RGC Ref. No.:

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(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines: 1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Microbiological and Metagenomic Analysis of the Microbiome and Antibiotics

Resistance Genes in Manure from Organic Farms (Phase 1)

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	MO, Wing-yin / Assistant Professor	Department of Applied Sciences, School of Science and Technology, HKMU
Co-Investigator(s)	CHAN, Ping-lung / Assistant Professor	Department of Health Sciences, School of Nursing and Health Studies, HKMU
	TSUI, Stephen, Kwok-wing / Professor	School of Biomedical Sciences, CUHK
	MARAFA, Lawal Mohammed	Department of Geography and Resources Management, CUHK
	NG, Tsz-yan	Trade and Industry Department, HKSAR Government
Others		

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	1st Jan 2020		
Project Completion Date	31st Dec 2021	30th Jun 2022	5th Oct 2021
Duration *(in month)*	24	30	5th Oct 2021
Deadline for Submission of Completion Report	31st Dec 2022	30th Jun 2023	5th Oct 2021

Part B: The Final Report

5. Project Objectives

5.1   Objectives as per original application

1. to characterise the evolution of the microbiome and the ARGs in composting manures, soils fertilised with these manures, and the produces grown from these soils using microbiological and metagenomics methods;

2. to correlate and identify different physicochemical factors in composting manures and soils fertilised with manures with the evolution process of the microbiome and ARGs in the two matrices; and

3. to examine the effect of different correlating physiochemical factors in the evolution of the microbiome and the ARGs in manures, soils fertilised with manures, and the produces grown from these soils.

5.2   Revised objectives

Date of approval from the RGC: N.A.

Reasons for the change: N.A.

1. N.A.

2. N.A.

3. 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 – Three piles of organic compost was composted on a local farm using aerobic composting methods using chicken manure, spent coffee grounds, and grass straws in May 2021. Compost samples were collected from the top, middle, and bottom layers of the compost during Day 0, Day 3, Day 5, Day 17, and Day 52, and DNA was extracted. The evolution of the microbiome and resistome of the composts during the composting process was characterised using metagenomics sequencing followed by principal correlation analysis (PCoA). The count of ten bacteria species with food safety significance in these samples was determined using traditional microbiological methods.

Objective 2 – The physicochemical factors (temperature, total organic carbon, total Kjeldahl Nitrogen, C:N ratio, water content, pH, electrical conductivity, nitrite, nitrate, ammonium, total phosphorus, orthophosphate and available potassium) of the samples stated in the above were determined using corresponding standard methods. The changes in these factors during the composting process were characterised and visualised using principal component analysis (PCA). The physicochemical factors associated with the differences in the microbiome and the resistome of the above samples were identified by the redundancy analysis method (RDA).

Objective 3 – Based on the result obtained from the above two parts, it was found that pH was associated with the changes in the microbiome and resistome. To achieve Objective 3, a controlled experiment was conducted to examine the effect of pH on the changes in ARGs. Six piles of organic compost were prepared as stated in Objective 1 in March 2022. Lime was added to three of these six compost piles to increase the compost's pH, while the remaining piles were the controls. Compost samples were collected on Day 52, and DNA was extracted. The abundance of selected antibiotic resistance genes (*tetM*, *sul2*, *ermB*, and *int1*) in the two groups was measured using q-PCR and compared using Student's t-test.

The microbiome and resistome in soil fertilised with the compost and the produces grown from these soils were not determined because of the limitation of the funding (**since the budget allocated was smaller than the proposed amount and was insufficient to cover the cost of the metagenomics sequencing for all the planned samples**). Although the microbiome and resistome of the soil fertilised with the composts and of the produces grown from the soil were not characterised due to insufficient funding, the project team has already mastered the techniques and skills, and in achieving these tasks, therefore, the project team plans to acquire additional funding in the coming round of FDS to characterise the effect of compost on the microbiome and resistome of farm soils and the organic produces and to delineate the underlying biogeochemical molecular mechanisms.

