



UNIVERSITI PUTRA MALAYSIA

**SURVIVAL OF BIFIDOBACTERIA AND OTHER SELECTED
INTESTINAL BACTERIA IN TPY MEDIA SUPPLEMENTED WITH
CURCUMIN AS ASSESSED *IN VITRO***

SEYED DAVOUD JAZAYERI

FBSB 2007 20



DEDICATION

I dedicate this work especially to my Parents for their continuous prayer, encouragement and patience. They sacrificed of their health and time to provide me with a suitable environment

Also, I dedicate this work to my dear sisters. Their patience and encouragement was the drive for me.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment
of the requirement for the degree of Master of Science

**SURVIVAL OF BIFIDOBACTERIA AND OTHER INTESTINAL BACTERIA
IN TPY MEDIA SUPPLEMENTED WITH CURCUMIN**

By

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October 2007

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Bacteria belonging to the genera *Bifidobacterium* and *Lactobacillus* are most often used as probiotic. They exert beneficial properties with regard to human health, such as inhibition of growth of exogenous and/or harmful bacteria, stimulation of immune functions, anti-tumour properties, cholesterol reduction, aid in digestion and/or absorption of food ingredients/minerals and synthesis of vitamins.

Foods are thought to be among the major factors that can affect the gut microbial balance. The gut is the site of active bioconversions and absorption of foodstuffs that have not been absorbed in the upper gastrointestinal tract. These include components especially plant-derived such as phenolic and other aromatics components. One of the most common sources of phenolic and aromatics in diets is curcumin derived from turmeric (*Curcuma longa* L.).



The growth of two *Bifidobacterium* strains (*Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4) and other selected intestinal bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* shirota, *Enterococcus faecalis* JCM 5803 and *Escherichia coli* K-12) were studied in 10 ml basal TPY medium containing various concentrations of curcumin (0.0025g, 0.005g, 0.0075g and 0.01g/10ml). Viable cell counts of the bacteria and their pH medium were determined during incubation period of 12h, 24h, 36h and 48h at 37°C anaerobically. The presence of curcumin did not change the pH of the medium as compared to the basal TPY without curcumin. In the absence of curcumin, growth of all the bacteria tested occurred within 12h except that of *B. pseudocatenulatum* G4 within 24h, after which cultures entered into early stationary phase and finally into death phase. Therefore depending on the bacteria, no growth occurred after 12h or 24h incubation. Maximum carrying capacity attained by all organisms before entering into stationary phase was about 10 log CFU/ml. However, in the presence of curcumin, cultures showed various degrees of growth inhibition in comparison with basal TPY.

E. faecalis and *B. longum* BB536 survived better than the other bacteria tested. The growth reduction percentage of *E. faecalis* grown in TPY medium supplemented with 0.0025g, 0.005g, 0.0075g and 0.01g of curcumin and incubated for 12h was 1%, 7%, 24% and 36%, respectively. At incubation period of 24h, the percentage of growth reduction was almost the same as for 12h of incubation. Interestingly, the percentage of growth reduction was greatly reduced to only 1%, 2%, 14% and 25% when incubated for 36h and 1%, 2%, 7%, and 8% when incubated for 48 h.



B. longum BB536 was ranked second in term of their survivability in basal TPY medium supplemented with curcumin. After 12h of incubation, 0.0025g of curcumin inhibits 10% *B. longum* BB536 growth. At concentrations of 0.005, 0.0075 and 0.01g, *B. longum* growth was inhibited at 16, 44 and 48%, respectively. At incubation period of 24h, the percentage of reductions was not much different from 12 h for 0.075, and 0.01g of curcumin supplemented in the media, but slightly increased for the concentrations of 0.0025, and 0.005g curcumin. Interestingly, the incubation of the *B. longum* culture for 36 and 48h showed lower growth inhibition as compared to the 12 and 24h.

