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Original Article

The Enzymatic Activity and Molecular Characterization of a Secreted Subtilisin-Like Protease in *Microsporum gypseum* and *Trichophyton vanbreuseghemii*

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Abstract

Background: Subtilisin –like proteases are the group of proteases including keratinases found in dermatophytes which degraded keratin. Determination of the proteases activity of *Trichophyton vanbreuseghemii* isolates which were obtained from soil and clinical and soil isolates of *Microsporum gypseum* in Iran and characterization of their genome were aim of present study.

Methods: Ezymatic activity was determined by use of chromogenic substrates. The genes, which coded subtilisin-like proteases in above-mentioned dermatophytes, was identified and amplified by using specific primers in PCR.

Results: The highest yield of enzyme production was observed in only one isolate of *T. vanbreuseghemii* Ir-84 whereas low enzyme activity was observed in *M. gypseum* isolates. Homology study of obtained nucleotide as well as amino acid sequences indicated different rates of homology with other subtilisin-like proteases genes in other pathogenic dermatophytes.

Conclusion: Intra-strain differences were observed in production of serine proteinases and molecular characterization of genes encoding such enzymes could be of great interest for studies on pathogenicity and other purposes.

Keywords: *Keratinases, subtilisin-like protease,* Trichophyton vanbreuseghemii, Microsporum gypseum

Introduction

Since the beginning of enzymology science, microbial proteases were the enzymes which have been of great interest particularly for industries of leathers, food and poultry that produce, large quantities of keratinc wastes constitute a growing problem(1). Keratinolytic enzymes from microorganisms may have important uses in biotechnological process involving keratin-containing wastes from poultry and leather industries through the development of non-polluting processes (2). Subtilisin- like proteases belong to proteases such as keratinases found in dermatophytes which degrade keratin. They have been reported as having essential role on the pathogenesis of dermatophytes. The previous studies revealing that the virulence of dermatophytes was based on the ability of secretion of these enzymes (3, 4). However, such enzymes are not exclusively associated with pathogenic dermatophytes since they have also been found in some geophilic species which degrade keratinous material including hair, nail and horns normally found in nature (5). Therefore, studying the enzymatic activity and also the genome which code these enzymes in the pathogenic and non-pathogenic soil strains of dermatophytes is absolutely essential to determine their biological significance.

Thus investigation of the enzymatic activity from

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T. vanbreuseghemii and *M. gypseum* and also determination of genes, which code the enzyme, are the subject of the present study.

Material and Methods

Microorganisms and culture conditions

This comparative cross sectional study was carried out using soil isolates of *T. vanbreuseghemii* (32 strains)and *M. gypseum* (7 strains) from north-east of Iran and also one clinical isolate of *M. gypseum* from Tehran during 3 yr from 2004 to 2006 (6). The fungi were propagated on yeast extract /peptone /dextrose agar (YPDA) plates at 25º C. Inoculums were prepared from 7 day-old culture by flooding with approx one ml of sterile distilled water and scrapping off the agar plate.

Culture for enzyme production

All fungi were grown in modified Czapek Dox liquid medium (MCLM) containing glucose, 10g ; K_2 HPO_4, 1g ; $MgSO_4$, 0.5g; KCl,0.5g; $FeSO_4$- $7H_2O$, 0.0lg; chloramphenicol l0g and bacteriological peptone 0.1% w/v as the nitrogen source.

For enzyme production, triplicate cultures of fungi were carried out in 100 ml sterile flasks containing each 50ml of MCLM which were incubated at 25 °C for two weeks. Following incubation, they were filtered through 0.45µm-pore- size pre- tarred membranes (Millipore) and serine proteinase activity was assayed using azocasein as the general substrate and the specified chromogenic substrates of serine proteinase in culture filtrate.

Determination of protein concentration

The protein content of culture filtrate was determined as described by Bradford (7), using BSA as standard.

