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Identification of FM001 as Plant Growth-Promoting Substance from *Acremonium strictum* MJN1 Culture

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Abstract A plant growth-promoting substance, FM001, was isolated from the culture broth of *Acremonium strictum* MJN1. The purification steps included solvent extraction, adsorption chromatography using Diaion HP20, TLC on silica, and HLPC using a C-18 column. The purified FM001 enhanced rice seedling growth by 11.1% and 34.0% of the dried weight of the shoots and roots, and also radish growth by 26.5% and 23.7% of the top length and dried weight. FM001 also significantly promoted the growth of red pepper by increasing 32.7% of fruit weight and 11.3% as regards the height. FM001 consisted of C, H, O, N, and S, and its molecular weight was determined to be 537.78 Da. The structure of FM001 resembled brassinosteriods, and it would appear to have great potential as an effective bio-fertilizer.

Key words: *Acremonium strictum* MJN1, plant growth promoting substance, isolation, characterization

It is well known that certain microorganisms have the capacity to synthesize a large number of organic compounds with widely varying chemical structures which belong to different chemical classes [5]. Many of these natural compounds exhibit plant growth promoting activity and are responsive to environmental conditions imposed by plant root [25]. Certain bacteria, algae, actinomycetes, and fungi have plant growth promoting ability [1, 6-17, 22], for example, rhizosphere fungus forms a symbiotic relationship with host plant root. The fungus benefits by obtaining necessary nutrients from the plant root, while the uptake of water and minerals to the host is increased via the fungal hyphae [2, 20, 21]. Certain plant growth-promoting fungi (PGPF) also aid the host by possessing properties antagonistic towards plant pathogens [19] or by producing metabolites with phytohormonal activities [24]. Both cytokinins and auxins have been isolated from mycorrhizal fungal filtrates [3, 25, 26].

Previously, a plant growth-promoting substance was isolated from the mycelial extract of *Acremonium strictum* MJN1, a fungal strain isolated from the roots of *Panax ginseng* C.A. Meyer, and identified as D-adenosine [18]. However, the current authors earlier observed the presence of a plant growth-promoting substance in culture broth of *A. strictum* MJN1. In the current study, isolation of this plant growth-promoting substance from *A. strictum* MJN1 and partial characterization was carried out.

The fungal strain *A. strictum* MJN1 was originally isolated from the roots of *P. Ginseng* C.A. Meyer and identified through KCTC (Korean Collection For Type Cultures). *A. strictum* MJN1 was cultured in a medium containing 15 g of glycerol, 6 g peptone, 5 g NaCl, 5 g KCl, 0.015 g $FeSO_4$, 0.02 g $CuSO_4$, 0.3 g KH_2PO_4, 0.05 g $MgSO_4$, and 0.1 ml of $MnSO_4$ (0.2% solution), in 1 l of distilled water at pH 6.0. After mycelium of a 7-day-old culture was broken by homogenization at 10,000 rpm (IKA T25, IKA LABORTECHNIK Co.), 5% (v/v) of the homogenate was inoculated into 3.5 l of media in a 5 l jar fermentor (Korea Fermentor Co.). The mycelium was then cultured at 25°C for 14 days with an agitation speed of 200 rpm and 0.5 vvm air supply.

The fermentation broth (1 l) was separated into the mycelial mat and filtrate by filtration through cheese cloth. Supplementation of the filtrate at 10^{-4} dilution resulted in 21.9% and 20.2% increase in the shoot and root weight of rice seeds, respectively, and at 10^{-6} dilution resulted in 42.4% and 35.8% increase in the shoot and root length of lettuce, respectively (Table 1). The filtrate was extracted twice by incubating with 250 ml of methylene chloride for 12 h, and the active aqueous layer was applied to a 80 ml

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Table 1. Plant growth promoting effects of culture broth from *A. strictum* MJN1 on rice and lettuce seedling growth.

