

RGC Ref. No.: <u>UGC/FDS16/M07/18</u> (please insert ref. above)
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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 six months of the approved project completion date. 2. Completion report: within 12 months of the approved project completion date.
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Part A: The Project and Investigator(s)

1. Project Title

Investigating the Potential of Using Probiotic Bacteria in Making Traditional Chinese Foods
with Health-promoting Effects and Reduced Carcinogenicity

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr SZE Eric Tung-po / Associate Professor	School of Science and Technology / Hong Kong Metropolitan University
Co-Investigator(s)	Dr EL-NEZAMI Hani / Associate Professor	School of Biological Sciences / The University of Hong Kong
Others		

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval (must be quoted)
Project Start Date	1 January 2019	1 January 2019	
Project Completion Date	31 December 2020	31 December 2021	7 May 2020
Duration (in month)	24	36	7 May 2020
Deadline for Submission of Completion Report	31 December 2021	31 December 2022	7 May 2020

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

- 1. To isolate potential probiotic candidates from various commercially available sources of Chinese fermented foods and to characterize their probiotic properties*
- 2. To determine the biogenic amine levels and carcinogenicity of fermented foods produced in-house with the use of commercially available probiotic species and the isolated probiotic candidates*
- 3. To optimize fermentation conditions to minimise biogenic amine levels in the production of fermented foods.*
- 4. To evaluate correlations between biogenic amine levels and the carcinogenicity of fermented foods*

5.2 Revised objectives

Date of approval from the RGC: N.A.

Reasons for the change:

- 1.
- 2.
3.

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)

For objective #1, a total of nine different traditional Chinese fermented soy bean products/semi-products were obtained from two different local manufacturers. Parameters such as pH, salinity and % moisture were examined. The microbial species from these products/semi-products were isolated by using plate count agar and de Man, Rogosa and Sharpe (MRS) agar. Colonies with different morphologies were selected individually and the DNA of each pure bacterial culture was extracted. A total of 24 and 23 species were isolated from plate count agar and MRS agar respectively. The 16S region of each sample was sequenced and identified. Besides, several probiotic properties of the isolated species, including haemolytic analysis, tolerance to harsh conditions, antibiotic resistance,

antimicrobial activity, were evaluated. Besides, meta-sequencing of the microbials in the nine different commercially available traditional Chinese fermented soy bean products/semi-products were also conducted.

For objective #2, laboratory made fermented soy beans were produced by the use of individual commercially available probiotic species, namely *Lactobacillus rhamnosus* GG (LGG), *Lactobacillus casei* Shirota (LcS) and *Escherichia coli* Nissle 1917 (EcN), and the isolated species in Objective #1, in both aerobic and anerobic conditions. Biogenic amines' level of the resulting fermented soy beans were evaluated.

For objective #3, by the use of principal component analysis and other statistical techniques to analysis the data from objectives #1 and #2, microbials isolated from the commercially available soy bean products/semi-products that may be responsible to the formation of biogenic amines were identified. A mixed culture of starter was formulated by the use of other microbials that would not generate significant level of biogenic amines.

For objective #4, effect in proliferation of tissue cell lines (i.e. NP69) of the extracts from laboratory made fermented soy bean products were examined by conventional testing methods such as the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay and the bromodeoxyuridine assay. The results were corelated with the biogenic amines level obtained in objective #2.

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 isolate potential probiotic candidates from various commercially available sources of Chinese fermented foods and to characterize their probiotic properties	✓	100%
2. To determine the biogenic amine levels and carcinogenicity of fermented foods produced in-house with the use of commercially available probiotic species and the isolated probiotic candidates	✓	100%
3. To optimize fermentation conditions to minimise biogenic amine levels in the production of fermented foods	✓	100%
4. To evaluate correlations between biogenic amine levels and the carcinogenicity of fermented foods	✓	100%

6. Research Outcome

6.1 Major findings and research outcome

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

In the beginning of the project, twenty-three raw soybean pastes and four bottled soybean pastes from local wet markets and supermarkets respectively and the biogenic amines' level of each sample were evaluated. All samples were found containing different levels of biogenic amines, which supported biogenic amines are prevailing in fermented soybean products, ranging from 39 to over 1,000 mg/kg.

On the other hand, the project investigated the potential of using selected bacteria species, including probiotic bacteria and bacteria isolated from soybean products/semi-products, in manufacturing traditional Chinese fermented soybean products, in minimizing biogenic levels and carcinogenicity. Traditional method using starters from microbiota of previous manufacturing batches without proper controls of microbial contamination would induce the risk of biogenic amines. A total of 47 species were isolated from 9 fermented soybean products/semi-products, and were identified by molecular technique according to 16S DNA sequence. The results showed that the appearances and textures varied among the use of different bacteria species. For the same bacterial species, fermentation conditions such as aerobic conditions and temperature conferred different flavor characteristics and biogenic amines level to soybean. Changes in flavor characteristics by different microbial species and fermentation conditions were also observed. Several isolated species would produce substantial biogenic amines in aerobic and/or anaerobic conditions, whereas the remaining species would not produce biogenic amines. Fermented soybeans made of starters formulated by non-biogenic amines causing species would not produce biogenic amines and cause carcinogenicity.

