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PRODUCTION OF EXTRACELLULAR FERULIC ACID ESTERASES BY *LACTOBACILLUS* STRAINS USING NATURAL AND SYNTHETIC CARBON SOURCES

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Background. Ferulic acid esterases (FAE, EC 3.1.1.73), also known as feruloyl esterases, cinnamic acid esterases or cinnamoyl esterases, belong to a common group of hydrolases distributed in the plant kingdom**.** Especially the fungal enzymes were very well characterised in the past whereas the enzyme was rarely found in the lactic acid bacteria (LAB) strains. It is well known that strong antioxidants free phenolic acids can be released from the dietary fiber by the action of intestinal microflora composed among others also of *Lactobacillus* strains. The aim of this study was to examine four *Lactobacillus* strains (*L. acidophilus* K1, *L. rhamnosus* E/N, PEN, OXY) for the ability to produce extracellular FAE on different synthetic and natural carbon sources.
Material and methods. The LAB strains were grown in the minimal growth media using German wheat bran, rye bran, brewers' spent grain, isolated larchwood arabinogalactan, apple pectin, corn pectin, methyl ferulate, methyl p-coumarate, methyl syringate or methyl vanillate as the sole carbon source. FAE activity was determined using the post-cultivation supernatants, methyl ferulate and HPLC with UV detection.
Results. The highest FAE activity was obtained with *L. acidophilus* K1 and methyl ferulate (max. 23.34 ±0.05 activity units) and methyl p-coumarate (max. 14.96 ±0.47 activity units) as carbon sources. *L. rhamnosus* E/N, OXY and PEN exhibited the limited ability to produce FAE with cinnamic acids methyl esters. Methyl syringate and methyl vanillate (MS and MV) were insufficient carbon sources for FAE production. Brewers' spent grain was the most suitable substrate for FAE production by *L. acidophilus* K1 (max. 2.64 ±0.06 activity units) and *L. rhamnosus* E/N, OXY and PEN. FAE was also successfully induced by natural substrates rye bran, corn pectin (*L. acidophilus* K1), German wheat bran and larchwood arabinogalactan (E/N, PEN) or German wheat bran and corn pectin (OXY).
Conclusions. This study proved the ability of *Lactobacillus* strains to produce FAE in the presence of a wide range of different ester-bound substrates. The highest enzyme activities obtained in the presence of synthetic phenolic acids methyl esters suggest that the bacteria were forced to produce FAE whereas in the presence of natural substrates other carbon sources were exploited. FAE is the enzyme of the minor importance during the de-

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composition of the food matrix during the intestinal absorption but the further characterisation of these enzymes should be carried on.

Key words: ferulic acid esterase, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, antioxidant

Abbreviations used: FAE – ferulic acid esterase, LAB – lactic acid bacteria, FA – ferulic acid, p-CA – p-coumaric acid, SA – syringic acid, VA – vanillic acid, MF – methyl ferulate, Mp-C – methyl p-coumarate, MS – methyl syringate, MV – methyl vanillate

INTRODUCTION

Dietary fiber is a rich source of phenolic compounds, with ferulic acid as most significant phenolic acid present in the cereal fibers. This phenolic acid is a very strong antioxidant *in vitro* [Cuvelier et al. 1992, Nardini et al. 1995, Maillard and Berset 1995, Maillard et al. 1996, Gerhäuser 2005] and a very effective anticarcinogenic compound *in vivo* as the previous studies revealed [Saija et al. 2000, Joshi et al. 2006, Xiao and Parkin 2007, Young et al. 2008, Itagaki et al. 2009]. Free ferulic acid effectively inhibited AAPH-induced hippocampal cell toxicity, moderated the oxidation of membrane lipids in synaptosomes caused by the hydroxyl radicals, completely prevented the synaptosomal system from the oxidation of proteins by the hydroxyl radicals. It was more potent than vanillic, coumaric or cinnamic acid [Kanski et al. 2002]. Also, free ferulic acid induced the resistance to β-amyloid-42 toxicity in the brain of mice what suggests that it can be useful against Alzheimer's disease [Yan et al. 2001]. During the last years, a considerable number of papers concerning the absorption of phenolic compounds from the gastrointestinal tract and transformations in blood plasma have appeared. Some papers prove that the free phenolic acids like ferulic acid are most easily absorbed from the intestinal tract among phenolic species [Nardini et al. 2006]. The ester bond of phenolic acids is hydrolysed followed by the absorption of these compounds in the small intestine [Scalbert and Williamson 2000, Rechner et al. 2001 a, Rondini et al. 2002, Olthof et al. 2003]. Ferulic acid originating from the food matrix was detected in the urine [Bourne and Rice-Evans 1998] and in blood at the considerable concentrations [Choudhury et al. 1999]. Phenolic acids, as simple compounds, are degraded in much lesser extent than the more complex phenolics so they can exert the antioxidant activity *in vivo* [Nardini et al. 1997, Hollman and Katan 1998, Scalbert and Williamson 2000, Deprez et al. 2000, Nardini et al. 2002, 2006, Rondini et al. 2002]. The more complex phenolic compounds are broadly consumed in foods, but they are easily degraded leading to the free phenolic acids formation [Gross et al. 1996, Clifford et al. 2000, Rechner et al. 2001 a, b]. In consequence, the *in vitro* antioxidant activities of more complex phenolics can be not relevant to their antioxidant activities *in vivo* due to their degradation. Although ferulic acid is present in the cereals mainly in the esterified form [Maillard and Berset 1995, Maillard et al. 1996], this phenolic acid is released by the FAE activity in the human gastrointestinal tract [Andreason et al. 2001, Couteau et al. 2001].

