

RGC Ref. No.: UGC/FDS25/M08/20 <p>(please insert ref. above)</p>
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**RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR**

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

<p><u>Submission Deadlines:</u></p> <ol style="list-style-type: none"> 1. Auditor's report with unspent balance, if any: within <u>six</u> months of the approved project completion date. 2. Completion report: within <u>12</u> months of the approved project completion date.
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Part A: The Project and Investigator(s)

1. Project Title

Investigating the functional role of aryl alcohol dehydrogenase in *Candida dubliniensis*
biofilm matrix synthesis via a proteomic approach

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	TSANG Wai-kei / Associate Professor	Technological and Higher Education Institute of Hong Kong
Co-Investigator(s)	SAMARANAYAKE Lakshman Perera / Professor	University of Sharjah
Co-Investigator(s)	BANDARA Hennaka Mudiyansele / Herath Nihal / Lecturer	University of Bristol
Others	N/A	N/A

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval (must be quoted)
Project Start Date	January 1, 2021	N/A	N/A
Project Completion Date	December 31, 2022	June 30, 2023	September 29, 2022
Duration (in month)	24	30	September 29, 2022
Deadline for Submission of Completion Report	December 31, 2023	June 30, 2024	September 29, 2022

4.3 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

N/A

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To generate proteomic profiles of *C. dubliniensis* wild type, *IFD6* null, complemented, and overexpression strains via proteomic approach in distinct stages of biofilm development; and
2. To identify proteins and cellular networks that characterize the changes in protein expression among these *C. dubliniensis* strains.

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Objective 1 (100% achieved):

Biofilms of the four *C. dubliniensis* strains were prepared in YNB medium into pre-sterilized, polystyrene, flat-bottomed microtiter plates at 37°C with agitation (80 rpm), and sessile cells were harvested by cell scraper at different development phases (i.e. early phase: 8 h; intermediate phase: 24 h; and maturation phase: 48 h). Cytoplasmic proteins (CPs) and extracellular proteins (ECPs) were isolated and purified independently. In the preparation of CPs, seven cycles of mechanical disruption were found to be not sufficient to complete cell lysis and we increased to ten cycles, as verified under microscope. Purified proteins were successfully obtained.

All CPs and ECPs were subject to TMT isobaric labeling and LC-MS/MS analysis. The MS data of each tryptic digest were analyzed using Proteome Discoverer and protein identification was performed using MASCOT to search against the *Candida dubliniensis* Uniprot database retrieved in November 2022 and updated with additional information in February 2023. Unmatched MS data were blasted against publicly available protein databases. The protein abundances were normalized within each sample to total peptide amount, scaled to the protein abundances of the common pool samples, then log₂ transformed to bring them closer to a normal distribution. Statistical significance for pairwise comparisons was determined using paired *t*-tests between the conditions of

interest. Statistical tests which multiple comparisons, or a continuous variable were conducted using linear regression models, fit independently to each protein using the *lm* package in R with log2 scaled abundance as the dependent variable. The *p*-values were FDR (False Discovery Rate) corrected using the Benjamini-Hochberg method. Finally, the proteomic profiles of the four *C. dubliniensis* strains at the three distinct biofilm development phases were generated.

Objective 2 (100% achieved):

We compared the proteomic profiles of all *C. dubliniensis* strains. Proteins were noted that showed significant up- or down-regulation in expression at different biofilm development phases. Specifically, the *ifd6Δ/ifd6Δ* strains (i.e. both alleles have been deleted) exhibited an up-regulation of ECPs at both the early and intermediate phases when compared with the wild type counterparts. Furthermore, the CdpNIM-IFD6 strains (i.e. overexpression of *IFD6* in *C. dubliniensis* biofilms) exhibited an up-regulation of CPs in the early, intermediate, and maturation phases, and down-regulation of CPs at the three phases respectively. Selected proteins were identified as mentioned above.