Enzyme activity assay

Serine proteinase activity was assayed using three different chromogenic substrate including N-Suc-Ala-Ala-Pro–Phe-ρNA, N-Suc-Ala-Ala-Pro-Leu-ρNA and N–Bz. Phel–Val-Arg- ρNA (Sigma) (where Suc is Succinyl, pNA is p-nitroanilide and Bz is benzoyl) titrating the chymotrypsin/subtilisin, elastase and trypsin activities, respectively.

Substrates were dissolved in dimethyl sulfoxide (DMSO) at stock concentration of 5 mM. The assay was performed on polystyrene micro titer plates and reaction mixture contained per well, l80 µl of culture filtrates and 20µl of chromogenic substrates (0.5 mM final concentration). After 30 min of incubation at 37° C, the amount of ρ-nitroaniline (ρNA) released was measured at 405 nm using a Titertec Multiscan spectrophotometer (Lab system).

Enzyme activity was expressed in nkat/ml; one nkat is being defined as the amount of enzyme which releases one mMole of ρNA in one second.

Azocaseinolytic activity was measured using azocasein titerating the serine proteinase activity. Azocasein was dissolved in 20 mMTris/HCl buffer at a stock concentration of 2% (w/v). The reaction mixture contained 250 µl of Azoeasein 2% (w/v), and l00µl of cultural filtrate. The mixture was incubated at 37° C for 1h in shaking condition. The reaction was stopped by the addition of 10% (w/v) trichloroacetic acid, held in an ice-water bath for 1 h. After centrifugation at 13000×g for 5 min in a cooled centrifuge, the absorbance value of the supernatant was measured at 405nm. One unit of enzyme activity was the amount of enzyme that caused a change of absorbance of 0.01 at 405 nm for 30 min at 37° C (8). Each assay was performed in duplicate.

Determination of the serine- proteinase's gene

Preparation of fungal genomic DNA

Fungal genomic DNA was prepared using a modification of the method of Rezaie et al. (9). Briefly the harvested mycelia mass was flash-frozen in liquid nitrogen and ground to a fine powder in a porcelain mortar. The powdered mycelia were suspended in DNA extraction buffer containing: 50 mM This- HCl (pH 8.0), 50 mM EDTA, 1ml SDS1M and 15 µl of proteinase (20 mg/ml). The suspension was then incubated at 60° C for 1 h, and the cellular debris was removed by centrifugation at 3000×g for 15 min. After addition of 25µl RNase (l0 mg/ 1 mol) the suspension was incubated at 37° C

for 30 min, extracted once with phenol-chloroform isoamyl-alcohol (25: 24: 1) and once with chloroform-isoamyl alcohol (24:1). The DNA was precipitated by adding an equal volume of isopropanol, followed by centrifugation at 15000× g for 30 min. The DNA pellet was rinsed with 70% ethanol and resuspended in distilled water.

PCR analysis

Pairs of 20 nt primers were designed from highly conserved regions of the serine- proteinase genes in other dermatophytes. PCR was performed by using of genomic DNA derived from *T. vanbreuseghemii* (Ir 84-ycc) and *M. gypseum* (Ir-7625) and synthetic oligonucleotide primers (MoS1: 5'-CATCACCAAGGCCATCCCTC-3' as sense and (MoAs1: 5'- GATGAGCTTGCTG-GTGC-3') according to a standard protocol (10). Briefly, 2.5µl of each primer (20 pm) was added to a volume of 50µl containing: 10µl of 10X buffer PCR, 2µl of dNTP, 3µl of $mgCl_2$, 1.5 µl of the genomics DNA of each fungi, 0.5 µl of Taq DNA polymerase and 27µl distilled H_2O.

The PCR protocol employed was 94° C for l min, 52° C for 1 min and 72° C for 75S, with 35 cycles by using thermal cycler (PerKin-Elmer, USA). PCR Products were analyzed by electrophoresis through 1% gel.