The other intestinal bacteria tested showed higher growth inhibition percentage as compared with *E. faecalis* and *B. longum*. Among the bacteria tested, *L. acidophilus* recorded the most sensitive to curcumin. The inhibition rates showed more than 80% values for 0.005, 0.0075, and 0.01g of curcumin applied. Moreover, the surviving cells of *L. acidophilus* in the culture medium supplemented with the lowest concentration of curcumin (0.0025 g) decreased to almost the same values of the higher concentrations.

Supplementation of the test medium with tween 80 increased the survivability of the bacteria tested. *E. coli* K-12 survived better than the other bacteria tested. The growth reduction percentage of *E. coli* K-12 grown in basal media supplemented with 0.01 ml of tween 80 and 0.0025 g of curcumin and incubated for 6h was 5% and 50%, respectively. At incubation period of 12h, the percentage of growth reduction in basal media supplemented with curcumin increased up to 60%, and with continuing incubation until 24h the percentage of growth reduction was reduced to

23%. At incubation period until 24h the percentage of growth reduction of *E. coli* K-12 in basal media supplemented with tween 80 was almost the same as for 6h of incubation (5%).

The ability of the bacteria to degrade curcumin was studied using spectrophotometric method. On ultraviolet visible spectrophotometric study (UV-Vis spectra), the maximum light absorption bands obtained in TPY media supplemented with different concentration of curcumin was observed at 400.4 nm. Maximum absorption bands of curcumin in TPY media after 48 h incubation with the bacteria tested without any change was at 400.4 nm. Comparing the wavelength of curcumin before and after incubation with the bacteria proved that all the bacterial tested at this experiment are unable to degrade curcumin and it remained intact in media.

To confirm the above finding, the amount of curcumin after 48 h of incubation with the bacteria was also calculated by standard curve. The absorbance of curcumin in wavelength of 400.4 nm in comparison with 0 h was reduced. Consequently, the amount of curcumin after incubation with all the bacteria tested decreased. The percentage reduction of 0.0025 and 0.0050 g of curcumin/10 ml was 56-60 and 18-24 % and for two other concentrations which were 0.0075 and 0.01g/10ml was 15-16 and 12-14 %, respectively.

Curcumin, as a common aromatic, stimulant and coloring property in diet was found to possess antibacterial activity toward *B. longum* BB536, *B. pseudocatenulatum* G4 and other selected intestinal bacteria (*L. acidophilus*, *L. casei* shirota, *E. faecalis* JCM 5803 and *E. coli* K-12). The number of the surviving cells of all tested micro-



organisms decreased drastically after 48 hours of incubation in comparison with the control in the basal TPY media.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KEMANDIRIAN BAKTERIA BIFIDO DAN LAIN-LAIN BAKTERIA USUS
DI DALAM MEDIA TPY YANG DISUPPLEMENTASI DENGAN
KURKUMIN**

Oleh

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Bacteria dari genus *Bifidobacterium* dan *Lactobacillus* sering digunakan sebagai probiotik. Bakteria-bakteria tersebut menyumbang kepada perkembangan manusia seperti perencatan pertumbuhan luaran dan/atau bacteria merbahaya , perangsangan tungsi imun, cirri-ciri anti-tumor,pengurangan kolestrol,membantu penghadaman dan/atau penyerapan kandungan makanan/minerals dan sintesis vitamin-vitamin.

Makanan adalah antara factor-faktor utama yang boleh memberi kesan kepada keseimbangan mikrob pert. Pert adalah tapak aktif biopenukaran dan penyerapan bahan-bahan makanan yang tidak terserap dalam bahagian atas usus. Ini termasuklah komponen-komponen terutamanya yang berasaskan fenolik dan komponen-komponen aromatic yang lain. Salan satu daripada punca-punca biasa fenolik dan aromatic dalam diet ialah curcumin yang berasal daripada turmeric(*Curcuma Longa* L.).



