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BIOTRANSFORMATION OF FERULIC ACID BY *LACTOBACILLUS ACIDOPHILUS* K1 AND SELECTED *BIFIDOBACTERIUM* STRAINS

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Background. Lactic acid bacteria (LAB) were pointed out to produce ferulic acid esterase. Except the release of phenolic acids from esterified forms, it was postulated that the biotransformations of these compounds can occur during the bacterial growth. In the presented work, the biotransformation of ferulic acid by *Lactobacillus acidophilus* K1 and three *Bifidibacterium* strains (*B. animalis* Bi30, *B. catenulatum* KD 14 and *B. longum* KN 29) was studied.
Material and methods. The microorganisms were grown in media containing methyl esters of phenolic acids as carbon sources. The feruloyl esterase activity as well as the contents of phenolic acids in supernatants were estimated using HPLC-DAD.
Results. The enzyme activity was evaluated using methyl ferulate exclusively, but p-coumaric acid and another chromatographic peak (probably caffeic acid, but its identity was not positively confirmed by the DAD analysis) were present in reaction mixtures containing the supernatants of *Lactobacillus acidophilus* K1 cultivars with methyl p-coumarate or methyl syringate. Both peaks of p-coumaric acid and another phenolic compound were also present in the solutions containing the supernatants of *B. catenulatum* and *B. longum* grown in the presence of methyl vanillate and the supernatants of *B. animalis* Bi30 grown using methyl p-coumarate, methyl syringate or methyl vanillate.
Conclusions. The results suggest a distinct ability of the studied LAB strains to transform free ferulic acid yielding p-coumaric acid and probably caffeic acid although no mechanism involved in this transformation was proposed and closer characterised in the frames of this work.

Key words: ferulic acid, biotransformation, *Bifidobacterium*, *Lactobacillus*, probiotic, antioxidant

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INTRODUCTION

In the previous decades, a considerable number of ferulic acid esterases (FAEs) of fungal origin were well characterised but the knowledge of the enzymes produced by lactic acid bacteria (LAB) is rather limited [Donaghy et al. 1998, Vardakou et al. 2007, 2008, Nsereko et al. 2008, Yuan et al. 2005, Wang et al. 2004]. Esterases (EC 3.1.1.x) is the class of hydrolytic enzymes broadly distributed in the plant and animal kingdom. Ferulic acid esterases produced by LAB present in human gastrointestinal tract can be a very interesting object of nutritional studies. Dietary fiber is a rich source of strong antioxidants- phenolic acids esterified to polymeric non-starch polysaccharides, with ferulic or p-coumaric acids known to be very strong antioxidants *in vitro* [Gerhäuser 2005, Maillard and Berset 1995, Maillard et al. 1996, Nardini et al. 1995] or *in vivo* [Tanaka et al. 1993, Itagaki et al. 2009, Joshi et al. 2006, Saija et al. 2000, Young et al. 2008]. Prior absorption, phenolic acids esters are deesterified from dietary fiber in the gastrointestinal tract due to the action of bacterial FAEs, also originating from LAB [Andreason et al. 2001, Couteau et al. 2001] followed by the transformations in the liver [Scalbert and Williamson 2000, Olthof et al. 2003, Plumb et al. 1999, Rondini et al. 2002, Hollman and Katan 1998, Nardini et al. 1997, Ghiselli et al. 2000]. A considerable number of works reveal that free phenolic acids, including ferulic acid, are very easily absorbed into blood plasma [Nardini et al. 2002, 2006] and excreted in urine [Choudhury et al. 1999, Bourne and Rice-Evans 1998].

Ferulic acid was pointed out as a very effective antioxidant and active biomolecule in the prevention of Alzheimer's disease and possibly other neurodegenerative disorders by inhibiting AAPH-induced hippocampal cells toxicity, significant moderation of hydroxyl radical induced oxidation of membrane lipids in synaptosomes, complete prevention of the synaptosomal system from hydroxyl radical induced oxidation of proteins leading to their crosslinking. Ferulic in its action was far more potent than vanillic, coumaric or cinnamic acid [Kanski et al. 2002]. Long term administration of ferulic acid to mice induced resistance to β-amyloid-42 toxicity in the brain what suggests that this compound can be a useful chemopreventive agent against Alzheimer's disease [Yan et al. 2001]. It should be stressed that phenolic acids undergo transformations in much lesser extent [Hollman and Katan 1998, Nardini et al. 1997, 2002, 2006, Rondini et al. 2002, Scalbert and Williamson 2000] than more complex phenolic compounds like flavonols and proanthocyanidins. The latter compounds are easily degraded yielding simple phenolic compounds including phenolic acids which are absorbed and excreted [Clifford et al. 2000, Gross et al. 1996, Rechner et al. 2001 a, b]. In consequence, simple phenolic acids can exhibit relevant antioxidant activity *in vivo* [Deprez et al. 2000]. Except FAE, other enzymes can be involved in the transformations of phenolic compounds in the gastrointestinal tract, for example demethylases [Micard et al. 2002]. The changes in the structure of phenolic compound can modify its absorption from the gastrointestinal tract and the antioxidant activity exhibited in the blood plasma. Taking under consideration these problems as well as the positive role of LAB in the gastrointestinal tract this work focused on the biotransformations of ferulic acid by selected *Lactobacillus* and *Bifidobacterium* strains originating from the human gastrointestinal tract.

