Intra-strain crossing in *Trichoderma harzianum via* protoplast fusion to enhance chitinase productivity and biocontrol activity

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ABSTRACT

Protoplasts were isolated from Trichoderma harzianum fungus using Novozyme 234 with 0.7 M KCl as osmotic stabilizer. Intra-strain T. harzianum protoplast fusion has been carried out using polyethylene glycol with STC (sorbitol, Tris – HCl, $CaCl_2$) buffer. The fused protoplasts were regenerated on colloidal chitin agar selective medium. Eighteen self-fusant strains were selected to study the chitinase production and biocontrol activity. Most of the fusants exhibited fast and vigorous mycelial growth on 2 % colloidal chitin agar compared to non-fusant and parent strains. High chitinase activity was measured in the culture filtrates of the self-fusant strains than the parent. Among the fusants, four (ATh1/9, ATh1/12, ATh1/14 and ATh1/17) produced maximum chitinase with a two – fold increase compared to the parent strain. Moreover, fusant ATh/7 produced 94.3 % more chitinase activity than the original strain. Most self – fusant strains exhibited increased antagonistic activity against Cephalosporium acremonium, Aspergillus niger and Rhizoctonia solani than the parent strain. The crude chitinase preparations of fusants ATh1/9, ATh1/12, ATh1/14 and ATh1/17 lysed the mycelia of T. reesei, T. viride and A. niger and released the protoplasts in higher numbers than the crude chitinase preparation of parent strain. Results demonstrated the significance of the protoplast fusion approach, as a technique to develop superior hybrid strains of filamentous fungi lacking inherent sexual reproduction.

Key words: *Protoplast fusion, chitinase, biocontrol, Trichoderma harzianum.*

INTRODUCTION

Trichoderma is among the most exploited fungal biocontrol agents in agriculture for the management of plant diseases caused by a wide spectrum of fungal pathogens (Mathivanan *et al.*, 2000). Production of extracellular hydrolytic enzymes is one of the biocontrol mechanisms exerted by *Trichoderma* towards fungal pathogens. Several biocontrol agents alleviate the growth of pathogenic fungi by producing extracellular chitinase, which degrades the chitin polymers of fungal cell wall (Mathivanan *et al.*, 1998; Mathivanan *et al.*, 2000).

Trichoderma harzianum is a known producer of cellulolytic and chitinolytic enzymes that are extensively used for the degradation of cellulose and chitin materials particularly in textile and paper industries, besides its use in wastewater treatment (Elad, 2000; Prabavathy *et al.*, 2006).

Fungal protoplasts are important tool in physiological and genetic studies. Genetic

manipulation can successfully be achieved through fusion of protoplasts in filamentous fungi that lack the capacity for sexual reproduction (Peberdy, 1980 and 1989). Protoplast fusion is universally applied for enhancing genetic recombination and developing hybrid strains in filamentous fungi (Peberdy, 1980; Lalithakumari, 2000). Isolation, fusion and regeneration of protoplasts have been achieved in the genus *Trichoderma,* mainly to enhance its biocontrol potential (Sivan and Harman, 1991; Mrinalini and Lalithakumari, 1998). Das *et al.* (1988) demonstrated the intra-strain protoplast fusion for inducing genetic recombinations in *Aspergillus niger*. On the other hand, Ogawa *et al.* (1989) reported enhancement of cellulases production in *Trichoderma reesei* by inter-specific protoplast fusion, but not much work has been focused for increasing the production of other hydrolytic enzymes such as chitinase in *Trichoderma* using this technique. Therefore, the present work aimed to isolate protoplasts from *T. harzianum* and carry out self-fusion of protoplasts for investigating the possible enhancement of chitinase productivity. The antagonistic potential of the fusant strains was studied as well.

MATERIALS AND METHODS

Culture of *Trichoderma harzianum*

A number of *Trichoderma* strains was isolated from soil and compost samples collected from different parts of Kafr EL-Sheikh, Egypt by serial dilutions on *Trichoderma* selective medium (Papavizas and Lumsden, 1982). Qualitative and quantitative screening for extracellular chitinase resulted in selecting *T. harzianum* strain ATh1 for protoplast fusion programme and maintained on potato dextrose agar (PDA) slants.

