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Overexpression of chimeric chitinase42 enhances the antifungal activity of *Trichoderma harzianum* against *Fusarium graminearum*

M. Kowsari ✉
Microbial Biotechnology and Biosafety Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

M. R. Zamani
M. Motallebi
National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Abstract: Evidence for the role of chitinases in biocontrol by *Trichoderma* species has been well documented.Chit42 lacks a chitin–binding domain (ChBD) which is involved in its binding activity to insoluble chitin. The objective of the present study was to enhance antifungal activity of *T. harzianum* by overexpression of wild type and hybrid forms of Chit42 containing chitin binding domain. To produce chimeric chitinase with improved enzyme activity, the hybrid chitinase was constructed by the adding of ChBD from *Rhizopus oligosporus* to the C–terminal end of Chit42 cDNA from *T. atroviride* using SoEing PCR. The recombinant hybrid chitinase (Chit42–ChBD) displayed a 1.56 fold higher chitinase activity than Chit42. This increase suggests that the ChBDmay play a role in helping the enzyme to bind better to the insoluble chitin. Moreover, Chit42–ChBD transformants showed higher antifungal activity towards *Fusarium graminearum*, the causal agent of Fusarium head blight (FHB) disease in wheat.

Key words: Biocontrol activity, Chitin binding domain, transformation, SoEing PCR

INTRODUCTION

The genus *Trichoderma*, is one of the most important biocontrol agents against phytopathogenic fungi (Reithner et al. 2011; Mayo et al. 2015). *Trichoderma harzianum* with high reproductive capacity, strong aggressiveness against pathogens, ability to survive under difficult conditions, efficiency in promoting plant growth and defense mechanisms is a ubiquitous biocontrol agent (Zeilinger & Omann, 2007, Carreras–Villasen et al. 2012; Pereira et al. 2014). Parasitism, competition and antibiosis are the main mechanisms for biocontrol of *Trichoderma* sp. (Harman et al. 2004). Parasitism is a common cause for pathogen death. It appears that the main mechanism involved in biocontrol by *T. harzianum* is the release of lytic enzymes (Kubicek et al. 2011, Matarese et al. 2012). Chitinases are considered as the key hydrolytic enzymes in the lysis of fungal cell walls, which play an important role in biological control (Guthrie et al. 2005; Ryder et al. 2012). Among *Trichoderma* chitinases, Chit42 is essential for biocontrol activities against phytopathogenic fungi (Limon et al. 2004). The lytic activity of *Trichoderma* strains could be improved by heterologous gene overexpression combined with enzyme modification (Kowsari et al. 2014). Only a few of the fungal chitinases contain a chitin–binding domain (ChBD), which is linked to the catalytic site via a linker region (Limon et al. 2004). Chit42 in *T. harzianum* does not contain a ChBD (Yanai et al., 1992, Arakane et al. 2003). Studies have shown that ChBDs exhibited remarkably high specificity to chitin and its binding activity was reversible (Hashimoto et al. 2000). In our previous work, a chimeric chitinase was produced by fusing a ChBD from *T. atroviride* chitinase 18–10 to the N–terminal of Chit42 *T. harzianum* (Kowsari et al. 2014). It is expected that, owing to its small size, the ChBD would have minimal interference with the tertiary structure of the fusion protein (Chern & Chao 2005). The ChBD is a tunnel–like structure which facilitates chitinase binding, thus allowing the efficient degradation of chitin (Van Aalten et al. 2001, Hardt & Laine 2004).

The *Fusarium graminearum*, the causal agent of Fusarium head blight (FHB) in wheat, reduces yield and also produces trichothecene (DON) mycotoxins. DON is an inhibitor of protein synthesis with a broad spectrum of toxigenicity, which poses a serious threat to human and animal health and food safety (Bottalico & Perrone 2002).

Different strategies to reduce the impact of FHB are applied that include disease–free seeds, crop rotation and fungicide application. Additionally, biological control offers an alternative strategy in

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✉ Corresponding author e-mail: kowsari@abrii.ac.ir

order to manage this destructive disease that can be used as parts of an integrated management of FHB.