FAEs produced by lactic acid bacteria were previously studied by some authors [Donaghy et al. 1998, Wang et al. 2004, 2005, Nsereko et al. 2008]. The *in vivo* release

of phenolic acids in the gastrointestinal tract can be a very interesting aspect of the beneficial role of the lactic acid bacteria. The aim of this work was to study the ability of *Lactobacillus acidophilus* K1 and *Lactobacillus rhamnosus* E/N, PEN, OXY to produce FAE using different natural and synthetic carbon sources.

MATERIAL AND METHODS

Strains and materials

Lactobacillus acidophilus K1 was isolated from the adult human gastrointestinal tract and was a kind gift from Prof. dr hab. Maria Bielecka from Department of Food Microbiology, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn, Poland [Bielecka et al. 2002]. *Lactobacillus rhamnosus* E/N, PEN and OXY were from the collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities, Lublin, Poland. The strains were grown in the MRS broth (10 cm^3) at 37°C in the test tubes (30 cm^3) and every 24 hours they were repeatedly inoculated in the new medium broths using 3% (v/v) of the inoculum. These cultures were used for subsequent studies as described below.

Methyl ferulate, methyl p-coumarate, methyl syringate and methyl vanillate were purchased from Apin Chemicals Ltd., UK. Larchwood arabinogalactan (cat. no 10830), corn and apple pectin (cat. no 76282) and amylopectin from maize (cat. no 10120) were from Fluka Biochemika. German wheat bran (Bialscy Otręby, M. and P. Hillar, Rybno, Poland) and rye bran were purchased in the local shop. Brewers' spent grain was a gift from the local brewery "Perła" Browary Lubelskie S.A., Lublin. HPLC grade chromatography reagents as well as, other reagents of analytical grade were from POCh Gliwice, Poland.

Method of the cultivation for FAE

During the main experiment, the strains were grown at 37°C in the test tubes (10 cm^3), in the anaerobic conditions in the minimal growth medium containing (per 1000 cm^3): peptone 2.0 g, yeast extract 2.0 g, L-cysteine·HCl 0.5 g, NaCl 0.1 g, $NaHCO_3$ 2.0 g, K_2HPO_4 0.04 g, KH_2PO_4 0.04 g, $MgSO_4 \cdot 7H_2O$ 0.01 g, $CaCl_2 \cdot 6H_2O$ 0.01 g, Tween 80 2 cm^3. German wheat bran, rye bran, brewer's spent grain, apple pectin, corn amylopectin or larchwood arabinogalactan (3% (m/v)) or methyl esters of phenolic acids (0.25% (m/v)) were used as the sole carbon source after the sterilization. The methyl esters were dissolved in 0.05 cm^3 of 96% ethanol and toped up to 10 cm^3 prior to the addition to the broth. The growth medium was inoculated using 5% (v/v) of the inoculum grown as described above.

The samples (10 cm^3) containing the natural substrates were incubated at 37°C for 75 h after the addition of the substrate. The samples containing the methyl esters were incubated for 84 h after the addition of the carbon sources in the anaerobic conditions. The anaerobic conditions were created by the addition of $NaHCO_3$ solution (0.5 cm^3, 15% (w/v)) and pirogallol solution (0.5 cm^3, 20% (w/v)) into the cotton stoppers placed inside the test tubes. The tubes were then additionally aseptically closed with the rubber stoppers.

The bacterial growth was controlled by the spectrophotometric measurements of the optical density at 600 nm. The supernatants were centrifugated (10 000 g, 15 min, 4°C, SIGMA 4K14 Laboratory Centrifuges, Polygen) prior the determination of the FAE activity. The cultivations were duplicated and the results represent the mean values with standard deviations. Herein presented results refer to the total growth times including 24 h of the initial bacterial growth without the studied carbon sources. The samples containing no bacterial inoculum (blank samples) was run in duplicate and the FAE activity in these samples was estimated. The obtained FAE activity was subtracted from the results obtained for the studied samples.

FAE activity determination

The ability of the studied bacterial FAE to hydrolyse metyl ferulate was studied and the released ferulic acid was determined using HPLC with UV detection. Metyl ferulate was dissolved in the minimal volume of 96.0% ethanol ((v/v), 0.05 cm^3) followed by the dilution with Tris-HCl buffer (100 $mmol \cdot dm^{-3}$, pH 6.5) until the concentration of 6 $mmol \cdot dm^{-3}$ was obtained. The volume of 0.5 cm^3 of the sample supernatant after the centrifugation was added to 0.1 cm^3 of methyl ferulate working solution and the samples were incubated for 5 h at 37°C. The enzyme was then inactivated by boiling in water (5 min) followed by cooling and centrifugation (7000 g, 30 min, 6°C). The double blank samples (lacking the substrate or the supernatant solution) were run and analyzed simultaneously as described above. Both blank samples were subtracted from the corresponding studied samples. FAE activity was expressed in units (1 unit was equal to 1 nmol of ferulic acid released in 1 cm^3 of the reaction medium after 1 min of the incubation). Analyses were duplicated and the mean values with standard deviations were calculated.

Effect of pH on the FAE activity

The effect of pH on the FAE activity was examined using 0.1 $mol \cdot dm^{-3}$ Tris-HCl buffer at the following pH values: 4.4, 5.6, 6.3, 6.9, 7.6, 8.1, 8.6, 9.0, 9.6. The samples containing 0.1 cm^3 of the sample supernatants, 0.1 cm^3 of the buffer and 0.2 cm^3 of methyl ferulate (1.2 $mmol \cdot dm^{-3}$) were incubated at 37°C for 5 h. The enzyme was then inactivated in the boiling water (5 min) and the concentration of free ferulic acid was determined using HPLC-UV as described below. Double blank samples were run simultaneously. The analyses were performed in duplicate.

