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Short communication

Effect of kynurenic acid on the viability of probiotics *in vitro*

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Abstract:
Probiotics are bacteria that are commercially available as dietary supplements. One of the important properties of probiotics is their ability to survive in the intestine. Recent evidence has identified kynurenic acid (KYNA) as a bactericidal constituent of intestinal fluid. These data led us to study the influence of KYNA on the viability of selected probiotics. We found that KYNA supported the growth of bacteria in the probiotics Acidolac (*Lactobacillus acidophilus, Bifidobacterium)* and Lakcid Forte (*Lactobacillus rhamnosus*) or retarded the growth of bacteria from the Acidolac, BioGaia (*Lactobacillus reuteri* Protectis), Dicoflor (*Lactobacillus rhamnosus* GG), Lacium (*Lactobacillus plantarum*) and Trilac (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus, *Bifidobacterium animalis* subsp. lactis) probiotics depending on its concentration. KYNA did not affect the viability of bacteria from the probiotic Linex (*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. lactis BB-12). Our results suggest a potential role of KYNA in the regulation of bacterial growth in the digestive system.

Key words:
probiotics, kynurenic acid, viability, *in vitro*

Introduction

Probiotics are defined by the FAO and the WHO as 'living microbial supplements that beneficially affect the host animal by improving its intestinal microbial balances' [2]. Most probiotics are bacteria similar to those naturally found in the human gut, and they are considered to naturally protect against numerous diseases (see [4] for review). Probiotics are industrially produced for both nutritional and pharmaceutical use. The consumption of these products is rising around the globe.

Kynurenic acid (KYNA) is known as a neuroprotectant, an antagonist of ionotropic glutamate receptors [1] and an antagonist of cholinergic α-7 nicotinic receptor [3]. KYNA is also an agonist of orphan G-protein-coupled receptor (GPR35), which is predominantly located on enterocytes and various subpopulations of immune cells [11]. Recently, KYNA was shown to be a constituent of intestinal fluid. It was found that the concentration of KYNA increases along the digestive system, reaching a concentration of 16 μM in the distal part of the rat small intestine [6, 12]. A previous study from our laboratory found that

exposure of bacteria to KYNA in micromolar concentrations resulted in a bactericidal effect. Therefore, we hypothesized that KYNA may help to control bacterial growth *in vivo* [5]. In this study, we focused on the effect of KYNA on the viability of selected probiotics *in vitro*.

Materials and Methods

Substances

Kynurenic acid (KYNA) was obtained from Sigma-Aldrich, St. Louis, MO, USA.

Bacteria

Lactobacillus acidophilus ATCC4356 and *Lactococcus lactis* subsp. cremoris ATCC19257 were purchased from MicroBiologics, Inc. (St. Cloud, MN, USA). The following probiotics were used: Acidolac (*Lactobacillus acidophilus, Bifidobacterium*; Polpharma, Stargard Gdański, Poland), BioGaia (*Lactobacillus reuteri* Protectis; BioGaia AB, Stockholm, Sweden), Dicoflor (*Lactobacillus rhamnosus* GG; Dicofarm, Roma, Italy), Lacium (*Lactobacillus plantarum*, NP Pharma, Ostrów Mazowiecki, Poland), Lakcid Forte (*Lactobacillus rhamnosus*; Biomed, Lublin, Poland), Linex Forte (*Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. lactis BB-12; Lek Pharmaceuticals, Ljubljana, Slovenia) and Trilac (*Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. bulgaricus, *Bifidobacterium animalis* subsp. lactis; Allergon AB, Angelholm, Sweden).

Conditions of bacterial growth

The cultures of commercially available probiotic bacteria were subcultured (three times in 5 ml of non-acidified Lacobacilli MRS broth; pH 6.8, Difco, Detroit, MI, USA) for 11–12 h at 37°C. The overnight cultures of the probiotic bacteria and the reference strains *Lactobacillus acidophilus* ATCC4356 and *Lactococcus lactis* subsp. cremoris ATCC19257 were used to inoculate Lactobacilli MRS broth (to a final concentration of 10^3 CFU/ml) supplemented with the appropriate concentration of KYNA and were incubated at 37°C for 48 h. Cell growth was estimated in 96-well plates (Kartell, Noviglio, Italy) by recording changes in optical density at 650 nm (OD_{650}) using a microplate reader (ASYS UVM 340, Asys Hitech, Eugendorf, Austria). The viability of the cultures was controlled by determination of colony forming units in MRS agar plates incubated anaerobically (bioMerieux GENbag microaer; BioMerieux SA, Marcy-l'Etoile, France) for 48 h at 30°C.

Statistics

Data are presented as the mean values and standard deviations (SDs). Statistical analysis was performed using one-way ANOVA and the Tukey-Kramer *post-hoc* test. Significance was accepted at $p < 0.05$.

Results

The growth of bacteria from the Acidolac and Lakcid Forte probiotics was enhanced in the presence of 0.5 μM KYNA (Tab. 1). The viability of bacteria from the BioGaia probiotic was decreased in the presence of KYNA at concentrations ranging from 5 to 500 μM. The higher KYNA concentrations of 250 and 500 μM decreased the growth of bacteria sourced from the Acidolac, Dicoflor, Lacium and Trilac probiotics (Tab. 1). The viability of the bacteria from the Linex Forte and the Lakcid Forte probiotics was not lowered by exposure to the concentrations of KYNA studied (Tab. 1).