The objective of this study was to improve the antagonistic effect of a biocontrol agent *T. harzianum* using construction of a chimeric chitinase by adding a chitin–binding domain from *R. oligosporuschi1* to the C–terminal of Chit42 from *T. atroviride* to improve its enzyme activity. In our previous work, we used other chiting binding domain with different source at the N– terminal of Chit42. The findings of these researches can show that expressed chimeric chitinases displayed higher chitinase and antifungal activity than both the wild type and transformants that overexpressed the native chitinases. The antifungal activity of the constructed chimeric was studied to evaluate the effect of ChBD on chimeric chitinase.

MATERIALS AND METHODS

Microorganisms and plasmids

Trichoderma harzianum (ABRIICC T8–7MK), *Rhizopus oligosporus* (ABRIICC Ro101) and *F. graminearum* (ABRIICC Fg21) were provided by the Agricultural Biotechnology Research Institute of Iran, type Collection Culture. Plasmid p3SR2 carrying the *amdS* gene from *Aspergillus nidulans*, which encodes for the acetamidase as a selectable marker and the pLMRS3 plasmid carrying the constitutive promoter *pki1* from *T. reesei* and the *cbh2* terminator from *T. reesei cellobiohydrolaseII* were kindly provided by Prof. Dr. M. Hynes from Melbourne University of Australia and Prof. Dr. R. L. Mach from Vienna University of Austria, respectively. Total genomic DNA was isolated from freeze–dried mycelia according to the method of Lee and Taylor (Lee & Taylor 1990). The fungal RNA from powdered mycelia was isolated using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations. Molecular biology procedures such as bacterial tansformation, cloning and digestion were performed following the standard protocols (Sambrook & Russell 2001).

Growth media

Fungal strains were maintained on PDA (Potato Dextrose Agar) slants. Colloidal chitin agar (CCA) selective medium contained (g/l): colloidal chitin, 5.0; sucrose, 1.0; $NaNO_3$, 2.0; K_2HPO_4, 1.0; KCl, 0.5; $MgSO_4$, 0.5; $FeSO_4$, 0.01; agar 15 at pH 6.5. *Trichoderma* salt minimal medium, MM (Penttilä et al. 1987) supplemented with 20 g/l glucose was inoculated with spores. The MM was buffered using 0.2 M MES (2–Nmorpholino–ethanesulfonic acid)–KOH pH 6.0, or 0.2 M Tris pH 8.0. The selective medium for *amdS* expression was MM containing 10 mM acetamide as the sole nitrogen source and 12.5 mM CsCl (MMA). The DH5α *E. coli* strain was grown in a Luria Bertani (LB) medium at 37 °C, and media were supplemented with ampicillin (SIGMA, 100 g/ml). All chemicals and antibiotics were purchased from Merck (Germany). DNA modifying enzymes were obtained from Fermentase (German) and Roche (Mannheim Germany) Biochemical.

Construction of chimeric chitinase

The sequence of *chit42* cDNA (Accession number; DQ022674) and ChBD of *R. oligosporuschi1* (Accession number; IFO 8631) were retrieved from NCBI. To produce a chimeric chitinase containing linker+ChBD at the C–terminal end of *chit42* cDNA, the fragment containing *chit42* cDNA was amplified using Pf1/Rr1–1 primers. This fragment (F1) contained the coding sequence of the protein of Chit42 with signal peptide and prepro region without stop codon. The other fragment containing linker and chitin binding domain (F2) was amplified using the genomic DNA of *R. oligosporus* as template and Fr2–1/Rr2–2 as primers. This fragment (243bp) contained 120 bp as linker and 123 bp as ChBD. The chimeric chitinase was constructed from the F1 and F2 fragments (Fig. 1) using Splicing by Overlap Extension (SOEing) PCR (Horton 1995).The chimeric gene was purified and cloned into *Xba*I site of pJET1.2. The nucleotide sequence of the chimeric gene was verified by DNA sequencing. Expression plasmids for the chitinase genes were constructed using pLMRS3 as the plasmid vector. The *chit42* cDNA and the chimeric chitinase were cloned into the *Xba*I site of pLMRS3 to yield pLMRS3–chit42 and pLMRS3–chit42 R ChBD, respectively. All DNA manipulations were performed using standard methods (Sambrook & Russell, 2001).

