ADHESIVE PROPERTY OF DIFFERENT STRAINS OF LACTOBACILLI IN THE PRESENCE OF RESVERATROL*

V. Jarosova[1], I. Doskocil[1], T. Volstatova[1], J. Havlik[2]

[1]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Microbiology, Nutrition and Dietetics, Prague, Czech Republic
[2]Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Food Quality and Safety, Prague, Czech Republic

The ability of bacteria to adhere to the intestinal epithelial cells is one of the main criteria for selection of new probiotic strains. Some dietary polyphenols have been proven to affect bacterial adhesion, providing a rationale for the use of mixtures of polyphenols and probiotics. Resveratrol, a naturally occurring stilbene in plants, has been shown to have a number of beneficial biological effects. The adhesion ability of four *Lactobacillus* strains (*Lactobacillus brevis*, *L. fermentum*, *L. gasseri*, and *L. plantarum*), in the presence of resveratrol, has been investigated in an *in vitro* model based on mixed co-culture of Caco-2 and HT29-MTX intestinal epithelial cells. The effective concentration of resveratrol used in the adhesion experiment has been selected based on cytotoxicity test. Resveratrol at three physiologically low concentrations (4.5, 2.25, and 1.125 μg ml^{-1}), added together with the bacterial suspension, had no statistically significant influence on the adhesion of any strain ($P < 0.05$). Since the health benefits of polyphenols are often associated with the composition of gut microbiota, the knowledge of interactions between known bacteria and polyphenols would be of high scientific value.

bacterial adherence, Caco-2, HT29-MTX, microbiota, stilbenes, polyphenols

DE GRUYTER OPEN

doi: 10.2478/sab-2018-0036
Received for publication on February 6, 2018
Accepted for publication on April 11, 2018

INTRODUCTION

Stilbenes are phenolic compounds characterized by the presence of a 1,2-diphenylethylene nucleus with hydroxyls substituted on the aromatic rings (Fresco et al., 2006; Han et al., 2007). The most studied and well-known is resveratrol (3,4′,5-trihydroxystilbene), which is found in a wide range of plants including wine grape skin (*Vitis vinifera*), peanuts (*Arachis hypogaea*), and several medicinal plants (Calamini et al., 2010; Lin et al., 2011; Piotrowska et al., 2012). Historically, resveratrol from red wine has been associated with the French paradox (Renaud, de Lorgeril, 1992), a low incidence of cardiovascular disease despite high saturated fat intake in Mediterranean diet. Currently, many studies have confirmed its antioxidant, antibacterial, antifungal, anti-atherogenic, cardioprotective, neuroprotective, and antitumour or chemopreventive activity *in vitro* (Fremont, 2000; Park et al., 2001; Piotrowska et al., 2012; Biais et al., 2017). Moreover, resveratrol may have a neuroprotective effect against Alzheimer's disease due to its ability to inhibit the aggregation of amyloid-β peptide (Aβ) (Riviere et al., 2007).

* Supported by the Internal Grant Agency of the Czech University of Life Sciences Prague (CIGA), Project No. 20162015, and the Czech Science Foundation (GA CR), Project No. 16-07193S.

Currently, resveratrol is marketed as various food supplements.

Based on rodent studies, resveratrol intake has been associated with healthy body weight, visceral adipose weights, and blood glucose and lipid levels (Qiao et al., 2014; Bird et al., 2017). It has been shown to change the microbiota composition, inhibit growth of non-beneficial bacteria, and potentiate the growth of probiotic bacteria (Queipo-Ortuno et al., 2012). As the health effects of polyphenols are often associated with the composition of gut microbiota (Bustos et al., 2012; Cueva et al., 2017), any additional knowledge of the interaction between known bacteria and polyphenols would be of high scientific value.

Diet has an important influence on intestinal microbiota and polyphenols are an abundant group of phenylpropanoid constituents in plant-based food. Several studies from recent years indicate the effect of polyphenols on compositional changes of intestinal microbiota *in vitro* and *in vivo* (Parkar et al., 2008; Anhe et al., 2015; Ritchie et al., 2015; Valdes et al., 2015; Volstatova et al., 2017). Parkar et al. (2008) hypothesized that one of the mechanisms could be selective influence on the adhesion of microorganisms, and he reported increased adhesion in an *in vitro* model in the presence of apple polyphenols phloridzin and rutin. Similarly, epigallocatechin and epigallocatechin gallate were shown to increase adherence of lactobacilli in Caco-2 model (Bustos et al., 2012).