MATERIAL AND METHODS

Strains and reagents

Bacterial strains were isolated from gastrointestinal tract either from infants (*B. longum* KN29) or adults (*B. catenulatum* KD14, *B. bifidum* Bi30, *L. acidophilus* K1) by Prof. dr hab. Maria Bielecka from the Department of Food Microbiology, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Poland [Bielecka et al. 2002]. Methyl ferulate, methyl p-coumarate, methyl syringate and methyl vanillate were obtained from Apin Chemicals, Oxon, UK. Garche's and MRS broths were purchased from BTL Ltd. Zakład Enzymów i Peptonów, Poland. All other reagents were from POCh Gliwice, Poland. Chromatography reagents (POCh Gliwice, Poland) were of HPLC grade.

Culture conditions

Bifidobacterium strains were grown using the slightly modified method of Biedrzycka et al. [2003]. The bacteria were thermostated in Garche's medium (10 cm^3) in test tubes at 37°C in anaerobic conditions (0.5 cm^3 of 15% (w/v) $NaHCO_3$ solution and 0.5 cm^3 20% (w/v) pirogallol solution injected into cotton stoppers followed by the aseptical closing of the tubes using rubber stoppers). Every 24 hours, cultures were repeatedly inoculated in a new broth using 5% (v/v) of inoculum. *Lactobacillus acidophilus* K1 was grown using MRS broth (10 cm^3) at 37°C in 30 cm^3 test tubes and then every 24 hours it was repeatedly inoculated using new MRS broths and 3% (v/v) of inoculum. These bacterial cultures were used for subsequent studies as described below.

Method of cultivation for FAE

All bacterial strains were cultivated at 37°C in tubes with cotton and rubber stoppers, in anaerobic conditions, as described above, in 10 cm^3 of minimal growth medium composed of (g in dm^3): peptone – 2.0, yeast extract – 2.0, L-cysteine·HCl – 0.5, NaCl – 0.1, $NaHCO_3$ – 2.0, K_2HPO_4 – 0.04, KH_2PO_4 – 0.04, $MgSO_4 \cdot 7H_2O$ – 0.01, $CaCl_2 \cdot 6H_2O$ – 0.01, Tween 80 – 2 cm^3. As a carbon source, 0.25% (w/v) of methyl syringate, methyl vanillate, methyl ferulate or methyl p-coumarate were used. The growth medium was inoculated using 5% (v/v) of inoculum previously grown for 24 hours on corresponding broth as described above. Presented results refer to the total growth times including 24 hours of inoculum growth on the minimal broth described above. The bacterial cultures were incubated at 37°C and feruloyl esterase activity was determined after centrifugation (10 000 × g, 6°C, 15 min, SIGMA 4K14 Laboratory Centrifuges, Polygen) as described below. The growth of bacterial cells was monitored by measurements of the optical density at 600 nm. In the presentation of the results, incubation times characterised by no feruloyl esterase activities were omitted. The cultivars were duplicated and the enzyme activities as well as phenolic acids concentrations are mean values with standard deviations.

FAE activity determination

The ability of FAE to hydrolyze methyl ferulate was used and the free ferulic acid content was determined using HPLC with Diode Array Detection. Methyl ferulate was dissolved in minimal volume of 96.0% ethanol (v/v, typically 0.05 cm^3) followed by the dilution using Tris-HCl buffer (100 mM × dm^{-3}, pH 6.5) until 6 mM × dm^{-3} concentration was obtained. 0.5 cm^3 of the studied sample was added to 0.1 cm^3 of substrate solution and the samples were incubated at 37°C for 5 hours. The enzyme was then inactivated in boiling water (5 min) followed by the cooling and centrifugation (7000 × g, 30 min, 6°C, centrifuge MPW-365, Mechanika Precyzyjna, Warsaw, Poland). Free phenolic acids and methyl ferulate in studied and double blank samples (lacking substrate or supernatant solution) were separated and analysed simultaneously as described below. Both blank samples were subtracted from corresponding studied samples. FAE activity was expressed in units (1 unit is equal to 1 nM of ferulic acid released in 1 cm^3 of reaction medium after 1 min of incubation). Analyses were duplicated and mean values with standard deviations were calculated.