Protoplast preparation and cotransformation

Protoplast preparation and transformation were carried out according to the method of Penttila et al. (1987). *Trichoderma harzianum* T8–7 MK wild type was cotransformed with chitinase–containing plasmids pLMRS3–chit42R ChBD and pLMRS3–chit42 with the plasmid p3SR2. Plasmid p3SR2 carries the *amdS* gene from as *A. nidulans*, which encodes for acetamidase as a selectable marker. Cotransformation was conducted with a 1:10 (p3SR2/pLMRS3–chit42 & pLMRS3–chit42R ChBD) plasmid ratio, and 200–1000 µl aliquots of the transformed protoplasts were plated in 0.75% selective top Agar containing 1 M sorbitol as the osmotic stabilizer. The selective medium for *amdS* expression was MM glucose containing 10 mM acetamide as the sole nitrogen source instead of $(NH_4)_2$ SO_4 and 12.5 mM CsCl. Individual colonies were randomly chosen for *amdS* in the selective medium and incubated at 28 °C after 5 days. Protoplasts were placed on a 2% CCA selective medium. The protoplast regeneration and the development of colonies were observed on plates that were incubated at room temperature. Regenerated transformants were selected based on their growth rate on selective medium. One mycelial disc (5 mm) of each transformant was inoculated on 0.5% CCA and PDA media and incubated at 28 °C for four days.

Chit42 transcripts analysis by quantitative real–time RT– PCR

Chit42 transcripts in transformants and control strains under repressive conditions with glucose were quantified by real–time quantitative RT–PCR. RNA was isolated from mycelia grown for 48 h at 28 °C in MM with 20 g/l glucose. Total RNA was isolated from 100 mg of freeze–dried mycelia powder derived from single spore of selected transformants and wild type using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The cDNA was synthesized from 1µg of total RNA using a cDNA synthesis kit with an oligo (dT) primer. One µl of the cDNA was used in the PCR reaction with the (chiF/chiR) and (βtubuF/βtubuR) as specific primers. Real–time PCR was performed using an ABI system with a SYBR green master mix. All PCRs were performed in triplicate in a total volume of 10 µl for 40 cycles under the following conditions: denaturation, 95 °C, 45 s; annealing, 60 °C, 1 min; extension, 72 °C, 1 min. The number of cDNA transcripts was normalized against the expression of the housekeeping β tubulin gene (Glass and Donaldson 1995). Data were expressed as $2^{-\Delta\Delta CT}$ (Livak & Schmittgen 2001).

Chitinase activity

Chitinase activity was assayed according to the method of Boller & Mauch (1988). To test the effect of a ChBD on chitinase activity, insoluble chitin was used as a substrate. Strains were grown for 60 h at 28 °C in pH 6–buffered MM with 20 g/l glucose (for repressive condition); 250 µl concentrated supernatant or cell–free extract of each strain was incubated with insoluble chitin. Chitin (10 g/l) was resuspended in a 70 mM potassium phosphate buffer pH 6.0. Activity was assayed in continuous shaking at 30 °C for 1 h. The released N–acetyl–glucosamine (GlcNAc) was measured according to the procedures set out by Reissig et al. (1955). A unit was defined as the amount of enzyme that released 1 µmol GlcNAc per 60 minute. Chitinase activity data are the average of three experiments. Specific activity was expressed in units per microgram protein. The protein content in the culture filtrates was estimated using Bradford's method (Bradford, 1976).

***In vitro* antagonism**

In vitro tests were conducted to evaluate the antagonistic effect of Chit42 and Chit42RChBD transformants against *F. graminearum*on a PDA medium using the dual culture technique (Dhingra & Sinclair 1985). One mycelial disc (5 mm) of recombinants and one disc (5 mm) of test pathogen were simultaneously placed on opposite sides of a PDA Petri dish and incubated at 26 °C. Three plates (replications) were used for each transformant and tests were based on a completely randomized design. The plates that received only the mycelial disc of pathogen served as control. The colony interaction was assayed as the percentage of inhibition on the PDA plate after four days of incubation following the formula suggested by Sundar et al. (1995). Inhibition of growth (%) = X –Y/ X×100 where, X = mycelial growth of pathogen in the absence of *Trichoderma* (control), Y = mycelial growth of pathogen in the presence of transformants.

