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Effects of microorganisms on thatch decomposition and turf growth-promotion in golf course field

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ABSTRACT

Effects of microorganisms on thatch decomposition and turf growth-promotion in golf course field

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Thatch is a layer of partially decomposed organic matter between turf green shoots and the soil surface. It is formed primarily from periodically sloughed-off roots and residue of horizontal stems (stolon and rhizomes), stubbles, and mature leaf sheaths and blades. Excessive thatch (>5.07cm) makes an adverse effect on turfgrass management that causes difficulties in mowing, fertilization, irrigation, and pest control. Since golf fields are maintained at low mowing heights, golf course greenkeepers face the difficult task of producing a high quality, stress-tolerant turfgrass with an acceptable putting distance or speed. There are numerous dethatching techniques including various mechanical practices such as vertical mowing, core cultivation, grooming, and topdressing to reduce thatch accumulation. However, these techniques are less effective and require high cost, time consuming and labor-intensive practices. This study aimed to select microorganisms that can be used for the biological decomposition of the thatch layer. For this, soil samples were collected from eight golf-course fields from

which a total of 53 soil bacteria were isolated. Among these bacterial isolates, ten isolates showed cellulose-degrading activity in carboxymethyl cellulose assay at 25^{o}C, among which five were selected for further studies because of their cellulose-degrading activity regardless of the temperature conditions from 21~28 ^{o}C. These bacterial isolates were identified as members of the genus *Bacillus* based on 16S rRNA gene sequencing analysis and morphological characteristics revealed by electron microscopy. Among these, three isolates (A1, F1, and H4) were more efficient for moisture retention, root growth and plant density promotions, which may be related to the reduction of thatch accumulations. These activities were validated in nursery experiments: For the thatch layer thickness, the three bacterial isolates were more efficient in reducing its thickness same as a commercial microbial product, Thatch Manager (TM) than the non-treatment control without the promotion of the green quality like putting speed and distance, but for the matter of plant-growth characteristics, bacterial treatments showed higher chlorophyll index related to visual quality of the green at one week after treatment than TM and non-treatment control; root length increased continuously more in bacterial treatments and control than TM. All of the aspects suggest that the three bacterial isolates may be developed as microbial products for the reduction of thatch layers in greens and for increasing the green visuals as well. The bacterial isolates are the residents of the golf course fields, so there will be no problem for them to be established to colonize the golf course fields.

Keywords: Bacteria, decomposition, thatch, turfgrass.

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INTRODUCTION

In Korea, golf courses are mainly composed of two types of grasses, warm-season and cool-season grasses in most fairways and greens, respectively. The golf course green is an area surrounding the hole, of which the firmness (referring to hardness) that controls the speed of ball roll in the green is a prime requirement for golf clubs to attract and retain their members. For this, the greens should be closely trimmed to make the firm ground with fast green speed composed of relatively even and smooth surfaces for the players to make precision strokes to the holes. The creeping bentgrass is mainly used in the green as it provides a dense, smooth uniform surface and smooth ball rolls along the greens (Salaizet al., 1995). There are a variety of ways to improve the firmness of the greens, one of which is to reduce thatch accumulation because too much thatch can be detrimental to putting green speed and smoothness.

Thatch is an intermingled layer of living and dead plant tissues which develops between the green vegetation and soil surface (Decker, 1974). Thatch is derived from dead (or partially dead) and blighted plant tissues such as sloughed-off roots, horizontal stems (stolon and rhizomes), stubbles, and mature leaf sheaths and blades (Engel, 1954; Roberts and Bredakis, 1960). When turfgrass produces organic matter exceeding the decomposition rates, thatch accumulates (Beard, 1973). Any climatic, edaphic and biotic factors inducing excessive plant growth result in the increase of plant death in turn and contribute to thatch development and accumulation (Hurto et al.,

1980).

Excessive thatch accumulation commonly influences negatively on physical and biological conditions of the ground profile such as reductions in hydraulic conductivity (Harris, 1978), decreased water infiltration (Murray and Juska, 1977), increased localized dry spots (Cornman, 1952), and reduced tolerance to cold temperatures (White, 1962; Beard, 1973; Thompson, 1967), all of which influence negatively on the green quality and make adverse effects on turfgrass management that causes difficulties in mowing, fertilization, irrigation, and pest control. More thatch accumulation is sometimes derived from turfgrass diseases such as large patch of zoysiagrass caused by *Rhizoctonia solani* (Park et al., 1995).

Mechanical practices are commonly used for controlling thatch accumulation in greens, which usually include aeration, vertical mowing, core cultivation and topdressing, which may provide some benefits such as improved water infiltration rates, surface compaction, and increased soil oxygen contents (Carrow et al., 1987; Dunn et al., 1995; Ledeboer and Skogley, 1967; Shildrick, 1985; White and Dickens, 1984; Williams and McCarty, 2005). However, these mechanical practices for maintaining low green height, which improve the green speed, disrupt the green surface and influence negatively on plant health and strength, reducing the tolerance of the turfgrass to abiotic and biotic stresses. Efficient ways other than mechanical practices are required to reduce thatch accumulation without disruption of the green surface. Chemical or biological control practices or both would be practical alternatives to the

mechanical management of the greens to reduce thatch accumulation (McCarty, 2007). Therefore, this study aimed to select and characterize microorganisms dwelling in the golf course fields that are efficient for reducing thatch accumulation without damaging the green surface and without concerning about their establishment and colonization in the golf course fields as they are the field residents.

MATERIALS AND METHODS

1. Isolation and identification of dethatching microbes for biological control of thatch accumulation

1-1. Sample collection

Isolation of bacteria from soil samples of golf course fields were conducted. For this, 8 soil samples from several locations in Anyang Country Club, Gunpo, Korea were used in this experiment, from which a total of 53 bacteria were isolated by pour-plating of the serially diluted soil suspensions (10^{-3} to 10^{-5}) on nutrient agar (NA), incubating at 28°C for 72 hours to form bacterial colonies on NA. The single bacterial colonies were re-inoculated for each on fresh NA and incubated at 28°C for 72 hours to obtain pure bacterial cultures.