5.4 Summary of objectives addressed to date

Objectives (as per 5.1/5.2 above)	Addressed (please tick)	Percentage Achieved (please estimate)
1. To generate proteomic profiles of <i>C. dubliniensis</i> wild type, <i>IFD6</i> null, complemented, and overexpression strains via proteomic approach in distinct stages of biofilm development; and	√	100%
2. To identify proteins and cellular networks that characterize the changes in protein expression among these <i>C. dubliniensis</i> strains.	√	100%

6. Research Outcome

6.1 Major findings and research outcome

(Maximum 1 page; please make reference to Part C where necessary)

1. Generation of proteomic profiles of *C. dubliniensis* strains in three different stages of biofilm development

CPs and ECPs were purified successfully with high quality from the four *C. dubliniensis* strains grown to three distinct stages (i.e. early, intermediate, and maturation) of biofilm development. They were subsequently subject to TMT isobaric labeling and LC-MS/MS analysis. The MS data were analyzed using Proteome Discoverer and identified using MASCOT to search against a *Candida* genome database (<http://www.candidagenome.org/>) as mentioned above in 5.3. We obtained the proteomic profiles of the four *C. dubliniensis* strains at the three distinct biofilm development phases. A summary of the experimental scheme and steps is depicted in Fig. 1.

2. Identification of proteins whose expressions are related to the three different stages of biofilm development

Proteins were noted that showed significant up- or down-regulation in expression at different biofilm development phases. The *ifd6Δ/ifd6Δ* strains exhibited an up-regulation of three ECPs at both the early and intermediate phases; while other proteins (early phase: 149; intermediate phase: 63; maturation phase: 26) were down-regulated when compared with the wild type counterparts. The CdpNIM-IFD6 strains exhibited an up-regulation of 84, 36, and 45 CPs in the early, intermediate, and maturation phases; while 90, 67, and 125 proteins were found down-regulated at the three distinct biofilm development phases. Representative results for extracellular (Fig. 2a) and intracellular (Fig. 2b) proteins are shown.

The identities of the three ECPs which were found up-regulated in the *ifd6Δ/ifd6Δ* strains at both the early and intermediate phases are: glucoamylase 1 (involved in carbohydrate metabolism and polysaccharide degradation), protein disulfide isomerase (involved in protein folding), and an uncharacterized protein ynl208w homologue. We also analyzed the identities of the up- and down-regulated CPs in the CdpNIM-IFD6 strains. Most proteins are related to energy metabolism and ER-related proteins, suggesting a global elevation of energy demand in biofilm development and turnover of protein pool. Three proteins received attention: cell surface hydrophobicity-associated protein, 37 kDa cell surface protein, UTP-glucose-1-phosphate uridylyltransferase as they were found up-regulated in *C. dubliniensis* biofilms. Cell surface hydrophobicity-associated protein is an important virulence factor in *Candida* fungi. The 37 kDa cell surface protein was characterized in *C. albicans* and the protein is essential for virulence in systemic infections induced in mice and the ability to adhere to polystyrene. UTP-glucose-1-phosphate uridylyltransferase is regulated by phosphorylation by PAS kinase, and phosphorylation is crucial for control of cell wall synthesis.

Taken together, we have identified proteins which are associated with *IFD6* in *C. dubliniensis* biofilm development which shed light on the establishment of novel strategies in antifungal interventions via modulation of the functions of these proteins.

These new findings will be published in a manuscript, which is currently under preparation.