RESULTS

Transformation of *T. harzianum* by chitinase genes

The chimeric chitinase was cotransformed with the plasmid p3SR2 and the pLMRS3 derivatives containing either native *chit42* (pLMRS3–chit42) or hybrid chitinase (pLMRS3–chit42RChBD) as shown in Fig. 1. The prediction of the ChBD glycozylation site by NetOGlyc 3.1 server showed five glycozylation sites in the Ser–Thr rich linker (data not shown). The glycozylation of linker which separated the catalytic domain from the binding domain prevents the chimeric enzyme from proteolysis (Limon et al., 2004). The ChBD was added to the C–terminal of *chit42* through the SOEing PCR approach (Fig. 1).

A selective medium containing acetamide (MMA) was used for selection of stable transformants .About 100 *amdS*$^{+}$ stable transformants were selected on the basis of their ability to grow on the selective medium containing 2% colloidal chitin (2%CCA) for each construct (pLMRS3–chit42 andpLMRS3–chit42R ChBD). For further study, 16 out of 100 transformants were selected based on mycelial growth on the selective medium which designated as Chit42–1 to 16and Chit42RChBD–1 to 16, respectively.

The growth rate of the selected colonies was examined for 48 h on 0.5% CCA medium. Table 1 shows eight selected fast growing transformants for each construct for subsequent study.

Presence, stability and expression of chimeric gene

The presence and stability of chitinase genes, in *Trichoderma* were confirmed using PCR. During four months fungi containing chimeric gene were subcultured 20 times in the non–selective medium. After that using specific primers (Fr2–1–M13R), (Fr2–1–Rr2xba2) and (M13F– Rr2xba2) the presence of the recombinant gene was confirmed by PCR. We had expected fragments, respectively 1373 bp, 243 bp and 2338 bp in the trasformants while there was not in wild type.

Chit42 and Chit42RChBD transcripts were quantified in the selected transformants by real time quantitative RT–PCR. The cDNA was prepared from the RNA of transformants and nontransformants (as negative control) grown in insoluble chitin under repressive conditions for endogenous chitinase repression. Based on calculations using the $2^{-\Delta\Delta CT}$ method and *β–tubulin* as an internal reference gene, differential expression level of *chit42* from 2.8 fold for Chit42–15 to 4.6 fold for Chit42–9 and chimeric chitinase from 3.1 fold for Chit42RChBD–1 to 4.9 fold for Chit42RChBD–3 were detected in transformants (Table 1).

