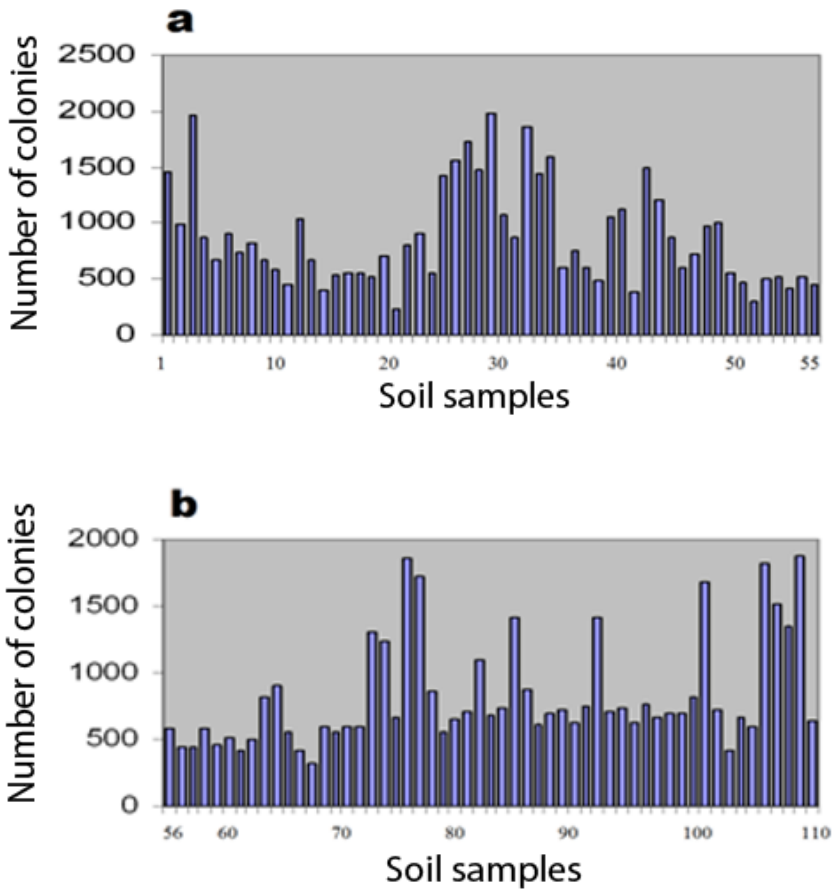
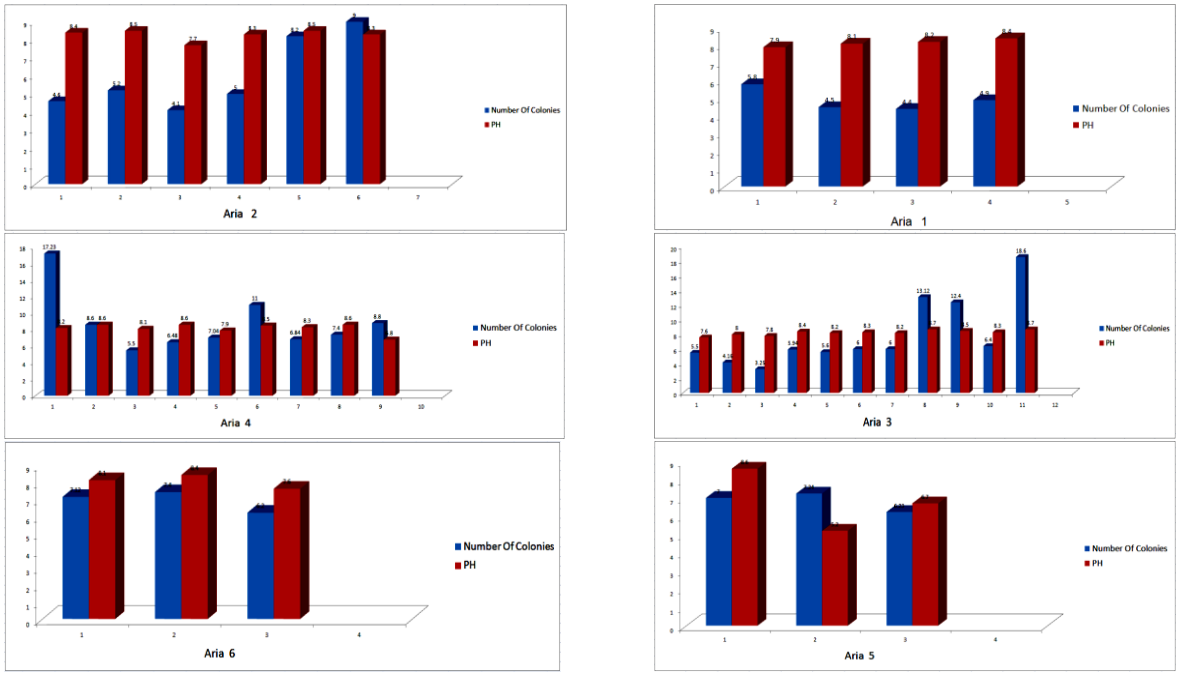


