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Research Article

Binding of heterocyclic aromatic amines by lactic acid bacteria: Results of a comprehensive screening trial

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Aim of the present study was a comprehensive investigation of the detoxification capacities of lactic acid bacteria (LAB) towards heterocyclic aromatic amines (HCA) formed during cooking of meat. It has been postulated that LAB prevent genotoxic and/or carcinogenic effects of HCA in laboratory rodents and humans via direct binding mechanisms. We measured the removal of the most abundant cooked food mutagens (AαC, PhIP, IQ, MeIQx, DiMeIQx) by eight LAB species. From each species, twelve strains were tested in liquid binding experiments with HPLC coupled with coulometric electrode array detection. The highest removal rates were observed with the representatives of the *L. helveticus* and *S. thermophilus* groups, which were seven to eight times more effective than L. kefir and L. plantarum. Strong and statistically significant differences were seen in the binding behaviour of the individual amines, the ranking order of detoxification being AαC > DiMeIQx > MeIQx > IQ > PhIP. Results of Salmonella/microsome assays with strain TA98 showed that the binding of AαC and PhIP to LAB correlates with the reduction of their mutagenic activities. This study may contribute to the development of strategies concerning the adverse health effects of HCA utilizing highly protective LAB for the production of fermented foods.

Keywords: Detoxification / Heterocyclic aromatic amines / Lactic acid bacteria

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1 Introduction

Heterocyclic aromatic amines (HCA) are pyrolysis products of amino acids that are formed during the cooking of meats. A number of studies indicate that these compounds are involved in the etiology of colon cancer in humans [1, 2]. Therefore, strong efforts have been made during the last decades to identify dietary factors that protect against DNA-damage caused by HCA (for review see [3–6]). For example, it was shown that tea catechins as well as coffee diterpenoids and glucosinolates in Brassicas elicit chemoprotective properties via interaction with the metabolism of HCA [7]; another important mechanism is direct binding, which was found with pigments, fibers and bacteria [8–23].

The inactivation of HCA by lactic acid bacteria (LAB), which are contained in fermented foods and are also a part of the intestinal human microflora, has been reported in several articles and it has also been shown in animal studies that the bacteria prevent induction of DNA-damage and preneoplastic lesions by certain HCA ([24, 25], for review see [11]). In a recent investigation, it was also found that the induction of DNA-migration in colon cells caused by the consumption of HCA-containing fried meats, can be reduced by the uptake of foods which contain Brassica vegetables and LAB [26] and it was also shown in human as well as in animal studies that the consumption of LAB reduces the urinary excretion of HCA [27, 28].

All currently available studies with LAB and HCA were carried out with a low number of strains and with a few selected HCA. Therefore, they do not provide firm information about the differences of the binding capacities of different genera and species. Furthermore, some of the HCA which were studied, such as tryptophan (TrpP1, TrpP2) and

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Abbreviations: **AαC**, 2-amino-9H-pyrido[2,3-b]indole; **CEAD**, coulometric electrode array detection; **DiMeIQx**, 2-amino-3,4,8-trimethyl-imidazo[4,5-f]quinoxaline; **HCA**, heterocyclic aromatic amine(s); **IQ**, 2-amino-3-methyl-imidazo[4,5-f]quinoline; **LAB**, lactic acid bacteria; **MeIQx**, 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline; **PhIP**, 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine

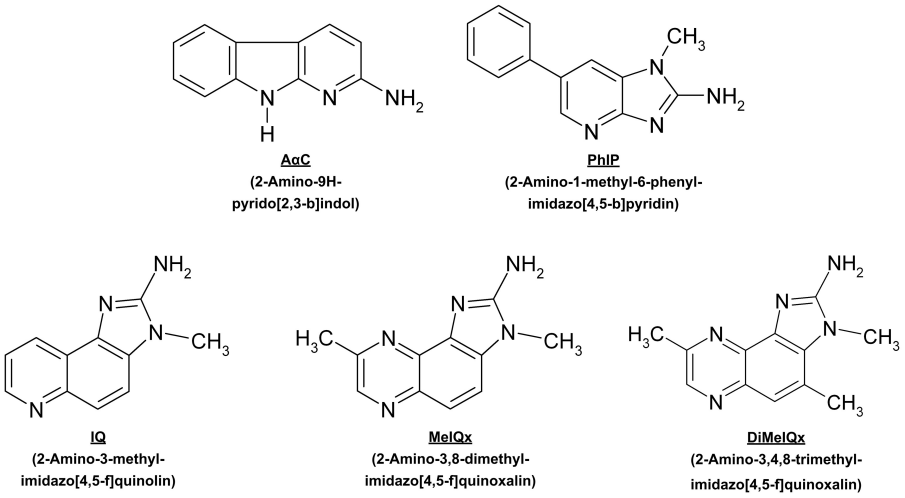

Figure 1. Structural formulas of the different heterocyclic aromatic amines.

glutamic acid pyrolysates (GluP1) are contained only in minor amounts in fried meats [29].

The aim of the present study was to conduct a comprehensive investigation concerning the binding capacities of different LAB species that are either contained in fermented foods or in the human gastrointestinal tract. In order to find out which groups have the highest inactivation capacities for HCA, 12 representatives of eight important species (*B. longum, L. acidophilus, L. bulgaricus, L. casei, L. helveticus, L. kefir, L. plantarum* and *S. thermophilus*) were tested under identical experimental conditions. All experiments were carried out with five HCA, namely 2-amino-9H-pyrido[2,3-b]indole (AαC), 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP), 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethyl-imidazo[4,5-f]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethyl-imidazo[4,5-f]quinoxaline (DiMeIQx), which are the most frequently found cooked food mutagens in fried red meat [30]. To verify that the effects, which were measured chemically with HPLC-CEAD, reflect the prevention of DNA-damage (which is considered to play a key role in the induction of cancer by these compounds), additional bacterial mutagenicity tests (*Salmonella*/microsome assays) were conducted with selected LAB strains. TA98 was used in these experiments, as it is most sensitive towards the mutagenic effects of HCA as the other *Salmonella* tester strains used in routine testing of compounds [31].

2 Materials and methods

2.1 Chemicals and media

IQ, 8-MeIQx, 4,8-DiMeIQx, 4,7,8-TriMeIQx, PhIP and AαC were purchased from the Nard Institute (Amagasaki, Japan). The molecular structures and full names are shown in Fig. 1. HCA stock solutions were prepared by dissolving 10 mg of each compound in 10 mL methanol in an ultrasonic bath. These stock solutions were used to prepare concentrations of 20 μg/mL of each HCA in bi-distilled water. All solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA, USA); glucose-6-phosphate, NADP, inorganic and organic salts were from Sigma (St. Louis, MO, USA). De Man-Rogosa-Sharpe (MRS) broth powder, Nutrient Broth Nr.1 and BactoAgar were purchased from Oxoid (Basingstoke, UK), Elliker broth powder was obtained from Difco (Detroit, MI, USA) and Aroclor 1254-induced rat liver S-9 homogenate from Biomedica (Vienna, Austria).

2.2 Lactic acid bacteria

LAB strains were kindly provided by Lactosan (Vienna, Austria) and the Institute of Milk Hygiene, Milk Technology and Food Science (University of Veterinary Medicine Vienna, Vienna; Austria) and had been isolated from the human colonic microflora or from fermented foods. The bacteria were coded (LA from Lactosan, MW from the University of Veterinary Medicine) and stored deep-frozen at –80°C and cultured in different media. *L. acidophilus, L. bulgaricus, L. casei, L. helveticus* and *L. plantarum* were grown in MRS broth, *S. thermophilus* in Elliker media and *L. kefir* in non-autoclaved MRS. For *B. longum* MRS broth containing 0.03% w/v L-cysteine was used. All strains were grown anaerobically using an Anaerocult A system from Merck (Darmstadt; Germany). Cell densities were determined with turbidimetry (600 nm, for details see [32–34]).