Pertumbuhan dua strain *Bifidobacterium* (*Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4) dan lain-lain bakteria usus (*Lactobacillus acidophilus*, *Lactobacillus casei* shirota, *Enterococcus faecalis* JCM 5803 dan *Escherichia coli* K-12) di dalam 10ml media TPY asas yang mengandungi pelbagai kepekatan kurkumin (0.0025g, 0.005g, 0.0075g dan 0.01g) telah dikaji. Jumlah sel bakteria yang hidup dan pH medianya ditentukan semasa tempoh inkubasi 12jam, 24jam, 36jam dan 48jam pada suhu 37°C secara anaerobik. Kehadiran kurkumin tidak mengubah pH media yang digunakan dimana pHnya sama seperti media TPY asas tanpa kurkumin. Dengan ketidakhadiran kurkumin, semua bakteria yang diuji menunjukkan pertumbuhan yang baik dengan julat log purata cfu/ml diantara 8.35 hingga 9.45 selepas diinkubasi selama 48jam. Walaubagaimanapun, dengan kehadiran kurkumin, kultur menunjukkan pelbagai darjah perencatan pertumbuhan berbanding media TPY asas.

E. faecalis dan *B. longum* BB536 bermandiri dengan lebih baik berbanding bakteria-bakteria ujian yang lain. Peratus penurunan pertumbuhan *E. faecalis* yang tumbuh di dalam media TPY yang disuplementasikan dengan 0.0025g, 0.005g, 0.0075g dan 0.01g kurkumin dan diinkubasikan selama 12jam ialah 1%, 7%, 24% and 36% masing-masing. Pada tempoh inkubasi 24 jam, peratus penurunan pertumbuhan adalah lebih kurang sama dengan tempoh inkubasi selama 12jam. Peratus penurunan pertumbuhan menurun dengan ketara iaitu kepada hanya 1%, 2%, 14% dan 25% apabila diinkubasikan selama 36jam dan 1%, 2%, 7% dan 8% dengan penginkubasian 48jam.

B. longum BB536 berada pada kedudukan kedua terbaik selepas *E. faecalis* dalam aspek kemandiriannya dalam media TPY asas yang disuplementasikan dengan kurkumin. Selepas diinkubasikan selama 12 jam, 0.0025g kurkumin merencat pertumbuhan *B. longum* BB536 sebanyak 10%. Pada kepekatan 0.005, 0.0075 dan 0.01g pula, pertumbuhan *B. longum* direncat sebanyak 16, 44 dan 48% masing-masing. Pada tempoh penginkubasian 24jam, peratus penurunan pertumbuhan tidak menunjukkan perbezaan yang ketara dengan 12jam masa inkubasi untuk kepekatan kurkumin 0.0075 dan 0.01g, tetapi peratus penurunan meningkat dengan sedikit untuk kepekatan kurkumin 0.0025 dan 0.005g. Penginkubasian kultur *B. longum* selama 36jam dan 48jam menunjukkan perencatan pertumbuhan yang lebih rendah berbanding tempoh penginkubasian selama 12jam dan 24jam.

Bakteria usus ujian yang lain menunjukkan peratus perencatan pertumbuhan yang lebih tinggi berbanding *E. faecalis* dan *B. longum*. Diantara semua bakteria yang diuji, *L. acidophilus* merupakan bakteria yang paling sensitif terhadap kurkumin. Kadar perencatan untuk kurkumin berkepekatan 0.005, 0.0075 dan 0.01g adalah lebih daripada 80%. Selain itu, kemandirian sel *L. acidophilus* dalam media kultur yang disuplementasikan dengan kepekatan kurkumin yang paling rendah iaitu 0.0025g menurun kepada nilai yang sama dengan kepekatan yang tinggi.

