DEVELOPMENT OF ULTRA RAPID ASSAY SYSTEM FOR MULTIPLEX DETECTION OF AIR-BORNE FUNGI BY CORIOLIS MICRO AND MULTIPLEX PCR BASED DNA MICROARRAY

Takayoshi Ootsu, Atsunori Isshiki
Toyo Seikan Group Holdings, Ltd., Corporate Research & Development, Yokohama, Japan

/ CONTEXT

Air-borne fungi can cause biodeterioration and health problems. To avoid these damages, it is important to check the mycoflora in indoor environments such as factories, museums and other public facilities. Yet conventional methods for checking fungi need a cultivation process step.

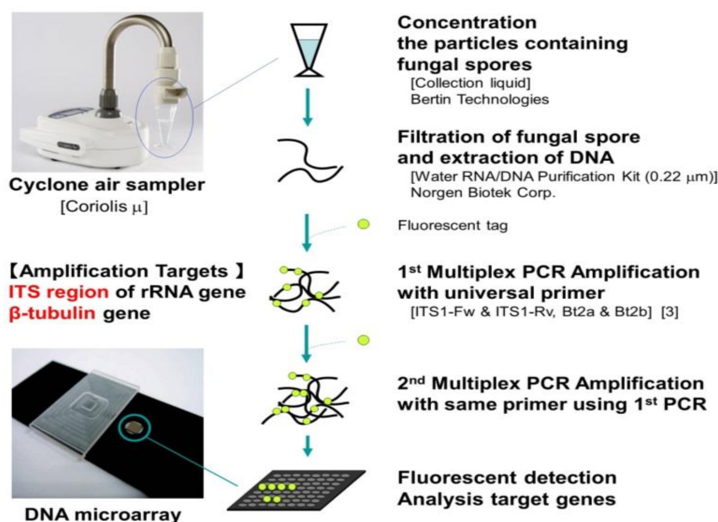
Studies [1] have reported that the Coriolis® µ concentration capability was enough to detect single fungal species by PCR amplification without cultivation process.

In our study [2], we evaluated a ultrarapid assay system for multiplex detection of air-borne fungi composed of the multiplex PCR-based DNA microarray detection system GENOGATE [3] and the Coriolis® µ.

/ MATERIALS

- Coriolis® µ, sterile cones, 15mL of collection liquid (Bertin Technologies)
- Multiplex PCR-based DNA microarray detection system GENOGATE (Toyo Seikan)

/ PROTOCOL



/ RESULTS

The performance of the DNA microarray with Coriolis® µ was validated by comparison with the results obtained from ITS sequencing analysis.

Table 1. Detection limit of DNA microarray analysis

Reference fungal strain	Strain No.*	Detection limit template DNA (n=3) 1st PCR	2nd PCR
Aspergillus penicillioides	JCM 22961T	25 pg	0.024 pg
Aspergillus versicolor	JCM 10258T	25 pg	0.098 pg
Aspergillus vitricola	NBRC 8155T	25 pg	0.098 pg
Cladosporium cladosporioides	NBRC 6348	25 pg	0.006 pg
Penicillium chrysogenum	NBRC 32030T	25 pg	0.098 pg

*JCM: Japan Collection of Microorganisms, NBRC: NITE Biological Resource Center

Table 2. Comparison of 2nd Multiplex PCR-based DNA microarray analysis with Coriolis µ results and ITS sequencing analysis results after culturing fungal colonies collected by air IDEAL sampler (bioMeriux).

	1st field trial		2nd field trial	
	DNA Microarray	Cuture & Sequencing	DNA microarray	Culture & sequencing
(air volume)	1500 L[a]	100 L[c]	1000 L[b]	100 L[c]
(number of colonies[d])	750 cfu	62 cfu	469 cfu	79 cfu
A. penicillioides	Detected	N.D.	N.D.	N.D.
A. restrictus	Detected	N.D.	N.D.	N.D.
A. vitricola	Detected	N.D.	N.D.	N.D.
A. versicolor	Detected	Detected (20 cfu)	N.D.	N.D.
Cladosporium spp.	Detected	Detected (7 cfu)	Detected	Detected (70 cfu)
Eurotium spp.	N.D.	Detected (4 cfu)	N.D.	N.D.
P. expansum	N.D.	N.D.	N.D.	Detected (1 cfu)

[a] flow rate 300 L / min, 5 min
[b] flow rate 250 L / min, 4 min
[c] flow rate 100 L / min, 1 min
[d] M40Y medium (ϕ 90) 25℃ 7days

Ultrarapid assay system with DNA microarray and Coriolis µ sampler successfully detected multiple air-borne fungal species without cultivation process.

/ CONCLUSION

The combination of the Coriolis® µ and the multiplex PCR platform GENOGATE allows the analysis of predominant airborne fungal species, even in highly contaminated environment. This system is a valuable tool for estimating the source of pollution, monitoring of specific harmful fungi, early diagnosis and discovering indoor environment changes. This combination can also have an interest for cosmetic, food and beverage industries as well as academic research.

bertin INSTRUMENTS

Authorised distributor

In Australia:
For customer service, call 1300-735-292
To fax an order, use 1800-067-639
To email an order, ordersau@thermofisher.com

In New Zealand:
For customer service, call 0800-933-966
To fax an order, use 0800-329-246
To email an order, ordersnz@thermofisher.com

ThermoFisher SCIENTIFIC

CNIM group