1-2. *In vitro* examination on thatch-degrading ability of the bacterial isolates collected from the golf course fields

The ability of thatch decomposition was examined by their abilities to hydrolyze cellulose that is one of the main components of thatch. The bacterial isolates collected from the golf course fields were inoculated in nutrient broth and incubated 28℃ for 2 days with shaking at 200 rpm in an incubator. Examination of the cellulose-degrading activity of bacterial isolates was performed by dropping 2 μl bacterial suspensions (1 x

10^9 colony forming units (CFU)/ml) on carboxyl methyl cellulose agar (CMCA) composed of 1□g KH_2PO_4, 0.5□g NH_4SO_4, 0.5g L-asparagine, 0.2 g crystalline $MgSO_4$, 0.1 g $CaCl_2$, 0.5 g yeast extract, 10 g carboxyl methyl cellulose and 20 g agar in 1 L sterile distilled water (SDW) (pH 6.8–7.2) (Jeon et al., 2003). For the first experiment on cellulose-hydrolytic activities of all of the bacterial isolates, cellulose-hydrolytic activities of the bacterial isolates were determined by white halos formed in or around the bacterial colonies after 3 days of incubation at 25℃, followed by the staining of the whole CMCA with 0.1% Congo-Red solution for 30 min, followed by treatment of 1 M NaCl for 5 min, plate surface rinsing by SDW, and the treatment of 5% acetic acid for 5 min to improve visibility, termed as CMC assay. The degrees of their cellulose-degrading activities were measured by the time and area of the while halo formed around the bacterial colonies on CMCA. For the second experiment on cellulose-hydrolytic activities, ten bacterial isolates selected in the first experiment as their positive activities on cellulose hydrolysis were subjected to the same CMC assay mentioned above, but in the different temperature conditions within the usual range of temperatures in the golf course fields (21~28℃), specifically at 21℃, 25℃ and 28℃.

1-3. Identification of the bacterial isolates efficient for cellulose hydrolysis.

The bacterial isolates selected because of high efficiency in cellulose hydrolysis were identified mainly by molecular-genetical and morphological characteristics. For molecular-genetical characteristics, 16s rRNA gene sequences were analyzed. For this, genomic DNA of the bacterial isolates was prepared according to Paspiech and

Neumann (1995). Using the 27mF and 1492mR primers, 16S rDNA was amplified by PCR and amplified fragments of 16S rDNA were purified by agarose gel electrophoresis and sequenced on an Applied Biosystems DNA sequencer (model ABI 3700) (Brosius et al., 1978; Weisburg et al., 1991). The resulting sequences were analyzed using Blast search program in GenBank and compared with other sequences of 16S ribosomal DNA listed in GenBank.

To examine the morphology of the bacterial cells, a colony grown on NA was picked with a spatula and placed in distilled water on a Formvar-coated copper grid, dried, and stained with 2% uranyl acetate briefly (for about 30 sec) for negative staining. The bacterial isolates preparation on the grids were examined under a JEM 1010 electron microscope (JOEL, Japan) at 80 kV.

2. Biological control activity of thatch accumulations *in vivo*

2-1. Pot experiments

Pot experiments were conducted twice. Five bacterial isolates (A1, F1, H4, D2, E4) selected in the above *in vitro* experiment on cellulose-hydrolytic activities were used in the first pot experiment. Water was used as negative control. In one pot, three hole cups of turfs {Shark-Creeping bentgrass (*Agrostis palustris*)} with a diameter of 108 mm and the average mowing height of 3 cm were planted with three replications of pots. On each cup of turf, 100 ml of each bacterial suspension with the concentration of

10^5 CFU/ml was applied and the turfs were grown in room temperature, watering twice in a week to field capacity with no fertilizers. Moisture contents (%) of the turf soil, root length (cm) of the grass and plant density (number of grasses/cm^2) were examined at intervals of one week after treatment (WAT). For the second pot experiment, three bacterial isolates (A1, F1 and H4) with higher efficiency in promoting turf quality in the first pot experiment using the same grass and under the same growth conditions to examine the same characteristics related to turf qualities by the same methods as in the first pot experiment.

2-2. Nursery experiment

In this experiment, the same three bacterial isolates as in the second pot experiment were applied to the nursery of Anyang Country Club planted with Shark Creeping Bentgrass with 1 L of the bacterial suspensions at the concentrations of 10^3 and 10^5 CFU/ml together with a commercial microbial product named Thatch Manager (TM) as a positive control and water as negative control. The nursery turf mowed every day to maintain the average mowing height of 2.6 mm without watering and fertilizers during the experimental period of time. Moisture content, chlorophyll index, thatch layer thickness (cm), plant density (number of grasses/cm^2), hardness and root length (cm) were examined at 3:00 pm at intervals of 7 days after treatment as follows.

Moisture content was evaluated using a soil moisture meter (FieldScout TDR150 with 3-inch rods, Spectrum Technologies, Inc., East Planifield, IL, USA), which was applied randomly on three places within a nursery plot. Chlorophyll index, which is

commonly considered as an important aspect of visual turfgrass quality, was measured by a Chlorophyll Meter (FieldScout CM 1000, Spectrum Technologies, Inc., East Plainfield, IL, USA) on three places within a plot at intervals of 7 days. For thatch layer thickness, the uncompressed soil cores collected from two points within a plot by an Accuform soil profile sampler (Par Aide Products Co., Hugo, MN, USA), which is designed to get an undisturbed soil profile of 1.3 cm (in thickness) x 7.6 cm (in width) x 15.2 cm (in height) and measured between soil surface and green shoots at 5 points by a ruler (Smith, 1979). Surface hardness was assessed using a Turf Firmness Meter (FieldScout TruFirm, Spectrum Technologies, Inc., East Plainfield, IL, USA), for which the weighted hammer was dropped from a height of 0.69 m to the turfgrass surface to provide the travel depth, for which three places were taken to measure at intervals of 7 days. Root length was measured using the undisturbed soil profiles of 1.3 cm (in thickness) x 7.6 cm (in width) x 15.2 cm (in height), collected by an Accuform soil profile sampler (Par Aide Products Co., Hugo, MN, USA), for which the root lengths from soil layers (below thatch layers) to root tips were measured randomly at 5 points by a ruler and averaged. For plant density, the whole grasses were taken at three points per plot by a soil repair tool of 1 cm^2 and the number of grasses with two or more true leaves was counted.