The primary objective of this study was to determine the effect of resveratrol on the adhesion of four potential probiotic *Lactobacillus* strains (*Lactobacillus plantarum*, *L. gasseri*, *L. fermentum*, and *L. brevis*) in colon epithelial cell model. The secondary objective was to assess the adherence potential of the four potentially probiotic strains in a Caco-2/HT29-MTX model. To our knowledge, this is the first study focused on bacterial adhesion in this model in presence of resveratrol.

MATERIAL AND METHODS

Bacterial strains

Bacterial strains (obtained from Czech Collection of Microorganisms) used in this experiment were: *L. plantarum* (MILCOM 195; unknown), *L. gasseri* (DSMZ 20243; human), *L. fermentum* (CCM 91; unknown), and *L. brevis* (CCM 3805; human faeces).

Cell cultures

Of the human epithelial intestinal cell lines, Caco-2 cell line was obtained from the American Type Tissue Collection (Rockville, Maryland, USA), and the HT29-MTX cell line was purchased from Sigma-Aldrich (Prague, CZ). Caco-2 cells were used in experiment at passages 20–25 and the HT29-MTX cells were used at passage 35–40. Both cell lines were grown in Dulbecco's modified Eagles medium (DMEM-F12) supplemented with 10% foetal bovine serum (FBS), 1% nonessential amino acids, 100 U ml^{-1} penicillin, and 100 µg ml^{-1} streptomycin, all obtained from Sigma-Aldrich. Cell culture bottles were kept at 37°C in a thermostat with humidified atmosphere containing 5% (v/v) CO_2. The medium was changed every 2–3 days.

Preparation of bacterial suspension

The lactobacilli were grown overnight in Wilkins-Chalgren anaerobe broth (Oxoid Ltd., Basingstoke, UK) at 37°C under anaerobic conditions. Prior to the assay, bacteria were centrifuged (2000 rpm, 10 min) and washed three times with phosphate buffer saline (PBS) (Sigma-Aldrich). The bacteria were then resuspended in PBS at concentration of 10^8 colony-forming units (CFU) ml^{-1} determined from their optical density at 600 nm (Infinite M200; Tecan Austria GmbH, Grödig, Austria).

Preparation of resveratrol

Resveratrol was obtained from Sigma-Aldrich and the stock solution of concentration 10 mg ml^{-1} was prepared in dimethylsulfoxide (DMSO) (Sigma-Aldrich). Prior to the adhesion experiment, working solutions of resveratrol were prepared in DMEM, without any sup-

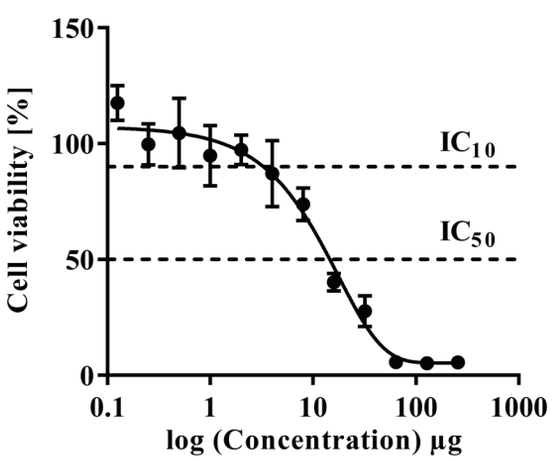

Fig. 1. Dose-response cytotoxicity curve showing inhibitory concentrations IC_{10} and IC_{50} of resveratrol towards mixed co-culture of Caco-2 and HT29-MTX cell lines. Values are expressed as mean of six repetitions ± standard deviation ($P < 0.05$)

plement, at concentrations of 5, 2.5, and 1.25 µg ml^{-1}, and aliquots were applied to the cells. The final concentrations of resveratrol after addition of bacterial suspension have been 4.5, 2.25, and 1.125 µg ml^{-1}.

MTT cytotoxicity assay

The viability of the mixed co-culture Caco-2/HT29-MTX was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) cytotoxicity assay. Cell lines were seeded in 96-well plates at a density of 4 × 10^4 cells per well and incubated for 24 h at 37°C in a 5% CO_2 -humidified atmosphere. Two-fold serial dilution of resveratrol (0.125–256 µg) was applied for 72 h. Subsequently, the medium was replaced by MTT reagent (1 mg ml^{-1}) in DMEM, and plates were incubated for additional 2 h. The culture supernatants were then aspirated and the formazan product was dissolved in DMSO. Absorbance was measured at 555 nm using a Tecan Infinite M200 reader (Tecan Austria GmbH). The absorbance values were then plotted against cell mortality and used to determine the inhibition concentrations IC_{50} and IC_{10} values.