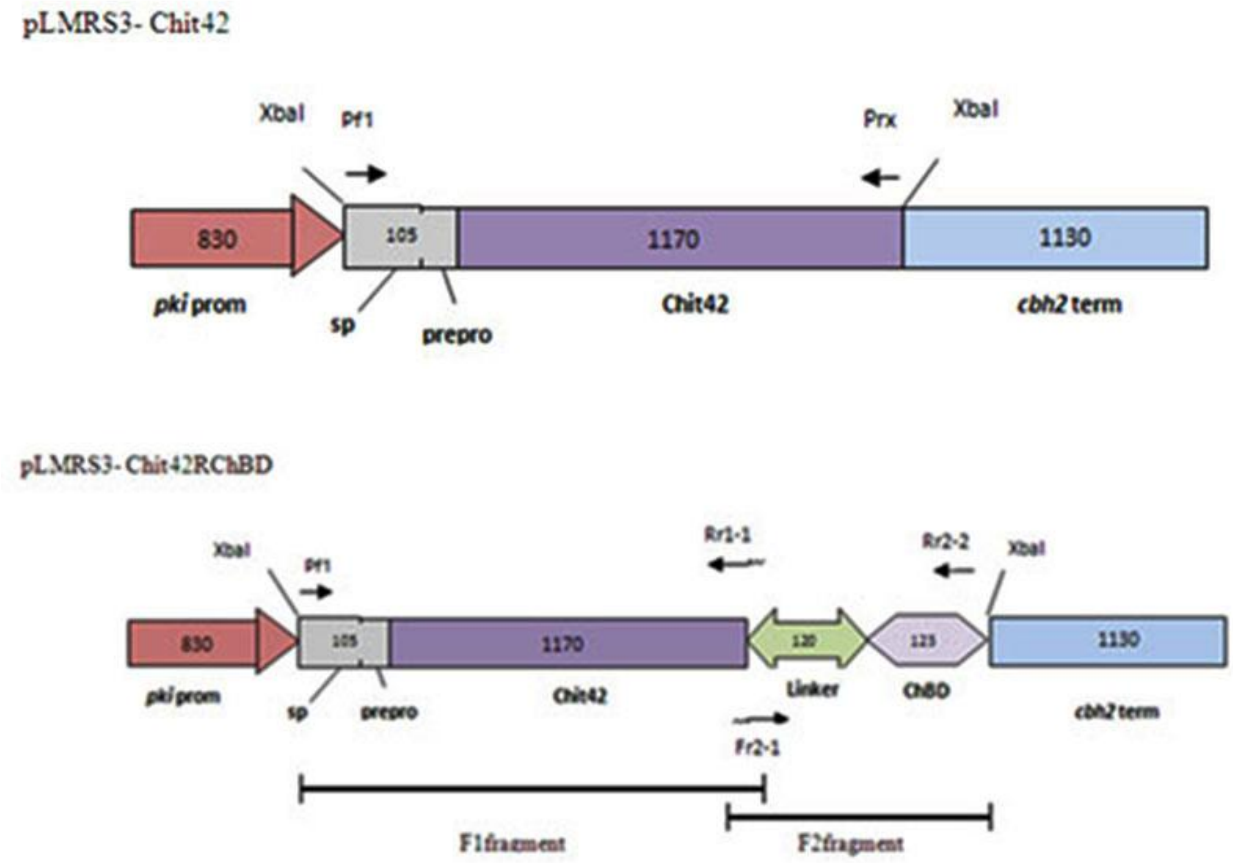

Fig. 1. Scheme of gene constructions. Vectors pLMRS3–Chit42 and pLMRS3–Chit42RChBD were constructed to produce a protein containing the signal peptide, prepro region, mature *chit42*, , linker region and ChBD. *pki* prom, Pyruvate kinase promoter from *Trichoderma reesei*; sp, signal peptide; prepro, preproregion; ChBD–Linker, Chitin binding domain and linker of chi1 *Rhizopus oligosporus*; Chit42 cDNA encoding mature protein; *cbh2* term, terminator of *cellobiohydrolases*II from *T. reesei*. Numbers inside the boxes show fragment sizes; Arrows indicate primers for PCR and SOEing PCR amplification.

Chitinase activity

The enzyme activity of Chit42 and chimeric chitinase was detected in repressive condition to study the effect of ChBDon chitinase activity. Compared with the *chit42* transformants, the chimeric chitinase transformants increased chitinase activity. While the highest enzyme activity in the chit42transformants was 3.01 U/ml (Chit42–9), the Chit42RChBD–3 transformant showed improved chitinase activity of 5.1 U/ml (Table 1). The minimum and maximum chitinase specific activity of Chit42 and chimeric chitinase was 98–212 U/mg and 136–307 U/mg, respectively (Table 1). These results indicate that the presence of a ChBD can increase chitinase specific activity.

