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NOVEL FERULIC ACID ESTERASES FROM *BIFIDOBACTERIUM* SP. PRODUCED ON SELECTED SYNTHETIC AND NATURAL CARBON SOURCES

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Background. Ferulic acid esterases (or feruloyl esterases), a common group of hydrolases are very well distributed in the plant kongdom. The fungal feruloyl esterases were very extensively studied whereas probiotic lactic acid bacteria as the source of this enzyme were generally omitted. Free phenolic acids – strong antioxidants can be released from the dietary fiber by the action of intestinal lactic acid bacteria. The aim of this study was to examine the three probiotic *Bifidobacterium* strains to produce extracellular FAE on different synthetic and natural carbon sources.
Material and methods. Studies were carried out using *Bifidibacterium* strains (*B. animalis* Bi30, *B. catenulatum* KD 14 and *B. longum* KN 29). The strains were cultivated using minimal growth media containing selected natural and synthetic carbon sources: German wheat bran, rye bran, barley spent grain, isolated larchwood arabinogalactan, apple pectin, corn pectin, methyl esters of phenolic acids. The production of extracellular feruloyl esterase was estimated using the post cultivation supernatants and methyl ferulate. The concentration of ferulic acid released from the ester was determined using HPLC with DAD detection.
Results. The most efficient bacterial strain for FAE production was *B. animalis* cultivated in the presence of methyl p-coumarate and methyl ferulate as the main carbon sources (14.95 nmol·ml^{-1}·min^{-1} and 4.38 nmol·ml^{-1}·min^{-1}, respectively). In the case of each FAE, the highest activity was obtained at 37^{o}C (pH 6.3) in Theorell/Steinhagen buffer (*B. animalis* Bi30) or in Tris/HCl buffer (*B. catenulatum* KD14 and *B. longum* KN29). Taking under consideration all results, it should be noticed that the highest feruloyl esterase activities were obtained using synthetic methyl esters of phenolic acids.
Conclusions. The presented results broaden the knowledge about the production of the feruloyl esterase by probiotic bacteria. Although the enzyme is only accessory during the hydrolysis of food components during intestinal digestion, some conclusions on the role of lactic acid bacteria in the release of food antioxidants phenolic acids can be established.

Key words: ferulic acid esterase, ferulic acid, *Bifidobacterium*, probiotics, antioxidant

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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

Scientific databases provide only limited knowledge on ferulic acid esterases (FAEs) produced by bacteria, especially by lactic acid bacteria [Wang et al. 2004, Nsereko et al. 2008]. Esterases (EC 3.1.1.x) are very popular hydrolases in the plant and animal kingdom and the fungal enzymes were very well characterised in the past. FAEs produced by probiotic strains present in the human gastrointestinal tract can be a very interesting subject of the studies. Dietary fiber is a rich source of bound phenolic acids, for example ferulic or p-coumaric acid – strong antioxidants *in vitro* [Gerhäuser 2005] and *in vivo* [Saija et al. 2000, Joshi et al. 2006, Young et al. 2008, Itagaki et al. 2009]. Prior the absorption, bound phenolic acids are released in the small intestine followed by the transformations (mainly in the liver) [Scalbert and Williamson 2000, Rechner et al. 2001, Rondini et al. 2002, Olthof et al. 2003]. It was previously shown that esterified ferulic acid can be released from dietary fiber fractions due to the action of bacterial esterases [Andreason et al. 2001, Couteau et al. 2001]. It must be noticed that the more complex phenolic compounds like flavonols and proanthocyanidins are very easily degraded in comparison to simple phenolic acids [Gross et al. 1996, Rechner et al. 2001]. As a result, simple phenolic acids can exhibit antioxidant activity *in vivo* [Deprez et al. 2000] On the other hand, the possible *in vivo* antioxidant activities of more complex phenolic compounds are not relevant to their antioxidant activities measured *in vitro* due to their degradation. The role of complex phenolic compounds as antioxidants *in vivo* must be revised and this problem is not existing in the case of simple phenolic acids. The aim of the presented work was examination of three probiotic *Bifidobacterium* strains to produce extracellular FAE on different synthetic and natural carbon sources.