HPLC-DAD identification of phenolic acids and methyl ferulate

The HPLC system consisted of two Gilson 306 Separation Module piston pumps, Gilson PhotoDiode Array Detector 170, Gilson loop (0.02 cm^3), manometric module Gilson 805, dynamic mixer 811C. Waters Symmetry C_{18} column (USA, 250 mm, 4.6 mm i.d., 5 μm), and Waters Symmetry C_{18} pre-column (5 μm, 8 × 20 mm) were used for separations. The method of Kim et al. [2006] was used. Eluents used were: A-1% (w/v) acetic acid solution in DDI water. Eluent B- 50% HPLC-grade acetonitrile in DDI water. Signals were monitored at 320 nm, 280 nm, 260 nm and 360 nm. The program applied was as follows: START 92% A, 8% B 0-10 min; 70% A, 30% B 10-40 min; 60% A, 40% B 40-55 min; 92% A, 8% B 55-70 min. The effluent flow was 0.8 $cm^3 \times min^{-1}$ (17 MPa). Phenolic acids concentrations were calculated using the calibration curves plotted using the series of HPLC grade phenolic acids standards individually injected into HPLC-DAD system. Diode Array spectra of studied chromatographic peaks were obtained and compared with spectra of phenolic acids standards in the database (Gilson Unipoint ver. 3.01).

RESULTS AND DISCUSSION

All supernatants studied in the frames of this work, obtained from the cultivations of *Lactobacillus acidophilus* K1 and *Bifidobacterium*, exhibited the ability to release ferulic acid from methyl ferulate determined as described in Methods chapter (results not shown). Anyway, HPLC-DAD results revealed the presence of significant concentrations of free p-coumaric acid (matching coeff. 900 of 1000, Gilson Unipoint ver. 3.01) in the reaction mixtures containing methyl ferulate but no methyl p-coumarate as a substrate for the enzyme activity. Besides, another chromatographic peak (retention time 30 min) was obtained. The retention time of this peak was equal to the retention time of caffeic acid standard but the spectral analysis did not match the caffeic acid standard spectrum (matching coeff. 760 of 1000, (Gilson Unipoint ver. 3.01). An example chromatogram obtained during the enzyme activity determination is presented in Figure 1.

Methyl ferulate concentration in the enzyme reaction mixture was significantly higher than the concentrations of the compounds originating from the studied supernatants. If this is the case, it can be concluded that both p-coumaric acid peak and unidentified peak originate from methyl ferulate. The unidentified peak can be a caffeic acid derivative as well as other phenolic acid but no further identification was performed. What is obvious, quantitative analysis of this compound was also omitted in the results.

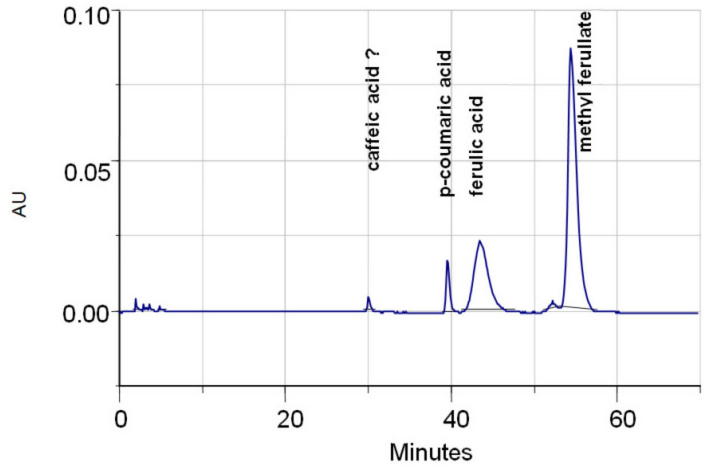

Fig. 1. An example chromatogram obtained after the separation of phenolic acids and methyl ferulate during ferulic acid esterase activity determination

Free p-coumaric acid was present in the reaction mixtures containing the post-cultivation supernatants of *Lactobacillus acidophilus* K1 after 42 hours of cultivation followed by the continuous increase in the phenolic acid concentration until 60 hours (Fig. 2). As it can be seen, free ferulic acid was released from methyl ferulate as a result of FAE activity in the reaction mixtures containing post-cultivation supernatants except the last hours of bacterial growth (84 hours of cultivation). It is interesting that p-coumaric acid concentrations were higher than corresponding ferulic acid contents in post-cultivation supernatants and that after 84 hours of cultivation, no free ferulic acid was detected in the solution.

