Food Sci. Biotechnol. Vol. 15, No. 5, pp. 817 ~ 819 (2006)

RESEARCH NOTE

Food Science and Biotechnology

Antimicrobial Effects of 8-Quinolinol

Young-Mi Kim, Eun-Young Jeong, Jeon-Hyeon Lim, and Hoi-Seon Lee*

Faculty of Biotechnology and Center for Agricultural Science and Technology, College of Agriculture, Chonbuk National University, Jeonju, Jeonbuk 561-756, Korea

Abstract 8-Quinolinol and other quinolinol derivatives were evaluated with regard to their growth-inhibitory effects against intestinal bacteria, using the paper disk-agar diffusion method. The observed growth responses varied according to the chemicals and dosages used, as well as the bacterial species tested. 8-Quinolinol showed a significant inhibitory effect against *Clostridium difficile*, *C. perfringens*, and *Escherichia coli*, at 5, 2, 1, and 0.5 mg/disk, and also exhibited a very strong inhibitory effect at 0.25 mg/disk. At low concentrations, 8-quinolinol had strong inhibitory effects against *C. perfringens* at 0.1 and 0.05 mg/disk; 8-quinolinol also manifested a moderate inhibitory effect against *C. perfringens* at 0.025 mg/disk. Furthermore, 8-quinolinol revealed moderate and weak growth inhibition against *C. difficile* and *E. coli* at concentrations of 0.1 and 0.05 mg/disk, respectively, but 2-quinolinol, 4-quinolinol, and 6-quinolinol evidenced no growth inhibition against *B. bifidum*, *B. longum*, *C. difficile*, *C. perfringens*, *E. coli*, or *L. casei*. The inhibitory effects of 8-quinolinol against *C. difficile*, *C. perfringens*, and *E. coli* lead to its consideration as a possible therapeutic modality for the treatment of diseases associated with harmful intestinal bacteria.

Key words: intestinal bacteria, 8-quinolinol, *Clostridium difficile*, *Clostridium perfringens*, *Escherichia coli*

Introduction

The intestinal microflora are generally classified into beneficial and harmful bacteria. In particular, harmful bacteria, including *Clostridium*, *Escherichia coli*, *Pseudomonas*, *Staphylococcus*, and *Veillonella*, not only generate carcinogenic substances *de novo*, but also have the ability to transform metabolites from dietary sources into tumor initiators or promoters (1-3). The infectious diseases induced by harmful bacteria are characterized by a broad spectrum of clinical severity, ranging from mild outpatient illness to sudden death. Among the clostridia, *C. perfringens* is associated with sudden death, toxicity, and gastrointestinal disease in humans (1, 3). On the other hand, the bifidobacteria are often considered to be useful indicators of human health under the majority of environmental conditions. These bacteria play important roles in metabolism such as amino acid and vitamin production, facilitate defense against infections, and are also associated with longevity, pathogenic inhibition, and immunopotentiation (4-6). Accordingly, it is desirable to both inhibit the growth of potential pathogens, such as the clostridia, and to increase the numbers of bifidobacteria within the human digestive system. Inhibitors of the growth and survival of harmful bacteria are especially important in regard to human health, as the intake of such inhibitors can normalize disrupted physiological functions, thereby preventing and treating a variety of diseases induced by gastrointestinal pathogens (5).

Recently, a great deal of attention has been focused on bacterial growth inhibitors/promoters for use in the intestine, based on the notion that many of these materials are relatively nontoxic to humans (2, 7, 8). As many of these compounds are largely free from adverse effects and exhibit desirable pharmacological activities, this might lead to the development of new classes of safer antimicrobial agents (2, 7, 8). However, despite their excellent pharmacological activity (8), relatively little work has been carried out on the effect of quinolinol derivatives on the growth of intestinal microorganisms. Therefore, we have evaluated the growth-inhibitory effects of 8-quinolinol, which is found in *Sebastiania corniculata* roots, and other quinoline derivatives against six intestinal bacteria.

*Corresponding author: Tel: 82-63-270-2544; Fax: 82-63-270-2550
E-mail: hoiseon@chonbuk.ac.kr
Received April 20, 2006; accepted August 8, 2006

Materials and Methods

Chemicals 2-Quinolinol, 4-quinolinol, 6-quinolinol, and 8-quinolinol were purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). All other chemicals used in this study were of reagent grade.

