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

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ABSTRACT: 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 TPY medium containing various concentrations of curcumin (0.025, 0.050, 0.075 and 0.1% w/v). Viable cell counts of the bacteria and their respective pH medium were determined during incubation period of 12h, 24h, 36h and 48h incubated at 37°C. In the presence of curcumin, cultures showed various degrees of growth inhibition compared to TPY medium without curcumin. E. faecalis and B. longum BB536 were survived better than the other bacteria tested. Among the bacteria tested, L. acidophilus recorded the most sensitive to curcumin. The presence of curcumin did not change the pH of the medium as compared to the basal TPY. The ability of the bacteria to degrade curcumin after 48h incubation was studied using spectrophotometric method measured at 400.4 nm wavelength. The overall percentage reduction of 0.025, 0.050, 0.075 and 0.1% of curcumin by the bacteria tested was 56-60, 18-24, 15-16 and 12-14, respectively.

KEYWORDS: Curcumin, Growth Inhibition, Intestinal Bacteria, Percentage Reduction

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INTRODUCTION

The human gastrointestinal tract is a kinetic micro ecosystem that enables normal physiological functions of the host organism unless harmful and potentially pathogenic bacteria dominate it. Many species of bacteria have evolved and adapted to live in the human intestine, 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 bacterial (Berg, 1996). The microbiota of an adult human gut predominantly consists of facultative anaerobes and obligate anaerobes such as Bacteroides, Bifidobacterium, Eubacterium, Escherichia, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, and Proteus (Simon and Gorbach, 1984). These gut microbiota play an important role in both human health and disease, (Guarner and Malagelada, 2003). Therefore, maintaining a proper equilibrium of the microbiota is very important and may be ensured by systematic supplementation of the diet containing probiotics.

The most widely used definition of probiotic has been proposed by Fuller (Fuller, 1989; Fuller, 1992) i.e. a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. Yet, for human nutrition, Salminen et al. (1998), proposed the following definition: 'a live microbial food ingredient that is beneficial to health'. Among the health advantages associated with probiotic intake are: (1) alleviation of symptoms of lactose malabsorption; (2) increase in natural resistance to infectious diseases of the intestinal tract; (3) suppression of cancer; (4) reduction in serum cholesterol level; (5) improved digestion; and (6) stimulation of gastrointestinal immunity (Collins and Gibson, 1999). However, well-characterized strains with proven clinical effects are not numerous. Bacteria belonging to the genera Bifidobacterium and Lactobacillus are most frequently used probiotics. They exert beneficial properties with regard to human
health, such as inhibition of growth of exogeneous and/or harmful bacteria, stimulation of immune functions, anti-tumor properties, cholesterol reduction, aid in digestion and/or absorption of food ingredients/minerals and synthesis of vitamins (Gibson, 1998).

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 compounds. One of the most common sources of phenolic and aromatic in diets is curcumin derived from turmeric (Curcuma longa L.). Curcumin is an active ingredient in turmeric, which is widely used as a food colorant and preservation due to the fact that natural dyes can often inhibit the growth of microorganisms without toxicity (Kang et al., 1996; Miyaoka and Miyaoka, 2001; Han, 2000; Yong et al., 1999; Park and Nam, 2003). Moreover the appeal of turmeric as a coloring, food preservation and flavoring is global. However, the metabolism of these food components may be produces benefit to the host or detrimental either to the host or the gut microbiota. Moreover, Generally, studies investigating the effect of curcumin on the growth and survival of intestinal bacteria are still lacking. Therefore, the purpose of this research is to study the inhibitory activity of curcumin on the growth of selected intestinal bacteria. The relationship between curcumin concentration and its antimicrobial activity was also investigated.

MATERIALS AND METHODS

Bacterial strains, culture conditions and chemicals

Bifidobacterium longum BB536 was obtained from Morinaga Milk Industry (Minato-ka, Japan). Bifidobacterium pseudocatenulatum G4 local isolate and Escherichia coli K-12 were obtained from the Department of Microbiology Culture Collection Center of University Putra Malaysia (UPM). Enterococcus faecalis JCM 5803 was obtained from Japan Collection of Microorganisms (Riken, Japan). Lactobacillus acidophilus and Lactobacillus casei Shiroti were isolated from fermented drinks Vitagen and Yakult, respectively. Strains were grown in TYP Broth (Scardovi, 1986). E. faecalis JCM 5803 and E. coli K-12 were incubated for 24 hours at 37°C aerobically. B. longum BB536, B. pseudocatenulatum G4, L. acidophilus and L. casei Shirotia were incubated for 24 hours at 37°C anaerobically (BBL GAS Pak System, Becton Dickinson, USA). Pure natural curcumin powder was purchased from Sigma Chemical Company (USA).

