Physiology of *Rhizoctonia solani* AG2-2(Ⅳ), *Trichoderma harzianum*, and *Chaetomium cochliodes*, and their Utilization of Thatch-related Carbohydrate in *Zoysia japonica*

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Rhizoctonia solani AG2-2(Ⅳ), *Trichoderma harzianum* and *Chaetomium cochliodes*의 생육생리와 이들 미생물들의 한국잔디 대취층 관련 탄소원 이용도 조사

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ABSTRACT

Cellulose-degrading fungi were idenfied as *Rhizoctonia solani* AG2-2(Ⅳ), *T. harzianum* and *C. cochliodes*. *Rhizoctonia solani* AG2-2(Ⅳ) grows better in the acidified media of pH 4 and 5 than pH 6 and 7. Mycelial growth of *T. harzianum* and *C. cochliodes* was also higher in pH 4 and 5 than in pH 6 and 7. In order to relate the above findings to nutrient utilization, mycelial growth of *R. solani* AG2-2(Ⅳ) are evaluated with various carbon sources. *R. solani* AG2-2(Ⅳ) grows well in the order of mannose, cellobiose, glucose, xylose and arabinose. However, mycelial dry weights of *T. harzianum* were 98.7, 78.0, 72.3, 43.7 and 32.3 ㎎ in glucose, mannose, cellobiose, xylose, and arabinose, respectively. Mycelial dry weight of *C. cochilodes* was 118, 65, 57, 49, and 16 ㎎ in mannose, cellobiose, xylose, glucose, and arabinose, respectively. Result of cellulase assay of *R. solani* AG2-2(Ⅳ) and soil fungi was reffered as, *R. solani* AG2-2(Ⅳ) produced more cellulase on CMC substrate than on CEL and secreted more enzyme in floated condition than in water-immersed condition. *T. harzianum* secreted less amount of cellulase than *R. solani* AG2-2 and *C. cochliodes*. *T. harzianum* produced no enzyme on CEL under water-immersed condition. *C. cochliodes* produced similar amounts of cellulase on either CMC or CEL under both water-immersed and floated condition.

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INTRODUCTION

The most difficult problem to manage the zoysiagrass ground is the disease of large patch caused by *Rhizoctonia solani* AG2-2(Ⅳ) (Yukiko, 1989; Burpee and Brace, 1992; Shim, 1995). Once established, turfgrass solis are hardly ever renovated and thereby organic matters are deposited to form thatch layers, which is distinctly different from agricultural field (Smiley and Fowler, 1986; Davis and Smitley, 1990; Dell *et al.*, 1994). Pathogen population in golf course is the highest in thatch layer (Shim, 1995), which is a layer of tightly intermingled live and dead stems, and leaves, and organic debris that accumulates between the managed turfgrass and soil surface (Potter *et al.*, 1990).

Current management practices to control the large patch mainly depended on the fungicide application which in turn cause environmental polution (Yukito, 1991; Kwanabe, 1991). Moreover, this chemical approach results in a limited control efficacies due to the fact that fungicidal spray protects the turfgrass from endemic pathogen infection only for a short period and also difficult to contact the target pathogen in thatch layer (Smiley *et al.*, 1985; Smiley and Fowler, 1986; Smiley and Uddin, 1993).

Carbohydrates in thatch layer are the important nutrient sources for the increasing population of pathogen (Sartain and Bivolk, 1984; Berndt, 1990; Andrew *et al.*, 1994). Of these carbohydrates, cellulose is an important factor for the growing of microbes in thatch and soil layer (Kim, 1985; Sakamoto *et al.*, 1989). This cellulose is hydrolysed to cellodextrin, cellohexose, cellotetriose, cellotriose, and cellobiose, and finally to glucose by β-glucosidase (Collins and Ferrier, 1995). So far there is no report on the carbohydrate competition between pathogen and soil microbes for the control of large patch. Therefore, in this study, it was attempted to estimate microbial utilization of thatch carbohydrates for reducing large patch by nutrient competition. In addition, mycelial growth was tested on various pH media to define adaptation of microbes in thatch and soil. Recently, many researchers (Harris *et al.*, 1993; Green, 1994; Shim, 1995) reported the integrated control of plant diseases. However, the effective control methods have not been established yet.