2.3 *Salmonella* tester strain

All experiments were conducted with the *Salmonella typhimurium* frame shift tester strain TA98, which was obtained from B.N. Ames (University of Berkeley, CA, USA). The strain was stored deep-frozen at –80°C and cultivated on master plates, which were restreaked every 6 weeks. The

characteristics of the tester strain TA98 (*ΔuvrB, rfa*, pKM101) were confirmed before the start of the experiments as described by Maron and Ames [35].

2.4 Binding assays

The binding assays were carried out according to the protocol developed by Gerbl *et al*. [36] with minor modifications. Briefly, LAB strains were grown in liquid overnight cultures. After the determination of the cell densities, the suspensions were centrifuged (12 000 rpm, 10 min) and the pellets resuspended in physiological saline. Of these suspensions (containing approximately 4×10^9 cfu), 250 μL were incubated with 50 μL of a HCA stock solution (containing 1.0 μg of each amine) and 700 μL of sodium phosphate buffer (50 mM, pH 5.0). The mixtures were incubated at 37°C with soft agitation (500 rpm) for 4 h. Subsequently, the mixtures were centrifuged (12 000 rpm, 10 min, 4°C) and the concentrations of the unbound HCA quantified in the supernatants.

The samples (supernatants or standard solutions) (200 μL) were diluted with 800 μL mobile phase and 20 μL of this solution was injected into a HPLC system, which consisted of two high-pressure pumps (ESA model 420, Chelmsford, MA; USA), a gradient mixer (Contron model M800, Zurich; Switzerland), and an autosampler (ESA model 465). The analytical column (alpha-chrome C8 2×150 mm, fitted with a precolumn of the same material 2×10 mm, Upchurch Scientific, Oak Harbour, WA, USA) was thermostated at 30°C. The HCA were eluted isocratically with a flow rate of 0.6 mL/min. The mobile phase consisted of acetonitrile/methanol/buffer/distilled water (10:15:10:65; v/v/v/v). The buffer was prepared by dissolving 3.7 g sodium acetate and 4.9 g trichloroacetic acid in 70 mL water; subsequently, 30 mL glacial acetic acid was added. Electrochemical detection was carried out with a coularray electrode system (ESA model 5500). The 4,7,8-TriMeIQx was used as an internal standard. The potentials of the working electrodes were adjusted to +100, +375, +400, +425, +450 and +525 mV, at which AαC was detected at +375 mV, PhIP at +525 mV and the other compounds at +400 mV. Peak heights were determined with ESA CoularrayWin software (Chelmsford, MA, USA). Concentrations were calculated with calibration curves, which were established for each individual amine. All measurements were performed in triplicate.

2.5 *Salmonella*/microsome assays

The antimutagenic effects of selected LAB strains towards PhIP and AαC were investigated in bacterial mutagenicity assays. Prior to experiments with the LAB strains, dose-response curves were established with the two HCA in plate incorporation assays. Subsequently, cell suspensions of LAB were preincubated with the two HCA. The mixtures contained 700 μL sodium phosphate buffer (50 mM, pH 5.0), 250 μL LAB-suspensions in physiological saline, 50 μL HCA solutions containing 1.0 μg of AαC or PhIP, respectively, (total volume 1.0 mL containing 1.0 μg of the respective HCA and 10^9 cfu) and were incubated at 37°C. After 4 h, the suspensions were centrifuged (12 000 rpm, 10 min) and 100 μL (for PhIP samples) or 200 μL (for AαC samples) of the supernatants were mixed with 100 μL of a stationary phase overnight culture of strain TA98 (ca. 1–2×10^8 cells), 0.5 mL S-9 mix prepared according to the standard recipe of Maron and Ames [35] and 2.0 mL of top agar, and were transferred to histidine-free selective media plates. In all experiments, solvent control plates were included, in which HCA-solutions were replaced by sodium phosphate buffer. The positive control doses of AαC and PhIP were 200 and 100 ng/ plate, respectively. After incubation in the dark at 37°C for 2 days, the number of his^+ revertants was counted manually. For each experimental point, three plates were prepared in parallel.