Sequencing of the PCR products

PCR products were extracted by using DNA gel extraction kit (Qiagene). Sequencing of DNA fragment was performed with the dye Terminator cycle kit. Sequencing kit using the amplified double stranded DNA as template and synthetic 20 meric nucleotide primers mentioned above. Sequencing was repeated at least three times for both strands. The nucleotide sequence of DNA was Compared with the sequence in gene data banks in National centre for Biotechnology Information (NCB, NIH).

Results

Enzyme activity assay

The enzymatic activity of the different soil strains of *T. vanbreuseghemii* and *M. gypseum* and also the clinical strain of *M. gypseum* are presented in Table 1. As it evident the highest hydrolysis rate (533 nKat/ml) was obtained by the proteinase from only *T. vanbreuseghemii* 84-YCC toward N-Suc-Ala-Ala-Pro-Phe-pNA a specific substrate for chymotrypsin/subtilisin, where at the same time the lowest hydrolysis activity (13 nkat/ml) was observed with N-BZ-Phel-Val-Arg-pNA, the substrate specific for trypsin. This strain also demonstrated high rate (128.6 u/ml) of caseinolytic activity. More over the clinical isolate of *M. gypseum* Ir-C7625 showed minimum rate of proteinase activity against both casein and chormogenic subsrates. The serine-protease specific activities of both *T. vanbreuseghemii* Ir84-YCC and *M. gypseum* Ir-c 7625 towards the chromogenic substrate N-Suc-Ala-Ala-Pro-Phe-pNA are presented in Table 2.

Characterization of subtilisin serine proteinase genes after performing PCR for amplification of subtilisin-like serine proteinases genes in the mentioned dermatophytes, two PCR fragment with approximate size of 1.2 kbp have been amplified (Fig. 1). The result of sequencing revealed the size of PCR fragments as 1261 bp for *T. vanbreuseghemii* and 1107 bp for *M. gypseum* (Fig. 2, 3).

These sequenced DNAs containd 3 open reading fram of approximately 1095bp in *T. vanbreuseghemii* and l082 in *M. gypseum* encoding a 358 amino acids protein in *T. vanbreuseghemii* and a 333 amino acids protein in *M. gypseum.* Three introns have been indicated within open reading frame of the amplified genes in both dermatophytes.

Nucleotide sequence comparison in gene data bank (NCBI, NIH) for the amplified DNA fragments and their deduced amino acid sequence revealed significant homology with the subtilisin-like serine proteinase of other dermatophytes. The amino acid sequences of the encoded protein in *T. vanbreuseghemii* were identical to the sequence of encoded protein in *M. gypseum.* The mentioned amino acid sequences were about 85% to 92% identical to the sequence of subtilisin-like serine proteinase from *Arthroderma benhamia* and *T. verrucosum*. The amino acid compositions of these proteins indicate that they

were rich in glycine (12%-13%) and alanine (10% to 11%). In contrast, a lot content of cystein, tryptophan (1%) and prolin and histidin 2% were deduced from the sequenced genes.

Nucleotide and amino acid sequences of these newly characterized genes have been submitted to the National Centre of Biotechnology Information Gene Bank and are available for public access under the accession numbers DQ 923809 for *M. gypseum* and DQ 923810 for *T. vanbreusghemii*.

Table 1: The enzymatic activity of the different soil strains of *T.vanbreuseghemii* and *M. gypseum* using azocasein and Chromogenic synthetic substrates