Antifungal activity

To determine whether an increase in chitinase activity correlates with the antifungal activity of eight selected chimeric transformants, dual culture tests were performed using *T. harzianum* and *F. graminearum*. When phytopathogenic fungus and wild type or chimeric transformants of *T. harzianum* were grown in the same plates, a zone of lysis was produced in the pathogenic fungal mycelia. Different chimeric transformants showed varied reductions with the minimum of 32% inhibition for Chit42RChBD–5 and maximum of 76% for Chit42RChBD–3 (Fig. 2). No growth was detected when pieces of the overgrown area of the lysed and killed *F. graminearum* mycelia were transferred to fresh medium (data not shown). The Chit42RChBD–3 transformant demonstrating the highest growth inhibition of the pathogen was also showed the highest level of enzyme activity (307$\pm$ 2.9 U/mg) (Table1). To evaluate the effect of ChBD on the efficiency of enzyme and antagonistic activity of chimeric transformants the mean values of enzyme and antagonistic activity of eight chimeric transfor–mants, were compared with those of overexpressed transformants. The transformants that overexpressed the hybrid chitinases inhibited growth of pathogen more than both the wild type and Chit42 transformants expressing the native chitinases. Transformation of *T. harzianum* by *chit42* increased its inhibition 1.36 fold while transformation by chimeric chitinase increased the inhibition to 1.66 fold when compared with the nontransformant (Table 2). This indicates the positive effect of the ChBD by 54% on biocontrol activity.

Table 1. Growth rate, expression fold and chitinase activity of the Chit42 and Chit42RChBD transformants of *Trchoderma harzianum* (Results and standard deviations are the average of three replicates).

Isolate	Diameter (mm/48h)	Expression fold	Chitinase activity U/ml	specific activity U/mg
Control (Nontransformed)	17.2±0.3	1.0	0.047±0.001	18.9±0.4
Chit42–1	29.5±1.0	4.1	2.31±0.08	175±1.2
Chit42–4	30.0± 0.5	4.2	2.50±0.01	183±1.4
Chit42–5	24.0±0.3	3.0	1.43±0.01	128±1.2
Chti42–8	26.5±0.4	3.1	1.61±0.02	132±0.5
Chit42–9	30.5±0.9	4.6	3.01±0.07	212±1.3
Chit42–10	25.4±0.2	2.9	1.17±0.02	109±0.8
Chit42–12	28.5±0.7	3.4	2.16±0.05	161±0.9
Chit42–15	25.5±0.3	2.8	1.16±0.01	98±0.4
Chit42RChBD–1	25.0±0.2	3.1	1.57±0.07	136±0.7
Chit42RChBD–3	32.0±0.6	4.9	5.10±0.09	307±2.9
Chit42RChBD–5	24.8±0.7	3.2	1.76±0.02	198±1.6
Chit42RChBD–7	31.0±0.1	4.2	4.12±0.06	261±2.7
Chit42RChBD–10	28.0±0.6	3.6	2.83±0.09	237±2.6
Chti42RChBD–11	29.5.5±0.5	4.0	3.03±0.09	243±1.4
Chit42RChBD–13	27.4.0±1.0	3.4	2.45±0.09	221±1.8
Chit42RChBD–14	26.0±0.7	3.5	2.60±0.05	228±1.0

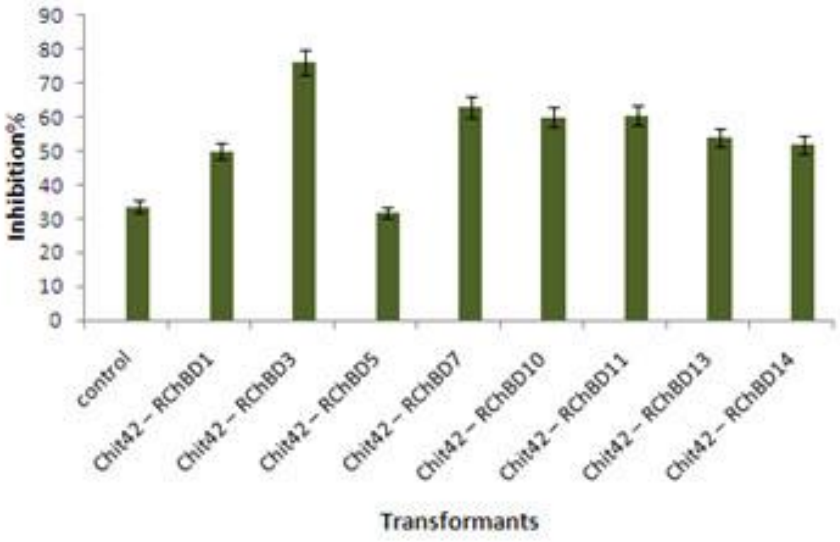

Fig. 2. Analysis of the growth inhibition (%) by selected Chit42 RChBD transformants of *Trichoderma harzianum* against *Fusarium graminearum*. Each value represents the mean (± standard error) of three independent experiments.