2.6 Statistics

To assess the significance of differences between the eight species regarding the overall HCA binding capacities the Student's *t*-test for two independent samples was used; for differences between the five HCA, Student's *t*-tests for paired samples were conducted. To justify the use of the *t*-test, the normal distribution of the data was analyzed by the use of the F-test. The Mann-Whitney test for two independent samples was applied to compare the results of the chemical binding studies with those obtained in the *Salmonella*/microsome assay. All tests were considered significant with $p = 0.05$.

3 Results

The results of the chemical binding assays with the individual compounds are depicted in Figs. 2a–e. The x-axis indicates the different species (from each group 12 strains were tested), the y-axis shows the decrease of the HCA-concentrations after incubation in percent. The strongest inactivation of PhIP was seen with the *L. helveticus* group (reduction by 78%), followed by *S. thermophilus* (reduction by 50%) and *L. bulgaricus* (reduction by 44%); relatively weak effects were observed with *L. kefir* strains (reduction by 7%). A similar pattern of binding was seen with AαC but the overall inactivation was generally higher and *S. thermophilus* strains bound the aminocarboline more efficiently than the imidazopyridine. Figures 2c, d and e show the results obtained with IQ and the two quinoxalines. The overall binding capacities decreased in the order DiMeIQx > MeIQx > IQ.

Results of Fig. 2 were mathematically transformed to calculate the total binding capacities of the individual spe-

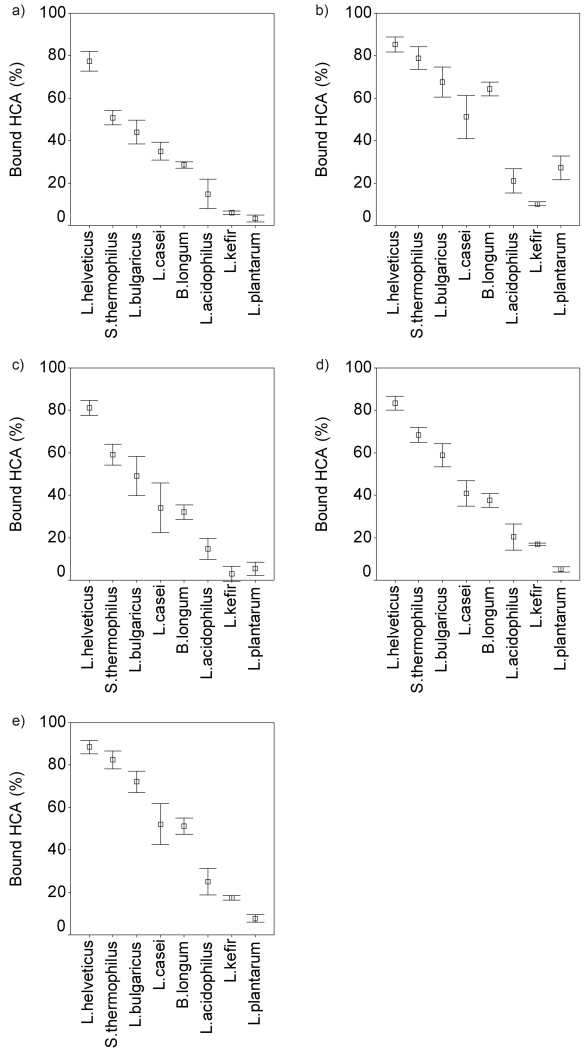

Figure 2. Binding capacities of eight different LAB species towards PhIP (a), AαC (b), IQ (c), MeIQx (d) and DiMeIQx (e). From each group, 12 different strains were tested. The measurements were carried out by HPLC-CEAD as described in Section 2. With each individual strain, three measurements were made in parallel. The y-axis indicates the reduction of the HCA-concentrations (in %) after incubation with LAB for 4 h.