n	Species	Strains	Azocaseinolytic Activity (U/ml)	Serine proteinase Activity nkat/mmol		
			Asocasein	Phe-ρNA	Leu- ρNA	Arg- ρNA
1	*M. gypseum*	Ir-c7625	11.6	42	16	14
2	*M. gypseum*	Ir-86ycc	3	25	14	10
3	*M. gypseum*	Ir-124ycc	4	32	11	14
4	*M. gypseum*	Ir105ycca	2	42	12	15
5	*M. gypseum*	Ir-171ycc	5	36	12	17
6	*M. gypseum*	Ir-191ycc	9	9	3	7
7	*M. gypseum*	Ir-175ycc	5	39	12	18
8	*M. gypseum*	Ir-361ycc	6	22	4	8
9	*T.vanbreuseghemii*	Ir-84ycc	128.6	533	92	13
10	*T.vanbreuseghemii*	Ir-89ycc	12	3	8	20
11	*T.vanbreuseghemii*	Ir142ycc	9	2	4	48
12	*T.vanbreuseghemii*	Ir-150ycc	5	22	2	46
13	*T.vanbreuseghemii*	Ir-117ycc	4	7	0	16
14	*T.vanbreuseghemii*	Ir-119ycc	12	6	0	16
15	*T.vanbreuseghemii*	Ir-45ycc	14	4	1	13
16	*T.vanbreuseghemii*	Ir-61ycc	13	10	2	5
17	*T.vanbreuseghemii*	Ir-78ycc	11	23	0	32
18	*T.vanbreuseghemii*	Ir-11ycc	16	0	2	32
19	*T.vanbreuseghemii*	Ir-94ycc	10	0	2	5
20	*T.vanbreuseghemii*	Ir-02ycc	11	9	3	7
21	*T.vanbreuseghemii*	Ir-21ycc	12	7	2	18
22	*T.vanbreuseghemii*	Ir-42ycc	3	2	4	48
23	*T.vanbreuseghemii*	Ir-17-ycc	6	11	2	46
24	*T.vanbreuseghemii*	Ir-33ycc	9	15	0	16
25	*T.vanbreuseghemii*	Ir-252ycc	12	65	0	16
26	*T.vanbreuseghemii*	Ir-215ycc	15	43	0	13
27	*T.vanbreuseghemii*	Ir-115ycc	18	10	2	5
28	*T.vanbreuseghemii*	Ir-164ycc	10	53	0	32
29	*T.vanbreuseghemii*	Ir-019ycc	2	14	21	32
30	*T.vanbreuseghemii*	Ir-137ycc	5	75	21	25
31	*T.vanbreuseghemii*	Ir-133ycc	2	91	25	37
32	*T.vanbreuseghemii*	Ir-149ycc	9	51	18	42
33	*T.vanbreuseghemii*	Ir- 252ycc	12	2	4	48
34	*T.vanbreuseghemii*	Ir-189ycc	15	11	2	46
35	*T.vanbreuseghemii*	Ir-164ycc	3	71	0	16
36	*T.vanbreuseghemii*	Ir-174ycc	9	6	0	16
37	*T.vanbreuseghemii*	Ir-162ycc	12	4	1	13
38	*T.vanbreuseghemii*	Ir-245ycc	15	10	2	5
39	*T.vanbreuseghemii*	Ir-227ycc	4	23	0	32
40	*T.vanbreuseghemii*	Ir-257ycc	9	0	2	32

Table 2: The serine-protease activities from *T. vanbreuseghemii* and *M. gypseum* by measuring the rate of hydrolysis of the chromogenic substrate N-Suc-Ala-Ala-Pro-Phe-pNA

Culture filtrate (species)	Volume (ml)	Total Protein(mg)	Total Activity(nkat)	Specific Activity(nkat mg)	Yield (%)
T. vanbreuseghemii (Ir-84YCC)	4870	33.5	1,165,878	21,792	100
M.gypseum (Ir-c 7625)	4840	9.9	3,308	332	100