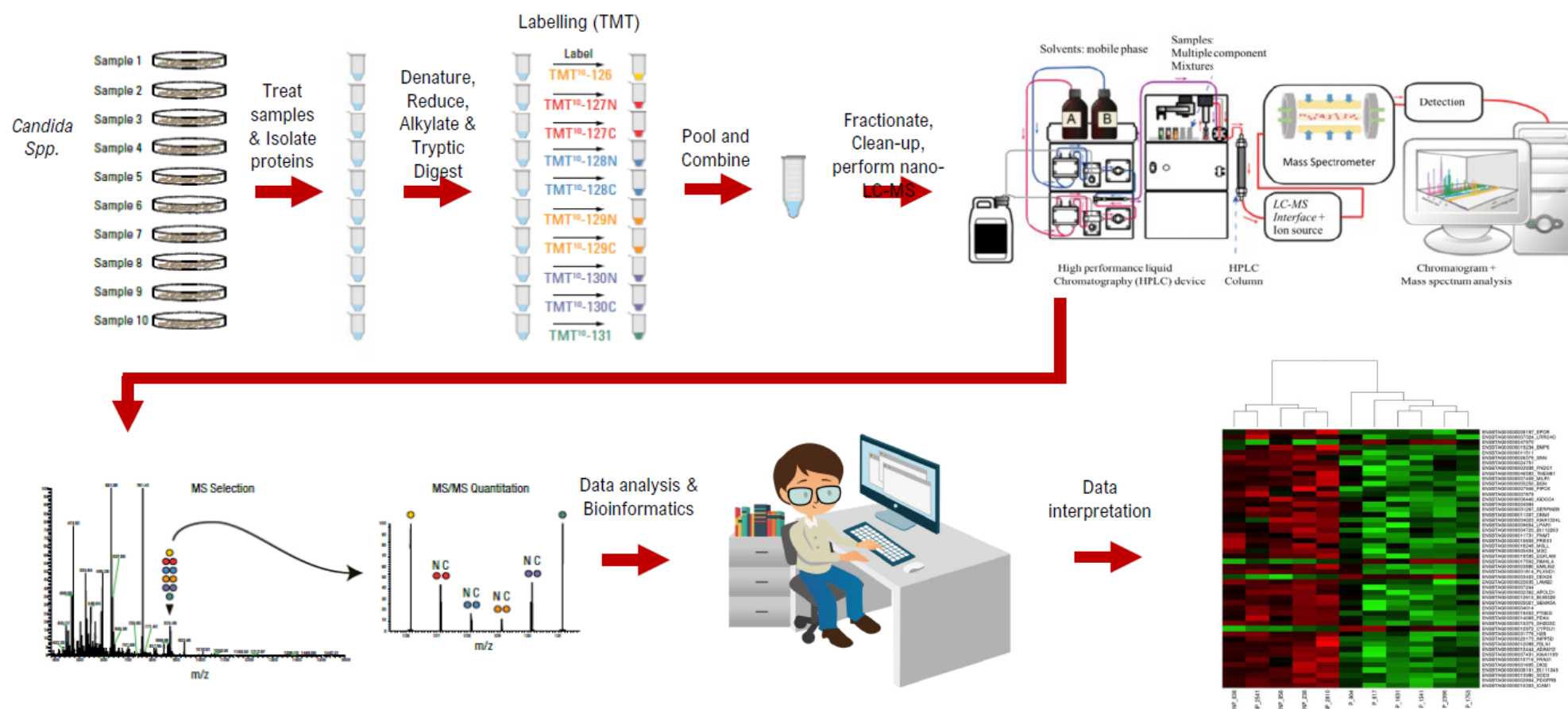


Fig. 1 Schematic diagram of the generation of proteomic profiles of *C. dubliniensis* strains. The isolated proteins are subject to tandem mass tagging, nano liquid chromatography, and mass spectrometry.