	Concentration*	Germination		Dried shoot weight		Dried root weight	
		N±SE**	%	Mg±SE	%	mg±SE	%
Rice seedling	Control	17.3±0.3	100	10.67±0.37	100	11.07±0.37	100
	10^{-2}	18.3±0.3	105.9	12.23±0.01	108.3	11.70±1.29	106
	10^{-3}	17.3±1.3	100	13.17±2.26	123.4	12.83±5.08	115.8
	10^{-4}	19.0±0.0	111.8	14.27±8.14	121.9	14.60±1.09	120.2
	10^{-5}	18.3±0.3	105.9	13.03±3.30	115.4	12.86±2.05	110
	10^{-6}	18.3±0.3	105.9	12.3±2.76	108.9	12.86±0.52	110
	Concentration	Germination		Shoot length		Root length	
		N±SE	%	mm±SE	%	mm±SE	%
Lettuce seedling	Control	18±0.3	100	54.0±15.7	100	121.0±23.4	100
	10^{-2}	18±0.5	100	69.0±11.4	127.8	146.5±26.5	107.4
	10^{-3}	19±0.2	94.7	67.9±12.6	125.7	158.8±19.1	128.3
	10^{-4}	20±0.6	111	60.0±11.9	111.1	130.0±18.6	111.6
	10^{-5}	18±0.4	94.7	61.6±12.1	114.2	133.8±19.3	115.2
	10^{-6}	20±0.3	111	76.9±11.7	142.4	160.0±9.8	135.8

*Culture broth was serial diluted in distilled water.
**SE means standard error.
The values are average of triplicate experiments of 20 seeds each. Therefore, each value is based on 60 seeds per treatment.

Diaion HP-20 column (Mitsubishi Chemical Corp., Japan) pre-equilibrated in distilled water (pH 3). After washing the column with 50 ml of degassed water (pH 3), a step gradient of methanol/water was applied (40, 60, 80, and 100% methanol). The 80% methanol eluate was concentrated by evaporation under reduced pressure at 45°C. The concentrate was extracted twice by incubating with 25 ml of butanol for 5 h. After concentrating the butanol layer by evaporation, the residue was dissolved in methanol and applied to a preparative TLC [Merck, precoated silica gel Kiesigel 60F; ethyl acetate:n-butanol: formic acid:water= 5:3:3:1 (v/v/v/v); with visualization by 5% H_2SO_4 in ethanol].

Table 2. Plant growth promoting effects of FM001 from *A. strictum* MJN1 on rice, radish, and red pepper growth.

	Concentration (ppm)	Dried shoot weight		Dried root weight	
		mg±SE	%	mg±SE	%
Rice	Control	20.8±0.76	100.0	13.5±1.20	100.0
	10^{-3}	23.1±0.87	111.1	18.1±1.50	134.0
	10^{-4}	22.1±0.39	106.2	17.7±0.83	131.1
	10^{-5}	20.7±0.68	99.5	13.5±0.88	100.0
	Concentration (ppm)	Dried shoot weight		Dried leaf weight	
		mg±SE	%	mg±SE	%
Radish	Control	628.3±22.5	100.0	755.9±3.3	100.0
	10^{-3}	795.1±51.3	126.5	935.7±35.3	123.7
	10^{-4}	669.2±80.4	106.5	813.0±91.0	107.5
	10^{-5}	637.9±10.4	101.5	815.5±2.6	107.8
	Concentration (ppm)	Fruit weight		Height	
		g±SE	%	mm±SE	%
Red pepper	Control	5.70±0.545	100.0	518±18.67	100.0
	10^{-3}	7.81±0.609	132.7	570±27.38	111.3
	10^{-4}	7.49±0.677	131.4	563±27.70	109.9
	10^{-5}	6.61±0.496	116.0	527±22.14	102.9

The values for rice are an average of triplicate experiments of 20 seeds each. Therefore, each value is based on 60 seeds per treatment.
The values for radish and red pepper are an average of triplicate experiments of 5 seeds each. Therefore, each value was based on 15 seeds per treatment.