Figure 3: Colony numbers from soil samples, a) number 1 to 55, b) number 56 to 110.



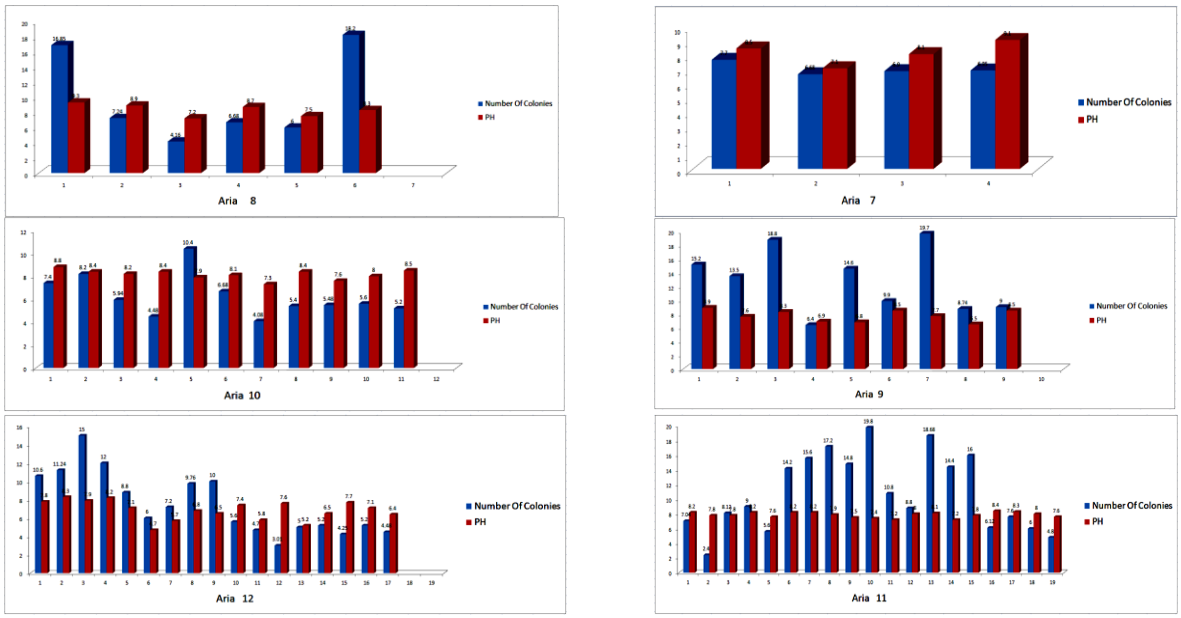


Figure 4: Graphs to compare the effect of pH on colony.