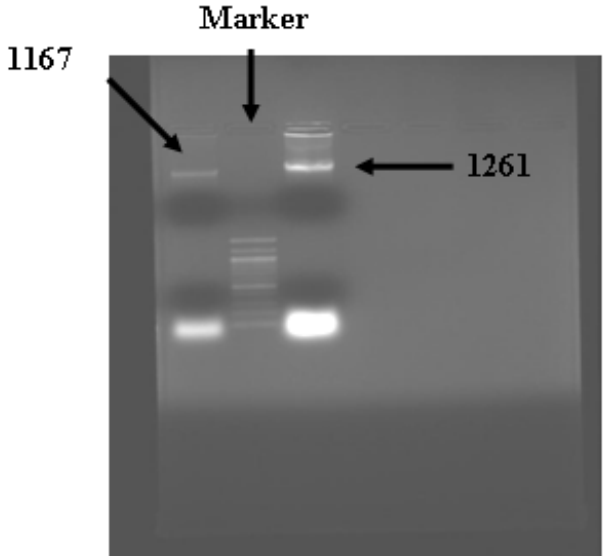

Fig. 1: The molecular weight of amplified DNAs in *T. vanbreuseghemii* and *M. gypseum*. From left to right:

Lane 1: Amplified DNA of *M. gypseum* (Ir-c7625), Lane2: Molecular weight marker VIII (Roche, Germany) with the size range of 67-1114 bp, Lane3: Amplified DNA of *T. vanbreuseghemii.*(Ir-84YCC)

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217    S   N   S   G   V   I   M   G   M   E   W   A   T   E   D   A   Q   Q   K   G   236
706   TCC AAC TCT GGT GTC ATC ATG GGT ATG GAA TGG GCT ACC GAG GAC GCC CAG CAG AAG GGT  765

237    A   D   T   S   V   V   N   M   S   H   R   V   A   F   S   Q   A   S   N   D   256
766   GCC GAC ACG TCC GTC GTC AAC ATG TCC CAT CGT GTT GCC TTC TCC CAG GCC TCC AAC GAC  825

257    A   A   A   A   I   A   Q   G   G   V   F   L   A   V   A   S   G   Q   D   N   276
826   GCC GCT GCA GCA ATT GCT CAG GGT GGT GTC TTC TTG GCC GTT GCC TCC GGT CAA GAC AAT  885

886    GTAAGTGCGTTCCGCCTCAGCAACGTAGCTACGGCATCCAACTTGCCGATCATGTTAGGGACAG               949

277    V   D   A   A   D   S   S   P   A   S   E   L   S   I   C   T   V   A   A   S   296
950   GTC GAT GCT GCT GAT TCC TCC CCA GCT TCC GAG CTC TCC ATC TGC ACC GTC GCC GCC TCC 1009

297    T   E   Q   D   G   K   A   D   F   S   N   F   G   Q   V                     311
1010  ACG GAG CAG GAC GGC AAG GCC GAC TTC TCC AAC TTC GGC CAA GTT G                   1055

1056   GTCACTGCTCCAGTTTATTCACCTCTACAGTTGACAGAAGGAAACAGCCAGAGCATGGTTTCTAG              1120

312    V   D   V   Y   A   A   G   D   S   I   T   S   D   K   P   G   G   G   S   Q   331
1121   TT GAT GTC TAC GCT GCC GGT GAT TCC ATC ACC TCG GAC AAG CCA GGT GGT GGA TCT CAG 1180

332    V   L   S   G   T   S   M   A   T   P   H   V   A   G   L   G   A   Y   L   I   351
1181  GTC CTC TCT GGT ACC TCC ATG GCC ACC CCA CAC GTC GCC GGC CTC GGC GCC TAC CTT ATT 1240

352    G   L   G   K   G   G   G                                                     358
1241  GGT CTC GGC AAG GGC GGC GGC                                                   1261
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Fig. 2: The nucleotide sequence of the DNA is inserted in *T. vanbreuseghemii*. The amounts of 1261bp had been sequenced which was contained 3 open reading frame of approximately 1095bp encoding a 358 amino acids protein and had 3 interons

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189    G   V   A   K   K   S   N   I   V   A   V   K   V   L   D   C   N   G   S   G   208
622    GGT GTT GCT AAG AAG AGC AAC ATC GTT GCC GTG AAG GTC CTT GAC TGC AAC GGG TCT GGC   681

209    S   N   S   G   V   I   M   G   M   Q   W   A   T   E   D   A   Q   S   K   G   228
682    TCC AAC TCT GGT GTC ATC ATG GGT ATG CAA TGG GCT ACC GAG GAT GCC CAG AGC AAG GGT   741