Furthermore, three isolated bacterial species from the commercially available fermented soybean products/semi-products were found fulfilling basic probiotic requirements such as non-haemolytic, tolerance to harsh conditions, antibiotic resistance and antimicrobial, and are potential to become probiotic bacteria.

The findings relating to literature review of biogenic amines in traditional Chinese fermented foods and study in reduction in biogenic amines in fermented bean by probiotic bacteria have been published in NFS Journal and PLOS One respectively (corresponding to items #2 and #1 of Part C Section 8), manuscript of other findings are now under preparation and will be submitted soon (corresponding to item #3 of Part C Section 8). Besides, a poster presentation of the findings for biogenic amines reduction by probiotic bacteria was presented in the 33rd European Federation of Food Science and Technology (EFFoST) International Conference (corresponding to item #1 of Part C Section 9). In addition, corresponding results of all findings have been reported in the M.Phil thesis of a postgraduate student (Corresponding to item #1 in Part C Section 10) who involved in this project.

6.2 Potential for further development of the research and the proposed course of action

(Maximum half a page)

The results and findings should aid future development of fermentation starters by the use of selected bacterial species that would not cause the formation of biogenic amines. The research team may conduct further organoleptic study of the fermented soybean products by using electronic tongue and/or traditional taste test. Since different fermentation conditions (e.g. aerobic vs anaerobic) may also contribute to the formation of biogenic amines, further study to the expression of enzymes in the microbial species under different fermentation conditions can be conducted in order to identify protein biomarkers for biogenic amines, by the use of molecular and proteomic techniques.

7. Layman's Summary

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

Traditional Chinese fermented soybean foods, including soybean paste and soy sauce, have been reported to have high levels of biogenic amines that are precursors of carcinogenic *N*-nitroso compounds. These molecules can be formed by the microbiological degradation of high-protein food during the fermentation process. Because of the adverse health impact of these substances, there is a growing interest to determine the cause(s) of biogenic amines formation in foods.

In this project, we have studied the use of probiotic bacteria in fermentation, which has demonstrated the suppression of biogenic amines in soybean products. Besides, microbials were isolated from commercially available soybean products/semi-products obtained from the factories, and were characterized the identities and biogenic amines levels different fermentation conditions. Those bacteria without producing biogenic amines were selected and mixed as starter culture for ripening and the resulting fermented soybean is non-carcinogenic. Among those non-biogenic amines causing species, three bacterial candidates were found fulfilling the requirements of non-haemolytic, tolerance to harsh conditions, antibiotic resistance and antimicrobial, and are potential to become probiotic bacteria. Further research is thus required to study the probiotic properties of these three candidates and other properties (e.g. organoleptic) of the fermented soybean products using selected bacterial starter cultures.

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)						
2020				Fiona Long Yan Fong; Ka Yam Lam; Chun San Lau; Kin Hei Ho; Yeuk Hei Kan; Mui Yee Poon; Hani El-Nezami ; Eric Tung Po Sze*	Reduction in Biogenic Amines in Douchi Fermented by Probiotic Bacteria/ <i>PLOS ONE</i>	No	Yes [Attachment 1]	Yes	Yes
2021				Fiona Long Yan Fong; Hani El-Nezami ; Eric Tung Po Sze*	Biogenic Amines – Precursors of Carcinogens in Traditional Chinese Fermented Food / <i>NFS Journal</i>	No	Yes [Attachment 2]	Yes	Yes
			Yes	Mui Yee Poon; Hani El-Nezami ; Eric Tung Po Sze*	Correlation of microbial and other fermentation conditions to the levels of biogenic amines in traditional Chinese fermented soybean foods	No	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 <i>(indicate the year ending of the relevant progress report)</i>	Attached to this Report <i>(Yes or No)</i>	Acknowledged the Support of RGC <i>(Yes or No)</i>	Accessible from the Institutional Repository <i>(Yes or No)</i>
11/2019 Netherlands	Reduction of Biogenic Amines in the Traditional Chinese Fermented Foods by Probiotic Bacteria	The 33 rd European Federation of Food Science and Technology (EFFoST) International Conference	Yes	Yes [Attachment 3]	Yes	Yes

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

(Please elaborate)

The research experience and capability built have been used extensively to both undergraduates' final year projects and postgraduate research. Besides, the technologies gained in fermentation and probiotic bacteria have been used in further research development, which also assist in nurturing of both undergraduate and postgraduate 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

12. Other Impact

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

NA

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	1			Type	No.

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.	