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<th><strong>Title</strong></th>
<th>Traditional Chinese medicinal herbs with bactericidal properties and their effects on the interactions of intestinal microbiota</th>
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### Introduction

3 Traditional Chinese Medicine (TCM) formulae, namely Ya-hom powder (保济丸), Ubat Po Chai Pills (保济丸), and Pil Chi-kit Teck Aun (保济丸), were widely used by the East Asians in the treatment of gastrointestinal discomforts and diarrhea. However, their effects on the intestinal microbiota were not fully understood. The aim of the project was to study their effects on 3 intestinal bacteria, namely Escherichia coli, Enterococcus faecalis, and Klebsiella pneumoniae, when they are in pure culture (monoculture) and coculture.

### Materials and Methods

- **Inoculation and Incubation of 3 bacterial cultures at 37°C for 14 hrs**
  - OD measurement and adjustment of colony forming units (CFU) to 1.8 x 10^8 CFU/ml
  - Monoculture: 3ul of each species added to 3ml 5X TCM
  - Dual-species coculture: E.coli and E. faecalis
  - E.coli, E. faecalis and K. pneumoniae
  - Added accordingly to 1:1/1:1:1 ratio respectively
  - Each monoculture and coculture was prepared in triplicates.

- **Inoculation and Incubation of 3 bacterial cultures at 37ºC for 14 hrs**
  - At 0-hr, 200ul of each inocula were used in serial dilutions and the appropriate dilutions were plated on UTI agar plates.
  - At 4-hr, 200ul of inocula were used in serial dilutions and the appropriate dilutions were plated on UTI agar plates as triplicates.

### Results

- **Effects of TCM on Relative Growth**
  - **E.coli**
    - In monoculture, TCM 1 and TCM 2 had an adverse effect on the growth of E.coli.
    - With E.coli and K. pneumoniae, the growth was enhanced by 5X TCM, vice versa.
    - Bacterial relative growth that coincided within the neutral zone, as shown by the red lines in the graphs, would be considered as negligible.

- **E. faecalis**
  - In monoculture, TCM 2 and TCM 3 imposed negligible growth effects on E. faecalis.
  - Only TCM 1 had a large bactericidal effect on E. faecalis.

- **K. pneumoniae**
  - Only when it was in multispecies coculture, its growth was being enhanced.
  - However, TCM 2 and TCM 3 imposed negligible growth effects on K. pneumoniae.

### Conclusions

- **E.coli**
  - In monoculture, the 3 TCM formulae imposed a negative growth on E.coli. However, in cocultures, its growth was enhanced instead with the exception of E.coli + E. faecalis coculture in the presence of TCM 1 and TCM 3. This could be due to positive interactions of E.coli in the presence of other bacteria, especially with K. pneumoniae.

- **E. faecalis**
  - In monoculture, TCM 1 and TCM 2 had an adverse effect on the growth of E. faecalis. Surprisingly, there were negligible effects on the growth of E. faecalis in cocultures.

- **K. pneumoniae**
  - Only TCM 1 had a large bactericidal effect on K. pneumoniae on both monoculture and cocultures. The bacteria might be sensitive to the presence of TCM 1. However, TCM 2 and TCM 3 imposed negligible growth effects on K. pneumoniae. Only when it was in multispecies coculture, its growth was being enhanced.

### Project Title: Traditional Chinese Medicinal herbs with bactericidal properties and their effects on the interactions of intestinal microbiota

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**Collaborators:** Chew Ley Byan and Khoo Bee Luan