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Influence of phenol, *p*-cresol and indole on growth and survival of intestinal lactic acid bacteria

Adriana Nowak*, Zdzislawa Libudzisz

Department of Biotechnology and Food Sciences, Institute of Fermentation Technology and Microbiology, Technical University of Lodz, ul. Wolczanska 171/173, 90-924 Lodz, Poland

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Abstract

Some intestinal bacteria can produce many genotoxic, mutagenic and carcinogenic substances. The major products of the bacterial aromatic amino acids fermentation—phenolic and indolic compounds which are responsible for colon cancer development are accumulated in the colon. The effect of phenol, *p*-cresol and indole (2, 20 and 100 μg/ml doses) on growth and survival of four strains of intestinal lactic acid bacteria was studied. Growth of bacteria was not affected by any of the concentrations of phenol and *p*-cresol tested. The growth of 2 strains was slightly inhibited by 100 μg/ml of indole. There was no influence of phenol and *p*-cresol on survival of lactic bacteria until 120 h and specific reaction to carcinogens depending on strain was observed after that incubation time. Indole concentrations 20 and 100 μg/ml appeared to be toxic for all tested strains but just after 24, 48 or 72 h of incubation depending on the strain. In total, 2 μg/ml of indole had a very little effect.

Keywords: Phenol; *p*-Cresol; Indole; Toxicity; Lactic acid bacteria

1. Introduction

The human gastrointestinal tract is inhabited by a very complex ecosystem of micro-organisms either facultative or strict anaerobes, of which the strict anaerobes are dominant. Within the colon the bacterial concentration is between 10^{10} and 10^{11} cells/g intestinal contents comprising at least 400–500 bacterial species including the strict anaerobes: *Bacteroides* sp., *Peptostreptococcus* sp., *Clostridium* sp., *Eubacterium* sp., *Fusobacterium* sp.; as well as the facultative anaerobic or aerobic group: *Bacillus* sp., *Enterobacteriaceae*, *Pseudomonas* sp., *Staphylococcus* sp. and the lactic acid bacteria (LAB) group: *Bifidobacterium* sp., *Lactobacillus* sp., and *Streptococcus* sp. *Enterococcus* sp. [1,2].

Intestinal bacteria play a significant role in nutrition and prevention of diseases. They help metabolize undigested polysaccharides, ''resistant starch'' and fibre what leads to production of SCFA (short-chain fatty acids) butyrate, acetate and propionate, which can reveal several healthful effects [3]. Moreover, intestinal bacteria are a crucial line of resistance to colonisation by exogenous microbes and as a result they protect from invasion of tissues by pathogenic bacteria—its the barrier effect [4]. What is more, they can produce vitamins and affect the host immune response. Intestinal bacteria can be either beneficial or harmful for human health. Beneficial bacteria, such as LAB, protect the intestinal tract from proliferation of harmful bacteria. In healthy subjects, the bacterial populations of the intestine are well balanced, and beneficial bacteria dominate [5].

The harmful bacteria can be responsible for colon cancer initiation by activation genotoxic, carcinogenic and tumour—promoting substances and converting procarcinogens to DNA reactive agents [6]. The harmful bacteria synthetize several enzymes (β-glucuronidse, β-glucosidase, nitroreductase, tryptophanase) involved in producing carcinogens (nitrosamines, heterocyclic aromatic amines, secondary bile acids, indoles, phenols, skatoles, cresols) which can cause colon cancer. Indolic and phenolic compounds, such as indole, phenol and *p*-cresol are products of aromatic amino acids (tyrosine, phenylalanine,

*Corresponding author. Tel.: +48 42 631 34 75; fax: +48 42 636 59 76.
E-mail address: adriana_nowak13@wp.pl (A. Nowak).

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tryptophan) metabolism descending from diet proteins [7–10]. Beside a variety of different diseases, all the compounds contributes to colon carcinogenesis [12,13]. It was shown that phenol is co-carcinogen, because enhances nitrosation of secondary amines (dimethylamine) by nitrite [14]. Indole is not a direct—acting mutagen, but seems to act like promoter of colon cancer and also enhances nitrosation [15–17]. Intestinal bacteria involved in protein degradation include *Escherichia coli*, *Proteus* sp., *Enterococcus faecalis*, *Staphylococcus* sp., *Bacteroides fragilis*, *Fusobacterium* sp. and *Clostridium* sp. [9,11].

Intestinal LAB may inhibit colon cancer, but the precise mechanism is unknown. It can include: binding of carcinogens, production of anticarcinogenic metabolites, degradation of potential carcinogens, increase in immune response, alteration of physicochemical conditions in the colon and faecal enzyme activity, stimulation of protective enzymes [6,18].