Effect of buffer on the FAE activity

The influence of the buffer (0.1 $mol \cdot dm^{-3}$) on FAE activity was evaluated using Theorell/Steinhagen (citric acid: H_3PO_4: boric acid), Britton/Robinson (acetic acid: H_3PO_4: boric acid) McIlvane (citric acid: Na_2HPO_4) or Tris/HCl buffer. All studied buffers were at optimal pH values for FAE activity. 0.1 cm^3 of the supernatant solution was incubated with 0.1 cm^3 of the appropriate buffer and 0.2 cm^3 of MF (1.2 $mmol \cdot dm^{-3}$) for 5 h at 37°C. The enzyme was then inactivated by boiling in water (5 min) and the concentration of free ferulic acid was determined using HPLC-UV as described below. Double blank samples were run simultaneously. The analyses were performed in duplicate.

Effect of the temperature on FAE activity

In order to assess the effect of the temperature on FAE activity, the sample supernatants were pre-incubated for 5 h at 22°C, 28°C, 37°C, 42°C or 55°C. The FAE activities in these sample supernatants were determined as described above. Double blank samples were run simultaneously. The analyses were performed in duplicate.

HPLC-UV separation and identification of ferulic acid 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 C18 column (USA, 250 mm, 4.6 mm i.d., 5 μm), and Waters Symmetry C18 precolumn (5 μm, 8×20 mm) were used for separations. The following eluents were used: 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 according to Kim et al. [2006] with the program 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 eluent flow was 0.8 $cm^3 \cdot min^{-1}$ (17 MPa). Ferulic acid concentrations were calculated using the calibration curve plotted using the series of the solutions of ferulic acid HPLC standard. Within-one-day repeatability (variation in measurements) and the laboratory reproducibility for ferulic acid was 3.4% and 6.0%, respectively.

The statistical analysis of the results was performed using STATISTICA 8.0 (StatSoft, Poland). The routine statistical tests were used (mean values, standard deviations, Tukey's HSD test, one-dimenstional and multidimensional analysis of variations). The $p \leq 0.05$ value was taken as the measure of the statistically significant results.

RESULT AND DISCUSSION

There is a limited number of works concerning the physical characteristics and the activity of FAE produced by *Lactobacillus* strains and the literature on this subject is scarce. In this study the overall highest enzyme activity was seen in the culture supernatants of *L. acidophilus* K1 after the bacterial growth with MF (23.34 ±0.05 units after 60 h of cultivation). Also, the high enzyme activities between 36 and 48 h of the cultivation with MF (8.74 ±0.29-13.42 ±0.02 units, respectively) were seen (Fig. 1). With Mp-C as the substrate, the considerable enzyme activities between 36 and 48 h were seen (14.96 ±0.47 to 9.56 ±0.10 units, respectively). After 84 h of cultivation, no enzyme activity was detected both in the supernatants produced with MF and Mp-C but this result was not further studied and needs explanation in the future. Also, the presence of FAE activity in the superanatants produced at 60 h using MS needs clarification because the presence of the enzyme at this cultivation time exclusively seems to be highly doubtful. It must be noted that FAE was produced by *L. acidophilus* K1 with cinnamic acid derivatives (MF and Mp-C) whereas the benzoic acid esters (MS and MV) were insufficient carbon sources for the enzyme induction (Fig. 1).

Other *Lactobacillus* strains (E/N, OXY, PEN) presented the limited ability to produce FAE with cinnamic acids methyl esters. In the case of E/N and PEN strains, both MF

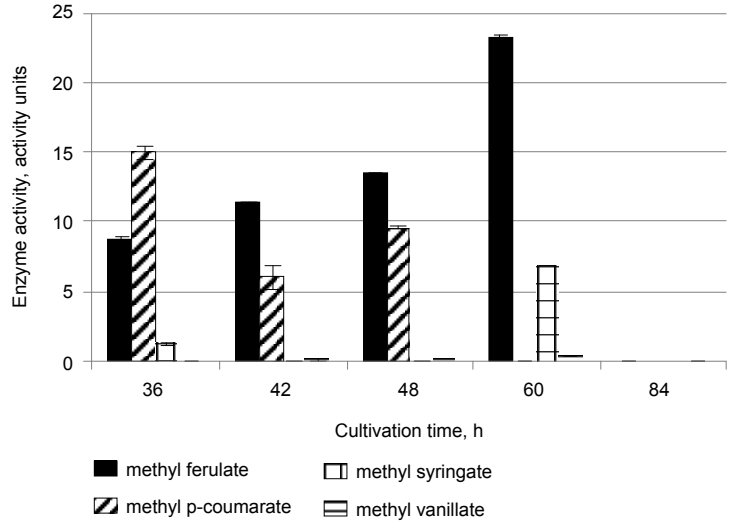

Fig. 1. FAE acivities produced by *L. acidophilus* K1 with the use of synthetic carbon sources