Suplementasi media ujian dengan 'tween' 80 meningkatkan kemandirian bakteria yang diuji. *E. coli* K-12 bermandiri dengan lebih baik berbanding bakteria-bakteria ujian yang lain. Peratus penurunan pertumbuhan *E. coli* K-12 dalam media asas yang disuplementasikan dengan 0.01ml 'tween' 80 dan 0.0025g kurkumin yang diinkubasikan selama 6jam ialah 5% dan 50% masing-masing. Pada tempoh inkubasi

12jam, peratus penurunan pertumbuhan dalam media asas yang disuplementasikan dengan kurkumin meningkat sehingga 60% manakala dengan penginkubasian berterusan sehingga 24jam menunjukkan penurunan peratus penurunan pertumbuhan iaitu kepada 23%. Pada tempoh inkubasi sehingga 24jam, peratus penurunan pertumbuhan bagi *E. coli* K-12 dalam media asas yang disuplementasikan dengan 'tween' 80 adalah hampir sama seperti tempoh penginkubasian selama 6jam iaitu sebanyak 5%.

Keupayaan bakteria untuk mendegradasi kurkumin dikaji menggunakan kaedah spektrofotometrik. Berdasarkan kajian spektrofotometrik ultraungu nampak (UV-Vis spectra), penentuan jalur penyerapan cahaya maksimum dalam media TPY yang disuplementasikan dengan kepekatan kurkumin yang berbeza diperhatikan pada panjang gelombang 400.4nm. Jalur penyerapan maksimum bagi kurkumin dalam media selepas 48jam penginkubasian dengan bakteria ujian tanpa sebarang perubahan adalah pada 400.4nm. Perbandingan antara panjang gelombang kurkumin sebelum dan selepas penginkubasian dengan bakteria membuktikan bahawa semua bakteria yang diuji dalam kajian ini tidak mampu untuk mendegradasi kurkumin dan kurkumin adalah kekal di dalam media.

Untuk menyokong penemuan di atas, kandungan kurkumin selepas penginkubasian selama 48jam dengan bakteria dikira menggunakan lengkung piawai. Penyerapan kurkumin pada panjang gelombang 400.4nm berbanding pada masa 0jam menunjukkan penurunan. Selain itu, kandungan kurkumin selepas diinkubasi dengan semua bakteria ujian turut menunjukkan penurunan. Peratus penurunan 0.0025 dan 0.0050g kurkumin/10ml ialah 56-60 dan 18-24% manakala untuk kedua-dua

kepekatan yang lain iaitu 0.0075 dan 0.01g/10ml ialah 15-16 dan 12-14% masing-masing.

Curcumin, sebagai aromatic yang biasa, cirri-ciri perangsang dan pewarna dalam diet telah didapati mengandungi aktiviti anti bacteria terhadap *B. longum* BB536, *B. pseudocatenulatum* G4 dan bacteria usus lain yang terpilih(*L. acidophilus*, *L. casei* shirota, *E. faecalis* JCM 5803 dan *E. coli* K-12). Bilangan sell yang Berjaya hidup daripada semua mikro-organisma yang telah diuji menurun secara mendadak selepas 48 jam pengeraman dalam perbandingan dengan kawalah dalam TPY media.

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I certify that an Examination Committee has met on to conduct the final examination of Seyed Davoud Jazayeri on his Master of Science thesis entitled "Survival of Bifidobacteria and Other Selected Intestinal Bacteria in TPY Media Supplemented with Curcumin as Assessed *In vitro*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SEYED DAVOUD JAZAYERI

Date:



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CHAPTER 1

INTRODUCTION

The human gastrointestinal tract is a natural habitat for a large and diverse microbial ecosystem. Many species of bacteria have evolved and adapted to live in the human intestinal, with a substantial part of these bacterial populations still awaiting discovery and characterization. The human gastrointestinal tract harbors a complex collection of microorganisms throughout its length, although it is the colon, which represents the main site of microbial colonization, providing residence for more than 500 different species of bacteria (Berg, 1996). The microflora of an adult human gut predominantly consists of facultative anaerobes and obligate anaerobes such as *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Esherichia*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Lactobaillus* and *Proteus* (Simon and Gorbach, 1984).