RESULTS

1. Isolation and identification of dethatching microbes for biological control of thatch accumulation

1-1. Sample collection

Soil samples were collected from 8 golf course fields as sampling sites (designated by A to H) including all areas of Anyang Country Club, Gunpo, Korea, which were the areas of mooring and teeing, the greens and fairways (Fig. 1), of which the soil dilutions with concentrations of 10^{-3} and 10^{-4} were pour-plated on nutrient agar (NA) and incubated at 28℃ for 3 days to form various bacterial colonies in shape and color. In each sampling site, the bacterial colonies with any different shapes and colors were selected and re-inoculated on NA and incubated for 3 days at 28℃ to form pure bacterial cultures without contamination of any other microorganisms. In this experiment, a total of 53 bacterial isolates were obtained from 8 sampling sites, including 7 isolates for each from A, C, D, E, F and H, 6 from B and 5 from G (Table 1).

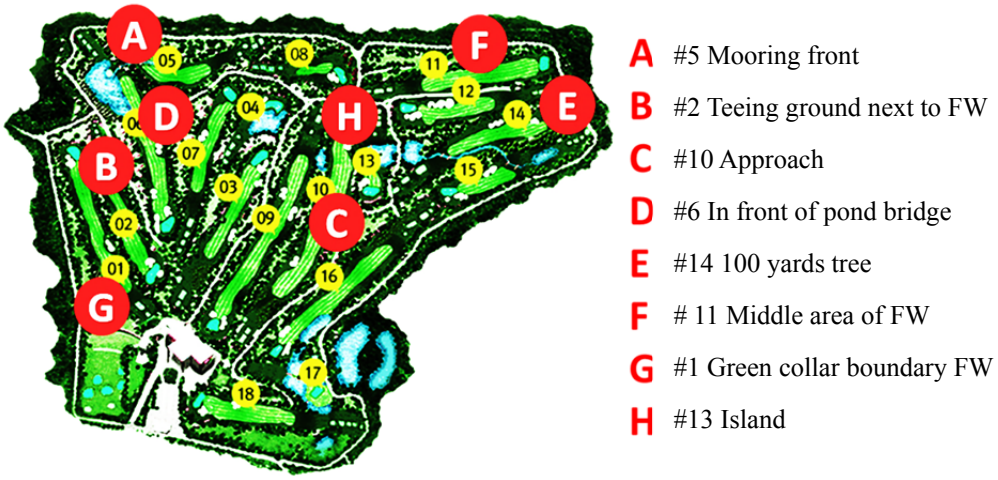

Figure 1. Sampling sites of golf course fields in Anyang Country Club, Gunpo, Korea

Table 1. Bacterial isolates obtained from sampling sites of turfgrass fields in Anyang Country Club, Gunpo, Korea

Sampling site	Isolates						
#5 Mooring front (A)	A1	A2	A3	A4	A5	A6	A7
#2 Teeing ground next to FW[1](B)	B1	B2	B3	B4	B5	B6	
#10 Approach (C)	C1	C2	C3	C4	C5	C6	C7
#6 In front of pond bridge (D)	D1	D2	D3	D4	D5	D6	D7
#14 100-yard tree (E)	E1	E2	E3	E4	E5	E6	E7
#11 Middle area of FW (F)	F1	F2	F3	F4	F5	F6	F7
#1 Green collar boundary FW (G)	G1	G2	G3	G4	G5		
#13 Island (H)	H1	H2	H3	H4	H5	H6	H7

[1]FW: Fair way

1-2. *In vitro* examination on thatch-degrading ability of the bacterial isolates collected from the golf course fields

All 53 bacterial isolates were subjected to the first assay on cellulose-hydrolytic activity in the temperature condition of 25℃, among which 10 isolates (A1, B2, B3, C4, D1, D2, E1, E4, F1 and H4) showed definite formation of white halos (Fig. 2). These bacterial isolates selected in the first experiment on their cellulose hydrolytic activities were subjected again to the same CMC assays, but in different temperature conditions of 21, 25 and 28℃, which were within the range of temperature conditions in open golf course fields of Anyang Country Club. In this experiment only 5 bacterial isolates including A1, D2, E4, F4, and H4 were selected as their cellulose hydrolytic activities were positive at all temperature conditions tested in this experiment, while other 4 isolates including B3, C4, D1 and E1were not active in cellulose hydrolysis at lower temperature of 21℃ and the other isolate (B2), not at higher temperature of 28℃ (Table 2).