Also, in the case of *B. animalis* Bi30, a distinct transformation of ferulic acid to p--coumaric acid was seen (Fig. 3). Supernatants obtained after 48-84 hours of *B. animalis* cultivation mixed with methyl ferulate caused the release of free ferulic acid and p-coumaric acid appeared in the studied samples. In opposition to the results obtained for *L. acidophilus* K1, the content of p-coumaric acid constantly decreased until the end of the experiment (84 hours of cultivation). It must be emphasised that all studied LAB strains produced FAE in the presence of methyl esters of cinnamic acids (p-coumaric or ferulic) more effectively than in the presence of methyl esters of benzoic acid derivatives (vanillic and syringic acid esters, results shown elswhere). Nevertheless, ferulic acid concentrations significantly changed during the bacterial growth (Fig. 2-4).

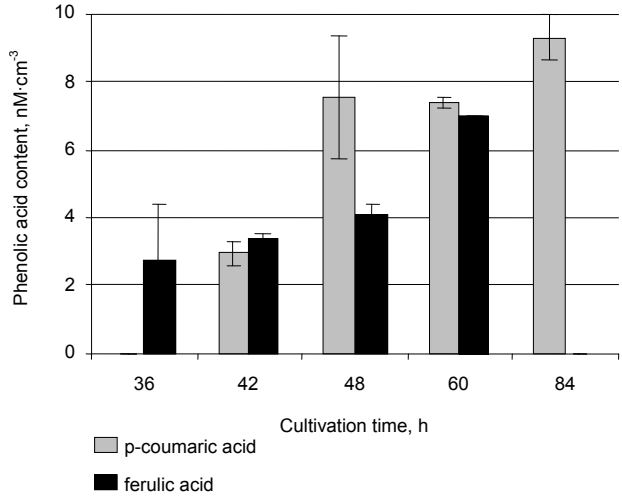

Fig. 2. Phenolic acids concentrations in reaction mixtures obtained during ferulic acid esterase activity determination (methyl p-coumarate was the carbon source for *L. acidophilus* K1 growth)

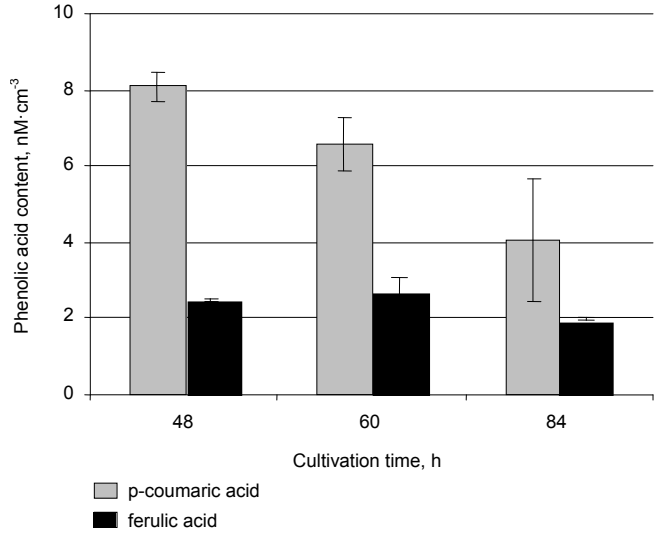

Fig. 3. Phenolic acids concentrations in reaction mixtures obtained during ferulic acid esterase activity determination (methyl p-coumarate was the carbon source for *B. animalis* Bi30 growth)

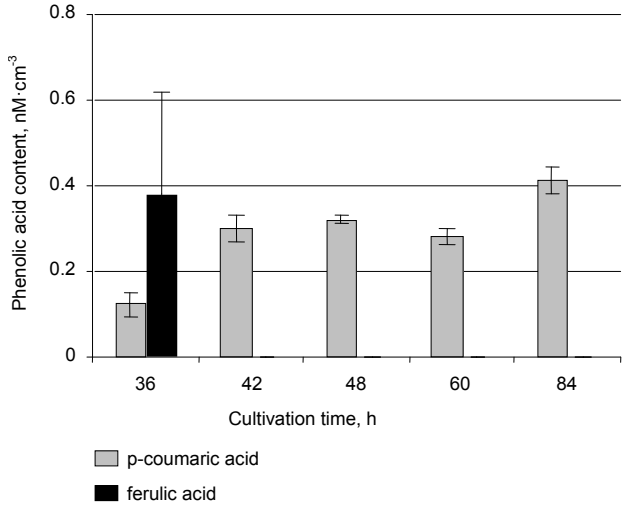

Fig. 4. Phenolic acids concentrations in reaction mixtures obtained during ferulic acid esterase activity determination (methyl syringate was the carbon source for *L. acidophilus* K1 growth)