The active fraction (Rf=0.26) was eluted with methanol, and then subjected to HPLC (SpectraSystem, Cosmosil C-18 column 4.6×150 mm; elution by 45% methanol at a flow rate of 0.7 ml/min; diode array detection; PC1000 software). The active substance with 14.7 min retention time, named FM001, was then obtained as a single peak (5 mg/l yield). FM001 was white powdered, soluble in methanol, butanol, and water, and it's maximum UV-absorption was at 210 nm. The molecular weight of FM001 was determined to be 537.78 Da by MALDI MS (Voager™ DE-STR, PerSeptive Biosystems, Inc.) and Tandem MS (JMS-HX 110A/HX110A, JEOL, Japan). The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA-300 (300 MHz ^{1}H, 75.5 MHz ^{13}C) spectrometer in deuterated chloroform ($CDCl_3$) with tetramethylsilane (TMS) as the internal reference (Table 3). The molecular formula of FM001 was $C_{27}H_{48}O_4N_3S_2$ determined by elemental analyzer (Flash EA 1112 Series, CE Instruments/ThermoQuest, Italia) and 2-dimension NMR. All the data obtained by instrumental analyses showed that FM001 had a brassinosteriods-like structure. The brassinosteriods consist of groups of biologically active natural products with steroidal structure. They exhibit multiple bioregulatory activities on plant growth and development-promoting effects in various bioassays [5].

A rice seedling test was performed by incubating seeds on a cubic plate (magenta ga-7 vessel, Sigma) containing Whatman No. 42 filter paper and 10 ml of serially diluted sample. The plate was incubated at 25°C for 10 days in a dark chamber until germination was complete. The germinated seedlings were collected, the shoots and roots of the seedlings were separated, and dried at 70°C to a constant weight to measure dry weight.

Table 3. ^{1}H and ^{13}C NMR chemical shift of FM001.

^{1}H (ppm)	^{13}C (ppm)
0.70	13.025
0.91	17.780
0.97d[a]	23.185
1.15m	24.237
1.39m	27.894
1.51m	28.669
1.54m	29.588
1.78m	31.204
1.83m	33.148
1.94m	34.262
2.11m	35.874
2.25m	35.916
2.95t	36.528
3.58t	36.605
3.79	36.902
3.94	40.487
	41.049
	42.987
	43.227
	51.512
	69.074
	72.901
	74.029
	176.604

[a]d=doublet; t=triplet; m=multiplet.

A lettuce seedling test was performed by incubating seeds on a glass pertri dish containing filter paper and 5 ml of the serially diluted sample. The petri dishes were incubated under 2,000 lux white light at 25°C for 3 days, then the lengths of the shoots and roots were measured.

To perform a radish growth assay, a polycarbonate tray (3×3×5 cm) filled with wet potting soil (Seoul Agriculture Co., Korea) was sown with radish seeds at a depth of about 0.2 cm and placed in a dark chamber at 25°C. After being sown, the tray was placed in a greenhouse and treated with 5 ml of the serially diluted sample. Seven days after the treatment, the shoots and roots were separated and dried at 70°C to a constant weight to measure dry weight.

For a red pepper growth assay, polycarbonate pots (24.5×12 cm) filled with 700 g of dried potting soil were sown with red pepper seeds (Youngwoong, No. VP-hy-227; Nongwoo Co.) at a depth of about 0.5 cm and placed in a growth chamber (light period of 15 h, 500 lux at 27°C; dark period of 9 h, at 25°C; average relative humidity of 75%). The pots sown with the red pepper seeds were watered every 2 days with tap water, and 50 ml of the water-diluted sample was sprayed on the leaves of the red pepper plants once a week after the four-leaf stage. Eight weeks after the initial treatment, the plants were harvested, and the height and fruit weights were measured.