The aim of this study was to evaluate the ability of intestinal lactobacilli to grow and survive in the presence of three carcinogens: phenol, *p*-cresol and indole and to estimate if there are any differences in reaction between strains of LAB to the compounds.

2. Materials and methods

In this work, the following bacterial strains were used: *Lactobacillus plantarum* WL from the own collection of Institute of Fermentation Technology and Microbiology (LOCK 105), Technical University of Lodz, Poland; *Lactobacillus casei/paracasei* KNE1 from Institute of Animal Reproduction and Food Sciences, Polish Academy of Sciences, Olsztyn, Poland; *L. casei* J/III from Institute of Industrial Biotechnology and Food Microbiology, University of Warmia and Mazury in Olsztyn; *L. casei* BD from Chemical—Technological University, Prague, the Czech Republic. All intestinal strains of LAB chosen appeared to be very resistant to low pH and bile salts, so they are able to survive in gastrointestinal tract during the transit time [Motyl, 2002—unpublished data].

2.1. Influence on growth of lactic acid bacteria

Phenol, indole and *p*-cresol were purchased from Sigma—Aldrich. To obtain stock solutions, phenol and *p*-cresol were diluted in water, indole was diluted in methanol, to obtain a concentration of 0.5%.

To define the influence of carcinogens on growth of LAB, the cells (3% inoculum) were incubated 24 h in MRS broth (BTL, Poland) containing glucose (20 g/l) at 37 °C in anaerobic incubator (WTBinder) in the presence of 5% v/v CO_2, with the addition of final concentrations of phenol, *p*-cresol and indole, which were: 2, 20 and 100 μg/ml. The control sample for each strain was culture of bacteria without toxic substances. To evaluate the influence of mutagens on growth of bacteria the Koch's plate method was performed. One milliliter of each of the culture was diluted in dilution liquid (0.85% NaCl) and the dilutions were poured on the plates (for each dilution from 4 to 8 plates) along with solid medium which was MRS with addition of 1.5% of agar. The cell number was estimated, at 0 h time and after 24 h of incubation. Every concentration was fourfold plated and for each one the standard deviation was estimated.

2.2. Influence on survival of lactic acid bacteria

After 24 h incubation in MRS broth at 37 °C in anaerobic incubator the cells were centrifuged (10,000*g* for 10 min), washed in 20 ml of sterile phosphate buffer (pH = 6.2–6.3), and centrifuged again. After that, the cells were suspended in the buffer with 2, 20 or 100 μg/ml concentrations of each mutagen and incubated 168 h (7 days) in 37 °C in anaerobic conditions. A control sample was cell suspension without mutagen.

In order to evaluate the survival of lactobacilli, the pour plate method was used. Every 24 h bacteria were plated using MRS broth (with addition of 1.5% agar) and incubated in 37 °C in anaerobic conditions. The colonies were counted after each 48 h incubation and as a result survival curves for each strain and mutagen concentration were obtained.

3. Results

3.1. Influence on growth of lactic acid bacteria

There was no reduction in the number of colonies of lactic bacteria on the plates (after 24 h incubation with carcinogens) as compared with the control (Table 1). None of the 2, 20 and 100 μg/ml concentrations of phenol and *p*-cresol influenced the growth of any strain of intestinal LAB tested. 2 and 20 μg/ml of indole did not affected the growth of lactic bacteria strains, but 100 μg/ml dosage of indole appeared to be slightly toxic for two strains *L. paracasei/casei* KNE1 ($\log \mathrm{CFU/ml} = 9.3 \pm 0.16$) and *L. plantarum* WL ($\log \mathrm{CFU/ml} = 9.4 \pm 0.06$) in comparison to the control sample ($\log \mathrm{CFU/ml} = 9.7 \pm 0.03$). Thus, all *Lactobacillus* strains were able to grow in the presence of phenol and *p*-cresol at doses 2, 20 and 100 μg/ml as well as at 2 and 20 μg/ml of indole. Some lactobacilli were resistant even at 100 μg/ml of indole.

3.2. Influence on survival of lactic acid bacteria

As is shown in Figs. 1–3, depending on the strain and dose, phenol, *p*-cresol as well as indole, had distinct impacts on survival of *Lactobacillus* strains. In the presence of all the concentrations of phenol, the number of living cells of *L. casei* BD and J/III was changing equally with the control up to 168 h. In the case of *Lactobacillus* strains KNE1 and WL, after 120 h a slight influence of phenol was observed and the concentration of surviving bacteria decreased with increasing phenol concentrations (Fig. 1).