A shallow layer of thatch may provide surface resiliency and wear tolerance, while an excessively thatch turf is subject to disease increase, localization of dry patch, and poor response to fertilizers and pesticides (Davis *et al.*, 1990; Kawanabe, 1991; Yukito, 1991; Handbook of Agrochemicals in Korea, 1996).

Thatch decomposition is generally initiated by fungi in soil (Smiley *et al.*, 1985; Dell *et al.*, 1994). However, intensive use of fungicide on turgrass often unexpectedly increases thatch, and alters the microbial population of thatch and underlying soil (Smiley *et al.*, 1985; Smiley and Flower, 1979). Current control practices in Korea depended mostly on fungicidal application, which resulted in reducing the saprophytic microbial population and thus promoting the thatch accumulation (Smiley *et al.*, 1985; Smiley and Flower, 1986). Such a situation is again conducive for this pathogen to be endemic in zoysiagrass fairway. Since the previous results showed that most of propagules of large patch pathogen inhabited in thatch layer (Shim, 1995), most feasible approach to reduce pathogen population would be either by providing a condition favorable to biodegradation of thatch layer by beneficial microbes and/or nutrient competition for thatch carbohydrate by using fast growing carbohydrate-guzzling microbes.

In this context, the study was conducted to investigate the utilization of thatch carbohydrate by selected fungi and to evaluate the competitive ability of microbes against *R. solani* AG2-2(Ⅳ) for thatch nutrients.

MATERIALS AND METHODS

Isolation, screening, and identification of antagonists

Various soil microbes were screened as nutrient competitive antagonist in thatch layer of zoysiagrass ground with *Rhizoctonia solani* AG2-2(Ⅳ), pathogen of large patch. *Trichoderma* spp. isolated from non-diseased area of DBGC (Dongrae Benest Golf Club) was screened with growth speed and activity in low temperature (no data). Isolation was accorded to the next method; a mixture of a thatch and soil, same as above, was diluted to $10^2 \sim 10^4$. The 0.1 ㎖ of aliquots was smeared on potato-dextrose agar added with 50 ㎍/㎖ of ampicillin (Sigma chemical co. A-9393) and 100 ㎍/㎖ of streptomycin (Sigma chemical co., S-6501). The most aggressive isolate was screened for rapidity of mycelial growth and over-growing against host pathogen, and then selected for the further studies. A screened isolate was identified by Rifai's method. This fungus was identified as *Trichoderma harzianum* (Table 1). *Chaetomium* sp. was kindly provided by Professor H. K. Kim from the Laboratory of Plant Pathology in Gyeongsang National University at Chinju, Korea and identified as *Chaetomium* cocliodes (Table 2) by Watanabe's method

Table 1. Morphological characteristics of *Trichoderma harzianum*.

Characteristics	*Trichoderma harzianum*	Isolated fungus
Branching system of conidiophore	Dendroid	Dendroid
Phialide	$6.0 \sim 7.0 \times 3.0 \sim 4.0$ ㎛ (plump)	$6.5 \sim 7.2 \times 3.1 \sim 4.0$ ㎛ (plump)
Conidia size	$2.6 \sim 3.1 \times 2.3 \sim 2.7$ ㎛ (subglobose)	$2.5 \sim 3.1 \times 2.5 \sim 2.9$ ㎛ (subglobose)
Surface	Smooth	Smooth

Table 2. Morphological characteristics of *Chaetomium cochliodes*

Characteristics	*Chaetomium cochliodes*	Isolated fungus
Apothecium	180~280×190~225 ㎛	164~272×140~200 ㎛
Ascus	72.5~85×8~11.3 ㎛	60~80×11~13 ㎛
Ascospore	7.5~10×6~8 ㎛	8.4~10×6~8 ㎛
Length and morphology of ascus	Above 70 ㎛ as lemon shaped, club type spore is not one.	Lemon shaped, spore in not on line.
Colony morphology on cellulose agar media	Dark olive green-dark olive gray	Dark olive green

(1993). And Morphological characteristics on these fungi were printed with Microworld (MW200, Display Devices Company in Samsung group, Korea). These isolates were tested the antagonism at the Turfgrass and Environment Research Institute of Samsung everland LTD.