Isolation of protoplasts from *T. harzianum*

About 1 ml of conidial suspension (5 x 10^8 conidia /ml) of *T. harzianum* strain ATh1 was inoculated into 50 ml of potato dextrose broth and incubated on a rotary shaker at 120 rpm at 28 ºC for 18 hr. The culture was harvested and mycelia were separated by filtration. A portion of 100 mg wet mycelia were washed with sterile distilled water followed by 0.1 M phosphate buffer, pH 5.8 and incubated with Novozyme 234 (Sigma Co.) at 10 mg/ml concentration prepared in phosphate buffer containing 0.7 M KCl as osmotic stabilizer. The enzyme-mycelial was incubated at 30 ºC with mild shaking and the release of protoplast was monitored regularly under phase contrast microscope. After 3 hr, the protoplast preparations were filtered through sterile cotton wad and centrifuged at 3000 rpm for 5 min at 4 ºC. The supernatant was discarded and the sedimented protoplasts were suspended immediately in buffer-osmotic stabilizer solution (EL-Bondkly, 2006). Self-fusion of *T. harzianum* protoplasts were carried out by a modified method of Stasz *et al.* (1988). Polyethylene glycol (PEG) MW 6000 prepared in buffer (STC) containing 0.6 M sorbitol, 10 mM Tris-HCl and 10 mM $CaCl_2$ (pH 6.5) was used as fusogen. Two ml of protoplast suspension were mixed with equal volume of 30 % (w/v) PEG solution and the fusion mixture was incubated at 30 ºC for 10 min. The mixture was diluted with equal volume of STC buffer. The PEG in the fusion mixture was washed away, using STC buffer and the fused protoplasts were collected by centrifugation at 3000 rpm for 5 min at 4 ºC, suspended in STC buffer and plated on 2 % colloidal chitin agar medium containing (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 20 and pH was adjusted to 6.5. Plates were incubated at 28 ºC and the

protoplast regeneration and development of colonies were observed.

Growth of parent and fusants on colloidal chitin medium

Eighteen regenerated self-fusants of *T. harzianum* were selected based on their fast growth on selective medium. Mycelial disc of each self-fusant and parent was inoculated on PDA and 0.5 % colloidal chitin agar media and incubated at room temperature. The mycelial growth and morphology were monitored after 3 – 5 days.

Preparation of culture filtrates of parent and fusants

The parent and self-fusant strains were grown in 50 ml of colloidal chitin broth in 250 ml Erlenmeyer flasks. Each flask was inoculated with 1 ml of conidial suspension and incubated at 28 ºC on a rotary shaker at 180 rpm. After 6 days, cultures were harvested, filtered through Whatman No. 1 filter paper and centrifuged at 10000 rpm at 4 ºC. The cell free culture filtrates of parent and fusants were used as enzyme sources for chitinase assay.

Protein estimation and chitinase activity

The protein content in the culture filtrates was estimated by the dye-binding method of Bradford (1976). The amount of protein was calculated using Bovine Serum Albumin (Sigma Co.) as standard. The chitinase assay mixture containing 1 ml culture filtrate and 1 ml suspension of colloidal chitin (0.1 % in 50 mM sodium acetate buffer, pH 5.0) was incubated at 37 ºC in a water bath with constant shaking. After 2 hr, the release of N-acetylglucosamine in reaction mixture was estimated by the method of Reissig *et al.* (1955). One unit chitinase activity was defined as the amount of enzyme, producing 1 µM of N-acetylglucosamine from colloidal chitin in the reaction mixture/ml under standard assay condition.

Antagonistic activity of parent and fusants against fungi

The antagonistic activity of *T. harzianum* against the pathogenic fungi *Cephalosporium acremonium*, *Aspergillus niger* and *Rhizoctonia solani* was determined by dual culture technique. Mycelial discs were cut out from actively growing self-fusant and parent cultures of *T. harzianum* and tested fungi, and placed at the opposite poles on PDA medium. Plates were incubated at 28 ºC and the mycelial growth of *T. harzianum* strains and tested fungi was estimated after 4 days and percent inhibition of mycelial growth of the pathogens were calculated.