Table 2. Comparison of antifungal (%) activity of improved Chit42RChBD and Chit42 transformants of *Trichoderma harzianum* and wild type as control.

	Control *	Overexpressed	$\frac{\text{Chit42}}{\text{control}}$ **	Chimer *	$\frac{\text{Chit42RChBD}}{\text{control}}$ **
Fusarium graminearum	33.6±0.5	45.7	1.36	55.9	1.66

*: Inhibition Mean;**: Fold

DISCUSSION

Biological control is an alternative method to reduce the impact of FHB to prevent toxins entering the food chain (Parry et al. 1995; Foroud & Eudes 2009; Matarese et al. 2012). Biological control agents have specific advantages over synthesis fungicide, including, fewer non–target, environmental effects, and reduction in resistance development probability (Harman 2006). *Trichoderma* sp. is a biocontrol candidate agent for protection of crop yields (Resende et al. 2015). Chitinases produced by *T. harzianum* play an important role in the antagonistic effect of this fungus (Limon et al. 1999; Mohamedy et al. 2015). Improvement of fungal strains by overexpression of chitinase gene in *Trichoderma* has significantly contributed to an intensive enhanced antifungal activity against fungal pathogens (Limon et al. 1995; Limon et al. 1999; Kowsari et al. 2014a). ChBD, found in some chitinases, could increase the chitin binding ability of chitinase and thus improve the chitinolytic activity of insoluble substrates (Limon et al. 2004). The linkage of ChBD with the chitinase

gene has been suggested to be a useful approach to improve *Trichoderma* chitinase activity (Limon et al., 2001).

In this study, transformants of *T. harzianum* that overexpressed either Chit42 or chimeric chitinase with a ChBD were obtained and their lytic and antifungal activities were experimentally investing–ated.The Chit42 and Chit42RChBD transformants were grown in buffered media to prevent the proteolysis of overexpressed chitinasesby acidic proteases (Delgado–Jarana et al. 2002). Furthermore, the predicted glycozylation at the linker region of the binding domain could protect the chimeric chitinase against proteolysis. Protection of the linker region by glycozylation has been demonstrated by Alfthan et al. (1995) and Limon et al. (2004). Significant differences were observed between the chitinase activity of Chit42 and chimeric transformants against insoluble chitin. The observed variations among the Chit42 or Chit42RChBD transformants in terms of enzyme activity (Table 1) probably are associated with the copy number of the transgene and/or their position in the genome. The effect of these two parameters could mainly be normalized when the means of data from two kinds of transformants were compared. The means of extracellular chitinase activity produced by the Chit42 and chimeric transformants were 1.93 and 3.01 U/ml, respectively (Table 3). The improved chitinase containing a chitin–binding domain showed higher chitinase activity than Chit42 (about 1.56fold) when grown in a glucose medium for repressing endogenous chitinases. This result confirmed an increase of about 56% in chimer chitinase activity over Chit42 expression (Table 3) suggesting the ChBD could impact on increasing enzyme activity. Our expression analysis demonstrates only a 5% increase of Chit42RChBD mRNA compared with that of Chit42 once analyzed by real time PCR emphasizing the role of the ChBD on enzyme activity (Table 3). Limon et al. added a ChBD from *Nicotianatabacum* to Chit42 and observed an approximately 36% increase in the chitinase activity of the chimeric enzyme in the presence of insoluble chitin (Limon*et al.,* 2001). In our previous work, a chimeric chitinase was produced by fusing a ChBD from *T. atroviride* chitinase 18–10 to Chit42. The improved chitinase containing a ChBD displayed a 1.7–fold higher specific activity than chit42. This increase (70 %) suggests that the ChBD may be helping the enzyme to bind better to the insoluble chitin, therefore, increasing enzyme activity (Kowsari et al. 2014). In the previous work, we could introduce the Chit42–ChBD15 as the best transformant with the highest chitinase activity (6.201 U/ml), specific activity (390 U/mg) and also an antagonistic effect. In this research, we can introduce the Chit42RChBD–3 as the best transformant with chitinase activity (5.10 U/ml), specific activity (307 U/mg). Our results showed that ChBD, could improve the chitinolytic activity. Fan et al. (2007) constructed a chimeric chitinase using the silkworm ChBD and *Beauveria bassiana* chitinase which showed increase in enzymatic activity in the presence of powdered chitin. The effect of a ChBD on chitin binding was also described by Hashimoto et al. (2000). They showed that deletion of the ChBD from chitinase A1 highly reduced .the efficiency of chitin degradation.