P-coumaric acid was also present when the reaction mixture was composed of methyl ferulate solution (substrate) and the supernatant obtained after the cultivation of *L. acidophilus* K1 (Fig. 4) or B. *animalis* Bi30 (Fig. 5) using methyl syringate as a carbon source.

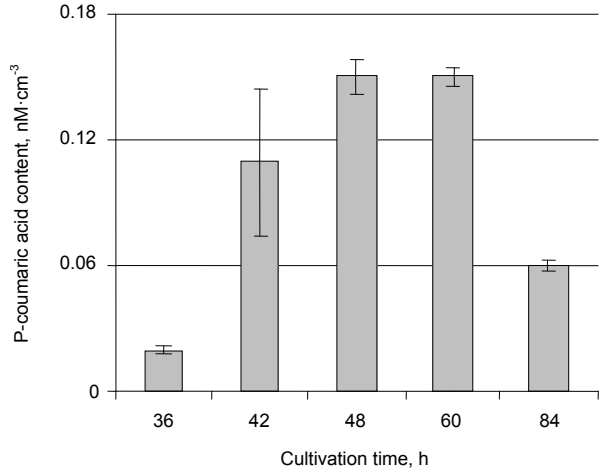

Fig. 5. P-coumaric acid concentrations in reaction mixtures obtained during ferulic acid esterase activity determination (methyl syringate was the carbon source for *B. animalis* Bi30 growth)

In the case of *L. acidophilus* K1 (Fig. 4), p-coumaric acid was present in the reaction medium during FAE activity determination beginning from the 36. hour of cultivation. The content of the acid significantly increased after 42. and 84. hour of cultivation. It must be pointed out that ferulic acid was released from methyl ferulate during enzyme activity determination. Nevertheless, it was not present in the reaction mixtures containing post-cultivation supernatants originating from the later stages of bacterial growth (42-84 hours).

Similarly, no free ferulic acid was detected in the reaction mixtures containing post-cultivation supernatants originating from all stages of *B. animalis* Bi30 growth (36-84 hours) when methyl syringate was the only carbon source for the bacterial growth (Fig. 5). After the initial increase in the content of p-coumaric acid in the reaction mixtures, the decrease after 84 hours of bacterial growth was seen what could suggest the corresponding changes in FAE activity or the loss of p-coumaric acid due to the bacterial decarboxylation of this acid yielding 4-ethylguaiacol. Anyway, this observation was not further elaborated within the frames of this study and needs a detailed explanation.

Other two LAB strains- *B. catenulatum* KD14 and *B. longum* KN29 exhibited the limited ability to transform ferulic acid into p-coumaric acid when cultivated with the use of methyl vanillate (Fig. 6). No ferulic acid originating from the hydrolysis of methyl ferulate was detected and the concentrations of p-coumaric acid in the case of both bacterial strains were similar especially at the later stages of cultivations.

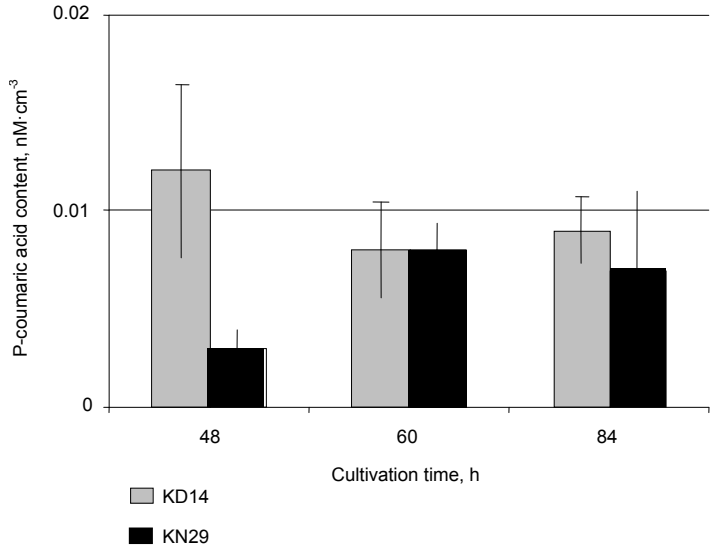

Fig. 6. P-coumaric acid concentrations in reaction mixtures obtained during ferulic acid esterase activity determination (methyl vanillate was the carbon source for *B. catenulatum* KD14 and *B. longum* KN29 growth)