Bacterial strains and culture conditions The bacterial strains used in this study were as follows: *Bifidobacterium bifidum* ATCC 29521, *B. longum* ATCC 15707, *Clostridium difficile* ATCC 9689, *C. perfringens* ATCC 13124, *E. coli* ATCC 11775, and *Lactobacillus casei* ATCC 393, which was isolated from human feces. Stock cultures of these strains were stored routinely on Eggerth-Gagnon (EG) liver extract-Field's slants at -80°C, and subcultured on EG agar (Eiken Chemical, Tokyo, Japan) when required. The plates were anaerobically incubated for 2 days at 37°C in an atmosphere containing 80% N_2, 15% CO_2, and 5% H_2 in an anaerobic chamber (Coy Lab., Grass Lake, MI, USA). The bacteria were subsequently grown in EG broth (pH 6.8).

Antimicrobial activity In order to assay the growth inhibitory effects of the test materials on the microorganisms of interest, one loopful of bacteria was suspended in 1 mL of sterilized physiological saline. An aliquot (0.1 mL) of these bacterial suspensions was then

seeded on EG agar. A 100 μL sample of the test compound in a methanol solution was applied to a paper disk (Advantec 8 mm diameter and 1 mm thickness) with a Drummond glass microcapillary. After the solvent had evaporated under a fume hood (25°C) for 1 hr, the disks were placed on an agar surface that had been inoculated with the test bacteria. All plates were anaerobically incubated for 2 days at 37°C. The control disks received 100 μL of methanol, which manifested no adverse effects against the tested organisms. All tests were conducted in triplicate. Inhibitory responses were classified as previously described: potent response, ++++, zone diameter >30 mm; strong response, +++, zone diameter 21-30 mm; moderate response, ++, zone diameter 16-20 mm; weak response, +, zone diameter 10-15 mm; and little or no response, -, zone diameter <10 mm.

Results and Discussion

8-Quinolinol and derivatives were evaluated with regard to their ability to inhibit the growth of six intestinal bacteria (Table 1). Growth responses varied according to the tested chemicals and dosages, as well as the bacterial strains. In tests conducted with *C. difficile*, 8-quinolinol effected a strong inhibition at concentrations of 2, 1, 0.5, and 0.25 mg/disk, and evidenced moderate and weak inhibition at 0.1 and 0.05 mg/disk, respectively. Furthermore, 8-quinolinol exerted a significant inhibitory effect against *C. perfringens* at concentrations of 2, 1, 0.5, 0.25, 0.1, and 0.05 mg/disk, and also manifested a moderate inhibitory effect at 0.025 mg/disk. This compound manifested strong activity against *E. coli* at 2, 1, 0.5, and 0.25 mg/disk, and showed moderate and weak activity at 0.1 and 0.05 mg/disk, respectively. However, at doses of 1 and 0.5 mg/disk, 8-quinolinol exerted weak or no growth inhibition against *B. bifidum*, *B. longum*, and *L. casei* (Table 1). These findings revealed that the growth-inhibitory effects of 8-quinolinol were more pronounced in *C. perfringens* and *E. coli*, compared to those observed with the bifidobacteria and lactobacilli. With regard to the structure-activity relationships of 8-quinolinol with *C. difficile*, *C. perfringens*, and *E. coli*, the growth-inhibitory effects of 8-quinolinol were compared with those of the quinolinol derivatives, 2-quinolinol, 4-quinolinol, and 6-quinolinol. At dosages of 2 and 1 mg/disk, 2-quinolinol, 4-quinolinol, and 6-quinolinol evidenced no growth inhibition against *B. bifidum*, *B. longum*, *C. difficile*, *C. perfringens*, *E. coli*, and *L. casei* (data not shown). Furthermore, the growth-inhibitory effects of 8-quinolinol were compared with those of tetracycline, which was used as a reference antimicrobial agent (data not shown). Tetracycline at 0.025 mg/disk significantly inhibited the growth of all bacteria, with the exception of *B. longum*, but 8-quinolinol at a concentration of 0.25 mg/disk did not inhibit the growth of *B. bifidum*, *B. longum*, or *L. casei*, although it did inhibit the growth of *C. difficile*, *C. perfringens*, and *E. coli*. The growth-inhibitory effects of tetracycline against *C. difficile*, *C. perfringens*, and *E. coli* were more pronounced than those of 8-quinolinol. However, 8-quinolinol exerted no adverse effects on the growth of bifidobacteria at a concentration of 0.25 mg/disk, thereby indicating that this compound possesses a selective activity against harmful bacteria.