MATERIAL AND METHODS

Strains and materials

B. animalis Bi30, *B. catenulatum* KD14 and *B. longum* KN29 were a kind gift of Prof. Maria Bielecka, Ph.D. (Department of Food Microbiology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland). Methyl esters of phenolic acids were obtained from Apin Chemicals, Oxon, UK. Pectin from apples (Fluka cat. no 76282), amylopectin from maize (Fluka cat. no 10120), (+)-arabinogalactan from larchwood (Fluka cat. no 10830) were used. German wheat bran and rye bran were purchased in the local shop, barley spent grain was a gift from a local brewery "Perła" Browary Lubelskie S.A., Lublin. Garchez broth was purchased from BTL Spółka z o.o. Zakład Enzymów i Peptonów, Poland. HPLC grade chromatography reagents as well as other reagents of analytical grade were from POCh Gliwice, Poland.

Culture conditions

Bifidobacterium strains were maintained in Garchez medium (10 cm^3) at 37°C in test tubes. The anaerobic conditions were obtained by the injection of 0.5 cm^3 of 15% (m/v) $NaHCO_3$ solution and 0.5 cm^3 of 20% (m/v) pirogallol solution in the cotton stoppers followed by the aseptical closing of the tubes with rubber stoppers). Every 24 h, stock cultures were repeatedly inoculated in a new medium using 5% (v/v) of inoculum. These cultures were used for subsequent studies as described below.

Method of cultivation for FAE

Bacterial strains were grown at 37°C in test tubes (10 cm^3), in anaerobic conditions in minimal growth medium containing in 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, barley 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 a carbon source. The growth medium was inoculated using 5% (v/v) of inoculum grown in Garchez medium for 24 h (37°C) as described above. Presented results refer to the total growth times including 24 h of initial bacterial growth on Garchez medium. The bacterial growth was simultaneously controlled by spectrophotometric measurements of the optical density at 600 nm. FAE activity was determined using the supernatants after centrifugation (10 000 g, 4°C, 15 min, SIGMA 4K14 Laboratory Centrifuges, Polygen). The cultivations were duplicated and the results represent the mean values with standard deviations.

FAE activity determination

The ability to hydrolyse MF by FAE was assessed and the free FA content was determined using HPLC-DAD. MF was dissolved in a minimal volume of 96.0% (v/v) ethanol followed by the dilution to 6 $mmol \cdot dm^{-3}$ using Tris-HCl buffer (0.1 $mol \cdot dm^{-3}$, pH 6.5). 0.5 cm^3 of the studied supernatant solution was added to 0.1 cm^3 of the substrate solution and the samples were incubated at 37°C for 5 h. The enzyme was then inactivated in a boiling water bath (5 min) followed by the cooling and centrifugation (7000 g, 30 min, 6°C, centrifuge MPW-365, Mechanika Precyzyjna, Warsaw, Poland). Free FA and MF were separated and analyzed using HPLC-DAD. Blank samples (lacking the substrate solution or the supernatant) were simultaneously incubated and analysed. FAE activity was expressed in units (u); 1 unit was equall to 1 nmol of ferulic acid released in 1 cm^3 of the reaction medium after 1 min of incubation. Analyses were duplicated from each cultivation and mean values with standard deviations were calculated.

Determination of optimal pH, buffer and temperature for FAE activity

Optimal pH for FAE activity was evaluated using the optimal buffer (pH 3.7, 4.4, 5.6, 6.3, 6.9, 7.6, 8.1, 8.6, 9.0 and 9.6). 0.1 cm^3 of the supernatant was incubated at 37°C for 5 h with 0.1 cm^3 of the corresponding buffer and 0.2 cm^3 of MF (1.2 $mmol \cdot dm^{-3}$). The influence of buffer (pH 6.3) on the enzyme activity was studied 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) and Tris/HCl buffers. 0.1 cm^3 of the supernatant solution was incubated for 5 h at 37°C with 0.1 cm^3 of the appropriate buffer and 0.2 cm^3 of MF (1.2 $mmol \cdot dm^{-3}$). The optimal temperatures for FAE activity were assessed after 5 hours of incubation of the supernatant samples (0.1 cm^3) with the optimal buffer (0.1 cm^3) and 0.2 cm^3 of MF (1.2 $mmol \cdot dm^{-3}$) at 22°C, 28°C, 37°C, 42°C and 55°C. Double blank samples were run simultaneously. FAE activities were calculated after HPLC-DAD analyses as described below.

HPLC-DAD analysis of FA and MF

The HPLC system consisted of two Gilson 306 Separation Module piston pumps, Gilson Model 170 PhotoDiode Array Detector, 0.02 cm^3 Gilson loop, Gilson 805 manometric module, Gilson 811C dynamic mixer. 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 eluents were: A – 1% (w/v) acetic acid solution in DDI water, B – 50% HPLC-grade acetonitrile in DDI water. Signals were monitored at 320 nm, 280 nm, 260 nm and 360 nm. The elution program 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 eluent flow was 0.8 $cm^3 \cdot min^{-1}$ (2050 p.s.i.).