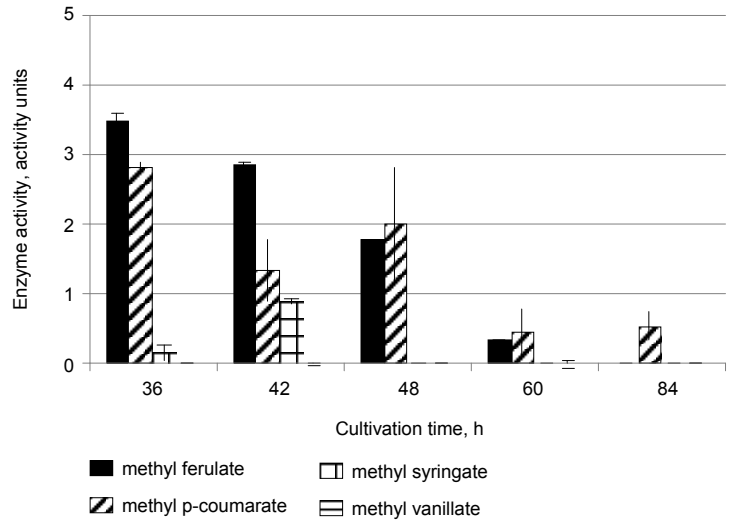

Fig. 2. FAE activities produced by *L. rhamnosus* E/N with the use of synthetic carbon sources

and Mp-C induced the strains to produce FAE to the similar extent (Fig. 2 and 4, respectively) whereas *L. rhamnosus* OXY probably preferred Mp-C as the carbon source (Fig. 3). Generally, the FAE activity in *L. rhamnosus* post cultivation supernatants was only 3.64 ±0.84 units (*L. rhamnosus* OXY, 36 h, with Mp-C) or considerably lower.

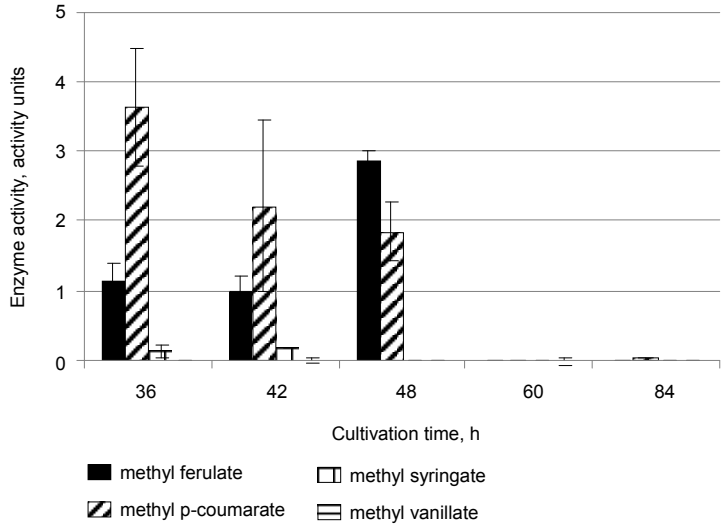

Fig. 3. FAE activities produced by *L. rhamnosus* OXY with the use of synthetic carbon sources

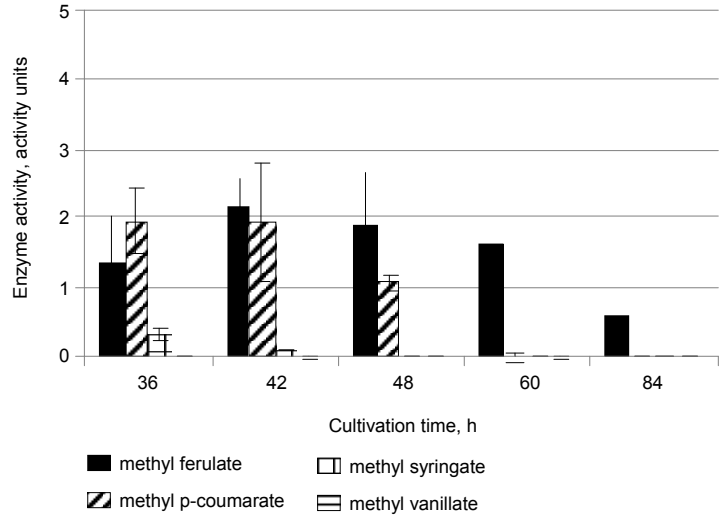

Fig. 4. FAE activities produced by *L. rhamnosus* PEN with the use of synthetic carbon sources

L. acidophilus K1 very effectively produced FAE in the presence of brewers' spent grain used as the carbon source (Fig. 5). The enzyme activity was high from the very beginning of the experiment and reached 2.64 ±0.06 units after 51 hours of cultivation. This result is interesting since brewers' spent grain is still not effectively exploited although it is a very cheap and a valuable by-product of the brewing industry. It is a rich

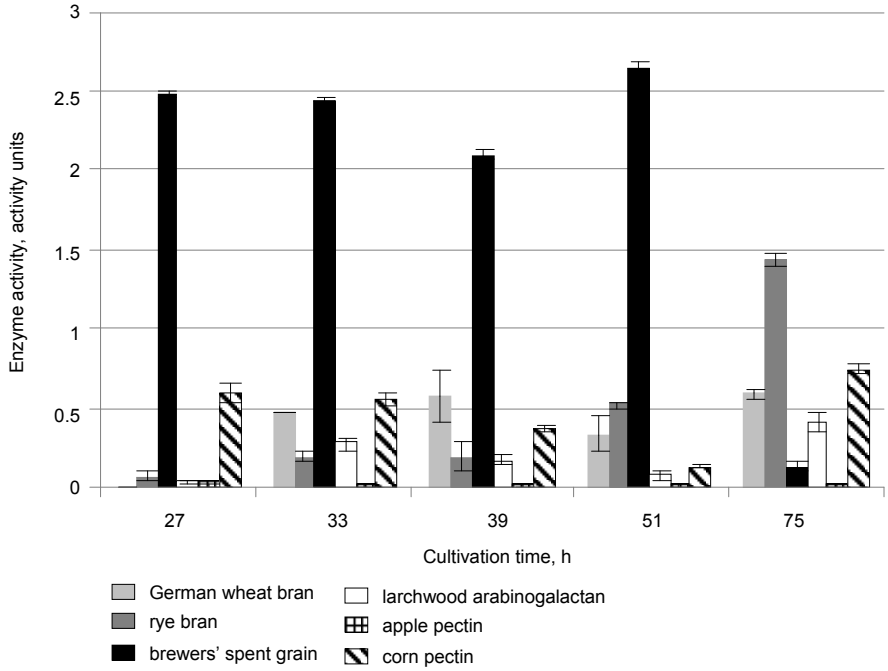

Fig. 5. FAE activities produced by *L. acidophilus* K1 with the use of natural carbon sources