Plant growth-promoting material from plants and microorganisms normally exhibits growth-promoting activity at concentrations below 0.1 mM in certain bioassay systems [5]. In particular, commercial synthetic L-adenosine with foliar application at 10^{-1}–10^{-5} ppm for 24 h increases the growth rate of rice seedlings, as measured by the total dry weight [23]. FM001 at 10^{-3} ppm enhanced rice seedling growth by increasing 11.1% and 34.0% of the dry weight of the shoots and roots, respectively, radish growth by increasing 26.5% and 23.7% of the shoot and leaf weight, respectively, and red pepper growth by increasing 32.7% and 11.3% of the fruit weight and height, respectively (Table 2).

The concentration effect of plant growth-promoing substance are usually grouped into 3 classes. First, concentration dependent plant growth-promoting substance, such as Giberellic acid [4], secondly, substance which is active in broad concentration range such as Thienodolin [6], and finally, substance which is active in specific concentration, like BSF-A [11]. FM001 showed the highest effect on our bioassay system at 10^{-3} ppm concentration, and this result indicates that FM001 needs specific concentration. But, more precise experiments are needed for the field application.

In summary, we describe isolation and partial characterization of the plant growth stimulator FM001. Physico-chemical and spectral data are insufficient to define precise structure of FM001, and further structural elucidation of FM001 using IR, HREI-MS, and multipulse NMR analysis will provide additional information on the functional group design and chemical synthesis of the plant growth regulator. Since FM001 exhibited growth stimulating activity of rice seedlings, radish, and red pepper, this indicates that FM001 has a great potential as a new effective bio-fertilizer.