Crude chitinase preparation and its effect on lysis of mycelia

The parent and fusants of *T. harzianum* were grown in colloidal chitin broth medium for six days as shaken cultures; and the culture filtrates were prepared as previously mentioned. The proteins in the culture filtrates were precipitated by salting-out with ammonium sulphate at 80 % saturation and left overnight at 4 ºC**.** The precipitate was collected by centrifugation at 10000 rpm, dialyzed against 0.1 M phosphate buffer (pH 6.0) and freeze-dried. The crude chitinase preparations (10 mg/ml) of *T. harzianum* parent and fusants (ATh1/9, ATh1/12, ATh1/14 and ATh1/17) were dissolved separately in 0.1 M phosphate buffer pH 5.8 containing 0.7 M KCl. Mycelia of *T. reesei*, *T. viride* and *A. niger* were incubated with the above enzyme preparation and also with commercial Novozyme 234 (Sigma Co.) and the released protoplasts were observed using a phase contrast microscope.

RESULTS AND DISCUSSION

Protoplast formation

Protoplasts have rapidly become acceptable tools for the fungal genetics with the development of new techniques of gene transfer such as protoplast fusion. Protoplasting requires specific conditions including cell age, temperature, medium, ……etc. The conditions for releasing the protoplasts were similar as reported by EL-Bondkly (2006). Complete lysis of mycelium and release of protoplasts were observed after 3 hr (Figure 1). The protoplasts released initially were smaller in size, but later enlarged to spherical structures. The protoplasts yield significantly affected by the concentrations of lysing enzymes. The lysis of fungal mycelium was confined only to the tip portion with a minimum release of protoplasts at low concentrations of lysing enzyme. On the other hand, at high enzyme concentrations, the mycelium lysed effectively yielding large numbers of protoplasts, but they bursted immediately after release and disintegrated. Among different concentrations of lysing enzymes tested, 10 mg/ml with 0.7 M KCl as osmotic stabilizer was optimal for the release of protoplasts from *T. harzianum*. Pe'er and Chet (1990) obtained highest protoplasts yield from *T. harzianum* using Novozyme 234 at 10 mg/ml with 0.6 M KCl, whereas Tschen and Li (1994) used 15 mg/ml of Novozyme 234 with 0.6 M sucrose to obtain maximum number of protoplasts from *T. harzianum* and *T. koningii*.

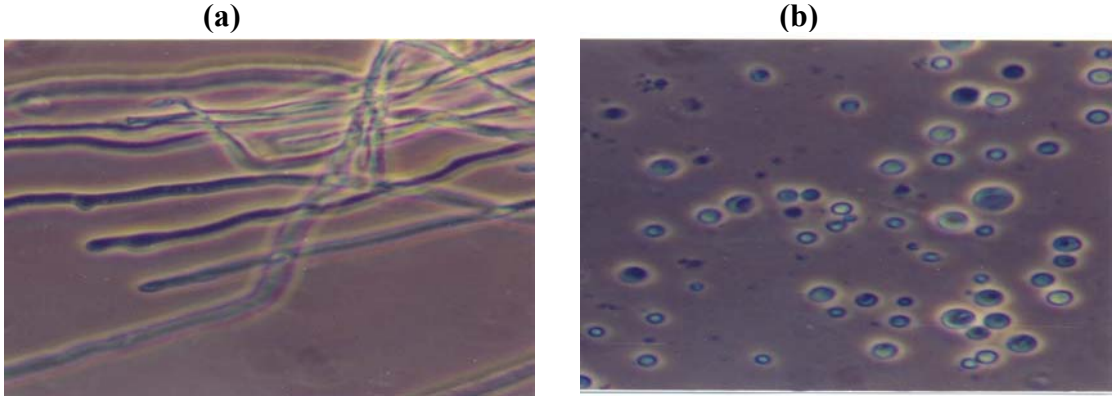

Fig. (1): The Trichoderma harzianum mycelium (a) and after three hours incubation with Novozymes 234 (b).