(a)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Accession Information		ID Metrics					Databases		Paired T-Test - 8h											
2	BestAccession	Protein names	Coverage	Protein FDR	# Peptides	# PSMs	# Unique Peptides	Marked as	Contaminant	Mutant v WildType				Overexpression v WildType				ComplementStrain v WildType			
3										Replicate	LogFC	P Value	FDR	Replicate	LogFC	P Value	FDR	Replicate	LogFC	P Value	FDR
4	Inf_Acc	Inf_Acc	Inf_ID.N	Inf_ID.N	Inf_ID.N	Inf_ID.N	Inf_ID.N	Inf_Data	Inf_Data	Stats_M	Stats_M	Stats_M	Stats_M	Stats_O	Stats_O	Stats_O	Stats_O	Stats_C	Stats_C	Stats_C	Stats_C
5	B9W6G7	Diphospho	3	High	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
6	B9W6H5	pH-respons	1	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
7	B9W6H8	60S riboso	14	High	4	7	4	Candida du	FALSE	1 v 1	0.26	NA	NA	1 v 1	-0.14	NA	NA	1 v 1	0.75	NA	NA
8	B9W6K1	Lactoylglut	2	High	1	2	1	Candida du	FALSE	1 v 1	0.10	NA	NA	1 v 1	0.53	NA	NA	1 v 1	0.11	NA	NA
9	B9W6M5	Vacuolar p	1	Medium	1	3	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
10	B9W6N8	Phospholip	1	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
11	B9W6P0	Mitochond	1	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
12	B9W6P7	Histone-lys	1	Medium	1	6	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
13	B9W6Q1	Origin rec	2	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
14	B9W6Q6	Multiprote	14	High	2	2	2	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
15	B9W6T2	Glycogenin	4	High	3	7	3	Candida du	FALSE	2 v 2	0.08	3.86E-01	8.97E-01	2 v 2	0.06	6.91E-01	9.93E-01	2 v 2	0.07	3.79E-01	8.56E-01
16	B9W6T3	40S riboso	22	High	2	2	2	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
17	B9W6U2	40S riboso	4	High	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
18	B9W6U3	DUF5353 d	8	High	2	2	2	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
19	B9W6V7	40S riboso	2	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
20	B9W701	Galactokin	2	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
21	B9W720	60S riboso	15	High	2	2	2	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
22	B9W735	Phosphom	11	High	3	3	3	Candida du	FALSE	1 v 1	0.02	NA	NA	1 v 1	0.33	NA	NA	1 v 1	0.58	NA	NA
23	B9W742	pyridoxal 5	8	High	2	2	2	Candida du	FALSE	1 v 1	0.71	NA	NA	1 v 1	0.63	NA	NA	1 v 1	0.42	NA	NA
24	B9W769	Mitochond	4	High	1	2	1	Candida du	FALSE	1 v 1	-0.02	NA	NA	1 v 1	0.23	NA	NA	1 v 1	-0.02	NA	NA
25	B9W780	Glucan 1,3	3	High	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
26	B9W782	60S acidic	7	Medium	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
27	B9W783	60S riboso	19	High	4	6	4	Candida du	FALSE	2 v 2	0.88	2.94E-02	4.06E-01	2 v 2	0.61	3.62E-01	9.93E-01	2 v 2	0.79	4.52E-02	7.59E-01
28	B9W792	60S riboso	19	High	4	7	4	Candida du	FALSE	2 v 2	0.21	1.64E-02	4.06E-01	2 v 2	0.21	6.47E-01	9.93E-01	2 v 2	0.55	1.07E-01	7.92E-01
29	B9W793	Uncharacte	3	High	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
30	B9W7A0	GPI-anchot	5	High	2	4	2	Candida du	FALSE	2 v 2	0.23	7.36E-01	9.66E-01	2 v 2	0.50	4.27E-01	9.93E-01	2 v 2	0.28	6.82E-01	9.05E-01
31	B9W7A3	14-3-3 prot	21	High	5	8	5	Candida du	FALSE	1 v 1	0.76	NA	NA	1 v 1	-0.04	NA	NA	1 v 1	0.59	NA	NA
32	B9W7A5	UPF0047 p	8	High	1	1	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA
33	B9W7B9	Trehalose-	1	Medium	1	2	1	Candida du	FALSE	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA	0 v 0	NA	NA	NA

Fig. 2 Identification of proteins associated with biofilm development in *C. dubliniensis* strains at the three distinct stages. (a) Representative results of paired *t*-test for extracellular proteins; (b) Representative results of paired *t*-test for cytoplasmic proteins.

(b)