Survival of microorganisms in TYP medium supplemented with curcumin

Experiments were conducted to determine the ability of test microorganisms to survive in TYP medium supplemented with different concentrations of curcumin. Non-supplemented TYP medium acted as a control. Curcumin was added to TYP medium to give final concentrations of 0.025%, 0.05%, 0.075% and 0.1% (w/v). Each medium was inoculated with approximately 7.6 log cfu/ml bacterial cultures. Incubation was carried out using the methods described earlier. Samples were taken at 0 h and at selected time intervals extending over a 48 h period. Changes in pH were determined using a Mettler-Toledo Delta 320 pH-meter (Mettler-Toledo Inc. Ohio, USA). Viable counts of the bacteria were determined in duplicate by spreading onto TYP agar and incubated under the condition as mentioned above. The mean values and standard deviations were calculated from the data obtained with triplicate experiments. The percent reduction of growth for each culture was calculated from the equation by Han and Yang [2005] as follows:

\[
\text{% Inhibition (H %)} = 100 \left(\frac{A-B}{A}\right)
\]

Where as: \( A = \) number of bacteria recovered from the control and \( B = \) is the number of bacteria recovered from TYP medium supplemented with various concentration of curcumin at time intervals.

Degradation of curcumin by bifidobacteria and other selected intestinal bacteria

The ability of the bacteria tested to degrade curcumin in TYP medium after 48 h of incubation was assessed. The spectrophotometric determination of curcumin was carried out on a lambda 3B UV-Vis 1601 spectrophotometer (Shimadzu Corp, Japan) with a band pass setting of 0.1 nm matched with 1 cm quartz cell. The uv-vis spectra of various concentrations of curcumin in TYP medium were measured from 200 to 800 nm. A linear calibration graph was constructed by measuring the absorbance of TYP medium supplemented with various concentrations of curcumin \((4.1 \times 10^{-2} , 3.7 \times 10^{-2} , 3.3 \times 10^{-2} , 3.1 \times 10^{-2} , 2.8 \times 10^{-2} , 2.6 \times 10^{-2} , 2.5 \times 10^{-2} , 2.3 \times 10^{-2} , 2.2 \times 10^{-2} , 2.0 \times 10^{-2} , 1.9 \times 10^{-2} , 1.8 \times 10^{-2} , 1.7 \times 10^{-2} , 1.6 \times 10^{-2} \% w/v\) at 400.4 nm (obtained from absorption spectra scan of curcumin in TYP medium) against a reagent blank (un-supplemented TYP medium).

Statistical analysis

Data were analyzed using one-way ANOVA MINITAB statistical software (Release 14 for Windows, 2006, Minitab Inc, USA) followed by Dunnett multiple comparisons to compare treatment groups with control. Probability levels of less than 0.05 were considered significant.

RESULTS

Inhibitory activity of curcumin on the growth rate of selected bacteria

Figure 1 shows the inhibitory activity of various concentrations of curcumin in TYP medium against selected intestinal bacteria B. longum, B. pseudocatenulatum, E. faecalis, L. acidophilus, L. casei Shirotia and E. coli. As shown in Figure 1a, the starting value of B. longum in the control medium and in the medium supplemented with various concentrations of curcumin was approximately 7.6 log cfu/ml. After 12 hours of incubation, 0.025% curcumin exhibited 10% inhibition of B. longum growth. At concentrations of 0.05%, 0.075% and 0.1%, B. longum growth was inhibited at 16, 44, and 48 %, respectively. At 24 hours of incubation, the percentage reduction was not much different as compared to 12
hours incubation with supplementation of 0.075 and 0.1% curcumin, but it was slightly increased at 0.025 and 0.050% curcumin. Interestingly, extending the incubation of the *B. longum* culture for 36 and 48 hours showed lower growth inhibition as compared to 12 and 24 hours.

Figure 1b shows the growth inhibition of curcumin against *B. pseudocatenulatum G4*. An inhibition rate of more than 50% was observed in all the incubation time tested when 0.075 and 0.1% of curcumin were supplemented. Concentration of 0.025 and 0.05% of curcumin were found to be less effective in inhibiting the growth of *B. pseudocatenulatum G4* with inhibition rate observed was less than 50% in all the incubation times tested. Among the bacteria tested, *L. acidophilus* was found to be the most sensitive to curcumin (Figure 1c). More than 80% inhibition was observed when 0.050, 0.075 and 0.1% of curcumin were supplemented into the TPY medium. Moreover, the surviving cells of *L. acidophilus* in the culture medium supplemented with the lowest concentration of curcumin (0.025%) were decreased to almost the same values as higher concentrations.