DISCUSSION

It is evident that lactic acid bacteria are able to transform the components of the dietary fiber fractions to the limited extent due to the production of some secondary enzymes like FAE [Wang et al. 2004]. Donaghy et al. [1998] detected FAE activities in 7 strains of lactic acid bacteria: 1 *Lactobacillus fermentum* and 6 *Bacillus subtilis* strains, but as many as 80 *Bacillus*-type strains and 50 gram positive bacteria (*Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Propionibacterium*) were studied in the frames of this work. *L. fermentum* NCF 1751 released free ferulic acid from methyl ferulate, methyl p-coumarate and a natural feruloylated oligomer-2-O-[5--O-(trans-ferulic acid)-β-L-ara-*f*]-D-xyl-*p*. In 2008, Nsereko et al. [2008] examined approx. 10 000 LAB strains for the ability to produce FAE and some FAE-producing microorganisms were selected for the further studies, including: *Lactobacillus buchneri*, *L. crispatus*, *L. reuteri*, *L. brevis* and one not identified *Lactobacillus* strain. Live *L. fermentum* 11976 cells were immobilized in alginate-poly(L-lysine)-alginate gel and the FAE activity was detected in the supernatant solution. The alginate microcapsules were used as the source for the enzyme in the *in vitro* experiments using the model of the gastrointestinal tract [Bhathena et al. 2008].

Some authors successfully used wheat bran, a cheap and valuable by-product, as natural carbon source for the LAB growth [Wang et al. 2005]. FAE activity was also detected in batch cultures of faecal microorganisms with water-unextractable feruloylated arabinoxylans from de-starched wheat flour [Vardakou et al. 2007] but no release or transformations of phenolic acids were studied in the frames of this work. *Lactobacillus* and *Bifidobacterium* were repeatedly pointed out as FAE producers in mixed batch cultures of faecal microorganisms containing water-unextractable (untreated and pretreated with fungal xylanase) feruloylated arabinoxylans from de-starched wheat flour [Vardakou et al. 2008]. The both citied works of Vardakou et al. focused on the ability of LAB strains to grow in the medium containing non-starch polysaccharides from wheat bran. The detailed analysis of the decomposition of polysaccharides was not performed and the phenolic acids concentrations in the growth media were not determined.

The analysis of the scientific databases can give a clear assumption, that the literature concerning the biotransformation of phenolic acids by lactic acid bacteria is incomplete. Phenolic acids side chains undergo biotransformations but the phenol ring remains unchanged what causes the modifications of biological activities of these phenolic compounds. Chlorogenic acid subjected to the action of the consortium of human faecal bacteria underwent epimerization to the mixture of 3-, 4- and 5-caffeoyl quinic acid followed by the hydrolysis yielding caffeic acid, then after 8 hours of incubation caffeic acid was reduced to dihydrocaffeic acid. After another 24 hours, dihydrocaffeic acid was dehydroxylated yielding 3-(3,4-dihydroxyphenyl)-priopionic acid [Rechner et al. 2004]. Taking under consideration individual bacterial strains, there is a considerable number of works concerning *Lactobacillus* strains. In the presented work it was shown that *Lactobacillus acidophilus* K1 was able to effectively release free ferulic acid but the post-cultivations supernatants contained significant concentrations of p-coumaric acid. Cavin et al. [1997] purified and characterised a p-coumaric acid decarboxylase from *Lactobacillus plantarum.* The enzyme activity was 60-fold higher towards p--coumaric acid than ferulic acid. The authors suggested the presence of at least two enzymes in the supernatant solution after bacterial growth. Rodriguez et al. [2008] de-