According to the above mentioned graphs areas with high alkaline pH have more Streptomyces species. In other words, alkaline soils have high potential to isolate the bacteria. For long-term storage of bacteria isolated in this study were used frozen bacteria in -80°C. Genomic DNA extracted from the bacteria Streptomyces wall peptide glycan very hard and resistant to harsh methods impossible; Therefore, using the fierce heat shock applied to alternate freezing and thawing, the extract was then broken wall Peptido glycan bacteria. Both direct and reverse sequences were done via BLAST 2 Sequences software. After obtaining the complete sequence of the fragment of the nucleotide sequence of the gene sequence databases by bioinformatics software were compared with Global Biotechnology (NCBI: National Center for Biotechnology information). BLAST results showed that 100% of these sequences show overlap with the highest similarity (96%) of 19 bacteria Streptomyces griseous chitinase enzyme coding gene sequences.

RESULTS AND DISCUSSION

Streptomyces bacterium, as one of the most well-known genus of Actinomycetes, due to its very large genome on its linear chromosome, as a result of frequent order by gene, is able to produce many secondary metabolites and thereby play an important role in medicine, agriculture, forestry, natural resources and biotechnology. The extraction of secondary metabolites and their ability to achieve commercial production can be an important step in the country's self-sufficiency in many aspects related to science and technology in developed countries. Chitinase enzyme is one of the secondary metabolites by Streptomyces, with many aspects of environmental and agricultural applications and many researchers have been investigating this case.

Brook and Madigan (1,7) concluded on the basis of different areas soils pH study that the samples of alkaline pH and neutral species have more Streptomyces. Civillo et al (7) sampled soils of different ecosystems in different seasons. They found that the spring samples have more Streptomyces.

On the other hand, DNA sequencing is an essential tool in molecular biology applications which can help to determine the exact nucleotides in a piece of DNA. Genome projects can identify unknown genes and map their position compared to known genes. Gene cloning and expression of recombinant proteins encoded by them, is one of the most important applications of gene sequencing and characterization of DNA. Production of transgenic plants those are resistant against plant pathogens are considered as one of the most important ways to control these pathogens, and developed countries pay special attention to it and invest in this area (15).

The need to reduce environmental pollution and endanger human health and other organisms due to use of chemical pesticides in agriculture and the need to reduce the high costs of these toxins requires that chemical pesticides replace with biological control materials from useful microorganisms and affordable and safe use of resources. Streptomyces bacteria with antifungal activity can be replaced by chemical pesticides from gardens and farm fields as biological control agents against plant pathogenic fungi used and increase agricultural products (11).

Chitinase enzyme is one of the most important secondary metabolites by Streptomyces bacteria. This enzyme breaks the cell wall chitin of fungi, the antifungal activity for a typical Streptomyces bacteria buildup. Streptomyces chitinase is divided in two 18 and 19 Qlycosil Hydrolyses family. 19 family Chitinase looks like the plant Chitinase and shows considerable antifungal activity (10,13).

As the result of Streptomyces resistance to the change of environmental conditions like moisture, temperature, acidity, salinity and spore production in adverse environmental conditions, formulas produced by the bacteria in this soil types and climates are less affected than other microorganisms. On the other hand, the diversity of secondary metabolites by Streptomyces causes the ability to use multiple controller mechanisms to an isolate. It can be said that Streptomyces sustainable development of the program, has the following benefits:

1. Enhance performance and help boost agricultural crops, especially in warm climates and soil salinity
2. Raising the standards of quality and safety of agricultural products to fertilizer and pesticide residues
3. Helping to increase exports
4. Reducing diseases caused by residue chemical fertilizers and pesticides in agriculture and surface water, including cancers
5. Job creation and development of small and great enterprises
6. Reduce foreign exchange outflow for import of fertilizers and chemical pesticides (11)

In previous years, the researchers had proven a number of chitinase genes in the genome of Streptomyces bacteria, but few of the tested genes belonging to 19 families Chitinase that have high antifungal activity. In total, 19 families Chitinase are relatively rare in prokaryotic organisms (4,5).