Figure 1d reports the result of inhibition of curcumin on the growth of *L. casei* strain shirata. A 0.025% curcumin causes 12% reduction after 12 hours incubation and the reduction rate increases proportionally with the increasing concentrations of curcumin used. The same patterns of growth inhibition were also observed when the culture was incubated for 24, 36, and 48 hours under the same incubation condition. Hence, better survival rate was observed when the culture was incubated for 48 hours. Figure 1e shows the results of the survival rate of *E. faecalis* in TPY medium supplemented with various concentrations of curcumin. The results showed that in all the curcumin concentrations used, the surviving cells of *E. faecalis* had reduced almost at the same rate when incubated.
for 12 and 24 hours. However, prolonged incubation of the culture resulted in decreased of more than half of the growth inhibition. As for *E. coli*, the inhibition rates were varied, with the lowest concentration of curcumin (0.025%) showed the lowest value, whereas the highest concentration of curcumin (0.1%) showed the highest inhibition value for all the incubation time tested (Figure 1f). The highest reduction percentage was achieved when the bacterium was inoculated into TPY medium supplemented with 0.1% curcumin and incubated for 48 hours.

**FIGURE 2.** Absorption spectra of different concentrations of curcumin in TPY medium.

![Absorption spectra](image)

**TABLE 1.** The pH of medium at 0 hour and after 48 hours incubation

<table>
<thead>
<tr>
<th>CULTURE</th>
<th>TIME</th>
<th>PH MEDIUM*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(H)</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>B. longum BB 536</td>
<td>48</td>
<td>4.20±0.02*</td>
</tr>
<tr>
<td>B. pseudocatenulatum G4</td>
<td>48</td>
<td>4.16±0.03*</td>
</tr>
<tr>
<td>L. casei Shiratai</td>
<td>48</td>
<td>3.95±0.02*</td>
</tr>
<tr>
<td>L. casei Shiratai</td>
<td>48</td>
<td>3.90±0.02*</td>
</tr>
<tr>
<td>E. faecalis JCM 5803</td>
<td>48</td>
<td>4.20±0.01*</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>48</td>
<td>4.90±0.01*</td>
</tr>
</tbody>
</table>

*Medium (control): TPY medium without curcumin; TPY medium supplemented with 0.025, 0.05, 0.075 and 0.1% curcumin. The mean values and the standard deviation (± SD) were calculated from the data obtained with triplicate trials. The superscript letter indicates no significant different (P > 0.05).

**Change of the pH medium**

Table 1 presented the results of the pH of the TPY medium measured at 0 and after 48 hours of incubation with the bacteria. At 0 hour, the pH for all of the media (control and TPY medium supplemented with different concentrations of curcumin) was approximately 6.29. After 48 hours of incubation, for lactic and acetic acid bacteria, the pH decreased to around 4 and for *E. coli* K-12 (non-acid producing bacteria), the pH decreased to around 4.9.

**Degradation of curcumin by the bacteria tested as studied by spectrophotometric method**

The probable peak(s) of the TPY medium without any supplementation of curcumin was carried out by spectral scan at wavelength ranges between 200 to 800 nm and no peak was observed. The maximum absorption for the TPY medium supplemented with curcumin was observed at 400.4 nm (λ max). The stability of the curcumin in TPY medium after autoclaving at 121°C for 15 min was also examined. It was observed that curcumin was stable during the autoclaving process and the absorbance at the wavelength of 400.4 nm still can be detected (data not shown). Therefore, the ability of bacteria to degrade curcumin was estimated by measuring the remaining curcumin in the medium spectrophotometrically at 400.4 nm. Calibration curve was constructed based on the absorbance of various concentration of curcumin in TPY medium (Figure 2) and a typical linear calibration curve for different concentrations of curcumin was established (Figure 3). Table 2 presented the data on the ability of the bacteria tested to degrade curcumin. The percentage reduction of 0.025% and 0.05% of curcumin was 56-60 and 18-24 % and for the other two concentrations which were 0.075% and 0.1% was 15-16 and 12-14 %, respectively. The maximum percentage reduction (60%) of 0.025% of curcumin after 48 h of incubation recorded when TPY medium supplemented with curcumin was inoculated with *E. faecalis* and *L. acidophilus* cultures.
TABLE 2. Percentage reduction of curcumin after 48 hours of incubation