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 characterise the evolution of the microbiome and the ARGs in composting manures, soils fertilised with these manures, and the produces grown from these soils using microbiological and metagenomics methods	✓	100%
2. to correlate and identify different physicochemical factors in composting manures and soils fertilised with manures with the evolution process of the microbiome and ARGs in the two matrices	✓	100%
3. to examine the effect of different correlating physiochemical factors in the evolution of the microbiome and the ARGs in manures, soils fertilised with manures, and the produces grown from these soils	✓	100%

6.   Research Outcome

6.1   Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

a.   Reduction of Antibiotics during Composting – We measured the concentration of 14 antibiotics from four classes, including tetracycline, macrolides, sulfonamides, and quinolone, in the composting materials (chicken faeces, spent coffee ground, and grass straws) and compost samples collected on Day 0, Day 3, Day 17, Day 30, and Day 52. Only a low concentration of tetracycline (261 ppb), oxytetracycline (4768 ppb), and enrofloxacin (6 ppb) was detected in the initial composts. The concentration of tetracycline decreased to 95 ppb in the final composts, while the others were not detected. ***The data also indicated that aerobic composting is an effective method for eliminating antibiotics in compost and was consistent with the findings from previous studies.***

b.   Changes of the Compost Resistome and Microbiome after Composting – The microbiome and resistome were characterised by metagenomics sequencing. Our result indicated that antimicrobial resistance genes (ARG) abundance decreased from 2 copies/cell to 1 copy/cell during the thermophilic phase of the composting process and maintained stable at 0.5 copy/cell from Day 17 (Fig 1a). Resistome from compost collected on Day 0 and 3 was more similar to chicken manure, which was dominated by resistance genes of aminoglycoside, macrolides, tetracycline, and sulfonamide. The resistome of compost collected on Day 52 was more similar to that of grass straws with an increased proportion of multi-drug resistance genes. The result of the PCoA analysis of the resistome was consistent with the observation (Fig 1b). The result indicated that composting is effective in reducing ARG load.

The microbiome of the initial composts was dominated by bacteria from the phylum of Firmicutes (92%), followed by Actinobacteria (4.4%) and Proteobacteria (3.9%). The relative abundance of Firmicutes decreased slightly on Day 3 but decreased sharply after the thermophilic phase (Day 17), with *Actinobacteria* becoming the dominating phylum (90%), followed by Firmicutes (5.6%) and

Chloroflexi (4.4%) (Fig 1c). At the species level, the dominating species were *Saccharomonospora virdis*, *Mycolicibacterium thermoresistible*, and *Sphaerobacter thermophilus*. PCoA analysis based on Bray-Curtis distance and ANOSIM also indicated that the microbiome of compost samples collected on Day 17, 30, and 52 significantly differed from that of Day 0 and 3 (Fig 1d).

c.   *Association between Resistome, Microbiome and Physicochemical Factors* – The association between resistome, microbiome, and other physicochemical factors was determined to identify factors affecting the resistome. Network analysis revealed the association between resistome and microbial taxa at the phylum and species level (Fig. 2a and b). Our result indicated that Actinobacteria was associated with bacitracin and β-lactam, while Firmicutes was associated with most classes of ARG. It was also noted that Proteobacteria and Ascomycota were associated with multi-drug resistant ARGs. These observation was consistent with the result of Procrustes analysis of the microbiome and resistome, which indicated that there was a significant correlation between microbiome and resistome (Fig 2c). Redundancy analysis was conducted to identify the physicochemical factors related to ARG abundance in the composts. The result indicated that pH, total organic carbon, ammonium concentration, and water content were significantly associated with the abundance of various ARG (Fig 2d). Variation partitioning analysis and structural equation modelling were conducted to differentiate the relative importance of microbiome and physicochemical factors in shaping the resistome. We found that resistome was shaped by the joint effect of physicochemical factors and microbiome (Fig 2e). We also found that while pH, NH_4, and microbial abundance directly affected the resistome, both NH_4 and water content also affected the resistome indirectly through microbial abundance (Fig 2f).

d.   *Effect of Increased pH on the Abundance of Selected ARGs* – A controlled experiment was conducted to confirm the effect of pH in reducing resistome load. Lime was added to the compost at the beginning of the composting process to increase the pH of the compost, and the abundance of ermB, ermF, tetM, sul2, and int1 in the compost of Day 0 and Day 52 were compared. However, no statistical significance was detected, suggesting that increasing pH alone cannot reduce these genes' abundance in the compost and that modulation of multiple factors may be needed.

