Variation in Antigenicity among *P. insidiosum* Isolates in Enzyme-Linked Immunosorbent Assay

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Introduction

Detection of *P. insidiosum*-specific-immunoglobulin-G antibody (*Pi*-Ab) levels by enzyme-linked immunosorbent assay (ELISA) is one of the common tools for vascular pythiosis diagnosis in Thailand. Currently, there are three *Pi*-Ab ELISA systems among three medical centers which utilize different strains of *P. insidiosum* as antigen: 1) Michigan State University-USA using type strain ATCC58643, equine origin 2) Ramathibodi Hospital-Thailand using “RAMA1”, Thai vascular isolate, and 3) King Chulalongkorn Memorial Hospital (KCMH), Thailand using “PC07”, Thai vascular isolate. It is of interesting that variability in *Pi*-Ab levels from the same serum samples has been observed depending on which ELISA system is used (Worasilchai N, unpublished data). We hypothesized that the divergence of *P. insidiosum* isolates used in these ELISA systems could potentially affect *Pi*-Ab levels, in addition to the different ELISA protocols.

Objective

To compare antigenicity of different *P. insidiosum* isolates as antigens in ELISA.

Methods

Crude antigens were extracted from six *P. insidiosum* isolates: reference strain (n=1; ATCC 58643TM, USA) and clinical isolates from patients (n=5; No. 1-5, Thailand). The antigenicity was examined by using *in-house* ELISA, established by Mycology unit, KCMH, against 3 groups of serum samples: 1) proven pythiosis cases (n=40), 2) fungal and higher bacterial infection patients (n=30; 5 samples each disease including candidiasis, cryptococcosis, aspergillosis, talaromycosis, histoplasmosis and nocardiosis), 3) healthy donors (n=20). To standardize the ELISA results for each testing batch, the OD₄₉₀ values representing antigenicity of each antigen were normalized to ELISA values (EVs) according to the following formula:

$$EV = (OD_{\text{sample}} - OD_{\text{background}}) / (OD_{\text{control}} - OD_{\text{background}}).$$

Results

Thai clinical isolates had significantly higher mean of EVs compared to the reference strain (7.3±0.4 vs 5.6±0.1 respectively; p <0.0001). In non-pythiosis serum samples, there was no significant difference in means of EVs among Thai clinical isolates and reference strain (p >0.05) (figure1, table1).

Conclusion

In this study, there is different antigenicity among Thai clinical isolates and reference strain (figure1, table1). The results indicate that different *P. insidiosum* isolates in ELISA assay affect EVs. It is very crucial to standardize ELISA assays among different institutions in Thailand particularly when EVs are used for disease monitoring.

Figure 1: The individual values of EVs among serum samples from pythiosis cases (•, n=40); pythiosis cases and patients with other fungal infections (n=30-5 serum samples each from candidiasis (▲), cryptococcosis (▲), aspergillosis (▲), talaromycosis (▲), histoplasmosis (▲) and nocardiosis (▲) against reference strain and Thai clinical isolates (No.1-5). The EVs were determined by established KCMH *in-house* ELISA.

Table 1: Mean±SD^c of EVs represented *P. insidiosum*-specific-immunoglobulin-G antibodies (*Pi*-Ab) level comparing type strain and clinical isolates.

Antigen					Antibody	
Antigen No.	Accession no.		Strain/Isolate	Source of isolate; type of pythiosis	Vascular pythiosis (n=81)	
	ITS ^a	CoxII ^b			Mean±SD ^c (Min-Max EVs) ^d	p-value ^e
Reference	ATCC58643	GQ451569*	MTPI19	Equine, Costa Rica	5.6±0.1 (5.5-5.8)	-
1	FJ917395	GQ451575	PC10	Thai human; vascular type	7.3±0.4 (6.5-8.0)	<0.0001
2	GQ260123	GQ451586	PMS01	Thai human; vascular type	7.3±0.4 (6.5-8.0)	<0.0001
3	GQ260121	GQ451591	PCM02	Thai human; vascular type	7.3±0.4 (6.5-8.0)	<0.0001
4	GQ260122	GQ451573	PC03	Thai human; vascular type	7.3±0.4 (6.6-8.0)	<0.0001
5	GQ260124	GQ451588	PC07	Thai human; vascular type	7.3±0.4 (6.5-7.9)	<0.0001

* strain contributed by Dr. L. Mendoza, MSU, USA ^a ITS = Internal Transcribed Spacer ^b CoxII = Cytochrome oxidase II ^c SD = Standard Deviation

^d Min = Minimum, Max = Maximum, EVs = ELISA Values ^e p-value calculated from EVs of each clinical antigen compared to EVs of reference antigen named “Reference”

References

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