RESULTS

Production of ferulic acid esterase on synthetic carbon sources

This preliminary study was undertaken in order to produce FAE by selected *Bifidobacterium* strains. The highest enzyme activity (14.95 units, 36 h) was obtained in the cultures of *B. animalis* Bi30 (Fig. 1) with Mp-C as the carbon source. On the other hand, in the presence of MF and *B. animalis* Bi30, the enzyme activity significantly increased in later hours of cultivations (60-84 h, 4.38 units, Fig. 1). No FAE activity was detected using MS or MV as carbon sources for *B. animalis* Bi30 growth.

FAE activities in the supernatants of *B. catenulatum* (KD 14) grown using Mp-C or MF are presented in Figure 2. As in the case of *B. animalis* Bi30, the highest activities were obtained after 36 h of cultivation with Mp-C (9.51 units). The significantly lower enzyme activity was observed when MF was used as the carbon source (6.74 units after 60 h of cultivation). It must be underlined that the differences in the highest FAE activities induced by MF and Mp-C were readily seen in the case of all three bacterial strains (Fig. 1, 2 and 3). In the case of *B. catenulatum* KD14 (Fig. 2) and *B. longum* KN29 (Fig. 3), the highest FAE activities were detected during the first hours of bacterial growth with Mp-C (9.51 and 13.53 units, respectively, after 36 h). In both cases, Mp-C turned out to be the more efficient carbon source than MF for the production of FAE.

It is interesting that *B. catenulatum* and *B. longum* produced FAE in the presence of MV as the carbon source (Fig. 4). However, FAE activities were significantly lower than the corresponding activities produced in the presence of the methyl esters of cinnamic acid derivatives.

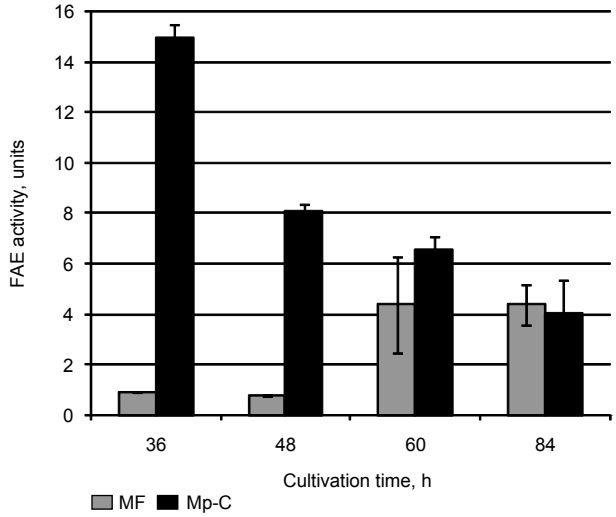

Fig. 1. Effect of cultivation time of *B. animalis* Bi30 with Mp-C or MF (as a carbon source) on FAE production, n = 4

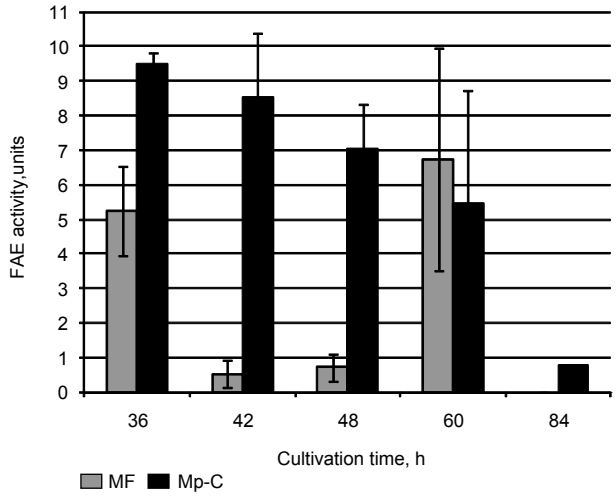

Fig. 2. Effect of cultivation time of *B. catenulatum* KD14 with MF or Mp-C (as a carbon source) on FAE production