Microbial growth at different pH

Fungal growth was tested at pH 4.1, 5.2, 6.1, and 7.1 in citrate-phosphate buffer (Dhingra, 1985), which was mixed with equal amounts of Czapek's broth. Medium (100 ㎖ of pH adjusted broth) was inoculated with mycelial disk (7 ㎜ in diameter) from the colonies of *Rhizoctonia solani* AG2-2(Ⅳ), *Chaetomium cochliodes*, and *Trichoderma harzianum*, grown for 3 days on 1/4 PDA, and incubated for 10 days at 28℃. And pellets were collected and dried in a mechanical convectional oven (Precision scientific, USA) at 80℃ for 5 hours, and the dry weight, of fungal mycelium was measured.

Measurement of monosaccharide utilization by fungi

R. solani, *T. harzianum*, and *C. cochliodes* were grown as follow; Czapek's medium (pH 7.3) was sterilized prior to introducing the solutions of L(+)-arabinose, D(+)-mannose, D(+)-xylose, D(+)-glucose, and D(+)-cellobiose to 0.5 %. The 7 ㎜ colony discs of mycelium grown for 3 days on PDA (Difco) at 28℃ were inoculated to 20 ㎖ of carbon-mineral broth and grown for 10 days at 28℃. Mycelia were harvested by centrifugation (Sorval RC-24) at 10,000 rpm at 0℃ for 10 minutes and dried in a mechanical conventional oven and the dry weight was measured.

Measurement of cellulase activity

Secretion of cellulase by fungi was estimated using plate method. Czapek's media including 0.5 % of crystalline cellulose (CEL, α-cellulose, Sigma C8002) or carboxymethyl cellulose sodium salt (CMC, YAKURI PURE CHEMICALS Co., Ltd) were prepared in 50 mℓ of flask and sterilized at 121℃ for 15 minute. Mycelium of *T. harzianum*, *R. solani* AG2-2(Ⅳ), and *C. cochliodes* grown for 3 days at 28℃ on 1/4 PDA was inoculated. After growing for 5 days at 28℃, cell pellet was discarded.

Assay plate was prepared by dissolving 1 % agarose and 0.2 % CMC (or cellulose) in distilled water and solidified at room temperature. 15 mℓ of 0.1 % Congo red solution was flooded to the plate and incubated for 20~30 minutes, and then washed twice with 15 mℓ of 1 M NaCl for 10 minutes. Diameter of cleaning zone (yellow in red plate) was measured with ruler.

RESULTS AND DISCUSSION

Identification of fungi

Fungi used for this test was identified to *Trichoderma harzianum*. and *Chaetomium cochliodes*(Table 1 and 2).

Effect of pH on the growth of *Rhizoctonia solani* AG2-2(Ⅳ) and other soil fungi

In *R. solani* AG2-2(Ⅳ), soil pH was one of the most important factors for the mycelial growth. This fungus grows better in the acidified media of pH 4 and 5 than pH 6 and 7 (Fig. 1). Mycelial growth of *T. harzianum* and *C. cochliodes* was also relatively higher in pH 4 and 5 than in pH 6 and 7 (Fig. 1).