termined the ability of *Lactobacillus plantarum* CECT 748^T strain to metabolize 19 food phenolic acids. Only p-coumaric, m-coumaric, ferulic and caffeic acids were metabolized. Caffeic and p-coumaric acids were transformed into their corresponding ethyl and vinyl derivatives, ferulic acid was transformed into 4-vinylguaiacol, and 3-(3-OH--phenyl) propionic acid was produced from m-coumaric acid. Gallic and protocatechuic acid were decarboxylated yielding pyrogallol and catechol, respectively. The enzymes involved in these transformations were inducible as verified in phenolic acids free bacterial cultures. On the other hand, the ability to metabolize the phenolic acids is strain-specific, because in another work [Landete et al. 2008] it was showed that only protocatechuic acid was metabolized to catechol (via decarboxylation) during green olives fermentation by a number of different *Lactobacillus plantarum* strains, including CECT 748^T strain. Alberto et al. [2001] showed that (+)-catechin and gallic acid were utilized from the very beginning growth of *Lactobacillus hilgardi* in the experimental FT80 medium containing tomato juice and phenolic standard. The bacterial cell number was higher in the medium containing gallic acid up to 100 mg·dm^{-3} compared to the cell number in control medium what suggested the use of this phenolic acid as the carbon source. When MRS broth was used for bacterial growth in the presence of gallic acid, no difference between studied samples and controls (without gallic acid) was seen. Unfortunatelly, the authors did not proceed with the closer characterisation of the gallic acid biotransformation by the studied *Lactobacillus hilgardii* strain.

Different LAB wild strains are able to modify different hydroxycinnamic acids and their derivatives during wine fermentations [Hernandez et al. 2007]. Phenolic acids like p-coumaric or ferulic acids released from their correspnding esters (mainly with tartaric acid) were further decarboxylated to corresponding 4-vinyl derivatives and reduced to 4-ethyl derivatives by the yeast [Chatonnet et al. 1995, Dugelay et al. 1993] or LAB species like *Lactobacillus brevis*, *L. plantarum*, *Pediococcus* [Cavin et al. 1993]. LAB strains from wine (*Oenococcus oeni*, *Lactobacillus brevis*, *L. hilgardii*, *L. plantarum*, *Pediococcus damnosus*) were also able to transform ferulic acid to vanillin. *Lactobacillus* sp. and *Pediococcus* very effectively degraded ferulic acid to 4-vinylguaiacol, and only *L. plantarum* was the only bacteria that was able to reduce 4-vinylguaiacol to 4--ethylguaiacol [Bloem et al. 2007]. On the other hand, De Las Rivas et al. [2009] used PCR assay for the identification of lactic acid bacteria able to produce volatile phenols from hydroxycinnamic acids. The authors determined that *Lactobacillus plantarum*, *L. brevis* and *Pediococcus pentosaceus* produced vinyl and ethyl derivatives from ferulic, caffeic and p-coumaric acids, but *L. hilgardii, L. mesenteroides* and *Oenococcus oeni* were not able to decarboxylate p-coumaric, ferulic or caffeic acid to corresponding vinyl or ethyl derivatives. The authors detected low concentrations of phloretic acid after the cultivation of *L. hilgardii* in the presence of p-coumaric acid. Vinyl derivatives (vinylphenol and vinylcatechol) originating from three phenolic acids were further reduced to corresponding ethyl derivatives, ethyl phenol and ethylcatechol. Besides, ferulic acid was partially reduced to hydroferulic acid. *Lactobacillus brevis* and *Pediococcus pentosaceus* were able to complete transform p-coumaric and caffeic acid only to their vinyl derivatives but ferulic acid was only partially transformed to vinylguaiacol by these two microorganisms [De Las Rivas et al. 2009]. Couto et al. [2006] screened 35 lactic acid bacteria strains (20 species) for the ability to produce volatile phenols from p-coumaric and ferulic acid. Thirteen strains produced volatile phenols from p-coumaric acid and 3 strains produced 4-ethylphenol as the final product of the p-coumaric acid metabolic pathway. The authors determined this ability in the case of

Lactobacillus brevis, *L. collinoides*, and *L. plantarum*. As for Pediococcus strains, most of these microorganisms transformed p-coumaric acid to 4-vinylphenol but not to 4--ethylphenol. The results concerning *Oenococcus oeni* strains and *Leuconostoc mesenteroides* were in oposition to some resultes citied above because these lactic acid bacteria did not produce any p-coumaric acid derivatives in the frames of this study [Couto et al. 2006]. In general, studied bacteria were predominantly able to transform ferulic acid and not p-coumaric acid to volatile phenols. In another work [Chatonnet et al. 1997] it was showed that only *Lactobacillus plantarum* was able to form considerable quantities of 4-ethylphenol and no 4-ethylphenol in medium containing *Pediococcus damnosus* or *Leuconostoc oenos*.