So, achieving bacteria Streptomyces chitinase gene family coding can lead to the identification of 19 strains is very important and effective antifungal defense. Because this gene is in the limited number of colonized and expressed Streptomyces bacteria. So the colonization and expression of this gene among Streptomyces strain appears so necessary for specification and differences and to select the best tag with more antifungal activity and its transfer to the plant which implant the most resistance to the Phyto pagan fungi.

In this study, the gene encoding the enzyme has been purified to a family 19 chitinase-producing bacteria Streptomyces enzyme was isolated from soil in different areas of Azerbaijan Shargi biological control potential of anti-fungal can be referred to as a factor.

Scientists have found that bacteria Streptomyces prefer alkaline and neutral pH to grow normally (3). PH range of the soil samples tested in this study was between 9 - 7/7 indicates that the soils tested in this study have potential in the presence of Streptomyces bacteria.

The research about the relative affinity between the colonized 19 Chitinase gene and corresponding Chitinase genes recorded in NCBI showed that Gene amplification of the isolates tested, shows the closest genetic distance Philo with S.griseus 19 chitinase gene family. This result confirms the validity of the chitinase enzyme coding gene families in the genomes of 19 strains isolated from soil.

In addition to the identification of bacteria by biochemical methods of identifying bacteria genes conserved sequence, 16SrDNA is a novel technique that can be used to identify the type of bacteria. The length of this gene is about 1500 bp in the most expressed spices containing high percentage of GC (6). Hoster et al (4) used cloned and sequenced the genes of bacteria 16SrDNA to identify the enzyme chitinase-producing bacteria isolated from the amplified (10).

Molecular identification of isolates discussed in this study, analysis of data from the sequencing of the cloned fragment was related to 16 srDNA showed the rational accordance in Streptomyces; the fragment was cloned so that 1519 bp to 100% recovery with 99% sequence similarity with genes of 16 srDNA of 43 strains of the genus Streptomyces. These results confirm the observations of morphological features Clooney, 19, confirmed that chitinase-producing bacterium isolated from soil of East Azerbaijan belongs to the genus Streptomyces.

CONCLUSION

In this research, 310 isolated bacteria were collected from 110 soil samples of different areas of Azerbaijan Shargi. Their selection was made on the basis of microscopically and macroscopically observations: 5 isolates showed positive result about the selection of enzymatic isolates of 19 Chitinase families. Antagonist test was performed on these isolates. Finally, 16srDNA sequencing proved that the strain belong to Streptomyces and has antifungal activity against plant pathogenic fungi. This bacterium has the potential to be introduced and used as bio-fertilizer.

Suggestions:

The need to reduce environmental pollution and endanger human health and other organisms from the use of chemical fertilizers and the need to reduce its high cost will require the use of biocontrol of the resource cost. Streptomyces bacteria with antifungal activity can take place in gardens and agricultural fields as fertilizers and biological control agent against fungal pathogens would only be used and increase agricultural products.

Streptomyces isolated from the natural environment or model though is not technically difficult, but time consuming and costly. However, you can follow some tips, refinement and selection of samples for isolation of the desired cut. So, first it should be studied carefully and used as specific conditions and selection elements. 19 chitinase gene expressions also can be purified enzyme purified in a suitable host to obtain the necessary studies done on it.

Laboratory and greenhouse enzyme can directly impact on pathogenic fungi and antifungal properties of the enzyme with careful precision measuring inhibition zone of fungal payment.

The enzyme optimization, its resistance to the temperature and special pH, causes its use in the different parts of industry and agriculture.

19 Chitinase enzymes are produced in the industrial scale.

It can be transported to the plant and be resistant to pathogenic fungi.

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