The maintenance of high levels of *Bifidobacterium* spp. and low levels of *C. perfringens* was found to facilitate good health and longevity in humans (9). Therefore, we elected to focus on the microorganisms *Bifidobacterium* spp. and *C. perfringens* for use in our screening for growth-modulator agents that might serve to improve the intestinal environment. Although there are little known regarding autogenic factors within the intestinal ecosystem, some health-promoting effects have been studied in association with several dietary components, and these components have been demonstrated to be effective in the control of normal microfloral growth (10, 11). Recently, a great deal of attention has focused on the inhibitory roles of compounds with regard to the suppression of the carcinogenic and mutagenic effects associated with the clostridia. Many compounds have been shown to function as modifiers of intestinal bacterial populations (2, 7, 8). In this study, the inhibitory activity of 8-quinolinol confirmed its superiority and usefulness as a lead compound for antimicrobial agents.

In conclusion, the results of this study clearly show that 8-quinolinol exerts an inhibitory effect *in vitro* against specific intestinal bacteria. This information should facilitate the elucidation and augmentation of the positive biological effects associated with 8-quinolinol. More importantly, the inhibitory effects of 8-quinolinol against *C. difficile*, *C. perfringens*, and *E. coli* might bear

Table 1. Growth-inhibiting activities of 8-quinolinol against intestinal bacteria

Compound	Dosage (mg/disk)	Bacterial strain[1)]					
		B. bifidum	*B. longum*	*C. difficile*	*C. perfringens*	*E. coli*	*L. casei*
8-Quinolinol	2.0	+[2)]	++	++++	++++	++++	-
	1.0	+	+	++++	++++	++++	-
	0.5	-	-	+++	++++	+++	-
	0.25	-	-	+++	++++	+++	-
	0.10	-	-	++	+++	++	-
	0.05	-	-	+	+++	+	-
	0.025	-	-	-	++	-	-

[1)]Bacteria were cultured on Eggerth-Gagnon agar at 37°C for 2 days in an atmosphere of 80% N_2, 15% CO_2, and 5% H_2.
[2)]Inhibitory zone diameter >30 mm, ++++; 21-30 mm, +++; 16-20 mm, ++; 10-15 mm, +; and <10 mm, -. Each assay was determined in triplicate.

implications for the potential development of novel therapeutic modalities for the treatment of diseases induced by harmful bacteria. However, further research will be necessary to determine whether this activity is still exerted *in vivo* after the actual consumption of 8-quinolinol by humans.

References

1. Bunting M, Lorant DE, Bryant AE, Zimmerman GA, McIntyre TM, Stevens DL, Prescott SM. Alpha toxin from *Clostridium perfringens* induces proinflammatory changes in endothelial cells. J. Clin. Invest. 3: 565-574 (1997)
2. Kim MK, Kim YM, Lee HS. Growth-inhibiting effects of *Juniperus virginiana* leaf-extracted components toward human intestinal bacteria. Food Sci. Biotechnol. 14: 164-167 (2005)
3. Goldman P. Biochemical pharmacology and toxicology involving the intestinal flora. pp. 241-263. In: Human Intestinal Microflora in Health and Disease. Hentges DJ (ed). Academic Press, New York, NY, USA (1983)
4. Mitsuoka T, Emeritus P. The human gastrointestinal tract. Volume I, pp. 68-78. In: The Lactic Acid Bacteria: The Lactic Acid Bacteria in Health and Disease. Wood BJB (ed). Elsevier Science Publishers, London, UK (1992)
5. Hentges DJ. Role of the intestinal microflora in host defense against infection. pp. 311-331. In: Human Intestinal Microflora in Health and Disease. Hentges DJ (ed). Academic Press, New York, NY, USA (1983)
6. Perdigon G, Alvarez S, Rachid M, Aguero G, Gobbato N. Immune system stimulation by probiotics. J. Dairy Sci. 78: 1597-1606 (1995)
7. Kim YM, Lim MY, Lee HS. *In vivo* evaluation of potato varieties (*Solanum tuberosum* L.) on fecal microflora of human volunteers. Food Sci. Biotechnol. 14: 420-423 (2005)
8. Cho JH, Lee CH, Lee HS. Antimicrobial activity of quinoline derivatives isolated from *Ruta chalepensis* toward human intestinal bacteria. J. Microbiol. Biotechn. 15: 646-651 (2005)
9. Mitsuoka T. Bifidobacteria and their role in human health. J. Ind. Microbiol. 6: 263-268 (1990)
10. Benno Y. Effect of diets on human fecal microflora. Bifidus 4: 1-8 (1990)
11. Mallett AK, Rowland IR, Bearne CA, Flynn JC, Fehilly BJ, Udeen S, Farthing MJG. Effect of dietary supplements of apple pectin, wheat bran or fat on the enzyme activity of the human fecal flora. Microb. Ecol. Health D. 1: 23-32 (1988)