	A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Accession Information			ID Metrics					Databases		Paired T-Test - 8h											
2	BestAccess	Protein names		Coverag	Protein	#	#	# Unique	Contami		Mutant v WildType				Overexpression v WildType				ComplementStrain v WildType			
3	ion			e	FDR	Peptides	PSMs	Peptides			Marked as	nant	Replicate	LogFC	P Value	FDR	Replicate	LogFC	P Value	FDR	Replicate	LogFC
4	Inf_Acce	Inf_Accession.Infor	Inf_ID.I	Inf_ID.I	Inf_ID.I	Inf_ID.I	Inf_ID.I	Inf_ID.I	Inf_Databa	Inf_Dai	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I	Stats_I
5	B9WAF2	Hexose transporter, pu	25	High	11	71	4	Candida dubli	FALSE	3 v 3	0.03	7.69E-01	9.01E-01	3 v 3	0.04	5.62E-01	8.83E-01	3 v 3	0.02	8.56E-01	9.45E-01	
6	B9WA05	Sulfate adenylyltransfe	48	High	24	58	24	Candida dubli	FALSE	3 v 3	0.04	8.47E-01	9.42E-01	3 v 3	-0.26	4.82E-01	8.75E-01	3 v 3	-0.10	4.52E-01	8.27E-01	
7	B9W8Q4	NA	15	High	8	21	8	Candida dubli	FALSE	3 v 3	0.19	1.11E-01	5.59E-01	3 v 3	0.26	1.36E-01	7.43E-01	3 v 3	0.16	1.06E-01	7.85E-01	
8	B9W957	NA	37	High	13	35	13	Candida dubli	FALSE	3 v 3	-0.02	8.38E-01	9.36E-01	3 v 3	-0.01	9.60E-01	9.88E-01	3 v 3	0.28	1.19E-01	7.85E-01	
9	B9WJ7J	NA	3	High	2	4	2	Candida dubli	FALSE	2 v 2	-0.26	4.17E-01	7.08E-01	2 v 2	0.02	9.52E-01	9.85E-01	2 v 2	0.00	9.97E-01	9.99E-01	
10	B9WDC4	NA	19	High	4	15	4	Candida dubli	FALSE	3 v 3	0.06	7.01E-01	8.72E-01	3 v 3	-0.01	8.72E-01	9.58E-01	3 v 3	0.08	2.10E-01	7.85E-01	
11	B9WD10	NA	55	High	16	84	16	Candida dubli	FALSE	3 v 3	0.07	6.28E-01	8.38E-01	3 v 3	0.02	8.08E-01	9.40E-01	3 v 3	0.19	1.09E-02	7.85E-01	
12	B9WA38	NA	14	High	6	15	2	Candida dubli	FALSE	2 v 2	0.07	5.81E-01	8.05E-01	2 v 2	-0.46	1.61E-01	7.65E-01	2 v 2	0.25	4.42E-01	8.27E-01	
13	B9WIC9	NA	5	High	3	5	3	Candida dubli	FALSE	3 v 3	0.24	1.60E-01	5.82E-01	3 v 3	-0.12	5.84E-01	8.85E-01	3 v 3	0.39	7.26E-02	7.85E-01	
14	B9WBX7	NA	11	High	5	9	5	Candida dubli	FALSE	3 v 3	0.09	5.82E-01	8.05E-01	3 v 3	0.38	1.96E-01	8.07E-01	3 v 3	0.02	9.25E-01	9.75E-01	
15	B9W8H3	Secreted protein	29	High	9	27	9	Candida dubli	FALSE	3 v 3	-0.21	2.82E-01	6.28E-01	3 v 3	-0.43	5.00E-02	6.84E-01	3 v 3	0.00	9.94E-01	9.99E-01	
16	B9WEG3	NA	10	High	6	13	6	Candida dubli	FALSE	3 v 3	0.02	8.17E-01	9.24E-01	3 v 3	-0.17	3.05E-01	8.72E-01	3 v 3	0.10	4.68E-02	7.85E-01	