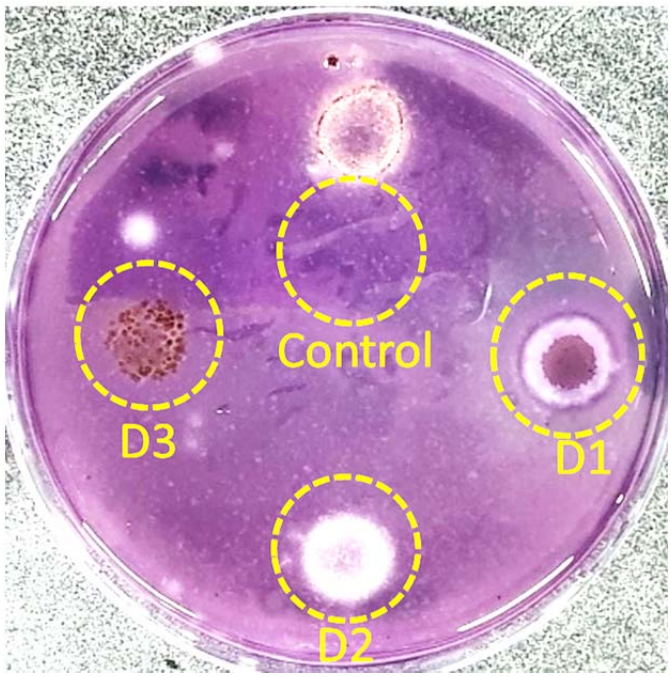

Figure 2. Cellulose-hydrolytic activities of bacterial isolates cultured on carboxyl methyl cellulose agar with Congo-red staining, showing middle (D1), high (D2), low (D3) and no (Control) activities, in which middle and high activities were regarded as positive reactions; but negative for the lower activities.

Table 2. Cellulose-hydrolytic activities of the bacterial isolates collected from golf course fields at different temperature conditions

Sampling site[1]	Temp.	Isolates (cellulose-hydrolytic activity)[2]						
A	25 ℃	A1 (+)	A2 (−)	A3 (−)	A4 (−)	A5 (−)	A6 (−)	A7 (−)
B	25 ℃	B1 (−)	B2 (+)	B3 (+)	B4 (−)	B5 (−)	B6 (−)	
C	25 ℃	C1 (−)	C2 (−)	C3 (−)	C4 (+)	C5 (−)	C6 (−)	C7 (−)
D	25 ℃	D1 (+)	D2 (+)	D3 (−)	D4 (−)	D5 (−)	D6 (−)	D7 (−)
E	25 ℃	E1 (+)	E2 (−)	E3 (−)	E4 (+)	E5 (−)	E6 (−)	E7 (−)
F	25 ℃	F1 (+)	F2 (−)	F3 (−)	F4 (−)	F5 (−)	F6 (−)	F7 (−)
G	25 ℃	G1 (−)	G2 (−)	G3 (−)	G4 (−)	G5 (−)		
H	25 ℃	H1 (−)	H2 (−)	H3 (−)	H4 (+)	H5 (−)	H6 (−)	H7 (−)

Temp.	A1	B2	B3	C4	D1	D2	E1	E4	F1	H4
21 ℃	(+)	(+)	(−)	(−)	(−)	(+)	(−)	(+)	(+)	(+)
25 ℃	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
28 ℃	(+)	(−)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

[1]Sampling sites: A; #5 Mooring front, B; #2 Teeing ground next to fair way, C; #10 Approach, D; #6 In front of pond bridge, E; #14 100-yard tree, F; #11 Middle area of fair way, G; #1 Green collar boundary fair way, H; #13 Island

[2]Cellulose-hydrolytic activity: (+); positive reaction (middle ~ high activities), (−); no and low activities

1-3. Identification of the bacterial isolates efficient for cellulose hydrolysis.

All five bacterial isolates (A1, D2, E4, F4, and H4) showing high cellulose-hydrolytic activities formed granular, round or irregular colonies colored whitish, grey-white or creamy with entire, less wavy or undulate margins on NA (data not shown). In transmission electron microscopy (TEM), all the isolates were rod-like shapes of about 1.0 x 2.0 μm in size with peritrichous flagella (Fig. 2). Analysis of 16S rRNA gene sequences of 4 bacterial isolates (A1, D2, E4, and F4) showed the highest similarity (99%) to NCBI GenBank accession no. NR_112636.1 of *B. megaterium*, and the other isolate H4 showed the highest similarity (99%) to NCBI GenBank accession no. NR_074540.1 of *B. cereus* (Table 3), indicating the former 4 isolates were identified as different isolates of *Bacillus megaterium* and the latter one as an isolate of *B. cereus*, all of which were named presumably as A1; *B. megaterium* A1, D2; *B. megaterium* D2, E4; *B. megaterium* E4, F4; *B. megaterium* F4, and H4; *B. cereus* H4, respectively, which were coincided with their cultural and morphological characteristics as shown in TEM (Fig. 2).

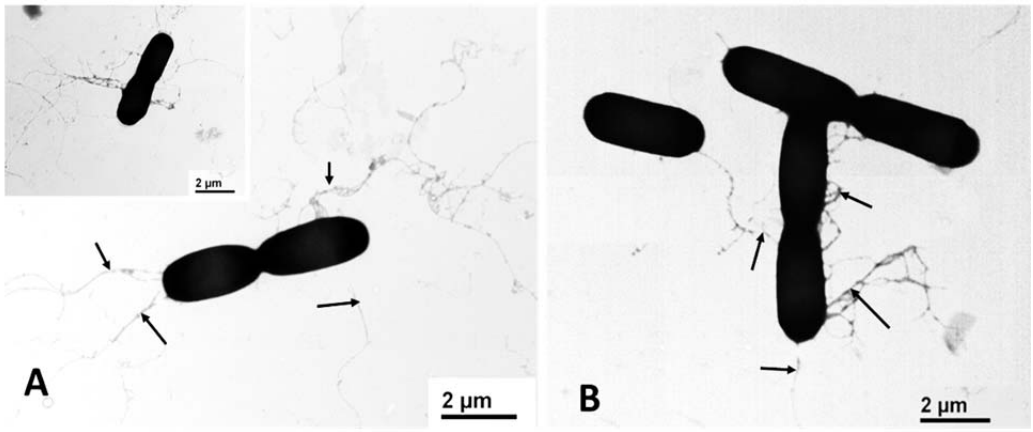

Figure 3. Electron micrographs of bacterial isolates (A: F1, B: H4), showing rod-like shape of about 1.0 x 2.0 µm in size with peritrichous flagella (arrows). Bar = 2.0 µm.