source of proteins, starch, dietary fiber, phenolic compounds [Xiros et al. 2008]. Also, a high FAE activity was obtained in the culture supernatants from the broth with rye bran (1.43 ±0.05 units) or corn pectin (0.74 ±0.04 units) after 75 hours of cultivation. It must be noticed that these enzyme activities were significantly lower than in the case of brewers' spent grain as a carbon source (Fig. 5). Other natural substrates used the induction of FAE from *L. acidophilus* K1 growth seem to be of minor importance in this context. These results are interesting since they suggest the possibility of *L. acidophilus* K1 to be used in synbiotic preparations containing brewer's spent grain. The activities of FAEs produced by *Lactobacillus rhamnosus* E/N, OXY and PEN (Fig. 6, 7 and 8, respectively) were significantly ($p > 0.05$) lower in the presence of the studied natural substrates in comparison to the corresponding results obtained for *L. acidophilus* K1. Nevertheless, it must be noticed that brewers' spent grain was once more the most suitable carbon source for the induction of FAE (E/N, OXY and PEN) followed by German wheat bran and larchwood arabinogalactan (E/N, PEN) or German wheat bran and corn pectin (OXY).

The optimal pH for FAE activity was 6.3 (*L. acidophilus* K1, *L. rhamnosus* OXY) or 6.5 (*L. rhamnosus* E/N, *L. rhamnosus* PEN; detailed data not shown, personal comm.: dominik.szwajgier@up.lublin.pl). In the case of all enzymes the considerable loss of FAE activity (40-50%) was observed with the decrease of pH to 4.4 followed by the further, near complete enzyme inactivation at pH 3.7. On the other hand, at pH 5.6 and pH 6.9, 80-90% of the original enzyme activity was retrieved.

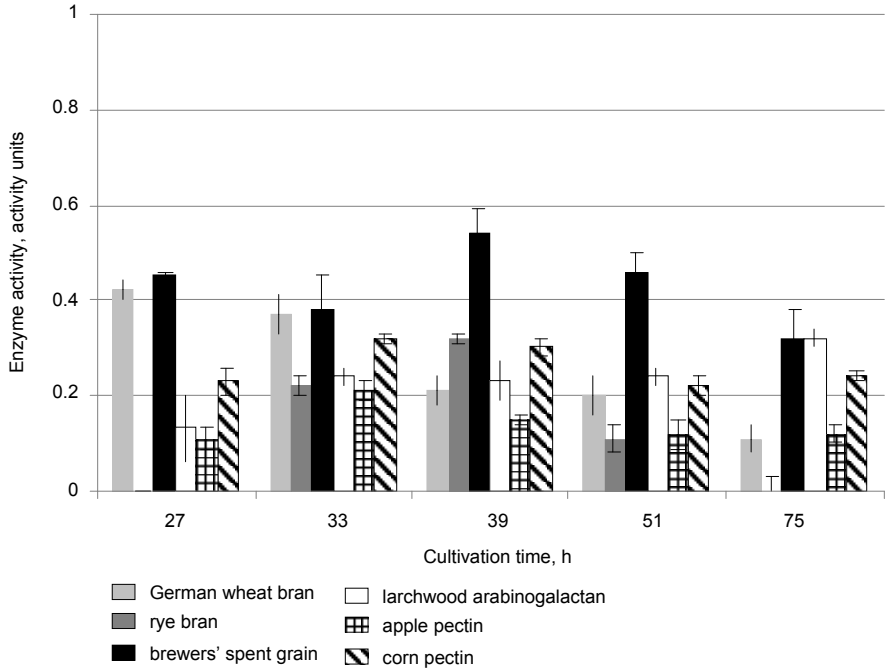

Fig. 6. FAE activities produced by *L. rhamnosus* E/N with the use of natural carbon sources

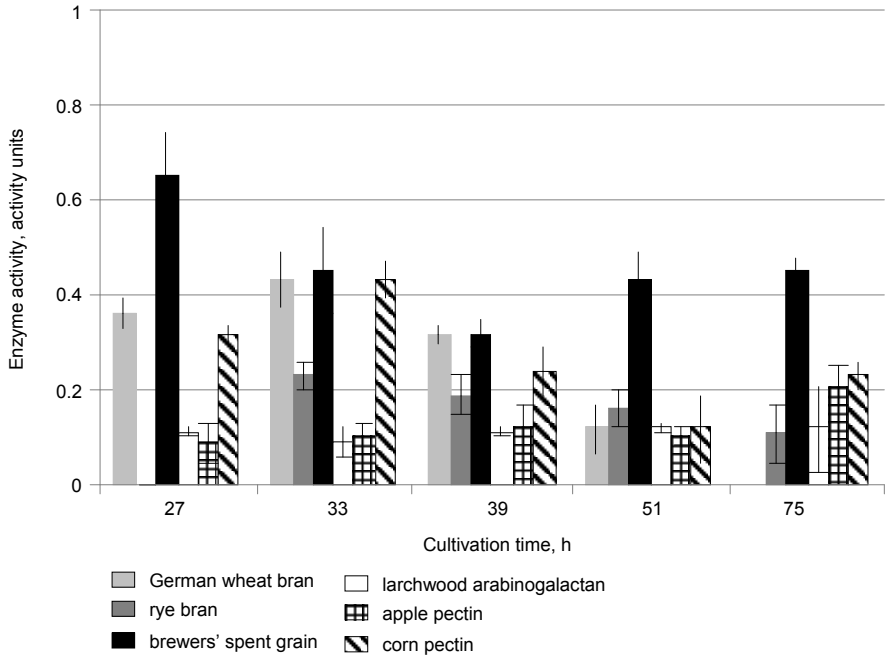

Fig. 7. FAE activities produced by *L. rhamnosus* OXY with the use of natural carbon sources