In herein presented work, methyl ferulate was used as the substrate for ferulic acid esterase activity and p-coumaric appeared in the post-cultivation supernatant solution. Within the frames of this work, caffeic acid was not identified although the retention time of unidentified chromatographic peak was in agreement with the retention time of caffeic acid standard. Other authors [Micard et al. 2002] detected the O-demethylation of ferulic acid by the anaerobic bacteria *Clostridium methoxybenzovorans* SR3 and *Enterobacter cloacae* DG6 yielding caffeic acid. The enzymes originating from both microorganisms were intracellular and both were unable to demethylate ferulic acid in the ester form in feruloyl-arabinoxylan. Other study also reported the ability of *Clostridium methoxybenzovorans* to o-demethylate vanillate, isovanillate, vanillin, anisate, ferulate and veratrate to their corresponding hydroxylated derivatives and to ferment the side chains to acetate and butyrate [Mechichi et al. 2005]. Veratrate was initially o--demethylated to vanillate and then to protocatechuate together with the production of acetate and butyrate from the side chains. Other human intestinal microorganism – *Peptostreptococcus productus* was also able to demethylate ferulic acid in the frames of the study involving the conversion of dietary lignan seicosolariciresinol in the system of synthetic stomach or intestinal juice [Clavel et al. 2006]. The results presented in the citied works prove that the problem of the biotransformation of phenolic acids and their derivatives by LAB strains is very interesting. In this context, the extended studies should be continued in this field, especially concerning probiotic strains belonging to the group of LAB.

SUMMARY

1. The results obtained within the frames of this study prove the ability of studied bacterial strains to transform ferulic acid methyl ester.

2. The supernatants of *Lactobacillus acidophilus* K1 contained p-coumaric acid and probably caffeic acid but the presence of the latter phenolic acid was not confirmed by HPLC-DAD spectrum analysis.

3. Similarly, supernatants of *B. catenulatum* and *B. longum* obtained using methyl vanillate and the supernatants of *B. animalis* Bi30 obtained using methyl p-coumarate, methyl syringate or methyl vanillate contained both free p-coumaric and caffeic acid as confirmed by HPLC-UV analysis.

4. The transformation of phenolic acids by lactic acid bacteria originating from human gastrointestinal tract becomes a very interesting research topic. Presented results can be useful in the understanding of the role of LAB in the biotransformations of simple phenolic compounds upon digestion.

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BIOTRANSFORMACJA KWASU FERULOWEGO PRZEZ BAKTERIE *LACTOBACILLUS ACIDOPHILUS* K1 ORAZ WYBRANE BAKTERIE Z RODZAJU *BIFIDOBACTERIUM*

Wprowadzenie. Bakterie kwasu mlekowego były w przeszłości wskazywane jako producent esterazy kwasu ferulowego. Poza uwalnianiem kwasów fenolowych z form estrowych, wskazywano na możliwość biotransformacji kwasów fenolowych w czasie wzrostu bakterii. W pracy badano zdolność bakterii *Lactobacillus acidophilus* K1 i trzech szczepów bakterii z rodzaju *Bifidibacterium* (*B. animalis* Bi30, *B. catenulatum* KD 14 i *B. longum* KN 29) do biotransformacji kwasu ferulowego.
Materiał i metody. Drobnoustroje hodowano w pożywkach zawierających metylowe estry wybranych kwasów fenolowych jako jedyne źródło węgla. Aktywności esterazy kwasu ferulowego oraz zawartości kwasów fenolowych w supernatantach określano za pomocą techniki HPLC z detekcją DAD.
Wyniki. Aktywność enzymatyczna była oznaczana wyłącznie z użyciem ferulanu metylu, ale w supernatantach pohodowlanych wszystkich bakterii stwierdzono obecność piku chromatograficznego kwasu p-kumarowego. Ponadto zarejestrowano dodatkowo jeden niezidentyfikowany pik (prawdopodobnie kwasu kawowego, jednak obecność tego związku nie została potwierdzona poprzez analizę widmową DAD) w próbach zawierających supernatant uzyskany po hodowlach bakterii *Lactobacillus acidophilus* K1 z użyciem p-kumaranu metylu lub syringanu metylu. Obecność obu pików (kwasu p-kumarowego oraz niezidentyfikowany) stwierdzono również w obrazie chromatograficznym w czasie analizy supernatantów uzyskanych po hodowlach *B. catenulatum* i *B. longum* na wanilianie metylu i *B. animalis* Bi30 z użyciem p-kumaranu metylu, syringanu metylu lub wanilianu metylu jako źródła węgla.
Wnioski. Powyższe wyniki wskazują na zdolność badanych bakterii mlekowych do przekształcania kwasu ferulowego do kwasu p-kumarowego i prawdopodobnie kwasu kawowego, ale w pracy nie podjęto próby bliższego scharakteryzowania mechanizmów enzymatycznych biorących udział w omawianych transformacjach.

Słowa kluczowe: kwas ferulowy, demetylacja, *Bifidobacterium*, *Lactobacillus*, probiotyk, przeciwutleniacz

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