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

a. *Development of protocols for reducing the burden of antimicrobial resistance genes in organic composts by modulating the physicochemical factors of the composting process*
In this project, we identified that pH, ammonium concentration, and water content were associated with the reduction of the abundance of the resistome in organic composts. The project team propose to develop protocols for modulating these factors during the composting process and to investigate the effect of these factors and protocols in reducing the burden of ARGs in organic composts.

b. *Multi-omics analysis of compost microbiome for modulating surviving microbes in composts*
Our data indicated that the genes of multi-drug resistance persisted in organic composts. Our data also showed an association between microbial taxa and the ARG types. These suggested that the persistence of ARGs of certain classes may be related to the microbial taxa surviving the thermophilic phase of the composting process, and the modulation of these surviving microbiota will be crucial for eliminating the remaining antimicrobial resistance genes. We, therefore, propose to conduct multi-omics (specifically transcriptomics and metabolomics) studies to elucidate the metabolic potential of the surviving microbes and to identify suitable metabolic pathways for modulating the microbiome of the composts, thus, in turn, the resistome of the composts.

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

Organic composts are made of animal manure and food wastes, harbouring antibiotic resistance genes (ARGs). The ARGs may be transferred to farm soil and organic vegetables, posing a risk of spreading antimicrobial resistance (AMR) to the environment and consumers of organic vegetables. As a result, reducing the ARGs' burden in organic composts is crucial to protect environmental health and food safety. One of the approaches to achieve this goal is to modify the composting process to create an environment favouring the elimination of ARGs in organic compost. However, the physicochemical factors contributing to the reduction of ARGs in organic composts during the composting process remain unclear. Therefore, this project aimed to identify physicochemical factors associated with the decrease in ARGs during the composting process and to examine the effect of modulating the identified physicochemical factors in reducing the ARGs burden in organic compost. Our result indicated that pH, ammonium, and water content were associated with the reduction of ARGs in organic composts. This project provided the essential information needed to modify the composting process to reduce the ARGs burden in organic compost, thus reducing the risk of AMR dissemination to the environment and the organic vegetable consumers.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising <u>Directly</u> 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)*						
2020				Kit-Ling Lam, Wai-Po Kong, Po-Yi Ling, Tsz-Ho Lau, Kin-Hang Ho, Fred Wang-Fat Lee, **Ping-Lung Chan***	Antibiotic-Resistant Bacteria in Hydroponic Lettuce in Retail: A Comparative Survey. *Foods.* 2020 Sep 21;9(9):1327. doi: 10.3390/foods9091327	Yes	**Yes [Attachment 1]**	**Yes**	**Yes**
2023				Jinpao Hou, Y.T. Chiu, K.L. Lam, K.Y. Kwong, J.H.L. Lau, L.M. Marafa, S.K.W, Tsui, **I.W.Y.Mo*, P.L. Chan***	High Copy Number of Multi-Drug Resistance Genes in Spent Coffee Grounds Used in Organic Composting/ *F1000Research*	No	**Yes [Attachment 2]**	**Yes**	**Yes**
			2023	Jinpao Hou, K.L. Lam, Y.T. Chiu, K.Y. Kwong, H.L. Lau, L.M. Marafa, S.K.W, Tsui, **I.W.Y.Mo*, P.L. Chan***	Urban Green Waste-based Bulking Agent but not Animal Manure is the Major Source of the Antimicrobial Resistance Genes that Persisted in Home Compost after Aerobic Composting / *Environmental International*	No	**Yes [Attachment 3]**	**Yes**	**Yes**
			2023	Yuen-ting. Chiu, K.L. Lam, K.Y. Kwong, J.H.L. Lau, L.M. Marafa, S.K.W, Tsui, **I.W.Y.Mo*, P.L. Chan***	Differential Changes in the Burden of Food Pathogens in Organic Composting after Aerobic Composting	No	No	**Yes**	**Yes**

9. **Recognised 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)*
August 2022 Hong Kong	Persistence of Human Pathogenic Bacteria during Composting	Virtual International Conference for Environment and Human Health	No	Yes **[Attachment 4]**	Yes	Yes
Jul 2023 Hamburg Germany	Physicochemical factors associated with the elimination of *Staphylococcus aureus* in the aerobic composting of organic compost	FEMS 2023	No	Yes **[Attachment 5]**	Yes	Yes

10. **Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning**
(Please elaborate)

Two student helpers were recruited to support the projects. Students participated in samples preparation, DNA extraction, culturing of pathogens, and measurement of antibiotics in composts. Students were also asked to join the weekly lab meeting to present their results.

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.			

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

The PI/Co-I (Dr Ping Lung Chan) was invited to be one of the speakers at an agricultural

forum supported by the Environmental Conservation Fund (Project Number: 2157) to

introduce the role and importance of microorganisms in composts to local farmers.

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	2 + 2(under-preparation)	2	NA	NA	Type	No.
					Forum speaker	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.