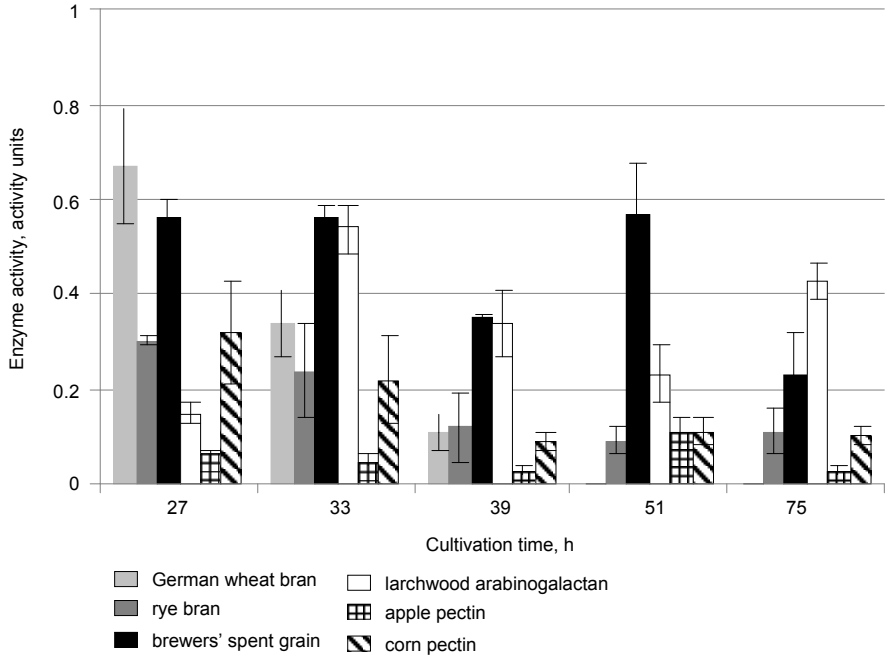

Fig. 8. FAE activities produced by *L. rhamnosus* PEN with the use of natural carbon sources

The optimal buffer for the FAE activity was Britton-Robinson buffer (*L. acidophilus* K1, *L. rhamnosus* OXY and *L. rhamnosus* PEN) whereas FAE from *L. rhamnosus* E/N was equally active in the Britton-Robinson and Theorell/Steinhagen buffers (detailed data not shown, personal comm.: dominik.szwajgier@up.lublin.pl).

The influence of temperature on FAE activity was studied at 22-55°C using FAE from *L. acidophilus* K1 in Britton-Robinson buffer (pH 6.3). The highest enzyme activity was seen at 37°C. The significantly lower enzyme activities were seen already at 28°C and 42°C and the complete inactivation of the enzyme activity was observed at approx. 55°C (detailed data not shown, personal comm.: dominik.szwajgier@up.lublin.pl).

In the herein presented study, an attempt was made to induce FAE by the use of different substrates that contained esters bonds. Previously, novel FAEs was detected produced by some *Bifidobacterium* strains [Szwajgier and Dmowska 2010]. In the case of the strains studied in the citied as well, as herein presented work, the most effective substrates were synthetic methyl p-coumarate and methyl ferulate followed by some natural substrates (above discussed in detail).

One of the first authors who studied the production of FAEs by lactic acid bacteria was Donaghy et al. [1998] who screened approx. 130 strains (*Enterococcus, Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Propionibacterium)* for the enzyme production. In this work, MF, Mp-C and 2-O-[5-O-(trans-ferulic acid)-β-L-ara-*f*]-D-xyl-*-p* were used as the carbon source for the bacterial growth. The most effective FAE producers were *Lactobacillus fermentum* NCF 1751 and 6 *Bacillus subtilis* strains. The physical properties of FAE produced by *Lactobacillus casei* L96 were characterized by

Choi and Lee [2001]. The optimal temperature and pH for the enzyme activity were 37°C and 7.0, respectively, although the enzyme was active in the range of 20-50°C and pH 4.0-9.0. Couteau et al. [2001] detected FAE activity in the post-cultivation supernatants of *Bifidobacterium* strains with chlorogenic acid as the carbon source. The authors also detected the release of ferulic acid in the intestines as the result of the activities of FAEs originating from intestinal microorganisms. Wang et al. [2004] was the first to isolate, purify and characterise the intracellular FAE produced by *Lactobacillus acidophilus,* a typical human intestinal bacteria. The optimal pH for FAE for this enzyme was 5.6 whereas our results prove that the optimal pH for FAEs from both *L. acidophilus* K1 and three *L. rhamnosus* strains was significantly higher (6.3 or 6.5) although at pH 5.6 the remnant enzyme activity was still high. Nevertheless, these authors underlined the enzyme was very active in the pH range 5.3-6.5. The purified FAE hydrolyzed free ferulic acid from O-(5-O-ferulic acid-α-L-ara-*f*)-(1→3)-O-β-D-xyl-*p*-(1→4)-D-xyl--*p* and 5-O-feruloyl-L-arabinofuranose originating from corn bran. Studies by Wang et al. [2005] concerned the induction of FAE produced by *L. acidophilus* using different carbon sources. The production of FAE was strongly stimulated by hemicellulosic compounds. The highest enzyme activity was obtained with the use of wheat bran as carbon source. In the culture supernatants grown with the simultaneous use of wheat bran and xylanase, the amount of the released free ferulic acid increased from 0 to 12 nmol·cm^{-3}. In the presence of α-L-arabinofuranosidase, the concentration of ferulic acid increased from 0 to 3.64 nmol·cm^{-3}.