cies towards all HCA tested, which are summarized in Fig. 3. Again, the most effective species (inactivation range between 72 and 89%) was *L. helveticus*, followed by *S. thermophilus*; the lowest effects were seen with *L. plantarum* and *L. kefir* strains (binding between 4 and 13%). The statistical analyses showed that the binding capacities of most groups differ significantly ($p = 0.05$) from each other; exceptions were the associations between *L. casei*/*B. longum* ($p = 0.83$) and between *L. plantarum*/*L. kefir* ($p = 0.62$).

Figure 4 summarizes the binding capacities of all LAB strains tested in the present study for the individual HCA.

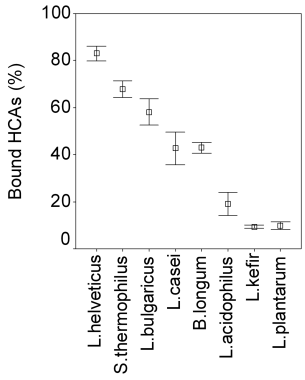

Figure 3. Total binding capacities of several LAB species to the sum of five HCA. Results from Fig. 2 were mathematically transformed to receive the total binding capacities to five HCA of the different species. The y-axis shows the reduction of the concentration of all HCA (in %).

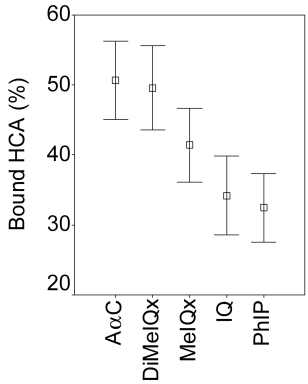

Figure 4. Differences of the binding efficiencies of 96 LAB strains towards the five HCA. From each amine, the adsorption values from Fig. 2 were used to calculate the overall binding capacities of all 96 strains tested. The y-axis shows the reduction of the HCA concentrations (in %).

In general, statistically significant differences in the binding behavior of the five amines were found except for IQ/PhIP ($p = 0.07$) and AαC/DiMeIQx ($p = 0.32$). It can be seen that the highest binding was found with AαC and DiMeIQx, the weakest effects were observed with PhIP and IQ.

In order to confirm the reproducibility of the individual experiments, several repetitive experiments were conducted with individual strains. Figure 5 shows the results of three repetitive experiments conducted with the *L. acidophilus* MWLA2 strain. It can be seen that the variations of the independent measurements were ≤9%.

Figure 6 depicts the results of *Salmonella*/microsome assays with PhIP and AαC. With both compounds, a dose-dependent induction of his^+ revertants was observed in strain TA98 in presence of liver S-9 homogenate. Based on the results of the dose-response experiments, concentrations of 0.1 µg PhIP and 0.2 µg AαC per plate were used.

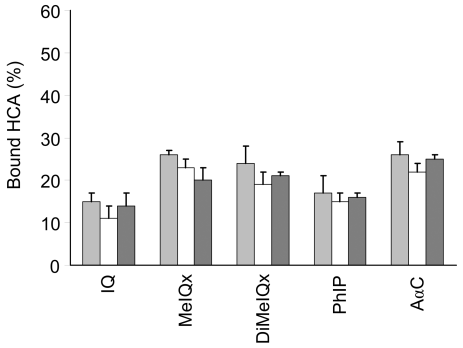

Figure 5. Comparison of results obtained with strain *L. acidophilus* MWLA2 in binding experiments with the five HCA. The bars depict means ± SD obtained in three independent experiments.