These gut microbiota plays an important role in both human health and disease (Guarner and Malagelada, 2003). They are considered crucial as first line of defence against colonization by pathogens (Servin, 2004). The other claimed beneficial effects of intestinal microbial include vitamins production, availability of minerals and trace elements, production of important digestive enzymes (e.g. β -galactosidase), prevention of different types of diarrhea (infectious diarrhea, traveler's diarrhea, children's acute viral diarrhea, antibiotic-associated diarrhea), cholesterol lowering effects, stimulation of the immune system, enhancement of bowel motility/relief from constipation adherence and colonization resistance, maintenance of mucosal integrity, lactose tolerance, and milk products (Wilhelm *et al.*, 2002).



Although human gut microbiota is considered pivotal for the maintenance of gut health, the gastrointestinal tract environment is susceptible to disturbances leading to the shift of the balance of the gut microflora away towards the predominance of potentially harmful or pathogenic microorganisms such as clostridia, sulphate reducers, and certain *Bacteroides* species. In healthy individuals, the composition of intestinal microflora is normally stable. However certain endogenous or exogenous factors can influence this equilibrium. Such factors include peristalsis disorder, cancer, surgical operation, radiation therapy, emotional stress, ageing, administration of antibiotics and nutrition (Borgia *et al.*, 1982; Colombel *et al.*, 1987; Siitonen *et al.*, 1990).

Foods are thought to be among the major factors that can affect the gut microbial balance. The gut is the site of active bioconversions and absorption of foodstuffs that have not been absorbed in the upper gastrointestinal tract. These include components especially plant-derived such as phenolic and other aromatics components. One of the most common sources of phenolic and aromatics in diets is curcumin derived from turmeric. Turmeric (*Curcuma longa* L.) that belongs to the Zingiberaceae family along with the other noteworthy members like ginger, cardamom and galangal are among popular plant flavoring used worldwide (Govindarajan, 1980). They belong to the genus *Curcuma* that consists of hundreds species of plants that possess rhizomes and underground root-like stems. Curcumin, the yellow pigment of turmeric, is produced industrially from turmeric oleoresin. Curcumin has been the subject of hundreds of published papers over the past decades, investigating its antioxidant, anti-inflammatory, and cancer chemo preventive and potentially chemotherapeutic properties (Sharma, 2005).



However, published information on the effects of curcumin on growth of microorganisms is hardly available. The appeal of turmeric as a colouring, food preservation and flavouring is global. However, the metabolism of these food components may be beneficial to the host or toxic or detrimental either to the host or the gut microflora.

Therefore the aim of the present study was to investigate the effect of different concentrations of curcumin on the survival and growth rate of selected intestinal bacteria: *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Escherichia coli*.

The specific objectives of this study were:

1. To determine the effect of curcumin at various concentrations on the growth of selected intestinal bacteria.
2. To evaluate the protective effect of tween 80 against antibacterial activity of curcumin.
3. To determine the ability of the bacteria to metabolic curcumin as measured by spectrophotometer procedure.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacterial populations inside the gastrointestinal tract

Bacterial populations inside the gastrointestinal tract have adapted such that numbers of each genera are fairly consistent, having their own individual growth niche. In order for the intestine to function optimally however, the 'balance' of the bacterial flora must be maintained, and this appears to be increasingly difficult, as lifestyles have changed. An increase in stress and modern day living, which makes a consequential demand on the immune system, can disrupt homeostasis in the gut. Similarly, the direct effects of a change in dietary patterns and eating habits can affect overall gut functionality.

Another contributory factor includes the consumption of pharmaceutical compounds, in particular antibiotics, which by design destroy bacteria, and therefore can have a harmful effect on the balance of the gut microbiota. All of these combine to shift the balance of the gut microflora away from potentially beneficial or health-promoting bacteria such as the lactobacilli and bifidobacteria, towards an increase in harmful or pathogenic microorganisms, like the clostridia, sulphate-reducers and proteolytic *Bacteroides* species. Predominance of the latter may pre-dispose towards a number of clinical disorders, including bowel cancer and inflammatory bowel diseases such as ulcerative colitis, whilst making the host more susceptible to infections by transient enteric pathogens such as *Salmonella*, *Campylobacter*, certain species of *Escherichia coli* and