17	B9WMM0	Cystathionine gamma-	37	High	14	73	14	Candida dubli	FALSE	3 v 3	-0.09	3.22E-01	6.58E-01	3 v 3	0.08	1.38E-01	7.43E-01	3 v 3	-0.02	7.46E-01	8.94E-01	
18	B9W8A4	NA	11	High	7	12	7	Candida dubli	FALSE	2 v 2	0.26	2.34E-01	6.10E-01	2 v 2	0.00	9.08E-01	9.70E-01	2 v 2	0.36	7.89E-02	7.85E-01	
19	B9WAY6	NA	5	High	3	6	3	Candida dubli	FALSE	3 v 3	0.15	3.30E-01	6.65E-01	3 v 3	-0.14	7.04E-01	9.09E-01	3 v 3	-0.02	9.36E-01	9.79E-01	
20	B9WAI2	NA	15	High	5	13	5	Candida dubli	FALSE	3 v 3	-0.09	9.83E-02	5.58E-01	3 v 3	-0.17	1.35E-01	7.43E-01	3 v 3	0.00	9.68E-01	9.93E-01	
21	B9WFE3	NA	40	High	10	24	10	Candida dubli	FALSE	3 v 3	-0.12	2.05E-01	5.92E-01	3 v 3	0.11	3.96E-01	8.75E-01	3 v 3	0.03	6.47E-01	8.68E-01	
22	B9WCR3	NA	2	High	2	5	2	Candida dubli	FALSE	2 v 2	-0.09	2.91E-01	6.36E-01	2 v 2	0.25	2.86E-01	8.69E-01	2 v 2	-0.04	8.09E-01	9.25E-01	
23	B9W7N1	NA	9	High	4	8	4	Candida dubli	FALSE	2 v 2	0.29	3.62E-01	6.78E-01	2 v 2	-0.55	2.13E-01	8.29E-01	2 v 2	0.30	5.56E-01	8.40E-01	
24	B9WK37	NA	3	Medium	2	4	2	Candida dubli	FALSE	2 v 2	-0.12	5.91E-02	5.34E-01	2 v 2	-0.10	5.41E-01	8.83E-01	2 v 2	0.04	7.42E-01	8.92E-01	
25	B9WA29	NA	13	High	5	12	1	Candida dubli	FALSE	2 v 2	0.20	2.95E-01	6.38E-01	2 v 2	-0.40	1.10E-01	7.17E-01	2 v 2	0.35	5.03E-01	8.34E-01	
26	B9WER4	NA	9	High	4	6	4	Candida dubli	FALSE	3 v 3	0.00	9.83E-01	9.96E-01	3 v 3	-0.01	8.41E-01	9.47E-01	3 v 3	0.03	7.10E-01	8.82E-01	
27	B9W806	NA	3	Medium	2	7	2	Candida dubli	FALSE	3 v 3	0.13	7.70E-01	9.01E-01	3 v 3	0.20	3.99E-01	8.75E-01	3 v 3	0.03	8.79E-01	9.55E-01	
28	B9W8L3	NA	34	High	18	57	18	Candida dubli	FALSE	3 v 3	0.30	9.96E-02	5.58E-01	3 v 3	0.08	8.12E-01	9.40E-01	3 v 3	0.12	6.54E-01	8.68E-01	
29	B9W729	NA	17	High	31	66	27	Candida dubli	FALSE	3 v 3	-0.01	9.18E-01	9.74E-01	3 v 3	-0.24	1.50E-02	6.51E-01	3 v 3	0.02	8.88E-01	9.59E-01	
30	B9WL11	NA	37	High	20	56	20	Candida dubli	FALSE	3 v 3	-0.09	3.96E-01	6.98E-01	3 v 3	0.23	4.56E-02	6.84E-01	3 v 3	-0.15	1.65E-01	7.85E-01	
31	B9WGA5	NA	26	High	6	12	6	Candida dubli	FALSE	3 v 3	-0.08	3.12E-01	6.50E-01	3 v 3	-0.93	3.90E-01	8.75E-01	3 v 3	-1.25	4.09E-01	8.23E-01	
32	P20930	NA	3	High	7	11	7		TRUE	2 v 2	-0.16	5.59E-01	7.98E-01	2 v 2	1.69	5.00E-01	8.75E-01	2 v 2	-0.46	7.15E-01	8.83E-01	
33	B9WCG2	NA	5	High	2	7	2	Candida dubli	FALSE	3 v 3	-0.26	4.89E-01	7.58E-01	3 v 3	0.56	3.95E-01	8.75E-01	3 v 3	0.42	6.23E-01	8.59E-01	

