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*Bse*GI Restriction of the Polymerase Chain Reaction Amplicon Th_{444} Is Required to Distinguish Biotypes of *Trichoderma aggressivum* Causing Serious Losses in Mushroom (*Agaricus bisporus*) Production

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Abstract. **Green mold is a serious disease of the cultivated mushroom causing losses in production of economical importance. In the present study, digestion of a Th_{444} amplicon with endonuclease *Bse*GI was useful to discriminate *Trichoderma aggressivum* f. *aggressivum* (*T.a.f.a*) from the *T. aggressivum* f. *europeanum* (*T.a.f.e.*). The informative restriction fragments of 260 and 300 bp were revealed in the corresponding reference strains *T.a.f.a.* and *T.a.f.e.* The 300-bp marker was found in all 28 Polish mushroom isolates tested.**

Trichoderma aggressivum f. *aggressivum* (*T.a.f.a.*) and *T. aggressivum* f. *europeanum* (*T.a.f.e.*) cause green mold disease in the cultivated mushroom (*Agaricus bisporus*) in North America and Europe, respectively. *T.a.f.a.* and *T.a.f.e.* were previously reported as *T. harzianum* biotypes Th4 and Th2 (Samuels et al., 2002). The disease is characterized by a rapid infestation of the compost by the pathogen and inhibition of *A. bisporus* fructification, resulting in serious yield losses worldwide in mushroom production (Ospino-Giraldo et al., 1999). Various molecular approaches were successfully used to characterize these isolates and distinguish them from non-aggressive Th1 (*T. harzianum*) and Th3 (*T. atroviride*) biotypes (Miyazaki et al., 2009; Ospino-Giraldo et al., 1999; Samuels et al., 2002). Based on Th4 genomic DNA, the polymerase chain reaction (PCR) marker of 444 bp was developed, which was amplified form both aggressive biotypes (Chen et al., 1999). To distinguish *T.a.f.a.* and *T.a.f.e.*, the random amplified polymorphic DNA technique was successfully used (Chen et al., 1999; Szczech et al., 2008). However, this technique often suffers from poor reproducibility. Hatvani et al. (2007) used restriction fragment length polymorphism of mitochondrial DNA to distinguish *Trichoderma* strains. We report a simple, reliable approach to recognize *T.a.f.a.* and *T.a.f.e.* by endonuclease restriction of the amplicon Th_{444}. Six isolates, CBS 466.94 (Th 1), CBS 693.94 (Th 3), CBS 100.528 (*T.a.f.a.*), CBS 450.95 (*T.a.f.a.*), CBS 689.96 (*T.a.f.e.*), and CBS 100.526 (*T.a.f.e.*), were obtained from the Centraalbureau voor Schimmelcultures (CBS) collection (Utrecht, The Netherlands). Twenty-eight Polish mushroom isolates of *T.a.f.e.* were received from the Research Institute of Vegetable Crops collection, Skierniewice, Poland (Table 1). Morphological and molecular studies of the *T.a.f.e* isolates are described in Szczech et al. (2008).

DNA was extracted from mycelium grown on malt extract agar according to Aljanabi and Martinez (1997). The PCR marker Th_{444} was visible as a strong band on ethidium bromide-stained agarose gels both in *T.a.f.a.* and *T.a.f.e.* and was not generated in *T. harzianum* biotypes Th1 and Th3 (data not shown), because it has been reported by Chen et al. (1999). Polymorphism in *T.a.f.a.* and *T.a.f.e.* was revealed by digestion of Th_{444} with the restriction enzyme *Bse*GI (Fermentas, UAB, Lithuania). The specific restriction fragment of ≈260 bp was informative for the *T.a.f.a.* reference isolates CBS 100.528 and CBS 450.95 (Fig. 1, Lanes 1 and 17, respectively), whereas the restriction product of 300 bp was visible in the reference isolates *T.a.f.e.* CBS 689.96 and CBS 100.526 (Fig. 1, Lanes 2 and 18, respectively) and all 28 *T.a.f.e.* Polish isolates (Fig. 1, Lanes 3 to 16 and 19 to 32). To our knowledge, it is the first example of the reproducible molecular markers that can considerably simplify detection of aggressive biotypes of *Trichoderma* in compost used in mushroom production.