Table 3. Analysis of 16S-ribosomal DNA sequences in the bacterial isolates showing cellulose-hydrolytic activities isolated from golf course fields in Anyang Country Club

Isolate	Identification	NCBI accession	
		Similarity	Accession No.
A1	*Bacillus megaterium* A1	99%	NR_112636.1[1]
D2	*Bacillus megaterium* D2	99%	NR_112636.1
E4	*Bacillus megaterium* E4	99%	NR_112636.1
F1	*Bacillus megaterium* F1	99%	NR_112636.1
H4	*Bacillus cereus* H4	99%	NR_074540.1[2]

[1]Partial sequences of 16S ribosomal RNA gene from *Bacillus megaterium* strain NBRC 15308 listed in GenBank

[2]Partial sequences of 16S ribosomal RNA gene from *Bacillus cereus* strain ATCC 14579 listed in GenBank

2. Biological control activity of thatch accumulations *in vivo*

2-1. Pot experiments

2-1-1. First pot experiment

For the first pot experiment, five bacterial isolates with positive cellulose-hydrolytic activities at all temperatures tested were applied on the turfs planted in pots. Three characteristics related to thatch decomposition such as soil moisture contents, root length and plant density were examined at intervals of 7 days. Moisture contents of soil increased continuously up to 3 weeks after treatment (WAT) for all bacterial treatments and control with no significant increase with increased WAT, but decreased sharply at 4 WAT (Fig. 3). However, the water content of the soil treated with A1 was significantly higher at $P \leqq 0.05$ from one WAT until three WAT than the other treatments and control, among which differences in moisture content among the others were not so highly significant, compared to A1. For H4, however, water content increased most significantly at 3 WAT compared to 1 and 2 WAT. The grass root lengths were not so significantly different among treatments and control as moisture contents during the experimental period, showing the root length greatest in H4 and the second greatest in A1 and E4 at 2 WAT, and the greatest in A1 and E4 at 3 and 4 WAT (Fig. 4). For all treatments except for the control, plant density increased, especially at one and two WAT in A1, F1 and H4 (Fig. 5).

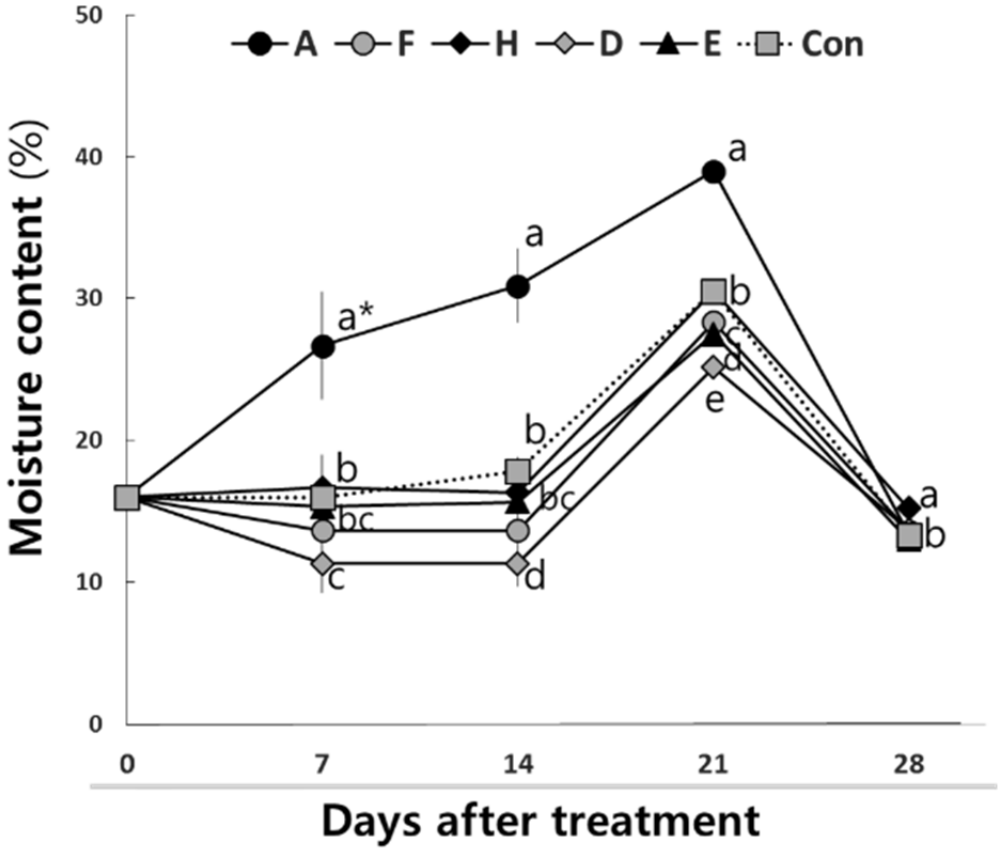

Figure 4. Result of moisture content in the first pot experiment. A: *Bacillus megaterium* A1, D: *B. megaterium* D1, E: *B. megaterium* E4, F: *B. megaterium* F1, H: *B. cereus* H4.

*Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

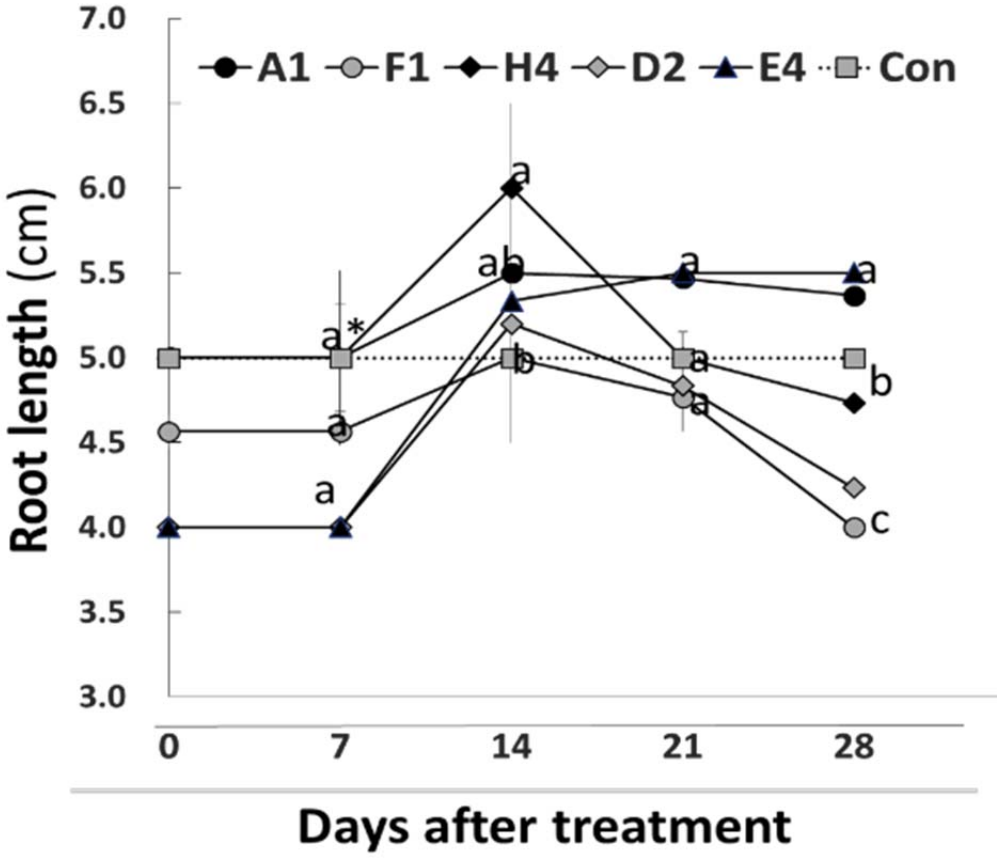

Figure 5. Result of root length in the first pot experiment. A: *Bacillus megaterium* A1, D: *Bacillus megaterium* D1, E: *Bacillus megaterium* E4, F: *Bacillus megaterium* F1, H: *Bacillus cereus* H4.

*Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

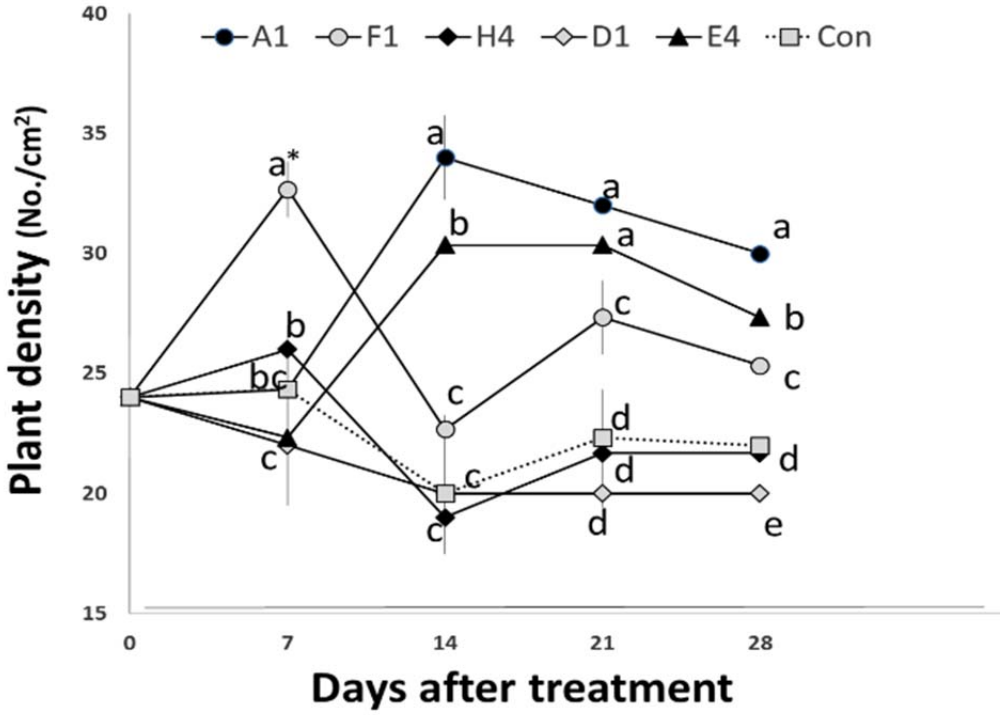

Figure 6. Result of plant density in the first pot experiment. A: *Bacillus megaterium* A1, D: *B. megaterium* D1, E: *B. megaterium* E4, F: *B. megaterium* F1, H: *B. cereus* H4.

*Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

2-1-2. Second pot experiment

For the second pot experiment, three bacterial isolates such as A1, F1 and H4 that showed the highest increase of plant density were applied on the turfs planted in pots. Effects of the microbial treatments on three characteristics related to thatch decomposition appeared to be similar to those of the first pot experiment (Table 4). Specifically, moisture contents increased significantly up to 3 WAT for all treatments and control as in the first experiment; for root length, no significant differences were shown among treatments and control and with time after inoculation; and especially plant density, which is related to green compactness (hardness) was always significantly higher in all treatments than the no treatment control (Table 4).