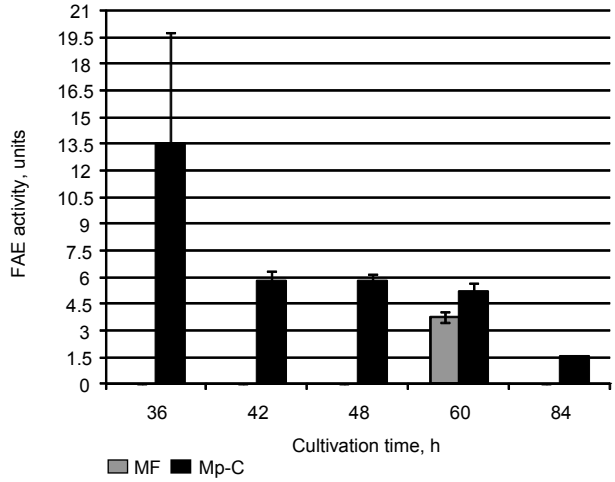

Fig. 3. Effect of cultivation time of *B. longum* KN29 with MF or Mp-C (as a carbon source) on FAE production, n = 4

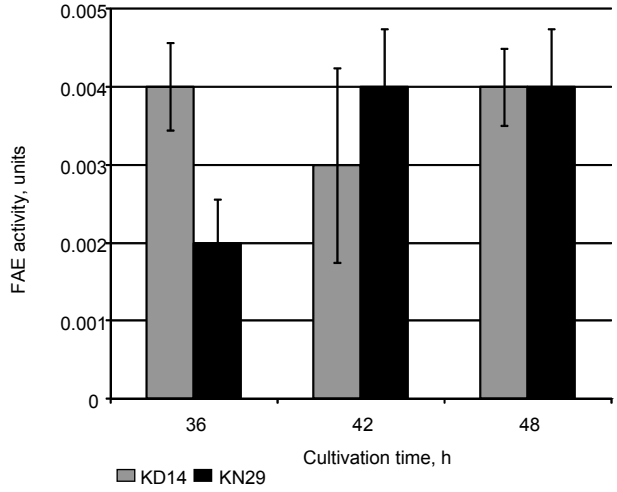

Fig. 4. Effect of cultivation time of *B. catenulatum* KD14 and *B. longum* KN29 *longum* KN29 with MV (as a carbon source) on FAE production, n = 4

No FAE activites were detected in the supernatant cultures of *B. catenulatum* KD14 and *B. longum* KN29 in the presence of MS.

Production of FAE on natural carbon sources

FAE activities detected in the supernatants of all *Bifidobacterium* strains cultivated using the natural carbon sources were significantly lower than the corresponding FAE activities obtained with the use of phenolic acids methyl esters. In the presence of German wheat bran, the highest enzyme activities were obtained after 27 h of cultivation (0.08 and 0.05 units in the case of *B. animalis* Bi30 and *B. catenulatum* KD14, respectively, Fig. 5) followed by the decreases of FAE activities until the end of the cultivation. In the case of *B. longum* KN29, the highest enzyme activity (0.13 units) was obtained after 51 h of the cultivation (Fig. 5).

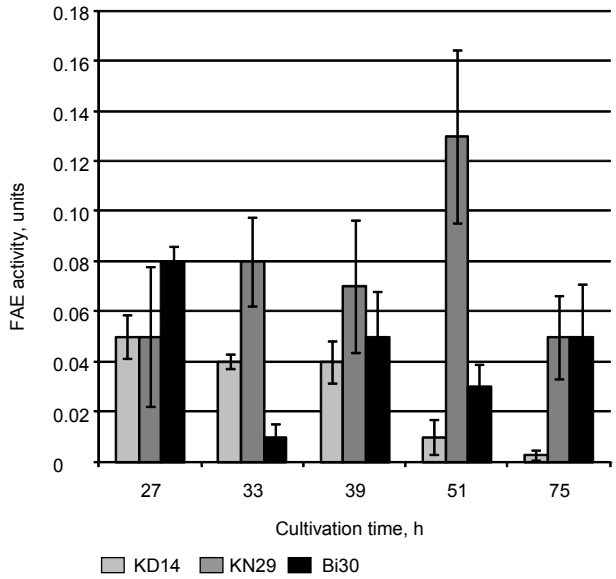

Fig. 5. Effect of cultivation time of *Bifidobacterium* strains with German wheat bran on FAE production, n = 4

The use of rye bran for FAE production by *B. catenulatum* KD14 and *B. longum* KN29 resulted in the presence of the highest enzyme activities (0.08 and 0.19 units in the case of KN29 and KD14, respectively) in the culture supernatants after 27 and 51 h of cultivation (Fig. 6). No FAE was produced by *B. animalis* Bi30 in the presence of rye bran, as well as no enzyme was detected in the case of *B. animalis* Bi30 cultivated in the medium containing barley spent grain.