<table>
<thead>
<tr>
<th>CURCUMIN CONCENTRATION (%w/v)*</th>
<th>PARAMETER</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. lon</td>
</tr>
<tr>
<td>0.025</td>
<td>C2 (%w/v)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>0.2335</td>
</tr>
<tr>
<td></td>
<td>%R</td>
<td>56</td>
</tr>
<tr>
<td>0.050</td>
<td>C2</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>0.4025</td>
</tr>
<tr>
<td></td>
<td>%R</td>
<td>18</td>
</tr>
<tr>
<td>0.075</td>
<td>C2</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>0.4211</td>
</tr>
<tr>
<td></td>
<td>%R</td>
<td>15</td>
</tr>
<tr>
<td>0.100</td>
<td>C2</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>0.4312</td>
</tr>
<tr>
<td></td>
<td>%R</td>
<td>12</td>
</tr>
</tbody>
</table>

*Initial concentration of curcumin in TPY medium; C2: Concentration of curcumin after 48 h of incubation; Abs: absorbance value at 400.4 nm after 48 h of incubation; %R: Percentage of curcumin reduction after 48 h of incubation; B. lon: B. longum BB536; B. pse: B. pseudocatenulatum G4; L. aci: L. acidophilus; L. cas: L. casei Shirotia; E. fae: E. faecalis JCM 5803; E. coli: E. coli K-12.

FIGURE 3. Linear calibration curve plotted based on absorbance of different concentrations of curcumin incubated for 48 hours in un-inoculated TPY medium measured at 400.0 nm wavelength.

**Calibration Curve**

\[
Y = 194.83 \times + 0.0002
\]

\[
R^2 = 0.9995
\]

DISCUSSION

Bioactive compounds are extra nutritional constituents that typically occur in small quantities in foods of plants origin. They are being widely studied to evaluate their effects on health. One of the active ingredients containing bioactive compounds that widely used in food is curcumin derived from turmeric (*Curcuma longa* L.). Pharmacological studies have demonstrated that curcumin used in traditional medicine exhibited anti-inflammatory, antifungal and anti-tumor activities (Yamamoto et al., 1997; Geoffrey et al., 1998). However, their inhibitory activity against intestinal bacteria is not well studied.

The results of this study showed that curcumin has inhibitory activity against selected intestinal bacteria namely *B. longum* BB536, *B. pseudocatenulatum* G4, *L. acidophilus*; *L. casei* Shirotia; *E. faecalis* JCM 5803 and *E. coli* K-12. This finding is in agreement with the previous study reported by Han and Yang (2005), who indicates that curcumin has antibacterial activity against intestinal bacterium *E. coli*. To our knowledge, there is no study on the antibacterial activity of curcumin against the so-called friendly gut bacteria like *B. longum*, *B. pseudocatenulatum*, *L. acidophilus*, *L. casei* and *E. faecalis* has been done. Although the interaction between the curcumin and gastrointestinal microbiota is still not well understood, it is worth bearing in mind that curcumin might have unpredictable effects on the microbial ecology of the human guts (Lakerbrink et al., 2000; Torres et al., 2001). Hence, more studies are required to substantiate the evidences. On the other hand, it is not feasible to initially carry out such experiments in *vivo* without conducting *in vitro* experiments first, and it should be considered that it is not right to count on the *in vitro* results if not followed by *in vitro* experiments.

From the present study, it is clear that the presence of curcumin in the growth medium has a major impact on the survival of the bacteria. The inhibitory activity increases when higher concentrations of curcumin were applied. Moreover, different bacterial species exhibited different sensitivities towards curcumin.
These variations may reflect differences in the cell surface structure among the micro-organisms ( Puuppomen et al., 2002). However, the exact mechanism for the antimicrobial activity of curcumin is not completely understood. Though, the existence of methoxyl and hydroxyl groups is believed to be responsible for its antimicrobial activity ( Krishnankutty and Venugopal, 1998; Liu et al., 1990; Gotoh et al., 1998; Tesaki et al., 1998; Han and Choi, 2000; Han and Choi, 2002).

Results of this study also indicate the ability of the bacteria to degrade curcumin. The degradative products, even though are not known from this study might contribute to the inhibitory action of curcumin. Moreover, physical and chemical properties of compounds in solution may play a role in determining the inhibitory effect, which depends largely on their ability to diffuse in the medium and eventually bind and penetrate the wall of cells. This result is consistent with the antibacterial activity of spices and essential oils reported earlier ( Sivropoulou et al., 1996; Naganawa et al., 1996).

CONCLUSION

Curcumin from turmeric, when introduced into TPY medium has inhibitory activity against selected intestinal bacteria. The inhibition rates vary among bacteria tested with the highest to the growth of L. acidophilus and the lowest to the growth of E. faecalis. However, more studies should be conducted on more intestinal bacteria to really determine the effects of curcumin on the growth of intestinal microbiota and subsequently affecting the human well-being.

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CONFLICT-OF-INTEREST STATEMENT

No conflict of interest between the researchers and funding agency (Ministry of Science, Technology and Innovation Malaysia) for research output intended for publication in scientific journals.

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