229    A   D   T   S   V   V   N   M   S   H   R   V   A   F   S   Q   T   S   N   D   248
742    GCC GAC ACG TCC GTC GTC AAC ATG TCC CAT CGT GTT GCC TTC TCC CAG ACG TCC AAC GAC   781

249    A   A   K   A   I   A   E   G   G   V   L   L   A   L   S   S   G   Q   D   N   268
782    GCC GCT AAA GCA ATC GCT GAA GGT GGA GTC TTG TTG GCC CTT TCC TCC GGT CAA GAC AAT   841

842    GTAAGTGCGTTCCGCCTCAGAAACGTAGCTACGGCATCCAACTTGCTAATCATTTTACCGACAG                905

269    V   D   V   A   E   A   S   P   A   S   E   L   S   I   C   T   F   A   A   S   288
906    GTC GAT GTC GCG GAA GCC TCC CCA GCT TCC GAG CTC TCC ATC TGC ACT TTC GCC GCC TCC   965

289    T   E   Q   D   G   K   A   D   F   S   N   F   G   Q   V                       303
966    ACG GAG CAG GAC GGC AAG GCC GAC TTC TCC AAC TTC GGC CAA GTT G                    1011

1012   GTAACTTTTCCCGTTTATTCACCTCTACAGGCGACAGAAGGAAACAGCCAGAACATAAATTCTAG               1076

304    V   D   V   Y   A   A   V   D   G   I   T   S   D   K   P   G   G   G   S   Q   323
1077    TT GAT GTC TAC GCT GCC GTT GAT GGA ATC ACC TCG GAC AAG CCA GGT GGG GGA TCT CAG 1139

324    V   Q   S   G   T   S   K   A   S   P                                           333
1136   GTC CAG TCT GGT ACC TCC AAG GCC TCC CCA CA                                       1167

189    G   V   A   K   K   S   N   I   V   A   V   K   V   L   D   C   N   G   S   G   208
622    GGT GTT GCT AAG AAG AGC AAC ATC GTT GCC GTG AAG GTC CTT GAC TGC AAC GGG TCT GGC   681

209    S   N   S   G   V   I   M   G   M   Q   W   A   T   E   D   A   Q   S   K   G   228
682    TCC AAC TCT GGT GTC ATC ATG GGT ATG CAA TGG GCT ACC GAG GAT GCC CAG AGC AAG GGT   741

229    A   D   T   S   V   V   N   M   S   H   R   V   A   F   S   Q   T   S   N   D   248
742    GCC GAC ACG TCC GTC GTC AAC ATG TCC CAT CGT GTT GCC TTC TCC CAG ACG TCC AAC GAC   781

249    A   A   K   A   I   A   E   G   G   V   L   L   A   L   S   S   G   Q   D   N   268
782    GCC GCT AAA GCA ATC GCT GAA GGT GGA GTC TTG TTG GCC CTT TCC TCC GGT CAA GAC AAT   841

842    GTAAGTGCGTTCCGCCTCAGAAACGTAGCTACGGCATCCAACTTGCTAATCATTTTACCGACAG                905

269    V   D   V   A   E   A   S   P   A   S   E   L   S   I   C   T   F   A   A   S   288
906    GTC GAT GTC GCG GAA GCC TCC CCA GCT TCC GAG CTC TCC ATC TGC ACT TTC GCC GCC TCC   965

289    T   E   Q   D   G   K   A   D   F   S   N   F   G   Q   V                       303
966    ACG GAG CAG GAC GGC AAG GCC GAC TTC TCC AAC TTC GGC CAA GTT G                    1011

1012   GTAACTTTTCCCGTTTATTCACCTCTACAGGCGACAGAAGGAAACAGCCAGAACATAAATTCTAG               1076

304    V   D   V   Y   A   A   V   D   G   I   T   S   D   K   P   G   G   G   S   Q   323
1077    TT GAT GTC TAC GCT GCC GTT GAT GGA ATC ACC TCG GAC AAG CCA GGT GGG GGA TCT CAG 1139