On the other hand, the supernatants obtained from *B. longum* KN 29 and *B. catenulatum* KD14 cultivations with barley spent grain exhibited FAE activities after 27 and 33 h (Fig. 7). It must be underlined that the enzyme activities induced by the presence of barley spent grain were significantly lower compared to those assessed in the supernatant solutions obtained from German wheat bran or rye bran.

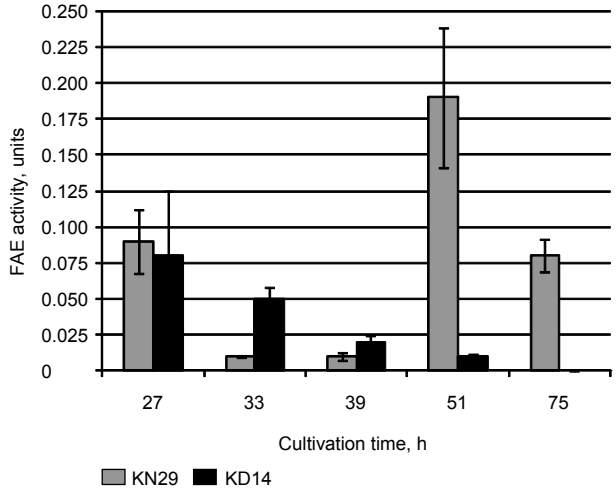

Fig. 6. Effect of cultivation time of *Bifidobacterium* strains with rye bran on FAE production, n = 4

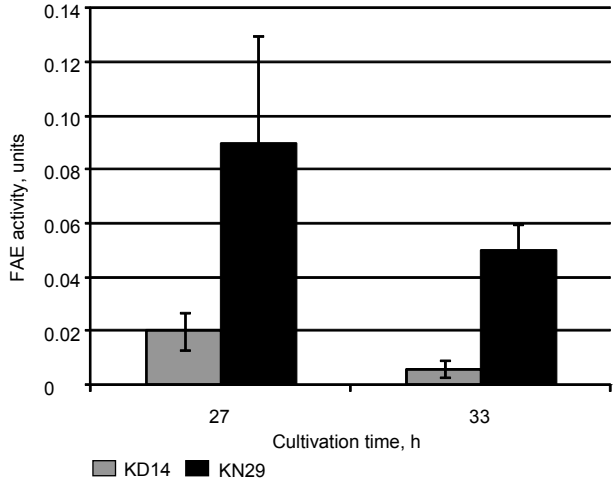

Fig. 7. Effect of cultivation time of *Bifidobacterium* strains with barley spent grain on FAE production, n = 4

Another natural component that induced FAE activity was larchwood arabinogalactan (Fig. 8). The only *Bifidobacterium* strain to produce the enzyme after 36-48 h of cultivation was *B. longum* KN29.

No FAE activities were detected in the supernatant solutions obtained after the cultivations of all *Bifidobacterium* strains in the presence of apple or corn pectins although the growth of bacteria was confirmed by the optical density measurements at 600 nm (data not shown).

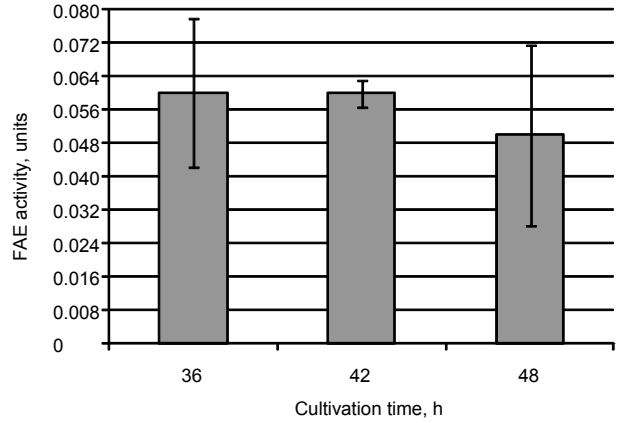

Fig. 8. Effect of cultivation time of *Bifidobacterium longum* KN29 with larchwood arabinogalactan on FAE production, n = 4

Effect of pH, temperature and buffer on FAE activity

Optimal pH for the activity of all studied ferulic acid esterases was 6.3 (Fig. 9). The highest enzyme activities were obtained in Tris/HCl buffer (*B. catenulatum* KD14 and *B. longum* KN29) or Theorell/Steinhagen buffer (*B. animalis* Bi30; Fig. 10). In the case of all *Bifidobacterium* strains, the optimal temperature for the enzyme activity was 37°C and a significant decrease of the activity at 42°C was seen. This result suggests the low enzyme thermostability (detailed data not shown).