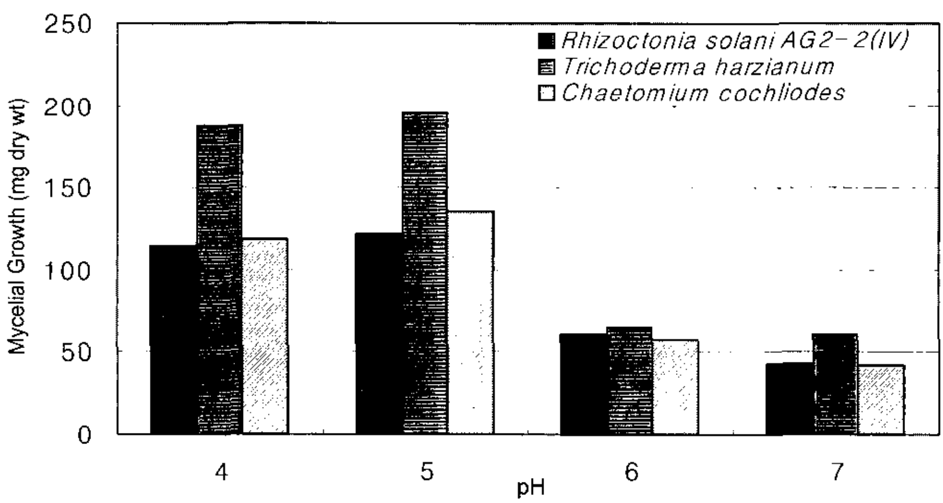

Fig. 1. Effect of pH on the mycelial growth of *Rhizoctonia solani* AG2-2(Ⅳ), *Trichoderma harzianium*, and *Chaetomium cochliodes*, cultured for 10 days at 28℃ in Czapek's broth adjusted with citrate-phosphate buffer.

James (1960) reported that the incidence of Rhizoctonia brown patch was significantly greater at pH 5.6~9.0 than 4.0. And the mycelial growth of *Rhizoctonia* sp. was optimum at pH 5.5 *in vitro* but infection of mung bean and pea seedlings occurred more severly in neutral and alkaline river sand than in the acidic sand (Hans *et al.*, 1987). Rhizoctonia brown patch was more severely developed at pH 5.6 and pH 9.0 than at pH 4.0 (James, 1960). Our results showed much higher mycelial growth of *R. solani* AG2-2 (Ⅳ) at pH 4 and 5 than pH 6 and 7. pH of soil and thatch layer on zoysiagrass ground is one of the most important environmental factors in turfgrass diseases occurrence because mycelial growth of soil and thatch microbes are affected by soil and thatch pH. However, more detailed study about occurrence of large patch will be needed to clarify the point.

Utilization of thatch-related carbohydrate by *Rhizoctonia solani* AG2-2(Ⅳ) and other soil fungi

Propagules of *R. solani* AG2-2(Ⅳ) in turfgrass soil were more in upper thatch layer than in lower thatch soil (Shim, 1995). In order to relate above findings to nutrient utilization, mycelial growth of *R. solani* AG2-2(Ⅳ) is evaluated with various carbon sources (Fig. 2). Czapek's media containing glucose, mannose, arabinose, xylose and

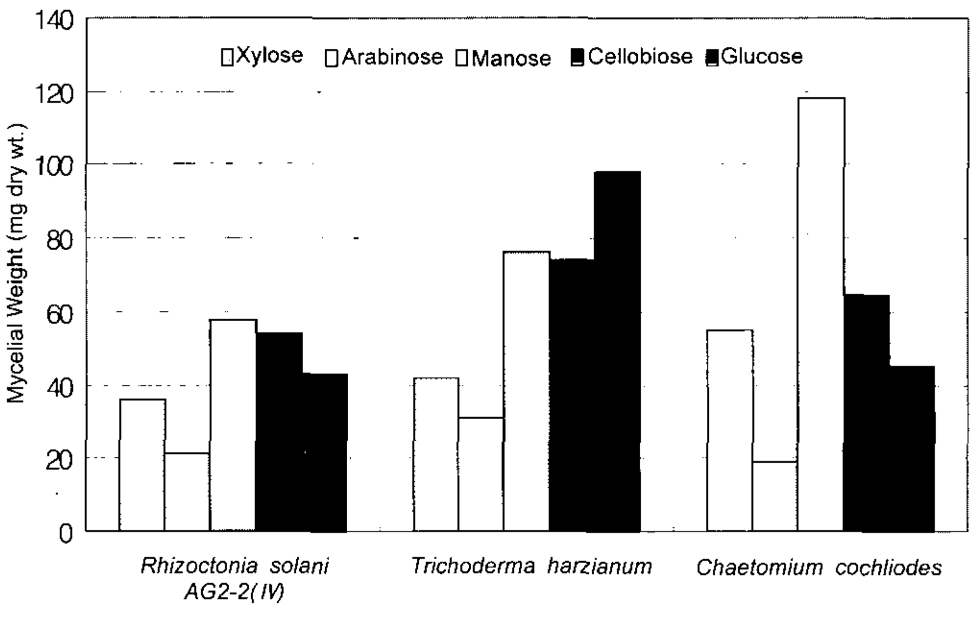

Fig. 2. Effect of carbon sources on the mycelial growth of fungi for 10 days at 28℃ on the Czapek's medium base supplemented with 0.5 % various carbon sources.

