



UNIVERSITI PUTRA MALAYSIA

**SYMBIOTIC RELATIONSHIP BETWEEN SELECTED PROBIOTIC
BACTERIA IN CHEMOSTAT CULTURE**

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BACTERIA IN CHEMOSTAT CULTURE**

By

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**Thesis Submitted in Fulfilment of the Requirements for the Degree of Master
of Science in Faculty of Food Science and Biotechnology
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of the requirements for the degree of Master of Science

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Faculty: Food Science and Biotechnology

The effect of culture combination on growth of three strains of probiotic (*Streptococcus faecalis* T-110, *Bacillus mesentericus* TO-A and *Bifidobacterium breve* G48) was examined using chemostat culture. Results of bacterial viable counts revealed that mixed culture of two probiotic microorganisms had higher growth rate compared to single culture of probiotic. The improvement of bacterial growth can be partly attributed to the production of certain growth factors by one microorganism that was capable of stimulating the growth of another bacterium. The metabolites in sample broth were determined using High Performance Liquid Chromatography



(HPLC). It can be postulated that succinic acid, arginine, vitamin B₁ and B₂ synthesised by *B. breve* G48 were used by *S. faecalis* T-110 to improve its growth. Furthermore, *B. mesentericus* TO-A was found to utilise vitamin B₆ and formic acid produced by the respective *S. faecalis* T-110 and *B. breve* TO-A. In return, *B. breve* G48 utilised vitamin B₁₂ produced by *B. mesentericus* TO-A. The effect of mixed culture of probiotic on growth of pathogenic *Escherichia coli* V517 was also investigated in chemostat culture. Results showed that mixed culture of probiotic exerted better inhibitory effect against *E. coli* V517 compared to single culture of probiotic. HPLC analyses showed that mixed culture of probiotic produced higher yield of lactic and acetic acids that are fatal to *E. coli* V517. Meanwhile, ammonia was found not to be an important inhibitory agent to *E. coli* V517. Studies on the effects of metabolites on growth of probiotic organisms as well as *E. coli* V517 were also carried out to validate the hypotheses made in previous experiments. The results revealed that growth of tested probiotic bacteria increased with certain level of substrate concentration. In contrast, growth of *E. coli* V517 decreased with increasing concentration of lactic and acetic acids. It was postulated that mixed culture of *S. faecalis* T-110 and *B. breve* G48 as well as co-culture of *S. faecalis* T-110 and *B. mesentericus* TO-A exhibited commensal relationship, in which only one party benefits and the other is neither harmed nor benefited. Meanwhile, mixed culture of *B. mesentericus* TO-A and *B. breve* G48 showed a mutualistic association whereby both organisms profit from each other. Finally, in mixed culture of *E. coli* V517 and probiotic organisms, a strong antagonistic relationship was observed.

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**PERHUBUNGAN SIMBIOSIS DI KALANGAN BAKTERIA PROBIOTIK
TERPILIH DI DALAM SISTEM KEMOSTAT**

Oleh

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Kesan penggabungan kultura ke atas pertumbuhan tiga strain mikroorganisma probiotik (*Streptococcus faecalis* T-110, *Bacillus mesentericus* TO-A dan *Bifidobacterium breve* G48) telah dikaji dengan menggunakan sistem pengaliran kultura berterusan atau kemostat. Keputusan pengiraan sel bakteria hidup menunjukkan bahawa campuran dua jenis strain mikroorganisma probiotik mempunyai kadar pertumbuhan sel yang lebih tinggi jika dibandingkan dengan hanya satu strain mikroorganisma probiotik. Peningkatan pertumbuhan mikroorganisma ini mungkin disebabkan oleh pengeluaran faktor perangsang pertumbuhan oleh satu organisma yang merangsang pertumbuhan organisma yang lain.

