Occurrence pattern of witches' broom disease in bamboo in Damyang and development of Aciculosporium take detection methods

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Abstract

Bamboo is used for making structures, bamboo crafts, furniture, and bamboo shoot and foods, tourism of ecology and culture are additional bamboo-farming culture as well as a source of food in Korea. In addition, 'Traditional land use structure in rural Damyang bamboo-field area' is formed in 'forest - bamboo fieldvillage-cultivation area-stream' formation, and rich agro-biodiversity and cultural landscape of bamboo farming and crafting are fruity results of the structure. One of the emerging diseases of bamboo in the country is the bamboo witches' broom (BWB). However, studies and information about BWB in the country remain lacking and largely unexplored. In this study, we bamboo leaf and stem samples which showed BWB symptoms were collected from in five major bamboo plantation area, and the samples were subject to high-throughput sequencing, for identify pathogen that causes BWB. One fungi which is Aciculosporium take was identified in bamboo libraries. A. take (Ascomycota; Clavicipitaceae) causes continuous shoot growth but maintains normal leafarrangement and branching patterns in the host plant, which eventually resulting in witches' broom disease of bamboo. Colonized shoots by A. take continue to grow in an acropetal sequence with very thin stems and little leaves, although normal bamboo shoots cease to grow when three to five leaves develop. For rapid and specific detection of A. take, we developed Taqman probe-based qPCR methods. Then, we investigate the occurrence patterns of BWB in Damyang. A total of 150 bamboo leaf samples, 150 bamboo stems samples, and 150 roots samples were collected randomly and tested for infection using qPCR. More than 50% of the tested samples were infected with A. take. The infection rate in collected leaf samples was 26.7%, stem samples was 27.3%, and root samples was 0.7%.



Fig. 4. Sankey visualization of the absolute read counts as estimated by Kraken/Bracken from the bamboo samples. D, Domain; P, Phylum; F, family; G, genus; D, domain.

Results



Fig. 1. The phenotypes of bamboo and map of Damyang areas surveyed in 2024. (A) Map of Damyang showing areas surveyed in 2024. (B) Symptoms of bamboo witches' broom (BWB) infection on bamboo in Korea.

Microbiome	Pathogen
analysis	identification

JN587272, Aciculosprium take strain MAFF-241224 indole-diterpene biosynthetic gene cluster

Consensus	1	2	,000	4,000	6,000	8,000	10,000	12,000	14,000	16,000	18,000	20,000	22,000	24,000	26,000	28,000	30,000	32,000	34,000	37,466
Identity									1			1				í				
Coverage		dh		~ ~ ~	r		A1 ->	-	A8		. A 1	5× A	_ <u>k</u> .	~~ <i>k</i>	ь п	mΔ	<u>k</u> .	• •	<u>م</u> م و	04 A

KC113319, Aciculosprium take strain MAFF-241224 RNA polymerase II largest subunit (rpbA) gene

	1	500	1,000	1,500	2,000	2,500	3,000	3,500	4,000	4,500	5,000	5,500	6,000	6,500	7,000	7,500	8,000	8,500	9,000 9,385
Consensus				****	= =	****		= = = = = == =											
ldentity	V ^N						والعاصب ساسه	ار المعالي ويسم ويسم	-										
Coverage	6,718 0±																		

Fig. 5. Read mapping to reference sequences using geneious program. Reference sequence: Aciculosprium take







Fig. 2. The experimental scheme. Overview of two workflows (1) pathogen identification and (2) microbiome analysis.

Α

28,308,697

sample_Pb1

13,780,561

644,114

Name 🍦	Number of raw reads	Classified reads	Chordate reads	Artificial reads	Unclass r	ified eads	Microbial 🝦	Bacterial +	Viral reads	Fungal 🕴	Protozoan reads
sampl2_Pb2	28,822,351	49.6%	2.68%	0.165%		50.4%	46.2%	0.987%	3.47%	0.304%	0.0291
sample_Pb1	28,308,697	48.7%	2.28%	0.122%		51.3%	45.1%	3.1%	0.321%	0.394%	0.0267
Name 🍦	Number of raw reads	Classified reads	Chordate reads	Artificial reads	Unclass r	ified eads	Microbial reads	Bacterial reads	Viral reads	Fungal reads	Protozoan reads
sampl2 Ph2	28 822 351	14 306 690	772 182	47 588	14	515 661	13 301 804	284 419	1 000 448	87 508	83

14,528,136

12,781,231

878,971

90,807

111,597

34,518



Fig. 6. DNA extraction results and real-time PCR results. (A) The analysis of 1% agarose gel electrophoresis of extracted DNA from bamboo samples (B) Real-time PCR amplification plot of the assay targeting A. take

	Region1	Region2	Region3	Region4	Region5	Total (ea)
Leaf	6	0	20	11	3	40
Stem	13	0	9	19	0	41
Root	0	1	0	0	0	1
Total	19	1	29	30	3	82

	Region1	Region2	Region3	Region4	Region5	Total (%)
Leaf	8.6	0.0	100.0	55.0	15.0	26.7
Stem	18.6	0.0	45.0	95.0	0.0	27.3
Root	0.0	5.0	0.0	0.0	0.0	0.7
Total	9.0	1.7	48.3	50.0	5.0	18.2



Total (%) Region2 Region3 Region5 Region1 Region4

Leaf Stem Root Total

7,556

Fig. 3 Classification results of bamboo samples. (A) Sample set summary (B) The Krona RSF display. The bacterioplankton metagenome from a vertical profiling of the North Pacific Subtropical Gyre was imported from METAREP and displayed using Krona.