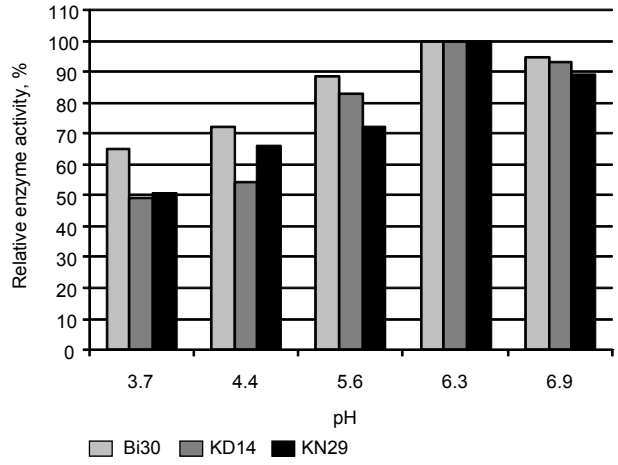

Fig. 9. The influence of pH value on FAE activities measured in the supernatant samples after *Bifidobacterium* growth, n = 4

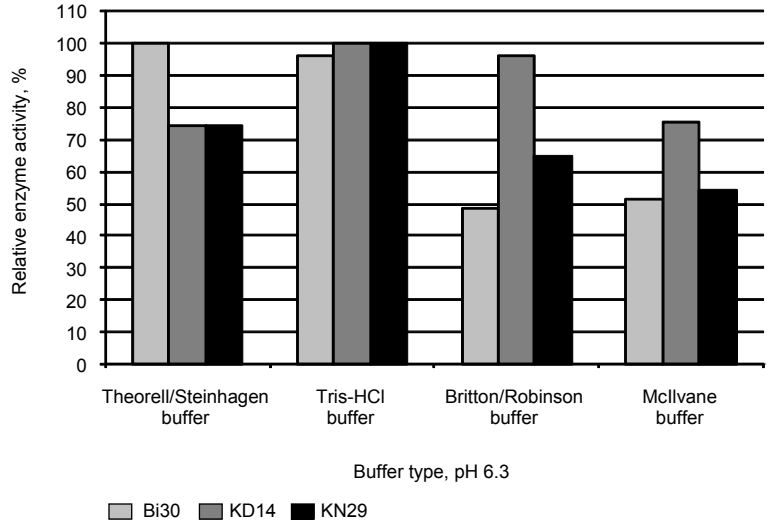

Fig. 10. The influence of buffer on FAE activities measured in the supernatant samples after *Bifidobacterium* growth, n = 4

DISCUSSION

In this study, the effect of different carbon sources on FAE production by selected *Bifidobacterium* strains was investigated. The future purification and a closer characterisation of these FAEs can be taken under consideration. In the case of all strains, the most effective carbon sources were synthetic methyl esters of cinnamic acid derivatives – Mp-C followed by MF. Generally, no FAE was produced in the presence of MV or MS in the frames of this study.

German wheat bran or rye bran were the most useful carbon sources for FAE production in the case of all bacterial strains. It must be underlined that German wheat bran was the only natural carbon source that induced FAE production by *B. animalis* Bi30 strain. Also, the differences in the induction times for the production of the enzymes on synthetic and natural carbon sources were seen.

There is a great number of works concerning the purification and characterisation of fungal FAEs but the papers studying FAEs produced by lactic acid bacteria are still lacking. Donaghy et al. [1998] screened 80 *Bacillus*-type strains and 50 gram positive bacteria (*Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Propionibacterium)* in agar-plate assay. The highest FAE activities were seen in the case of 1 *Lactobacillus fermentum* and 6 *Bacillus subtilis* strains. *Lactobacillus fermentum* NCF 1751 released ferulic acid from MF, Mp-C and 2-O-[5-O-(trans-ferulic acid)-β-L--ara-*f*]-D-xyl-*p*. The optimal pH and temperature for the enzyme activity were 6.5 and 30°C, respectively.