The growth of the reference strain, *Lactobacillus acidophilus* ATCC4356, was not affected by KYNA in concentrations ranging from 0.5 to 500 μM, whereas the presence of 500 μM KYNA decreased the growth of *Lactococcus lactis* subsp. cremoris ATCC19257 (Tab. 1).

Discussion

In this study, we found that KYNA at concentrations ranging from 0.5 to 500 μM affect the viability of bacteria present in probiotics *in vitro*. The growth of bacteria from the Acidolac and Lakcid Forte probiotics was enhanced in the lowest tested concentration of

Tab. 1. Growth of the probiotic bacteria and reference strains in the presence of kynurenic acid (KYNA)

Strain/Preparation	Kynurenic acid (μM)				
	0.5	5	50	250	500
Lactobacillus acidophilus ATCC4356	105.1 ± 7.1	100.7 ± 9.2	97.0 ± 2.5	96.5 ± 5.5	93.0 ± 6.4
Lactococcus lactis subsp. cremoris ATCC19257	108.8 ± 4.1	104.2 ± 2.7	99.0 ± 2.5	96.4 ± 6.0	87.0 ± 9.1*
Acidolac	112.6 ± 6.4*	99.0 ± 5.1	92.6 ± 2.6	89.5 ± 2.5*	86.7 ± 4.5*
BioGaia	106.3 ± 7.9	87.8 ± 7.4*	73.7 ± 5.3*	69.1 ± 4.6*	63.4 ± 6.2*
Dicoflor	100.2 ± 2.7	97.4 ± 3.6	96.8 ± 7.3	88.6 ± 6.0*	87.1 ± 7.5*
Lacium	99.3 ± 4.2	92.5 ± 7.2	90.6 ± 6.4	89.1 ± 5.9*	86.9 ± 6.7*
Lakcid Forte	114.9 ± 7.6*	99.1 ± 4.1	97.9 ± 7.8	94.1 ± 6.6	93.9 ± 5.9
Linex Forte	106.3 ± 6.3	94.6 ± 7.2	94.0 ± 6.3	92.2 ± 4.9	90.8 ± 7.8
Trilac	98.0 ± 4.1	94.3 ± 3.2	95.8 ± 4.0	86.5 ± 10.3*	88.3 ± 7.6*

Growth of bacteria is expressed as a percentage of the control culture. The mean value of the optical density (OD_{650}) in the respective controls (without KYNA) was considered as 100%. Data are the mean ± SD from six experiments. Statistical analysis was performed using one-way ANOVA and the Tukey-Kramer *post-hoc* test; * $p < 0.05$ *vs.* the respective control (100%)

KYNA (0.5 μM). In contrast, the viability of bacteria from the BioGaia probiotic was decreased in the presence of KYNA at a wide range of concentrations (5–500 μM), and the growth of bacteria present in the Acidolac, Dicoflor, Lacium and Trilac probiotics was lowered in the presence of KYNA in concentrations of 250 and 500 μM. The tested concentrations of KYNA did not affect the viability of bacteria from the Linex Forte probiotic. All of the tested probiotic preparations contained at least one strain of *Lactobacillus*. We found that the viability of the reference strain *Lactobacillus acidophilus* ATCC4356 was not affected by KYNA. The growth of another reference strain, *Lactococcus lactis* subsp. cremoris ATCC19257, which is a common lactic acid bacterium that is widely used as a starter in the dairy industry [8, 9], was slightly decreased by KYNA, but only in the highest concentration tested. These results agree with our previous finding that exposure of bacteria to KYNA at micromolar concentrations resulted in a bactericidal effect on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 [5].

In the present study, lower concentrations of KYNA, corresponding to "physiological" values were investigated. The concentration of KYNA in the rat intestine increases from 1.5 μM in the jejunum to 16.1 μM in the distal ileum [6]. The growth of bacteria from the BioGaia probiotic was clearly decreased in this range of concentrations. It should be noted, however, that the reported concentration of KYNA was investigated in rats deprived of food for 24 h [6] and the exact concentration of KYNA in the intestinal contents composed of both intestinal fluids and food has not yet been measured. It is known that KYNA is present in human bile obtained from the gallbladder (0.8 μM) and the extrahepatic bile duct (0.3 μM) [7]. KYNA has been found in the bile (1.1 μM) and pancreatic juice (0.8 μM) of pigs [7]. The high concentration of KYNA in bile and pancreatic juice indicates the contribution of these sources to the maintenance of a high KYNA content in the digestive system [7]. On the other hand, the presence of this compound in dietary components has been reported, and the highest concentrations have been found in potato (688 pmol/g), broccoli (2214 pmol/g) and honeybee products (948–8573 pmol/g) [10]. Some food products also contain a very low amount of KYNA, such as red paprika (6 pmol/g), fish (8 pmol/g), apples (12 pmol/g), beef (17 pmol/g) and pork (19 pmol/g) [10]. Thus, it can be expected that normally nourished subjects will display a final concentration of intestinal KYNA that may vary substantially from the concentration reported in fasting rats [6]. Therefore, we also investigated the influence of KYNA at the low concentration of 0.5 μM. Unexpectedly, the growth of probiotic strains present in the Acidolac and Lakcid Forte samples was improved under this condition.

Probiotics are commercially available as dietary supplements in the form of capsules, tablets, powders and other formulations. To reach the intestinal tract,

probiotics must survive the gastric and bile acids and colonize the host epithelium of the intestine. Here, we showed that KYNA, a constituent of intestinal fluid, may affect the viability of probiotics.

Taken together, our findings support a potential role of KYNA in the regulation of bacterial growth in the digestive system.

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