Fig. 2 Identification of proteins associated with biofilm development in *C. dubliniensis* strains at the three distinct stages. (a) Representative results of paired t-test for extracellular proteins; (b) Representative results of paired t-test for cytoplasmic proteins.

6.2 Potential for further development of the research and the proposed course of action (Maximum half a page)

We have identified new proteins which are associated with *IFD6* in *C. dubliniensis* biofilms but their cellular interactions and functional significance in biofilm development have not yet been investigated. Further studies at molecular level are warranted by creating double knock-out and / or overexpression mutants and evaluated the concerted effects, if any, on phenotypic determinants such as biofilm formation, virulence, and susceptibility to stressors and antifungal agents. It would shed light on novel cellular networking among these proteins with respect to biofilm development and / or pathobiology of this emerging human fungal pathogen.

Our proteome data could be employed as a new model system to conduct comparative studies between *C. dubliniensis* and *C. albicans* as their genome sequences are highly similar (> 80%), but they possess diverse phenotypic determinants. The results could pave the way for delineation of the evolutionary relationship between these two siblings in *Candida* pathobiology.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Invasive fungal infections (IFI) have been a major health problem in humans. The fungus, *Candida*, is an important opportunistic human fungal pathogen that lives harmoniously as commensal inhabitants in the human mouth, the digestive system, and the urogenital tract. However, these microbes can cause diseases (candidiasis) in individuals with impaired immunity with high morbidity and mortality. A notable virulence trait of *Candida* fungi is biofilms, and more than 80% of *Candida* infections are biofilm-associated. Thus, it can be envisaged that suppressing biofilm development a tangible antifungal approach through destruction of this protective barrier.

Recently, we characterized the functional significance of an aryl alcohol dehydrogenase (*IFD6*) in biofilm matrix production in *C. dubliniensis*. Here, we have generated the proteomic profiles of four different *C. dubliniensis* strains and compared the relative abundance of cytoplasmic (CPs) and extracellular proteins (ECPs) to identify proteins which are associated with *IFD6*. The protein expression levels of three ECPs were increased at the early and intermediate phases of biofilm development in the absence of *IFD6*; and the protein levels of three CPs were increased in *IFD6* overexpression strains. Collectively, these proteins are related to virulence trait, cell adhesion, and cell wall synthesis which warrant further investigations.

Part C: Research Output**8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project**

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) (denote the corresponding author with an asterisk*)	Title and Journal / Book (with the volume, pages and other necessary publishing details specified)	Submitted to RGC (indicate the year ending of the relevant progress report)	Attached to this Report (Yes or No)	Acknowledged the Support of RGC (Yes or No)	Accessible from the Institutional Repository (Yes or No)
Year of Publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)						
2024	N/A	N/A	Yes	TSANG WK*, SAMARANA YAKE LP, BANDARA HMHN	N/A	N/A	No	Yes	Yes

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

(Please attach a copy of each conference abstract)

Month / Year / Place	Title	Conference Name	Submitted to RGC (indicate the year ending of the relevant progress report)	Attached to this Report (Yes or No)	Acknowledged the Support of RGC (Yes or No)	Accessible from the Institutional Repository (Yes or No)
November / 2023 / Sendai, Japan	Proteomic analysis of <i>Candida dubliniensis</i> biofilms with overexpression of an aryl alcohol dehydrogenase	The 71 st Annual Meeting of Japanese Association for Dental Research	2024	Yes (Appendix 1)	Yes	Yes

A copy of the conference abstract is attached as Appendix 1.

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

The background, research plan and methodology, and results of this project have established suitable platform to decipher into the functional role and significance of *IFD6* in biofilm development of an emerging human fungal pathogen *C. dubliniensis*. The underlying principles, experimental designs, skills and techniques, and new knowledge of this project have been incorporated into the two teaching areas (i.e. Genetic engineering and microbial biotechnology; Microorganisms and human health) of a General Education Elective Module (Title: Journey to the Exotic World of Microorganisms) in which the PI is the Module

Convenor. The core concepts and crucial information are taught and discussed with the students.

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
N/A	N/A	N/A	N/A

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

This research project is a collaborative work between Technological and Higher Education Institute of Hong Kong, University of Sharjah, and University of Bristol. The skills and technique involved in this project was disseminated by the PI in an institute-wise Research Seminar Series for all academic and research staff and students. The CPD-seminar was held on May 2, 2024 via MS Teams with more than 30 participants. The title of the seminar was “The Matrix Revisited”: Learning More About Biofilms. The poster of the seminar is attached (Appendix 2).

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	1 (Under preparation)	1	0	0	Type	No.
					Seminar	1

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
N/A	N/A