Self - protoplast fusion and regeneration

The protoplasts were attracted to each others and pairs of protoplasts were observed when PEG solution was added to the protoplasts. Later, the plasma membrane at the place of contact dissolved and protoplasmic contents fused together, then followed by nuclear fusion in most cases. Finally, the fused protoplasts became single, larger and round or oval-shaped structures. In the present study, self-fusion of protoplasts in *T. harzianum* was achieved using 30 % PEG 6000. As similar concentration of PEG was reported as optimum for inter-specific fusion of protoplasts between *T. harzianum* and *T. reesei*, as well as intergeneric protoplast fusion between *T. harzianum* and *A. niger* (EL-Bondkly, 2002; EL-Bondkly, 2006). However, Pe'er and Chet (1990) used 33 % PEG for protoplast fusion in *T. harzianum*.

Chitinase activity in the culture filtrates

The fused protoplasts were plated on a high concentration (2 %) of colloidal chitin agar for further selection. Prominent colonies were observed after 4 days on 2 % colloidal chitin. Based on the mycelial growth, 18 fast growing colonies were selected. Though an initial set back in growth of self-fusants was observed, the colonies exhibited fast mycelium growth after three days. Moreover, most of the self-fusants grew very fast on PDA medium than the parent strain, indicating the enhanced growth vigour. The fusants also exhibited good growth on colloidal chitin agar medium, and some of them grew extremely compared to the parent strain, indicating quicker adapt-ability to the new environment. In addition, this growth pattern on colloidal chitin agar medium indirectly indicated the enhanced production of extracellular chitinase. This could be directly related to strain improvement in *T. harzianum*.

Table (1): Chitinase activity and protein content in culture filtrates of the parent and self- fusants of T. harzianum.

Strain	Chitinase activity		Protein content (µg/ml)
	U/ml	% From parent	
ATh1 (parent)	3.5	100.0	35.5
ATh1/1	4.3	122.9	37.5
ATh1/2	5.5	157.1	39.2
ATh1/3	4.6	131.4	37.5
ATh1/4	6.2	177.1	45.0
ATh1/5	4.7	134.3	37.5
ATh1/6	3.5	100.0	35.8
ATh1/7	6.8	194.3	47.0
ATh1/8	5.5	157.1	39.5
ATh1/9	7.0	200.0	50.0
ATh1/10	3.5	100.0	35.8
ATh1/11	4.5	128.6	37.5
ATh1/12	7.0	200.0	52.0
ATh1/13	6.5	185.7	46.0
ATh1/14	7.0	200.0	50.0
ATh1/15	3.5	100.0	35.8
ATh1/16	6.5	185.7	46.2
ATh1/17	7.0	200.0	50.0
ATh1/18	5.5	157.1	39.0

High protein content was recorded in culture filtrates of all the 18 fusants obtained, than the parent strain. The highest amount of protein (52.0 µg/ml) was estimated in the fusant ATh1/12, followed by the three fusants ATh1/9, ATh1/14 and ATh1/17 with 50.0 µg/ml. On the other hand, the lowest protein content of 35.8 µg/ml was recorded in the culture filtrates of the fusants ATh1/6, ATh1/10 and ATh1/15, but still more than the parental strain (Table 1).

Data in Table (1) present the enhanced chitinase production in self-fusant cultures. The enzyme activity remarkably increased 83.3 % in most self-fusant isolates, except strains ATh1/6, ATh1/10 and ATh1/15, which still produce chitinase activity as the same parent strain when compared to the parent ATh1 strain. Four fusants (ATh1/9, ATh1/12, ATh1/14 and ATh1/17) produced the maximum enzyme activity of 7.0 U/ml, representing two-fold the activity of the parent strain. Fusant ATh1/7 exhibited 94.3 % more chitinase activity than the parental ATh1 strain. Furthermore, two self-fusant strains ATh1/13 and ATh1/16 showed higher

chitinase productivity (85.7 %) more than the original culture ATh1. Self-fusants ATh1/4 produced 77.1 % more chitinase activity than the parental strain. Three self-fusants ATh1/2, ATh1/8 and ATh1/18 produced 57.1 % more activity than the original strain ATh1. The four fusants ATh1/1, ATh1/3, ATh1/5 and ATh1/11 produced > 22 % chitinase activity than the parent strain.

Table (2): Antagonistic activity of parent and self-fusants of T. harzianum against pathogenic fungi.