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References

1. Bernart, M. and W. H. Gerwick. 1990. 3-(Hydroxyacetyl) indole, a plant growth regulator from the Oregon red alga. *Prionitis lanceolata. Phytochemistry* **29:** 3697–3698.
2. Bowen, G. D. 1973. Mineral nutrition of ectomycorrhiza, pp. 207–230. *In* Marks, G. C. and T. T. Kozolowski (eds.), *Ectomycorrhiza.* Academic Inc., New York, U.S.A.
3. Craft, C. B. and C. O. Miller. 1975. Detection and identification of cytokinins produced by mycorrhizal fungi. *Plant Physiol.* **54:** 586–588.
4. Frankland, B. and P. F. Wareing. 1960. Effects of gibberellic acid on hypocotyl growth of lettuce seedling. *Nature* **4708:** 255–256.
5. Gross, D. and B. Parthier. 1994. Novel natural substances acting in plant growth regulation. *J. Plant Growth Regul.* **13:** 94–114.
6. Kanbe, K., H. Naganawa, K. T. Nakumura, Y. Okami, and T. Takeuchi. 1993. Thienodolin, a new plant growth regulating substance produced by a *Streptomycetes* strain II. Structure of thienodolin. *Biosci. Biotechnol. Biochem.* **57:** 636–637.
7. Kanbe, K., H. Naganawa, M. Okamura, T. Sasaki, M. Hameda, Y. Okami, and T. Takeuchi. 1993. Amidenin, a new plant growth regulating substance isolated from *Amycolatopsis* sp. *Biosci. Biotechnol. Biochem.* **57:** 1261–1263.
8. Kim, M. S., C. Kim, D. H. Jo, and Y. W. Ryu. 1999. Effect of fungal elicitor and heavy metals on the production of flavonol glycosides in cell cultures of *Ginkgo biloba. J. Microbiol. Biotechnol.* **9:** 661–667.
9. Kim, M. S., E. J. Huh, H. K. Kim, and K. W. Moon. 1998. Degradation of polycyclic aromatic hydrocarbons by selected white-rot fungi and influence of lignin peroxidase. *J. Microbiol. Biotechnol.* **8:** 129–133.
10. Kim, Y. S. and S. D. Kim. 1994. Antifungal mechanism and properties of antibiotic substances produced by *Bacillus subtilis* YB-70 as a biocontrol agent. *J. Microbiol. Biotechnol.* **4:** 296–304.
11. Kimura, Y., H. Fujioka, H. Nakajima, T. Hamasaki, and R. Nakashima. 1993. BSF-A, a new plant growth regulator produced by the fungus *Botrytis squamosa. Biosci. Biotechnol. Biochem.* **57:** 1584–1585.
12. Kimura, Y., H. Nakajima, and T. Hamasaki. 1990. Sescandelin, a new root promoting substance produced by the fungus *Sesquicillium candelabrum. Agric. Biol. Chem.* **54:** 2477–2479.
13. Kimura, Y., T. Matsumoto, H. Nakajima, T. Hamasaki, and Y. Matsuda. 1993. Dihydroampullicin, a new plant growth regulator produced by the *Ampulliferina*-like fungus sp. No 27. *Biosci. Biotechnol. Biochem.* **57:** 687–688.
14. Kimura, Y., T. Mizuno, H. Nakajima, and T. Hamasaki. 1992. Atechrommones A and B, a new plant growth regulator produced by the fungus *Alternaria* sp. *Biosci. Biotechnol. Biochem.* **56:** 1664–1665.
15. Kubo, I., M. Ochi, K. Shibata, F. J. Hanke, T. Nakatsu, K. S. Tan, M. Taniguchi, T. Kamikawa, Y. Yamagiwa, M. Arizuka, and W. F. Wood. 1990. Effect of a marine algal constituent on the growth of lettuce and rice seedling. *J. Nat. Prod.* **53:** 50–56.
16. Kubo, I., M. Sutisna, and K. S. Tan. 1991. Effect of nagilactones on the growth of lettuce and rice seedling. *Phytochemistry* **30:** 455–456.
17. Lee, J. Y., B. S. Kim, and B. K. Hwang. 1995. Numerical identification of *Streptomyces flaveus* producing antibiotic substances inhibitory to plant pathogenic fungi. *J. Microbiol. Biotechnol.* **5:** 324–334.
18. Lim, H. B., Y. J. Chung, J. Y. Bae, D. J. Kim, H. J. Kwon, I. H. Lee, B. C. Chung, W. S. Lee, and J. W. Suh. 2000. Plant growth promoting activity of *Acremonium strictum* MJN1 isolated from roots of *Panax ginseng. Agric. Chem. Biotechnol.* **43:** 104–108.
19. Lumsden, R. D. 1981. Ecology of mycoparasitism. pp. 295 – 328. *In* D. T. Carroll (ed.), *The Fungal Community,* Marcel Dekker Inc, New York, U.S.A.
20. Meyer, F. H. 1966. Mycorrihizae and other plnat synbiosis, pp. 171–255. *In* S. M. Henry (ed.), *Symbiosis*, Academic Inc., New York, U.S.A.
21. Miller, C. O. 1967. Zeatin and Zeatin riboside from mycorrhizal fungus. *Science* **157:** 1055–1057.
22. Nakajima, H., T. Hamasaki, S. Maeta, Y. Kimura, and Y. Takeuchi. 1990. A plant growth regulator produced by the fungus. *Cochiobolus spicifer. Phytochemistry* **29:** 1739–1743.
23. Ries, S. K. 1991. Triacontanol and its second messenger 9- β-L(+)-adenosine as plant growth substance. *Plant Physiol.* **95:** 986–989.
24. Shivanna, M. B., M. S. Meera, and M. Hyakumachi. 1994. Sterile fungi from zoysiagrass rhizosphere as plant growth promoters in spring wheat. *Can. J. Microbiol.* **40:** 637–644.
25. Strzelcyk, E. and A. Pokojska-Burdzeit. 1984. Production of auxin and gibberelin like substance by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus silverstris* L.). *Plant and Soil* **81:** 185–194.
26. Ulich, J. M. 1961. Effects of mycorrhizal fungi and auxins on root development of sugar pine seedling (*Pinus lambertiana* Dougl.). *Plant Physiol.* **51:** 871–874.