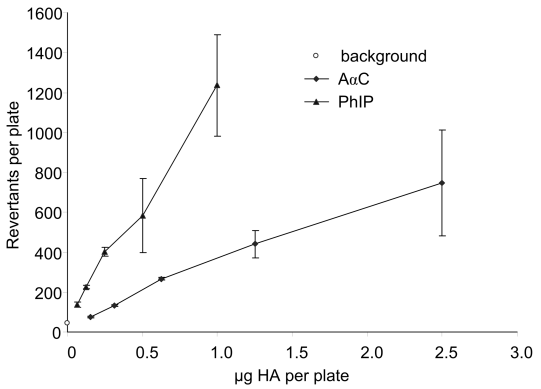

Figure 6. Induction of his$^+$ revertants in strain TA98 by PhIP and AαC in plate incorporation assays. The experiments were carried out as described by Maron and Ames [35]. For each experimental point, three plates were prepared in parallel.

The activities per µg HCA (in the concentration range used in the subsequent experiments) were calculated by linear regression analyses and were 1413 revertants/µg for PhIP ($R^2 = 0.999$) and 365 revertants/µg for AαC ($R^2 = 0.979$). These findings are similar to results reported in the literature [37, 38].

In order to investigate if the incubation of the two HCA with selected LAB strains, which have been used in the screening trial, results in a decrease of their mutagenic activities, experiments were carried out with strains which differed strongly in their binding properties (*L. plantarum* MWLP3, *S. thermophilus* MWST7, *L. casei* MWLC6, *L. helveticus* MWLH1, *L. helveticus* MWLH2). The bacteria were incubated with the two amines under identical experimental conditions as those used in the chemical binding experiments (*i.e.* 10^9 cfu/mL, 4 h incubation). The results of a representative experiment are summarized in Table 1; in a second trial, identical results were obtained (data not shown).

Table 1. Results of a representative experiment concerning the inhibition of induction of his$^+$ revertants by PhIP and AαC in *Salmonella* strain TA98 after incubation with selected LAB strains[a)]

Test condition	LAB strain/code	Total number of revertants per plate[b)]	Reduction of mutagenic activity in %[c)]	Reduction of the amine concentration in %[d)]
Negative control	none	33 ± 5	(100)	(100)
PhIP	None (positive control)	315 ± 12	(0)	(0)
	L. plantarum MWLP3	312 ± 14	1 ± 4	2 ± 1
	S. thermophilus MWST7	201 ± 12*	40 ± 4**	60 ± 2
	L. casei MWLC6	221 ± 3*	36 ± 3**	29 ± 2
	L. helveticus MWLH1	76 ± 6*	85 ± 2	84 ± 1
	L. helveticus MWLH2	74 ± 5*	85 ± 4	83 ± 1
AαC	None (positive control)	118 ± 3	(0)	(0)
	L. plantarum MWLP3	74 ± 5*	52 ± 4**	38 ± 2
	S. thermophilus MWST7	47 ± 10*	84 ± 8**	97 ± 0
	L. casei MWLC6	89 ± 4*	34 ± 3**	20 ± 2
	L. helveticus MWLH1	34 ± 5*	99 ± 4**	84 ± 0
	L. helveticus MWLH2	32 ± 2*	101 ± 2**	83 ± 1

a) Solutions of the amines (1 µg/mL of PhIP or AαC) were incubated with the different LAB strains (the final concentration 10^9 cfu/mL) for 4 h. Subsequently, the mixtures were centrifuged and the supernatants (0.2 mL of the PhIP mixtures and 0.1 mL of the AαC mixtures) were plated with the indicator bacteria and S-9 mix as described in Section 2. Positive controls contained 0.1 µg of PhIP and 0.2 µg AαC per plate in the corresponding experiments.

b) Revertants numbers are means ± SD of three plates by experimental point. * indicate statistical significance (p =0.05; Student's *t*-test for independent samples) compared to the effect with the amine in absence of LAB; ** indicate statistical significant differences in the results of the mutagenicity experiments and the findings of the chemical binding tests (p = 0.05; Mann-Whitney test for independent samples).

c) The decrease of the mutagenic activity was calculated on the basis of the difference of the revertants/plate (revertants of the negative control were subtracted) seen under identical conditions with or without pre-incubation of the HCA with LAB.

d) Numbers indicate the reduction of the concentrations in %, which were found in the analytical screening trials.