Strain	*Cephalosporium acremonium*			*Aspergillus niger*			*Rhizoctonia solani*		
	GA (cm)	GP (cm)	GIP (%)	GA (cm)	GP (cm)	GIP (%)	GA (cm)	GP (cm)	GIP (%)
ATh1 (parent)	6.5	2.5	72.2	6.0	3.0	66.7	6.8	2.2	75.6
ATh1/1	7.0	2.0	77.7	6.5	2.5	72.2	7.2	1.8	80.0
ATh1/2	7.5	1.5	83.3	7.2	1.8	80.0	7.7	1.3	85.6
ATh1/3	7.0	2.0	77.7	6.8	2.2	75.6	7.3	1.7	81.1
ATh1/4	8.0	1.0	88.9	7.5	1.5	83.3	8.2	0.8	91.1
ATh1/5	7.5	1.5	83.3	6.8	2.2	75.6	7.4	1.6	82.2
ATh1/6	6.8	2.2	75.6	6.2	2.8	68.9	6.9	2.1	76.7
ATh1/7	8.5	0.5	94.4	7.8	1.2	86.7	8.8	0.2	97.8
ATh1/8	7.6	1.4	84.4	7.2	1.8	80.0	7.8	1.2	86.7
ATh1/9	9.0	0.0	100.0	8.5	0.5	94.4	9.0	0.0	100.0
ATh1/10	6.7	2.3	74.4	6.2	2.8	68.9	6.9	2.1	76.7
ATh1/11	7.0	2.0	77.7	6.8	2.2	75.6	7.3	1.7	81.1
ATh1/12	9.0	0.0	100.0	6.5	8.5	94.4	9.0	0.0	100.0
ATh1/13	8.3	0.7	92.2	7.6	1.4	84.4	8.5	0.5	94.4
ATh1/14	9.0	0.0	100.0	8.5	0.5	94.4	9.0	0.0	100.0
ATh1/15	6.8	2.2	75.6	6.2	2.8	68.9	6.9	2.1	76.7
ATh1/16	8.4	0.6	93.3	7.6	1.4	84.4	8.5	0.5	94.4
ATh1/17	9.0	0.0	100.0	8.5	0.5	94.4	9.0	0.0	100.0
ATh1/18	7.5	1.5	83.3	7.2	1.8	80.0	7.8	1.2	86.7

GA = Growth of antagonist GP = Growth of pathogen GIP = Growth inhibition of pathogen (cm) = Size of fungal growth in Petri dish (9 cm).

Improvement of antagonistic activity in self-fusants was recorded against *C. acremonium*, *A. niger* and *R. solani*, compared to the parent strain. Complete inhibition (100 %) of mycelial growth of *C. acremonium* and *R. solani* was recorded with the four fusants ATh1/9, ATh1/12, ATh1/14 and ATh1/17 against 72.2 and 75.6 % for parent strain, respectively (Table 2). Also, they produced the highest chitinase productivity. On the other hand, all self-fusant strains show no complete inhibition of mycelial growth of *A. niger*. Whereas, all self-fusant strains inhibited at least 74.4, 68.9 and 76.7 % of mycelial growth of *C. acremonium*, *A. niger* and *R. solani*, respectively, compared to the parent strain which appeared 72.2, 66.7 and 75.6 % inhibition of fungal mycelia growth of *C. acremonium*, *A. niger* and *R. solani*, respectively. A clear correlation between the chitinase production and biocontrol activity of the fusant and parent strains were reported.

The crude chitinase preparation of *T. harzianum* parent ATh1 lysed the mycelia and released protoplasts from three filamentous fungi (*T.reesei*, *T. viride* and *A. niger*), but this activity was remarkably high with the crude enzyme preparation of the four highest chitinase activity self –fusant strains ATh1/9, ATh1/12, ATh1/14 and ATh1/17 (Table 3). However, the commercial lysing enzyme (Novozyme 234) effectively lysed the cell walls of *T. reesei*, *T. viride* and *A. niger* as well as released maximum protoplasts compared to the crude enzyme preparations of these fusants, because it was a multi-enzyme

mixture containing chitinase, cellulases and proteinase. Similarly, a lyophilized enzyme preparation of *T. harzianum* that contained chitinase and β-1,3-glucanase released the highest number of protoplasts from different fungi than the commercial enzymes (Kitamoto *et al.*, 1988).In conclusion, the self-fusion of protoplasts in *T. harzianum* resulted in an appreciable increase of chitinase productivity and biocontrol activity in most of the self-fusant strains. Results demonstrated the significance of the protoplast fusion technique, which could successfully be used to develop superior hybrid strains in filamentous fungi that lack sexual reproduction.