cellobiose were used for the mycelial growth. *R. solani* AG2-2(Ⅳ) grow well in the order of mannose, cellobiose, glucose, xylose, and arabinose. However, mycelial dry weights of *T. harzianum* were 98.7, 78.0, 72.3, 43.7 and 32.3 ㎎ in glucose, mannose, cellobiose, xylose, and arabinose, respectively. Mycelia dry weights of *C. cochliodes* were 118, 65, 57, 49, and 16 ㎎ in mannose, cellobiose, xylose, glucose, and arabinose, respectively.

Thatch layer contains a lot of carbohydrates (Berndt, 1990; Charles *et al.*, 1992; Andrew *et al.*, 1994). Kim (1985) reported that many carbohydrates including xylose, mannose, arabinose, glucose, sucrose, rhamnose etc. were utilized by *R. solani* AG2-2(Ⅳ). These results suggested that disease might be suppressed due to the insufficiency of carbohydrates in thatch layer when mannose, cellulose, and glucose in thatch layer of zoysiagrass are utilized by other soil microbes, Exhaustion of carbohydrate in thatch layer by other soil microbes might lead to the effective control method of large patch. *R. solani* AG2-2(Ⅳ) and *C. cochliodes* utilized mannose specifically, and *T. harzianum* utilized glucose, mannose, and cellobiose (Fig. 2). *R. solani* AG2-2(Ⅳ) preferably utilized mannose, cellobiose, and glucose. *T. harzianum* utilized glucose most preferably. This antagonist utilized glucose, mannose, and arabinose far better than the pathogen did. *C. cochliodes* specifically utilized mannose, which is the least availble in thatch layer (Fig. 2). Cellobiose, xylose, and glucose were good sources for both *C. cochliodes* and *R. solani* AG2-2(Ⅳ).

Cellulase assay of *R. solani* AG2-2(Ⅳ) and soil fungi

R. solani AG2-2(Ⅳ) produced more cellulase on CMC substrate than on CEL, and this fungus secreted more enzyme in floated condition than in water-immersed condition. *T. harzianum* secreted less amount of cellulase than *R. solani* AG2-2 and *C. cochliodes*. *T. harzianum* produced no enzyme on CEL under water-immersed condition. *C. cochliodes* produced similar amounts of cellulase on either CMC or CEL under both water-immersed and floated condition. This fungus, distinctly different from *T. harzianum* and *R. solani* AG2-2(Ⅳ), secreted remarkable amount of cellulase on natural substrate CEL. This result would be meaningful because zoysiagrass thatch contains about 25~40 % cellulose (Berndt, 1990).

Therefore, *C. cochliodes* might be more competitive in saprophytic survival in ecosystem when this fungus utilizes sufficiently other saccharide from thatch layer. *T. harzianum*, in spite of high antagonism in vitro, may not show its suppressive effect in wetted area because cellulose was not utilized in water-immersed condition (Fig. 3). And cellulase activity in vitro may be also differed in natural substrates because enzymic systems for cellulose hydrolysis is divided to tree major types: a) Endo-1,4-β-glucanase