In general, a good correlation between the reduction of the HCA-induced mutagenicity and the binding behavior of the individual strains was observed, *i.e.* strains that decreased the HCA concentrations to high extents caused also the strongest antimutagenic effects. However, correlations of the effects of the individual strains were in most cases not significant, as the reduction of the mutagenic effects was in most cases higher as expected on the basis of the chemical binding experiments. The strongest differences were seen with in this regard with *L. helveticus* MWLH1 and *L. helveticus* MWLH2.

4 Discussion

As mentioned above, the present study is the first comprehensive report concerning the inactivation of HCA by different LAB species.

The statistical analyses show that distinct differences exist in the binding behavior of the bacteria towards structurally different amines. In the present study, the binding capacities of the LAB declined in the order AαC > DiMeIQx > MeIQx > IQ > PhIP. PhIP and MeIQx are the most abundant HCA in fried meat [30]. AαC, which was most effectively detoxified, is formed less frequently but nevertheless is one of the most relevant amines. It is notable that the binding of individual HCA may be different from that seen in experiments with mixtures; however, since HCA occur in fried meat in mixtures it reflects the realistic situation better as investigations with individual compounds [39].

Our findings correspond partly with the results of earlier studies, but only a few strains were included in previous experiments. For example, Bolognani *et al*. [8] found a similar order but tested only two strains and did not include amino-carbolines. Also, in the investigation of Morotomi *et al*. [12], a stronger binding to MeIQx compared to IQ was seen, while Lankaputra *et al*. [40] observed more efficient binding with IQ as with PhIP and AαC. Extremely strong binding was observed in some investigations with tryptophan pyrolysates (TrpP1 and TrpP2) [13, 18, 19], but these compounds were not included in the present study as they are rarely found in fried meats [29].

As shown in Figs. 2a–e, the overall binding capacities of the different strains towards the individual amines showed a similar pattern. This can be taken as an indication that the inactivation of the amines is due to a common mechanism. In this context, it is notable that Sreekumar *et al*. [15], who used two *L. gasseri* strains in their study, postulated that the amines bind to carbohydrate moieties of the bacterial cell walls and Turbic *et al*. [19] hypothesized that amino moieties play an important role.

Different physicochemical properties of the amines such as the pKa-values, solubility and lipophility may have an impact on their elimination by LAB. Table 2 summarizes the characteristics of the different HCA that were tested in the present study.

Table 2. Physicochemical properties of selected HCA[a)]

	pKa	logP	Water solubility [g/L][b)]
IQ	6.2 ± 0.4	1.2 ± 0.3	9.5
MeIQx	2.5 ± 0.5	1.4 ± 0.5	0.21
DiMeIQx	2.9 ± 0.5	1.9 ± 0.5	0.12
PhIP	7.7 ± 0.3	1.3 ± 0.8	120
AαC	6.8 ± 0.3	3.0 ± 0.8	2.2
Trp-P-1	10.9 ± 0.3	3.0 ± 1.0	15
Trp-P-2	10.6 ± 0.3	2.6 ± 1.0	26

a) Values were calculated with Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (© 1994–2006 ACD/Labs); pKa-values published by Jägerstad *et al*. [30] were <2 for MeIQx and DiMeIQx, 6.6 for IQ, 5.7 for PhIP and 8.5 for Trp-P-1 and Trp-P-2.
b) At pH 5.0.