Table (3): Protoplasts released from mycelia of filamentous fungi by the crude enzyme preparations of parent and self-fusants of T. harzianum.

Treatment	Concentration (mg/ml)	No. of protoplasts / ml		
		T. reesei	*T. viride*	*A. niger*
Crude enzyme ATh1	10	5×10^4	4×10^4	2×10^3
Crude enzyme ATh1/9	10	1×10^5	8×10^4	5×10^3
Crude enzyme ATh1/12	10	1×10^5	8×10^4	5×10^3
Crude enzyme ATh1/14	10	2×10^5	8×10^4	6×10^3
Crude enzyme ATh1/17	10	1×10^5	8×10^4	5×10^3
Novozyme 234 (Sigma Co.)	10	8×10^7	5×10^7	5×10^5
Buffer control	0	0	0	0

REFERENCES

Bradford, M. M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248 – 254.

Das, A.; Gokhale, D. V. and Peberdy, J. F. (1988). Protoplast fusion and genetic recombination in intra- and interstrain crossing in *Aspergillus niger*. Enzyme Microb. Technol., 11: 2 – 5.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Prot., 19: 709–714.

EL-Bondkly, A. M. (2002). Genetic transformation in *Trichoderma reesei* for the improvement of cellulase production. Ph.D., Thesis, Faculty of Kafr EL-Sheikh, Tanta University.

EL-Bondkly, A. M. (2006). Gene transfer between different *Trichoderma* species and *Aspergillus niger* through intergeneric protoplast fusion to convert ground rice straw to citric acid and cellulases. Appl. Biochem. Biotechnol., 135 (2): 117 – 132.

Kitamoto, Y.; Mori, N.; Yamamoto, M.; Ohiwa, T. and Ichikawa, Y. (1988). A simple method for protoplast formation and improvement of protoplast regeneration from various fungi using an enzyme from *Trichoderma harzianum*. Appl. Microbiol. Biotechnol., 28: 445–450.

Lalithakumari, D. (2000). Fungal protoplasts-a biotechnological tool. Oxford & IBH Publishing Company Private Limited, New Delhi, India.

Mathivanan, N.; Kabilan, V. and Murugesan, K. (1998). Purification, characterization and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasite to groundnut rust, *Puccinia arachidis*. Can. J. Microbiol., 44, 646 – 651.

Mathivanan, N.; Srinivasan, K. and Chelliah, S. (2000). Biological control of soil-borne diseases of cotton, eggplant, okra and sunflower by *Trichoderma viride*. J. Plant Dis. Prot., 107: 235 – 244.

Mrinalini, C. and Lalithakumari, D. (1998). Integration of enhanced biocontrol efficacy

and fungicide tolerance in *Trichoderma spp*. by electrofusion. J. Plant Dis. Prot., 105: 34–40.

Ogawa, K.; Ohara, H.; Koide, T. and Toyama, N. (1989). Interspecific hybridization of *Trichoderma reesei* by protoplast fusion. J. Ferment. Bioeng., 67: 207–209.

Papavizas, G. C. and Lumsden, R. D. (1982). Improved medium for isolation of *Trichoderma spp*. from soil. Plant Dis., 66: 1019 – 1020**.**

Peberdy, J. F. (1980). Protoplasts fusion-a tool for genetic manipulation and breeding in industrial microorganisms. Enzyme Microb. Technol., 2: 23 – 29.

Peberdy, J. F. (1989). Fungi without coats-protoplasts as tools for mycological research. Mycol. Res., 93: 1 – 20.

Pe ́er, S. and Chet, I. (1990). *Trichoderma* protoplast fusion; a tool for improving biocontrol agents. Can. J. Microbiol., 36: 6–9.