Table 3. Comparison of overexpressed and chimer transformants of *Trichoderma harzianum* based on $2^{-\Delta\Delta CT}$ and chitinase activities

	Control	**Overexpressed**	**Chimer**	$\frac{\textbf{Chimer}}{\textbf{over}}$(fold)
$2^{-\Delta\Delta CT}$(Mean)	1.00	3.51	3.72	1.05
Chitinase activity (U/ml) (Mean)	0.044	1.93	3.01	1.56

Taken together, our results offered positive evidence that linkage of ChBD to chitinase is a useful way for strain improvement *Trichoderma* strains. *Trichoderma* with chimeric chitinase can offer much more antagonistic effect on *F. graminearum* than the overexpressed *chit42*. Among all the obtained transformants, Chit42RChBD-3 could be considered as a potential candidate to be applied for field assessment in order to check the ability in controlling of Fusarium head blight disease in wheat.

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افزایش توان بیوکنترلی *Tricoderma harzianum* در برابر *Fusarium graminearum* بوسیله افزایش بیان کیتیناز کایمر ۴۲

مژگان کوثری[۱]✉، محمدرضا زمانی[۲] و مصطفی مطلبی[۲]

۱– گروه بیوتکنولوژی میکروبی، پژوهشکده بیوتکنولوژی کشاورزی، سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران

۲– پژوهشگاه ملی مهندسی ژنتیک و زیست فناوری، تهران، ایران

چکیده: فعالیت بیوکنترلی قارچ تریکودرما به آنزیم های هیدرولازی مخصوصا کیتینازها وابسته است. آنزیم کیتیناز ۴۲ به عنوان کلیدی ترین عضو این مجموعه هیدرولازی است که جهت اتصال به سوبسترای کیتین (دیواره سلولی قارچ های بیمارگر) فاقد ناحیه اتصال به کیتین (ChBD) است. چشم انداز این تحقیق افزایش توان بیوکنترلی قارچ *Trichoderma harzianum* از طریق افزایش تعداد ژن کیتیناز ۴۲ و همچنین ایجاد ژن کیتیناز نوترکیب است. به منظور ایجاد کیتیناز کایمر با فعالیت آنزیمی بیشتر، ChBD ازقارچ *Rhizopus oligosporus* جداسازی شد و با استفاده از SOEing PCR به انتهای کربوکسیل کیتیناز ۴۲ قارچ *T. atroviride* متصل گردید. کیتیناز نوترکیب حاصل (Chit42–ChBD) ۱/۵۶ برابر بیشتر از کیتیناز ۴۲ معمولی فعالیت نشان داد. این افزایش فعالیت آنزیمی می تواند بیانگر نقش ChBD در اتصال بهتر آنزیم به سوبسترای کیتین باشد. یافته ها نشان می دهند که قارچ *T. harzianum* بهینه سازی مولکولی شده، فعالیت بیوکنترلی بهتری نسبت به شاهد در برابر قارچ بیمارگر *Fusarium graminearum*، عامل بیماری بلایت فوزاریومی سنبله گندم (FHB) دارد.

کلمات کلیدی: فعالیت بیوکنترلی، ناحیه متصل شونده به کیتین، تراریخت، SoEing PCR

مکاتبه کننده: مژگان کوثری Email: kowsari@abrii.ac.ir

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