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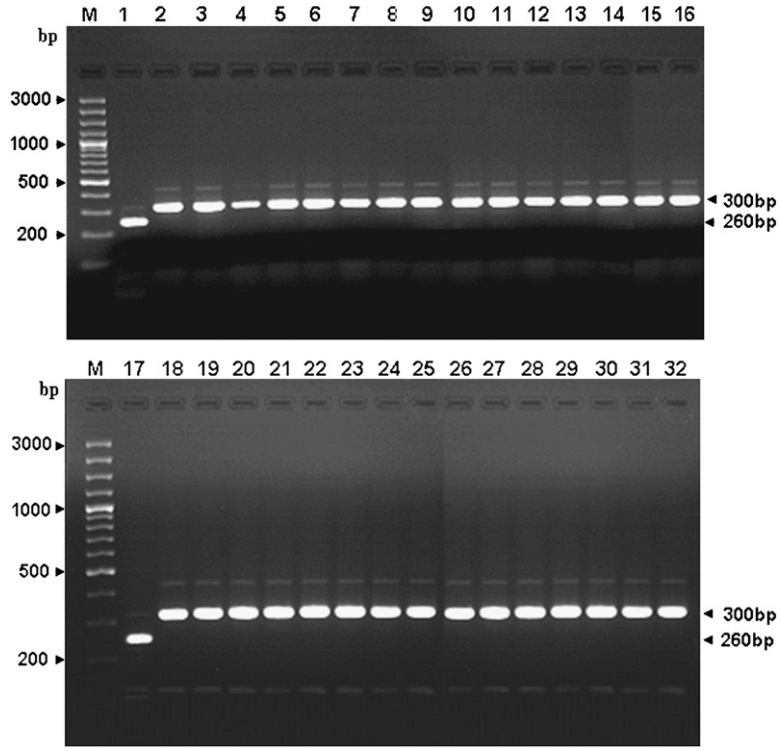

Fig. 1. Restriction patterns of the polymerase chain reaction marker Th_{444} amplified in the *Trichoderma aggressivum* reference strains and Polish isolates digested with *Bse*GI. Lanes 1 and 17: amplicons obtained in *T.a.f.a*. strains CBS 100.528 and CBS 450.95, respectively; Lanes 2 and 18: amplicons obtained in *T.a.f.e*. strains CBS 689.96 and CBS 100.526, respectively. Lanes 3 to 16 and 19 to 32, Polish isolates: 3- SP.22.07, 4-11.02, 5-28.05, 6-7.06, 7-16.11.2A, 8-13.02.B, 9-29.05.1B, 10-11.04, 11-PGU12, 12-2-P-9.08-4, 13-PGU25, 14-30.01, 15-11.03, 16-27.12.2, 19-19.09.E, 20-11.08.C, 21-2.02.A, 22-5-0-7.07-9, 23-4-0-9.08-1, 24-3-0-9.08-1, 25-14.12.2C, 26-T4/3.30.03, 27-3-9.08-4, 28-5-0P-7.07-4, 29-1-0P-9.08-3, 30-5.11, 31-5/3.30.03, 32-2-0P-9.08-1. M contains the 100-bp DNA ladder as a molecular size marker.

Table 1. Polish *T.a.f.e* isolates used in this study.

Code of isolates	Origin[z]	Location in Poland[y]
SP.22.07	Compost	Maków (łódzkie)
11.02	Compost	Częstochowa (śląskie)
28.05	Compost	Orzeł (wielkopolskie)
7.06	Compost	Skierniewice (łódzkie)
11.03	Casing	Mogielnica (mazowieckie)
16.11.2A	Compost	Lipinki (lubelskie)
27.12.2	Casing	Lipinki (lubelskie)
19.09.E	Casing	Szreniawa (wielkopolskie)
11.08.C	Casing	Żelki (warmińsko-mazurskie)
14.12.2C	Dust from cloth store	Wojnowo (lubuskie)
T4/3.30.03	Peat	Skierniewice (łódzkie)
13.02.B	Compost	Trzcianna (łódzkie)
2.02.A	Casing	Maków (łódzkie)
5/3.30.03	Dust collected from walls	Skierniewice (łódzkie)
29.05.1B	Compost	Krzepielów (lubuskie)
11.04	Compost	Polesie (lubelskie)
3-9.08-4	Dust collected from walls	Ignanie Nowe A (mazowieckie)
5-0P-7.07-4	Air sample	Kolonia Bolimowska (łódzkie)
5-0-7.07-9	Casing	Kolonia Bolimowska (łódzkie)
2-0P-9.08-1	Dust collected from walls	Ignanie Nowe B (mazowieckie)
4-0-9.08-1	Casing	Skórzec (mazowieckie)
1-0P-9.08-3	Dust collected from walls	Ignanie Nowe C (mazowieckie)
PGU12	Compost	Skierniewice (łódzkie)
2-P-9.08-4	Compost	Ignanie Nowe B (mazowieckie)
3-0-9.08-1	Casing	Ignanie Nowe A (mazowieckie)
5.11	Containers for transport	Skierniewice (łódzkie)
PGU25	Compost	Skierniewice (łódzkie)
30.01	Compost	Skierniewice (łódzkie)

[z]The cultivation was run on the mushroom compost that was made under the controlled fermentation process of the mixture of straw, chicken manure, gypsum, and water. In case of the casing soil, the mixtures of peat (low, medium, and high) with sugar beet lime and chalk were used.
[y]City; in parentheses the region of Poland. Isolates were collected in the years 2004 to 2008.