Prabavathy, V. R.; Mathivanan, N.; Sagadevan, E.; Murugesan, K. and Lalithakumari, D. (2006). Intra-strain protoplast fusion enhances carboxymethyl cellulase activity in *Trichoderma reesei*. Enzyme Microb. Technol., 38: 719–723.

Reissig, J. L.; Strominger, J. L. and Leoloir, I. F. (1955). A modified colorimetric method for the estimation of N-acetylamino sugars. J. Biol. Chem., 217: 959–966.

Sivan, A. and Harman, G. E. (1991). Improved rhizosphere competence in a protoplast fusion progeny of *Trichoderma harzianum*. J. Gen. Microbiol., 137: 23–9.

Stasz, T. E.; Harman, G. E. and Weeden, N. F. (1988). Protoplast preparation and fusion in two biocontrol strains of *Trichoderma harzianum*. Mycologia, 80: 141–150.

Tschen, J. S. M. and Li, I. F. (1994). Optimization of formation and regeneration of protoplasts from biocontrol agents of *Trichoderma sp*. Mycoscience, 35: 257–263.

الملخص العربي

التزاوج الداخلي عن طريق الدمج الخلوي في فطر الترايكوديرما هارزيانم لتحسين إنتاجيته من إنزيم الكيتينيز و قدرته على المقاومة الحيوية

أحمد محمد البندلى و فاطمة نبيه طلخان

معمل الوراثة الميكروبية التطبيقية – قسم الوراثة و السيتولوجى – المركز القومي للبحوث – الجيزة – مصر.

تناولت هذه الدراسة عزل البروتوبلاست من فطر الترايكوديرما هارزيانم باستخدام إنزيم النوفوزيم ٢٣٤ في محلول اسموزى منظم ٠,٧ مول كلوريد البوتاسيوم ثم الدمج الخلوي الداخلي (بين خلايا نفس السلالة) باستخدام البولي إيثيلين جليكول ٦٠٠٠ تلى ذلك إعادة نمو الخلايا المندمجة على بيئة انتخابية تحتوى على ٢ % كولديل كيتين. تم انتخاب ١٨ مندمجة خلوية بناء على سرعة و قوة النمو على البيئة الانتخابية مقارنة بالسلالة الأبوية ATh1، التي كانت اقل في السرعة و درجة النمو. تم اختبار هذه المندمجات الخلوية المنتخبة بالنسبة لإنتاجيتها من إنزيم الكيتينيز ووجد أن أربعا من المندمجات الخلوية (ATh1/9 و ATh1/12 و ATh1/14 و ATh1/17) لها قدرة عالية على إنتاج إنزيم الكيتينيز، حيث أعطت مرتين أعلى من السلالة الأبوية، بينما المندمجة الخلوية ATh1/7 أعطت ٩٤,٣ % كيتينيز أعلى من السلالة الأبوية. و لقد أظهرت معظم المندمجات الخلوية فاعلية و قدرة على تثبيط نمو بعض الفطريات (*C. acremonium* و *A. niger* و *R. solani*) أفضل من السلالة الأبوية و خاصة الأربع مندمجات الخلوية عالية القدرة في إنتاج إنزيم الكيتينيز، حيث أظهرت تثبيطا كاملا لاثنين من هذه الفطريات (*C. acremonium* و *R. solani*). كذلك تم دراسة تأثير إنزيم الكيتينيز المجهز من مستخلص نمو الأربع مندمجات الخلوية بالإضافة للسلالة الأبوية على تحليل الجدار الخلوي لثلاث من الفطريات (*T. reesei* و *T. viride* و *A. niger*) و تحرير البروتوبلاست منها حيث وجد أن هذه المندمجات لها قدرة أعلى من السلالة الأبوية على تحرير البروتوبلاست لكنها ما زالت أقل

من الإنزيم التجاري نوفوزيم ٢٣٤. أظهرت نتائج هذه الدراسة أهمية استخدام تكنيك الدمج الخلوي للحصول على سلالات ذات إنتاجية عالية و خاصة في الفطريات التي لا يعرف لها دورة تكاثر جنسي.