Table 4. Effects of microbial treatments on turf characteristics related to thatch decomposition with time after treatment

Characteristics	Isolate[1]	Days after treatment			
		0	7	14	21
Moisture content (%)	A1	17.1±0.3a[2]	16.7±1.5ab	19.2±1.7b	15.0±1.7a
	F1	17.5±1.5a	21.4±2.4a	23.2±0.6a	14.0±0.0a
	H4	16.0±1.0a	20.7±3.0a	21.5±0.9a	14.2±3.2a
	Con	16.7±1.6a	14.5±2.8b	24.0±0a	15.0±0.0a
Root Length (cm)	A1	4.5±0.15a	4.6±0.1a	4.5±0a	4.5±0.0a
	F1	4.2±0.0a	4.0±0.0b	4.0±0b	4.2±0.0b
	H4	4.1±0.1a	4.6±0.2a	4.5±0a	4.1±0.1c
	Con	4.1±0.4a	4.0±0.0b	4.5±0a	4.1±0.0c
Plant Density (No./cm^2)	A1	22.0±0.0a	23.0±0.0a	22.3±0.6a	18.0±1ab
	F1	21.3±1.5ab	23.0±0.0a	22.0±0.0a	19.3±0.6a
	H4	17.3±0.6c	19.3±2.5a	19.0±0.0b	16.7±1.5bc
	Con	20.0±0.0b	16.3±0.6c	18.0±0.0c	15.0±0.0c

[1]Bacterial isolates: A1 and F1; *Bacillus megaterium*, H4; *B. cereus*

[2]Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

2-2. Nursery experiment

In the nursery experiment, the same three bacterial isolates used in the second pot experiment and a commercial product “Thatch Manager” (TM) were used to examine effects of their treatments on thatch layer thickness and the characteristics related to the green speed such as plant density and turf hardness, and those related to plant growth such as chlorophyll index {related to photosynthesis for overall plant growth} and root growth affected by soil moisture and nutrient contents. Firstly thatch layer reduced in all microbial treatments and TM at one WAT, compared to the non-treatment control whose thatch layer was increased slightly; for plant density, no significantly different effects occurred by the microbial treatments and TM at one and two WAT; and turf hardness decreased generally in all bacterial treatments and varied depending on the bacterial isolates and their concentrations (Table 5). Secondarily for the matter of plant-growth characteristics, there are no significant differences in chlorophyll index among bacterial treatments, TM and control with time after treatment; however, bacterial treatments showed higher chlorophyll index at one WAT, compared to TM and non-treatment control (Table 6). Root length increased continuously more in bacterial treatments and control than TM (Table 6).

Table 5. Effects of microbial treatments on the thatch layer thickness and the characteristics related to the green speed with time after treatment in the nursery

Characteristics	Treatments[1]		**Days after treatment**		
			0	7	14
Thatch Layer thickness(cm)	A	10^3	5.0±0.0b[2]	5.0±0.0b	4.7±0.3bc
		10^5	5.0±0.0b	5.0±0.0b	4.8±0.2b
	F	10^3	5.0±0.0b	5.0±0.0b	4.5±0.3c
		10^5	5.0±0.0b	4.7±0.3c	4.7±0.2c
	H	10^3	5.3±0.1a	5.5±0.0a	5.5±0.0a
		10^5	5.0±0.0b	4.7±0.1c	4.7±0.1c
	TM		5.0±0.0b	4.8±0.2b	4.4±0.2c
	Con		5.0±0.0b	5.5±0.3a	5.4±0.1a
Plant Density (No./cm^2)[3]	A	10^3	34±4.6a	34±1.7a	37±1.0a
		10^5	33±2.0a	33±0.0ab	35±2.0a
	F	10^3	33±2.6a	34±1.0a	35±2.0a
		10^5	34±2.6a	33±2.6ab	34±1.7a
	H	10^3	32±3.6a	32±1.0ab	34±2.6a
		10^5	32±1.0a	31±1.0ab	33±2.0a
	TM		31±2.0a	32±1.0ab	36±2.6a
	Con		32±1.7a	33±2.0ab	34±3.0a
Turf Hardness[3]	A	10^3	546±16.0a	417±30.0d	431±11.8b
		10^5	499±18.2bc	451±10.1bc	459±12.5a
	F	10^3	523±13.5ab	468±11.8a	463±10.8a
		10^5	493±13.1c	445±15.2bcd	468±11.8a
	H	10^3	495±21.4bc	424±5.6cd	461 ±7.2a
		10^5	494±21.7bc	448±1.0bc	475±11.3a
	TM		492±17.3c	455±30.0b	465±10.8a
	Con		468±14.0c	437±7.8bcd	463 ±9.6a

*Figures are averages and standard deviations of three replications.

[1]Treatments: bacterial isolates (A and F; *Bacillus megaterium* A1 and F1, H; *B. cereus* H4) and concentrations (10^3; 10^3 CFU/ml, 10^5; 10^5 CFU/ml)

[2]Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

[3]One of the characteristics related to the green speed.

Table 6. Effects of microbial treatments on the characteristics related to plant growth with time after treatment in the nursery

Characteristics	Treatments[1]		**Days after treatment**		
			0	7	14
Chlorophyll[3] Index	A	10^3	253±14.4a[2]	257±6.6a	249 ±8.5a
		10^5	251±13.0ab	257±9.2a	258 ±6.1a
	F	10^3	243±13.2ab	248±5.6a	249±11.8a
		10^5	235±11.5ab	237±12.5b	236±11.4a
	H	10^3	240±16.1ab	244±13.0a	233±11.4a
		10^5	229±14.9b	238±8.9b	230 ±2.6a
	TM		218 ±8.2b	224±2.0c	224 ±4.2a
	Con		226±10.4b	228±3.6c	224 ±3.5a
Root Length(cm)	A	10^3	7.0±0.3c	7.0±0.2b	8.0±0.3b
		10^5	7.0±0.2c	8.0±0.2a	9.0±0.2a
	F	10^3	7.8±0.3a	8.3±0.5a	9.0±0.3a
		10^5	7.6±0.3a	8.1±0.2a	9.0±0.3b
	H	10^3	7.4±0.3b	8.0±0.1a	8.0±0.3b
		10^5	7.6±0.2a	8.1±0.3a	8.0±0.3b
	TM		7.0±0.2c	7.0±0.2b	7.1±0.3c
	Con		7.0±0.1c	8.0±0.2a	8.1±0.3b

*Figures are averages and standard deviations of three replications.