Comparisons of the pKa values with the results of the HPLC analyses (Fig. 4) indicate that they have apparently no strong impact on the binding behavior. The pKa value of PhIP (7.7), which is only weakly bound by the bacteria, and of AαC (6.8), which is inactivated to a substantially higher extent, are both substantially higher than the pH-value used under the present experimental conditions (pH 5.0). In addition, the solubility of the HCA at pH 5.0 does not appear to have a major impact on the binding capacities of the individual amines; it increases in the order DiMeIQx < MeIQx < AαC < IQ < PhIP and does not correlate with the ranking order seen in the binding experiments. A more important factor may be Van der Waals (hydrophobic) interactions. The central column of Table 2 shows the logarithms of the octanol/water partition coefficients. It can be seen that the most hydrophobic compounds like AαC and DiMeIQx were efficiently removed by the bacteria in the present study while IQ and PhIP, which are less hydrophobic, were bound to a smaller extent. In addition, the tryptophan pyrolysates, which are efficiently bound by bacteria according to earlier studies [13, 18–20], are more hydrophobic than the quinolines, quinoxalines and PhIP.

The elimination capacities of most species differed significantly. The ranking order found in the present study was *L. helveticus* (83) > *S. thermophilus* (68) > *L. bulgaricus* (59) > *B. longum* (44) = *L. casei* (42) > *L. acidophilus* (20) > *L. plantarum* (10) = *L. kefir* (9). Numbers in parentheses indicate in percent the overall inactivation of the different HCA (Fig. 2). Data from earlier investigations (in which only a few strains were tested) are in agreement with our observations. For example, Bolognani *et al*. [8] found stronger effects with *B. longum* than with *L. acidophilus* and Morotomi *et al*. [12] as well as Lankaputra *et al*. [40] reported that *L. bulgaricus* and *Bifidobacteria* strains are better binders than *L. acidophilus*.

The results of the bacterial mutagenicity experiments show, in agreement with earlier investigations [13, 23], that the inactivation of the amines with LAB results in a decrease of their mutagenic potential and correlates with the binding capacities of the individual strains. In the present study, we focused on AαC and PhIP since these amines are among the most abundant cooked food mutagens in fried meats, and the prevention of mutagenic effects of other HCA by LAB has been reported repeatedly in a number of earlier investigations [12, 13, 20]. This can be taken as an indication that removal of the amines from liquid media in the binding experiments, monitored in the HPLC-experiments, indeed results in a reduction of their mutagenic properties, which are considered to play a key role in regard to their carcinogenic effects. In the chemical analyses we found that AαC was generally eliminated more efficiently as PhIP; also in the mutagenicity experiments a stronger decrease of the mutagenic effects of the carboline were found after preincubation with LAB. Interestingly, we found a pronounced reduction of the mutagenic effects of AαC (*i.e*. by 52%) with the strain *L. plantarum* MWLP3, while only a marginal effect was seen with PhIP. One of the reasons for this may be the substantially higher lipophilicity of the carboline (see Table 2).

Notably, the current experiments were carried out under conditions relevant for humans, *i.e*. at a pH-value of 5.0, which is in the typical range for the small intestine [41] and was used in earlier studies [8, 17]. Furthermore, the concentrations of the bacteria in our incubation mixtures were 10^9 cells/mL and similar densities of LAB can be found in human colonic contents after consumption of fermented foods [42].

Taken together, the results of our comprehensive study show that strong differences exist in the detoxification capacities of LAB species towards HCA. For example, species such as *L. helveticus* and *S. thermophilus* were found to be seven to eight times more effective than *L. kefir* and *L. plantarum* strains. Furthermore, we also found that distinct differences exist in the efficiency of elimination of different HCA: AαC was removed by far (two to three times) more efficiently as PhIP and IQ by the bacteria. However, the individual representatives of each species showed comparable binding efficiencies. Representatives of LAB species with high binding capacities, such as *S. thermophilus* and *L. bulgaricus*, are contained in yoghurts; also, *L. helveticus* bacteria are often used for the production of fermented milk and probiotic supplements. Since there is an increasing evidence that LAB protect animals as well as humans against DNA damage and/or cancer induction by HCA due to direct binding mechanisms, our findings may contribute to the development of nutritional strategies concerning the prevention of adverse health effects of HCA.

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The authors have declared no conflict of interest.

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