324    V   Q   S   G   T   S   K   A   S   P                                           333
1136   GTC CAG TCT GGT ACC TCC AAG GCC TCC CCA CA                                       1167
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Fig. 3: The nucleotide sequence of the DNA is iserted in *M. gypseum.* The amounts of 1167bp had been sequenced which was contained 3 open reading frame of approximately 1095bp encoding a 333 amino acids protein and had 3 interons

Discussion

The growing concern for health and environmental protection coupled with the urgent search for potentially useful material such as proteinases has led us to undertake the present study. Proteases have been reported to be important in the invasion of skin and causing infection, therefore they could be considered as a virulence factor (2, 3, 11). In addition to dermatophytes, other non-pathogenic soil keratinolilic fungi are able to produce proteases (12). Therefore, the enzymatic activity of proteases and characterization of their genome in both pathogenic *M. gypseum* and non-pathogenic geophlic dermatophytes were determined. *T. vanbreuseghemii* and *M. gypseum* are both soil dermatophytes. The former fungus rarely causes ectothrix hair infection and it is now considered to be worldwide distributed (13, 14). This fungus also recently was recovered from soils of north part of Iran (6). The later fungus is a common geophilic dermatophytes widely distributed in soil globally as well as Iran. It causes ringworm of scalp and glabrous skin in human and animal (15).

Only *T. vanbreuseghemii* (Ir-84 Ycc) grown on Czapex –Dox liquid medium containing 0.1% bacteriological peptone and 1% glucose displayed strong protease activity after incubation for two weeks using three different chromogenic substrates. Observation of an obvious difference on hydrolysis rate of synthetic substrates in different strains of *T. vanbreuseghemii* and *M. gypseum* were observed, confirmed intra-strain differences in them, which is also in agreement with similar findings on the enzymatic activities of *A. fumigatus* (16), *T. mentagrophytes*, *T. rubrum* (17) and *T. tonsurans* (18). Among the three chromogenic substrates, the highest rate of hydrolysis obtained for N-Suc-Ala-Ala-Pro-Phe-pNA and revealed that the enzyme belongs to the chymotrepsin/ subtilisin family of serine proteinases. On the other hand the fact that the proteinase was not totally inefficient on these two other substrates seem to be a common feature of fungal subtilisin, since it has also been reported for similar chymotrypsin/subtilisin like serine proteinase purified from *A. fumigatus*, S*cedosporium apispormum*, *Microsporum canis*, *Fusarium calmorum* and *Myrothecium verrucaria* (16,19-23).

In this investigation, concerning enzymatic activity, soil isolate *T. vanbreuseghemii* (Ir- 84 ycc) and clinical isolate *M. gypseum* (Ir-c-7625), were selected to characterize the gene, which encoded serine-proteinase. Analysis of amino acids sequence derived from these genes in both fungi revealed a considerable identity with each other (94%) as well as with related proteinase from other dermatophytes such as those of *T. verrucusum* (93%), *Arthroderma benhamiae* (93%) and *T. rubrum* (92%) (23).

Homology between these two genes and their encoding proteinase with each other and also with other pathogenic dermatophytes may suggest the pathogenic role of these enzymes in mentioned dermatophytes. However probably in *M. gypseum,* an environmental inducer such as keratin is needed for secretion of this enzyme, which is quite in contrary to nutritional requirements of *T. vanbreusgehmii*.

In conclusion, this study provides the indication that not only the chemical composition of media may contribute to the variability of proteinase production, but this could be important for further investigation of *M. gypseaum* enzyme activities. Furthermore, molecular characterization of genes encoding serine proteinases in dermatophyte fungi and production of such proteases could be of great interest for both fundamental studies on pathogenicity and other purposes.

Acknowledgements

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The authors declare that they have no conflict of interests.

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