[1]Treatments: bacterial isolates (A and F; *Bacillus megaterium* A1 and F1, H; *B. cereus* H4) and concentrations (10^3; 10^3 CFU/ml, 10^5; 10^5 CFU/ml)

[2]Means with same letters are not significantly different at $P \leqq 0.05$ by least significant Difference test (LSD).

[3]One of the characteristics related to visual quality of the green.

DISCUSSION

The biological control of thatch accumulation using microorganisms has been little studied until now. Thatch decomposition other than mechanical practices has a tremendous value as the physical ways such as aeration, vertical mowing, core cultivation and topdressing are harmful to the green surface and decrease tolerance of grass against biotic and abiotic stresses by weakening turfgrass health (Ledeboer and Skogley, 1967). Murdoch and Barr (1967) could not find evidence of thatch reduction by a commercial microbial product in common bermudagrass {*Cynodon dactylon* (L.) Pers. Var. *dactylon*}. However, Berndt et al. (1990) noted thatch reduction on 'Kentucky' bluegrass (*Poa pratensis* L.) after a year of applying three commercial bio-organic products, but was unclear whether it was attributed by the microorganisms added or increased N fertility (McCarty, 2007).

Three bacterial isolates, which were identified as *Bacillus megaterium* A1, *B. megaterium* F1 and *B. cereus* H4 by 16S ribosomal RNA gene analysis and morphological characteristics revealed by TEM, were selected for their use in nursery field as their cellulose-hydrolytic activities were validated *in vitro* assays and because of their ability of efficient moisture retention, promotions of root growth and plant density that are related to the reduction of thatch accumulations, and improvement of turfgrass growth in pot experiments as excessive thatch accumulation reduces

hydraulic conductivity and water infiltration, but increases localized dryness (Cornman, 1852; Harris, 1978; Murray and Juska, 1977).

In nursery experiment, some characteristics related to thatch decomposition such as thatch layer thickness and plenty density were improved by all (for thatch layer) or one (F1) (for plant density) of the microbial treatments as TM in this study. On the other hand, one of plant-growth related characteristics, chlorophyll index (degree of greenness) was higher in all microbial treatments than TM, suggesting the bacterial treatments may not only improve the green speed by the reduction of thatch layers as TM but also strengthen turfgrass health and improve the visual appearance of the greens. All of these aspects of the effects of the microbial treatments suggest that these bacterial isolates may be used for the development of microbial products not only for the non-physical, biological thatch decomposition, but also turfgrass health promotion and visual appearance improvement of the greens.

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골프장 토양 미생물의 골프장 때치 분해와 잔디 생장 촉진 효과

정성택

초록

골프장의 때치는 잔디와 지제부 사이에 쌓인 불완전 분해 유기물을 일컫는다. 때치는 주로 주기적으로 벗겨져 나온 뿌리와 포복경 및 근경, 그루터기 및 노화한 엽초와 잎의 잔해물로부터 형성된다. 많은 때치가 축적될 경우 잔디 예지, 시비, 관수 및 병해충 방제 등 골프장 잔디 관리와 함께 낮은 예고로 골프장 그린을 유지하여야 하는 코스관리사들의 그린의 품질(퍼팅 거리와 속도)과 관련된 고품질 잔디생육 유지에 어려움을 겪게 한다. 때치 제거 방법에는 기계적 천공을 통한 잔디 제거, 통기와 손질 및 배토 등 여러 가지 방법이 있는데 이러한 방법은 때치 제거에는 유용하나 단기적으로 잔디의 표면을 손상시키고 생육을 약화시키는 등 다른 부작용을 야기한다. 그리하여 이러한 기계적 방법이 아닌 다른 화학적 또는 생물학적 때치 제거 방법이 필요하다. 이를 위해 본 연구에서는 안양 컨트리 클럽의 8 군데의 골프장 코스의 토양에서 총 53 세균을 분리하였고 이들 세균 중 25℃에서 때치의 주요 성분인 섬유소 분해 효과가 있는 10 균주를, 또 이들 균주 중 21~28℃에서 유효한 섬유소 분해 효과가 있는 5 균주(A1, D2, E4, F1, H4)를 선발하였다. 이들 균주들을 16S ribosomal RNA 유전자의 염기서열

분석 및 전자현미경에 의한 형태적 특성을 조사한 결과 4균주(A1, D2, E4, F1)는 *Bacillus megaterium*으로 동정되었고 다른 한 균주(H4)는 *B. cereus*(H4)로 동정되었다. 이들 다섯 균주의 현탁액을 포트에 심겨진 잔디에 처리하여 때치 분해와 관련한 특성들을(수분 함량, 뿌리 길이 및 잔디의 밀도) 조사한 결과 세 균주(A1, F1, H4)들의 효과가 더 높았다. 실제로 이 균주들의 현탁액을 잔디의 묘포에 처리하여 골프장의 때치 관리를 위해 상업적으로 개발된 제품(Thatch Manager, TM)과 비교한 결과 대조군에 비해 때치층의 두께는 TM과 마찬가지로 얇아졌으며, 그린의 품질(퍼팅 속도와 거리)와 관련된 특성의 개선 효과에는 차이가 없었으나, 잔디의 녹색 시각성과 잔디의 생육과 관련된 특성(뿌리 생장)은 대조군이나 TM보다 개선 효과가 큰 것으로 나타났다. 이러한 결과들을 종합하여 고려할 때 본 연구에서 선발된 세균들 즉 *B. megaterium*의 균주 A1과 F1 및 *B. cereus* H4는 현재 상품으로 개발된 TM과 유사한 때치 분해효과가 있을 뿐만 아니라 잔디의 녹색 시각성을 TM보다 더 제고하여 그린의 시각적 품질을 높이는 효과를 보였다. 또한 이들 균주들은 실제 사용된 골프장 토양에서 분리한 것으로 미생물을 처리하였을 때 골프장에 더 쉽게 정착하여 효과를 보일 것으로 생각된다.

주요어: 세균, 분해, 때치, 잔디

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