In the herein presented work it was shown that some natural substrates were good carbon sources for bacterial growth and the induction of FAE. Yuan et al. [2005] determined that the growth and the biomass yield of *B. bifidum* F-35 was improved by feruloylated oligosaccharides from wheat bran insoluble dietary fiber although the release of free FA or FAE activity was not studied in the frames of the citied work.

Some other authors studied the degradation of different non-starch polysaccharides but omitted the detailed description of FAE in the post cultivation broths. For example, Zeng et al. [2007] determined that *B. bifidum, B. adolescentis and B. infantis* degraded xylooligosaccharides and produced β-D-xylosidase, α-L-arabinosidase. Vardakou et al. [2007] proved that FAE was present in *in vitro* samples of the complex faecal slurry containing *Bacteroides*, *Bifidobacterium*, *Clostridium* and *Lactobacillus* strains and water-unextractable arabinoxylan fractions (containing a considerable content of esterified FA) but no enzyme activity was present in the samples containing water-extractable arabinoxylan fractions (with the low content of esterified FA). In other words, ferulic acid in the ester form induced the production of FAE in these strains. It must be also underlined that both arabinoxylan fractions stimulated the growth of *Bifidobacterium* but not *Bacteroides* or *Clostridium* strains. The authors did not study the individual ability of each strain to produce FAE as well as FAEs were not purified and characterised in this work. Similarly, FAE was not closer characterised in another work of these authors [Vardakou et al. 2008]. The extracellular xylanase and FAE activities were detected in the post cultivation supernatants obtained using *Lactobacillus* and *Bifidobacterium* strains and unextractable arabinoxylan isolated from wheat. This non-starch polysaccharides fraction induced the higher xylanase and FAE production by both LAB strains especially when the water unextractable, xylanase-pretreated arabinoxylan was used. Also, the improved growth of both LAB strains was seen with the use of the untreated or xylanase-treated arabinoxylan fractions. Similarly, Napolitano et al. [2009] studied the *in vitro* fermentation and release of ferulic acid from durum wheat dietary

fibre (DWF) by intestinal microbiota in a gut model mimetizing the human colonic environment. The authors stated that the degradation of durum wheat fibre with the enzyme preparation from *Trichoderma harzianum* strain 22 prior the inoculation with faecal slurry elevated the degree of utilisation of this substrate by faecal microbials. Although in both cases the release of free ferulic acid from DWF was seen, the pre-treatment of DWF with the enzyme preparation from *Trichoderma harzianum* strain 22 elevated almost 3-fold the content of free ferulic acid released in comparison to control. Also, the growth (count number) of *Lactobacillus* and *Bifidobacterium* strains enumareted in this work was stimulated by enzyme-treated DWF. No FAE was closer characterized within the frames of this work. In another work, Nsereko et al. [2008] studied the influence of ensiling perennial ryegrass with LAB strains on neutral detergent fiber degradation. Of 10,000 LAB strains, approx. 500 were FAE-positive and 8 strains were further studied, namely *Lactobacillus buchneri*, *L. crispatus*, *L. reuteri*, *L. brevis* and one not identified *Lactobacillus* strain). A similar work was performed by Kang et al. [2009] who conserved corn in mini silos with the use of *Lactobacillus casei* and *L. buchneri*, FAE-producing LAB. The authors evaluated the influence of esterase – producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage but the influence of FAEs produced during on the dry matter digestion of corn was not studied in detail. Kin et al. [2009] isolated from stool samples of diabetes-resistant rats a *Lactobacillus johnsonii* strain that displayed a strong FAE activity. The enzyme was purified and two proteins with cinnamoyl esterases produced by *Lactobacillus johnsoni were* isolated. The two proteins displayed 42% sequence identity and very effectively hydrolyzed a broad range of substrates such as ethyl ferulate and chlorogenic acid.

FAE produced by LAB can be considered as the enzyme of the minor importance in the context of the enzyme-assisted degradation of food components. Nevertheless, it can play a considerable role in the release of phenolic acids in the intestine. Ferulic acid and other ester-bound phenolic acids released in the gastrointestinal tract are easily absorbed into bloodstream and elevate the antioxidant status of the plasma. The knowledge on the ability of LAB strains to hydrolase the non-starch polysaccharides is still incomplete.

The results of the herein presented study suggest that *Lactobacillus acidophilus* K1 is able to effectively de-esterify some natural substrates and to use the organic carbon in the form of phenolic acid esters. Therefore, the production of FAE by *Lactobacillus* strains with the use of natural or synthetic carbon sources creates the possibility to use this bacteria for the production of probiotic and synbiotic preparations. Bhathena et al. [2008] immobilized *L. fermentum* 11976 cells in alginate-poly(L-lysine)-alginate gel microcapsules which were further used as the source of FAE in the *in vitro* experiments using the model of the gastrointestinal tract. FAE activity was detected in the supernatant solution of the mixed post-cultivation broth.