Couteau et al. [2001] studied FAEs produced by *Bifidobacterium* strains in the medium containing chlorogenic acid as a substrate and the authors proved that ferulic acid was released in the gastrointestinal tract from the dietary fiber due to the action of bacterial esterases. Indeed, *Bifidobacterium catenulatum* KD14 studied in our work re-

leased caffeic acid from chlorogenic acid (results not shown). Wang et al. [2004] characterised the intracellular FAE from *Lactobacillus acidophilus* after $(NH_4)_2SO_4$ precipitation followed by the double HIC and IEC what resulted in a very high degree of the enzyme purification. The molecular mass of the enzyme was 36 kDa (SDS-PAGE). The purified enzyme released 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* (FAXX) and 5-O-ferulic acid-L-arabinofuranose (FAA) from corn bran.

One year later Wang et al. [2005] presented the results concerning the release of FA from wheat bran by FAE produced by *Lactobacillus acidophilus*. The simultaneous use of bacterial FAE and fungal xylanase and L-arabinofuranosidase significantly increased the release of FA [Wang et al. 2005]. Zeng et al. [2007] proved that *B. adolescentis*, *B. infantis* and *B. bifidum* produced β-D-xylosidase, α-L-arabinosidase and degraded xylooligosaccharides but the ability to produce FAEs was not studied. The growth of *B. bifidum* F-35 was improved by ferulic acid oligosaccharides obtained from wheat bran insoluble dietary fiber [Yuan et al. 2005]. The biomass yield was higher in the presence of glucose, but it was not observed with arabinose or xylose as the sole carbon source. Also, *B. bifidum* biomass yield was higher in the presence of arabinoxylan oligosaccharides and not ferulic acid oligosaccharides what suggests the inhibitory effect of released FA on the bacterial growth. The release of free FA or FAE activity was not studied in this work. Nevertheless, these results support the thesis that LAB strains can at least partly degrade complex carbohydrates.

Vardakou et al. [2007] examined the influence of different water-unextractable (WU-AX) and water-extractable arabinoxylan fractions (WE-AX) on the growth of *Bacteroides*, *Bifidobacterium*, *Clostridium* and *Lactobacillus* strains after the inoculation with the faecal sample in the model of the human colon. FAE was present in *in vitro* samples of the complex faecal slurry containing WU-AX (treated and untreated with xylanase) but no enzyme activity was present in the samples containing WE-AX. Both WE-AX and WU-AX caused the increase in the bifidobacteria count numbers in the presence of xylanase but bacteroides and clostridia levels remained approximately constant. FAEs were not studied in the frames of this work. In 2008, Vardakou et al. studied the influence of dietary supplementation with prebiotics (water unextractable arabinoxylan from wheat) on the growth of lactobacilli and bifidobacteria in the batch cultures. The extracellular xylanase and FAE activities were detected in the batch cultures with arabinoxylan and faecal samples. The water unextractable arabinoxylan caused the increase of both xylanase and FAE activity especially when the water unextractable, xylanase-pretreated arabinoxylan was used. The use of the untreated or xylanase-treated arabinoxylan resulted in the increase of lactobacilli and bifidobacteria count numbers. No FAE or FAE-producing bacteria were studied in detail in the frames of this work.

Nsereko et al. [2008] screened 10000 lactic acid bacteria strains in order to detect FAE activity. Approx. 500 strains produced the enzyme and 8 of them were further studied (*Lactobacillus buchneri*, *L. crispatus*, *L. reuteri*, *L. brevis* and one not identified *Lactobacillus* strain). The work was not expanded with the closer characterization of any particular FAE. The influence of ensiling perennial ryegrass with lactic acid bacteria on neutral detergent fiber degradation was only studied. A complete description of the fermentation conditions including changes in pH and lactic acid bacteria cell numbers, levels of some metabolites such as lactate, acetate and total N were obtained.

In conclusions, it can be stated, that although FAE produced by LAB is only the accessory enzyme, it can be of a great significance in the context of the metabolism of low molecular, very broadly distributed phenolic acids. This paper reports on the ability of three *Bifidobacterium* strains to produce FAEs which were not previously detected.

FA released in the gastrointestinal tract can be easily absorbed followed by its antioxidant action in the plasma. Nowadays, *Bifidobacterium* strains are deeply studied but the knowledge of the ability to produce hydrolases acting towards non-starch polysaccharides is still not complete. A composition of the probiotics and prebiotics in a well balanced product can be an interesting offer for the nutritional market. Last but not least, purification of FAE produced by LAB can be useful for the industrial purposes. LAB are generally considered as harmless and different industrial by-products can be processed using FAEs yielding phenolic acids. These compounds could be then used as flavouring agents, antioxidant supplments or cosmetic components (e.g. UV filters). The presented work should be continued in order to purify and characterise the FAEs from *Bifidobacterium* strains in detail.