Dengan menggunakan teknik Kromatografi Cecair Berkeupayaan Tinggi (HPLC), beberapa sisa metabolit di dalam cecair sampel telah dapat dikesan. Daripada kajian tersebut, didapati bahawa *S. faecalis* T-110 menggunakan asid suksinik, asid amino arginine, vitamin B₁ dan B₂ yang telah dihasilkan oleh *B. breve* G48 untuk meningkatkan kadar pertumbuhannya. *B. mesentericus* TO-A juga didapati menggunakan vitamin B₆ yang dikeluarkan oleh *S. faecalis* T-110 dan asid formik oleh *B. breve* G48. *B. breve* G48 pula telah menggunakan vitamin B₁₂ yang dihasilkan oleh *B. mesentericus* TO-A. Kesan pencampuran kultura probiotik ke atas pertumbuhan mikroorganisma berbahaya *Escherichia coli* V517 juga telah dikaji menggunakan sistem kemostat. Campuran dua kultura probiotik merencat pertumbuhan *E. coli* V517 dengan lebih berkesan berbanding jika menggunakan hanya satu strain probiotik. Keputusan analisis HPLC menunjukkan bahawa campuran kultura probiotik menghasilkan asid laktik dan asetik dalam kuantiti yang lebih banyak hingga ke tahap yang berbahaya kepada *E. coli* V517. Sebaliknya ammonia didapati tidak memainkan peranan penting di dalam proses perencatan pertumbuhan *E. coli* V517. Seterusnya, kesan sisa metabolit ke atas pertumbuhan mikroorganisma probiotik serta *E. coli* V517 juga telah dikaji untuk memastikan keputusan-keputusan sebelum ini adalah tepat dan relevan. Keputusan kajian menunjukkan bahawa pertumbuhan mikroorganisma probiotik meningkat selaras dengan peningkatan kepekatan substrat pada tahap tertentu. Tetapi penambahan kepekatan asid laktik dan asetik merencat pertumbuhan *E. coli* V517.

Berdasarkan keputusan eksperimen-eksperimen di atas, beberapa teori tentang perhubungan simbiosis di kalangan mikroorganisma yang telah diuji dapat dijelaskan dengan lebih terperinci. Campuran kultura probiotik *S. faecalis* T-110 dan *B. breve* G48 serta ko-kultura *S. faecalis* T-110 dan *B. mesentericus* TO-A menunjukkan sifat perhubungan komensal, di mana hanya satu organisma yang mendapat kebaikan, manakala satu organisma lagi tidak mendapat apa-apa daripada perhubungan tersebut. Sementara itu, campuran *B. mesentericus* TO-A dan *B. breve* G48 didapati mempunyai sifat perhubungan mutualistik, iaitu kedua-dua organisma mendapat faedah daripada penggabungan itu. Akhirnya, di dalam campuran kultura probiotik dan *E. coli* V517, menunjukkan wujudnya perhubungan antagonistik atau yang bertentangan antara mereka.

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LIST OF ABBREVIATIONS

μg	:	microgram
CFU	:	Colony Forming Unit
h	:	hour
h^{-1}	:	per hour
i.d	:	internal diameter
L	:	Litre
N	:	Normality
mg	:	milligram
min	:	minute
ml	:	millilitre
mm	:	millimeter
μm	:	micrometer
mM	:	milliMolar
rpm	:	revolution per minute
v/v	:	volume/volume
μ	:	Specific Growth Rate
μ_m	:	Maximum Specific Growth Rate

CHAPTER I

INTRODUCTION

Since birth, there are varieties of microorganisms inhabit human bodies. For instance, it has been estimated that over 400 species of bacteria reside in human gastro intestinal tract (GIT). The GIT bacteria are characterised into two types; indigenous and transient. Indigenous bacteria refer to the microorganisms that colonise and become established in the gut, while transient bacteria are those that are continuously passing through the gut and they would disappear with a change of diet or stress. Indeed, transient bacteria are normally pathogens. The microflora function optimally when they are composed of particular species in the proper proportion at their particular sites of the gut. But, the health and integrity of the mixed populations which make up the normal GIT microflora can be affected by a variety of internal and external factors such as stress, diet or by the activity among the microorganisms themselves. Disturbed ecology of the GIT microflora may involve an excessive growth of undesirable microorganisms, or very sparse levels of the desirable residents.