Finally, the purification of FAE produced by LAB can be useful for the industrial purposes as LAB can be considered as GRAS (generally recognised as safe) microorganisms. The released phenolic compounds can be further converted into flavouring agents, cosmetic additives etc.

CONCLUSIONS

1. *Lactobacillus* strains (with the most effective *L. acidophilus* K1) exhibited the ability to produce ferulic acid esterases in the presence of synthetic and natural substrates. The highest enzyme activity was obtained with *L. acidophilus* K1, methyl ferulate and methyl p-coumarate after 60 h and 48 h of the cultivation, respectively.

2. *L. rhamnosus* E/N, OXY and PEN exhibited the limited ability to produce FAE with methyl esters of cinnamic acid derivatives. In the case of all strains, cinnamic acid derivatives were suitable carbon sources for the enzyme production whereas benzoic acid derivatives exhibited no ability to induce ferulic acid esterase.

3. In the case of *L. acidophilus* K1, the activities of ferulic acid esterases produced in the presence of natural substrates were significantly lower than the corresponding enzyme activities determined in the presence of methyl ferulate and methyl p-coumarate.

4. Among the natural substrates, the most suitable substrates for enzyme production were: brewer's spent grain (*L. acidophilus* K1, *L. rhamnosus* E/N, OXY, PEN), rye bran, corn pectin (*L. acidophilus* K1), German wheat bran and larchwood arabinogalactan (*L. rhamnosus* E/N, PEN) or German wheat bran and corn pectin (*L. rhamnosus* OXY).

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PRODUKCJA ZEWNĄTRZKOMÓRKOWYCH ESTERAZ KWASU FERULOWEGO PRZEZ SZCZEPY Z RODZAJU *LACTOBACILLUS* Z WYKORZYSTANIEM NATURALNYCH I SYNTETYCZNYCH ŹRÓDEŁ WĘGLA

Wstęp. Esterazy kwasu ferulowego (FAE, EC 3.1.1.73), znane jako esterazy ferulowe, esterazy cynamonowe należą do popularnej grupy hydrolaz rozpowszechnionych w króle-

stwie roślin. W przeszłości zostały scharakteryzowane zwłaszcza grzybowe esterazy, natomiast stosunkowo rzadko był charakteryzowany enzym produkowany przez bakterie kwasu mlekowego. Wiadomo że silne przeciwutleniacze – kwasy fenolowe mogą być uwalniane z frakcji błonnika pokarmowego w wyniku działania enzymów bakterii jelitowych, w skład których wchodzą również bakterie z rodzaju *Lactobacillus*. Celem pracy było zbadanie czterech mikroorganizmów (*L. acidophilus* K1, *L. rhamnosus* E/N, PEN, OXY) pod kątem uzdolnień do produkcji zewnątrzkomórkowej esterazy kwasu ferulowego w obecności wybranych syntetycznych i naturalnych źródeł węgla.

Materiał i metody. Drobnoustroje hodowano w pożywkach minimalnych z dodatkiem: otrąb z pszenicy orkisz, otrąb żytnich, wysłodzin piwowarskich, arabinogalaktanu z modrzewia, pektyn jabłkowych, pektyn kukurydzianych, ferulanu metylu, p-kumaranu metylu, syringanu metylu lub wanilianu metylu jako jedynego źródła węgla. Produkcję zewnątrzkomórkowej esterazy kwasu ferulowego określano w supernatantach pohodowlanych za pomocą ferulanu metylu jako substratu oraz HPLC z detekcją w zakresie UV.

Wyniki. Największą aktywność esterazy kwasu ferulowego stwierdzono w supernatantach po hodowli *L. acidophilus* K1 z ferulanem metylu (maks. aktywność 23,34 ±0,05 u) i p-kumaranem metylu (maks. aktywność 14,96 ±0,47 u). *L. rhamnosus* E/N, OXY and PEN wykazywały ograniczoną zdolność do produkcji esterazy ferulowej w obecności metylowych pochodnych kwasów fenolowych. Nie stwierdzono obecności esterazy, gdy syringan metylu lub wanilian metylu był jedynym źródłem węgla. Wysłodziny piwowarskie były najlepszym naturalnym substratem do produkcji esterazy ferulowej przez *L. acidophilus* K1 (maks. aktywność 2,64 ±0,06 u) i *L. rhamnosus* E/N, OXY and PEN. Esteraza była również produkowana gdy źródłem węgla były: otręby żytnie, pektyna kukurydziana (*L. acidophilus* K1), otręby z pszenicy orkisz, arabinogalaktan z modrzewia (*L. rhamnosus* E/N, PEN), otręby z pszenicy orkisz, pektyna kukurydziana (*L. rhamnosus* OXY).

Wnioski. W pracy wykazano uzdolnienie bakterii z rodzaju *Lactobacillus* do produkcji esterazy kwasu ferulowego w obecności wielu substratów zawierających wiązania estrowe. Największe aktywności enzymatyczne uzyskane w obecności syntetycznych pochodnych kwasu ferulowego i p-kumarowego sugerują, że bakterie musiały wykorzystać to jedyne źródło węgla w formie estrowej, natomiast w obecności substratów naturalnych było możliwe wykorzystanie innych źródeł węgla. Esteraza kwasu ferulowego jest enzymem o nieco mniejszym znaczeniu w degradacji składników żywności zachodzącej w czasie trawienia jelitowego. Pomimo to wydaje się celowe dalsze charakteryzowanie omawianych enzymów produkowanych przez bakterie kwasu mlekowego.

Słowa kluczowe: esteraza kwasu ferulowego, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, przeciwutleniacz

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