CONCLUSIONS

1. *Bifidobacterium* strains (mainly *B. animalis* Bi30 and *B. catenulatum* KD 14) effectively produced ferulic acid esterase in the presence of methyl ferulate and especially methyl p-coumarate and as the main carbon sources.

2. In all cases, methyl esters of cinnamic acid derivatives were more effective carbon sources than methyl esters of benzoic acid derivatives.

3. Ferulic acid esterase activities induced by the natural substrates were significantly lower than the corresponding enzyme activities obtained in the presence of synthetic methyl esters of phenolic acids.

4. Among the natural substrates, German wheat bran or rye bran were the most useful for FAE production (with *B. catenulatum* KD14 and *B. longum* KN24 as the most active) and no enzyme activity was induced by apple or corn pectins.

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NOWE ESTERAZY KWASU FERULOWEGO PRODUKOWANE PRZEZ BAKTERIE Z RODZAJU *BIFIDOBACTERIUM* Z UŻYCIEM WYBRANYCH SYNTETYCZNYCH I NATURALNYCH ŹRÓDEŁ WĘGLA

Wstęp. Esterazy kwasu ferulowego należące do pospolitej grupy hydrolaz są bardzo rozpowszechnione w świecie roślin. Grzybowe esterazy kwasu ferulowego były intensywnie badane w przeszłości, natomiast zwykle pomijano esterazy produkowane przez bakterie kwasu mlekowego. Wolne kwasy fenolowe – silne związki o charakterze przeciwutleniaczy mogą być uwalniane z frakcji błonnika pokarmowego pod wpływem aktywności esteraz kwasu ferulowego produkowanych przez jelitowe bakterie kwasu mlekowego. Celem pracy było zbadanie produkcji esterazy kwasu ferulowego przez wybrane bakterie z rodzaju *Bifidobacterium* w hodowlach prowadzonych z użyciem wybranych naturalnych i syntetycznych źródeł węgla.

Materiał i metody. Badania prowadzono z użyciem bakterii z rodzaju *Bifidibacterium* (*B. animalis* Bi30, *B. catenulatum* KD 14 i *B. longum* KN 29). Bakterie hodowano w pożywkach minimalnych zawierających następujące źródła węgla: otręby orkiszowe, otręby żytnie, wysłodziny piwowarskie, oczyszczony arabinogalaktan z modrzewia, pektyny jabłkowe, pektyny kukurydziane, estry metylowe kwasów fenolowych. Produkcję zewnątrzkomórkowej esterazy kwasu ferulowego określano, używając supernatanty pohodowlane i ferulan metylu. Stężenie kwasu ferulowego uwolnionego z estru określono za pomocą HPLC z detekcją diodową DAD.

Wyniki. Największe uzdolnienia do produkcji esterazy kwasu ferulowego wykazał szczep *B. animalis* Bi30 w obecności p-kumaranu metylu i ferulanu metylu jako jedynego źródła węgla (odpowiednio 14,95 $nmol \cdot ml^{-1} \cdot min^{-1}$ and 4,38 $nmol \cdot ml^{-1} \cdot min^{-1}$). W przypadku każdego z wyprodukowanych enzymów, najwyższą aktywność zanotowano w temperaturze 37°C (pH 6,3) w obecności buforu Theorell/Steinhagena (*B. animalis* Bi30) lub Tris/HCl (*B. catenulatum* KD14 i *B. longum* KN29). Biorąc pod uwagę wszystkie uzyskane wyniki, należy stwierdzić, że najwyższe aktywności enzymu uzyskano po użyciu syntetycznych estrów kwasów fenolowych jako źródła węgla.

Wnioski. Przedstawione wyniki wzbogacają wiedzę na temat produkcji esterazy kwasu ferulowego przez probiotyczne bakterie kwasu mlekowego. Pomimo że jest to jedynie poboczny enzym wykorzystywany przez mikroorganizmy do hydrolizy składników żywności, można sformułować wnioski na temat ważnej roli bakterii kwasu mlekowego w czasie uwalniania przeciwutleniaczy – kwasów fenolowych.

Słowa kluczowe: esteraza kwasu ferulowego, kwas ferulowy, *Bifidobacterium,* probiotyki, przeciwutleniacz

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