To remedy the altered gut microflora, some suggestions have been made such as antibiotic or drugs intake to kill the pathogens and probiotic intake to proliferate the friendly bacteria in the gut. In some cases, antibiotic intake is not a very promising solution. Despite killing undesirable bacteria, antibiotics also sometimes inhibit growth of beneficial bacteria as well. Therefore, until recently, probiotic approach seems to gain a reasonable interest from researchers and medical practitioners worldwide, as an alternative to antibiotic therapy. In fact, probiotic has been used extensively in Japan and Europe as a mean to correct the imbalance condition of the gut flora in diarrhoea patients. By definition, probiotic is a live microbial food/feed supplement, which beneficially affect the hosts by improving their intestinal microflora balance. The genus lactobacilli, streptococci, bifidobacteria and lactococci are the most common microorganisms used in probiotic preparation.

The probiotic preparation is either used in single or mixed culture. Mixed culture preparation is proven to have better result compared to monoculture. This claim is due to the establishment of symbiotic interaction between the microorganisms. Since the purpose of probiotic approach is to proliferate the growth of friendly bacteria, mixed culture will be most suitable to give higher yield of bacterial cell concentration compared to single culture. In mixed culture, one microorganism may produce necessary nutrient required for growth of another microorganism. In addition, one microorganism may also produce substances that are capable of neutralising toxic end product that may be harmful to another

microorganism. Microorganisms in mixed culture may also be capable to produce compounds or chemicals that complement each other to the exclusion of undesirable bacteria. Thus, these mechanisms may enhance the growth of desirable microorganisms and suppress the unwanted one.

Even though mixed culture is a common occurring phenomenon, not many studies have been done on the importance of the interaction between these bacteria and the mechanism of action that may explain the higher growth yield and stronger antibacterial action of the mixed culture. Therefore, the objectives of this study are as follows:

- 1) To investigate and compare the growth of single and mixed culture of probiotics.
- 2) To investigate and compare the antibacterial action of single and mixed culture of probiotics against selected pathogenic microorganisms.
- 3) To determine the metabolite products which may involve in the interaction among these bacteria.
- 4) To determine the type of interaction involved and to elucidate the mechanism of such interaction.

CHAPTER II

LITERATURE REVIEW

Probiotics

The Nobel Prize microbiologist, Elie Metchnikoff of the Pasteur Institute, initially introduced the idea of probiotics in the early 1900s (Metchnikoff, 1907). According to Metchnikoff, the ageing process is resulted from toxic substances produced by some pathogenic intestinal flora, which absorb into the bloodstream of human. Hence, in his famous 'theory of longevity,' he postulated that harmful effects produced by undesirable intestinal flora could be prevented by enriching the appropriate desirable microorganisms to replace or diminish the number of pathogenic microorganisms in the intestine (Tannock, 1997).

Even though the concept of probiotics was introduced by Metchnikoff, the term 'probiotics' was only coined in 1965 by Lily and Stillwell, whose referred the word 'probiotics' as the stimulation of growth of one microbe by another (Conway, 1996). Since then, numerous definitions of probiotics have been proposed. Today, it is generally agreed that a probiotic is a preparation of live microorganisms, which

applied to man and animals, will beneficially affect the hosts by improving their intestinal microbial balance (Hull, 1995).

The beneficial effects of the probiotic application to human health is believed first starting since the human consumed fermented milk or yoghurt (Fuller, 1989). In many parts of Europe and Japan, probiotics preparations have been used as drugs for intestinal regulation and treatment of diarrhoea and gastro-enteritis in man since ten years ago (Kozasa, 1989). Besides maintaining the balance of the gut microflora and restoring the protective effect against pathogens, probiotic microorganisms also offer several other benefits to human as well.

Probiotic microorganisms have been reported to help alleviate lactose intolerance people (Driessen and Boer, 1989). The person who suffers from this problem shows symptoms such as bloating, flatulence and diarrhoea. The lactose intolerance people are not capable of digesting lactose in milk because they lack the enzyme lactase or β -galactosidase, an enzyme responsible for the lactose digestion (Fuller, 1989). However, Gilliland and Kim (1982) reported that the lactose intolerance people could digest lactose in milk containing the probiotic strain *Lactobacillus acidophilus* better than the same amount of lactose in unsupplemented milk. Garvie *et al.* (1984) also showed that rats fed with yoghurt have increased the enzyme lactase in small intestine. These studies therefore suggest that some probiotic