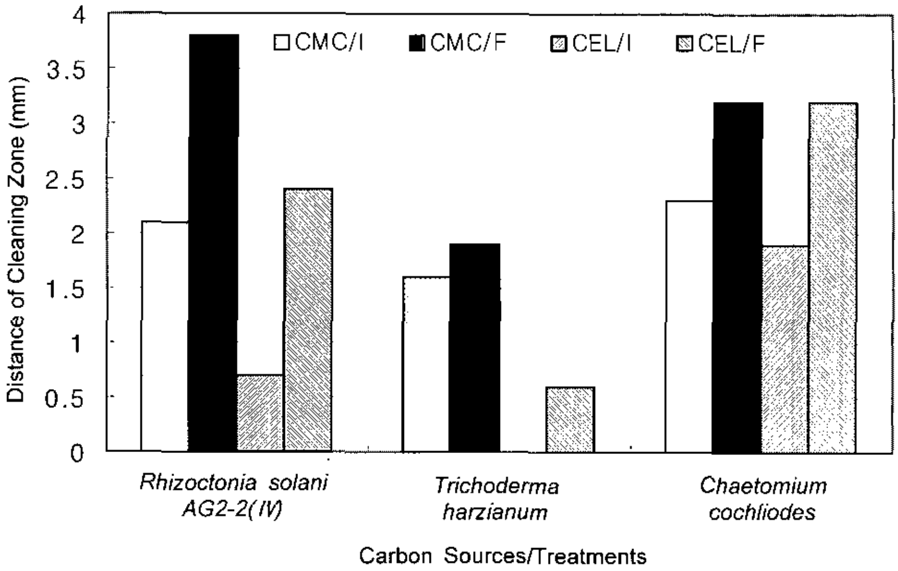

Fig. 3. Cellulase production by fungi. The fungi were grown on Czapek's broth supplemented either Carboxymethyl cellulose (CMC) or crystalline cellulose (CEL) as a sole carbon source. The culture was grown immersed (I) in 25 ㎖ of medium or floated(F) on 5 ㎖ of medium for 5 days at 28 ℃. Cellulase activity was measured of the culture supernatant using CMC/congo red method.

(EG) or endo-1,4-β-D-glucan 4- glucanohydrolase, which cleaves β-glucosidic bonds at random position in the cellulose polymer; b) Cellobiohydrolase(CBH) (1,4-β-D-glucan cellobiohydrolase), which attacks cellobiose molecule stepwise from the non-reducing ends, liberating cellobiose; c) β-Glucosidase(BGL) which hydrolyze cellobiose and low molecular weight cellodextrines into glucose.

요 약

토양에서 분리된 *Rhizoctonia* sp.와 *Trichoderma* sp.는 *R. solani* AG2-2(IV)와 *T. harzianum*로 동정되었고 셀룰로즈를 분비하는 진균은 *C. cochliodes*로 동정되었다. 이들 미생물의 생육력은 pH4와 5에서 가장 좋았고 탄소원 이용도는 *R. solani*의 경우, mannose, cellulose, glucose, xylose, arabinose순이었고 *T. hazianum*은 glucose, mannose, cellobiose, xylose, arabinose순이었으며 *C. cochliodes*는 mannose, cellobiose, xylose, glucose, arabinose순이었다. Cellulose 분해도는 수중 및 호기조건에서 *R. solani*와 *C. cochliodes*가 동일했으며 *T. hazianum*은 수중에서 cellulase 분비도가 매우 낮았다.

CONCLUSION

Rhizoctonia solani AG2-2(IV), *Trichoderma harzianum* and *C. cochliodes* known as large patch pathogen, antagonist of pathgen and high cellulase-secreting fungus, respectively, were grown well in pH 4 and 5 than in pH 6 and 7.

In ratio of carbohydrate utilization by these microbes, high utilizing ratio by *R. solani* were shown in mannose, cellulose, glucose, xylose, and arabinose, respectively. However, *T. hazianum* and *C. cochliodes* utilized glucose, mannose, cellobiose, xylose, and arabinose, respectively, and mannose, cellobiose, xylose, glucose, and arabinos ,respectively, Therefore, It was suggested when *T. hazianum* was considered as biocontrol agent, glucose as nutrient was, may be, so effect to control the pathogen. In *C. cochliodes* and *Rhizoctonia* sp., mannose was suggested so considerable agent.

It was shown that cellulose-degradation ratio of of *R. solani* and *C. cochliodes* in aerobic and aquatic condition were appeared as same condition relatively. However, *T. hazianum* could not degrade the celluose in a aquatic condition. Therefore, environmental factors of pysiological and chemical was may be, important conditions to control the turfgrass diseases.

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