Adhesion assays

The adhesion assay was performed according to Volstatova et al. (2017) with slight modifications. Mixed co-culture of Caco-2 and HT29-MTX cell lines was seeded in 24-well culture plate at concentration of 3.6 × 10^4 Caco-2 cells and 0.4 × 10^4 HT29-MTX cells per well. They were grown for 14 ± 1 days at 37°C in a 5% CO_2-humidified atmosphere in DMEM supplemented with 10% FBS, 1% nonessential amino acids, 100 U ml^{-1} penicillin, and 100 µg ml^{-1} streptomycin. The culture medium was changed every second or third day. Before the experiment, the medium was washed three times with PBS, without disrupting the monolayer. Thereafter, the monolayer was overlaid with 900 µl of resveratrol solution (5, 2.5, and 1.25 µg ml^{-1}), or medium (without supplements) as a control, and 100 µl of bacterial suspension in PBS at concentration of 10^8 CFU ml^{-1}. For each bacteria and concentration six replicates were set. The plates were incubated for 2 h at 37°C and 5% CO_2-atmosphere. After the incubation, wells were gently washed three times with PBS to remove non-attached bacteria, and trypsinised by the addition of 300 µl of 1% Triton-X100 (Sigma-Aldrich) per well for 30 s, followed by 700 µl PBS. The remaining suspensions with viable adhered bacteria were diluted (*L. brevis* and *L. fermentum* 50–500×, *L. gasseri* and *L. plantarum* 500–2500×) and seeded on petri dishes with Rogosa agar (Oxoid Ltd.). CFU were counted after 72 h of aerobic incubation at 37°C. The adhesion was determined as a percentage of adherent bacteria over the total bacteria added.

Statistical analysis

Statistical analysis for MTT tests was performed using Magellan^{TM} software (Tecan Group, Männedorf, Switzerland). Two-way analysis of variance (ANOVA; $P < 0.05$) was employed using STATISTICA 12 program to evaluate the statistical influence of resveratrol on bacterial adhesion. Data are expressed as mean ± standard error and the posteriori comparison was analysed by the Scheffe's test.

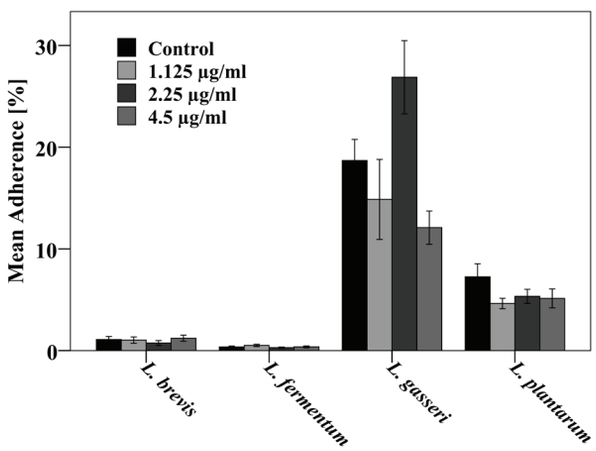

Fig. 2. Adhesion of four lactobacilli to the mixed co-culture of Caco-2/HT29-MTX cell lines in the presence of three concentrations of resveratrol. Values are expressed as percentage of bacterial adherence, after a 2-hour exposition of three concentrations of resveratrol, compared to the control. Values are expressed as means ± standard error of three independent assays ($P < 0.05$)

RESULTS

Resveratrol exhibited high cytotoxicity towards Caco-2/HT29-MTX co-culture, as seen from IC_{50} and IC_{10} values of 13.60 ± 0.16 and 3.56 ± 0.36 µg ml^{-1}, respectively (Fig. 1); these values have been used to design adhesion experiments with effective concentrations. There were significant differences in the adhesive properties of the tested strains (Fig. 2). The highest ability to adhere was shown by *L. gasseri* followed by *L. plantarum*; from the initial 10^7 cells per ml^{-1} applied on the monolayer, average adhesion was 18.96 and 7.26%, respectively. The other two strains, *L. brevis* and *L. fermentum*, showed very low adhesion of 1.09 and 0.36%, respectively.

Resveratrol at three physiologically low concentrations of 4.5, 2.25, and 1.125 µg ml^{-1}, added together with the bacterial suspension, had no statistically significant influence on the adhesion of any strain ($P < 0.05$).

DISCUSSION

The ability of bacteria to adhere to the intestinal epithelial cells is one of the main criteria for selection of new probiotic strains, together with other features, such as survival in simulated gastrointestinal conditions or production of antimicrobial substances (Makinen et al., 2012). *In vitro* cell models form a widely established platform for screening of bacterial adhesion capabilities, prior to *in vivo* trials. Different cell lines, used as an *in vitro* model of human colon, include Caco-2, HT 29, T-84, and others. However, the co-culture of Caco-2/HT29-MTX, seeded in the ratio of 9 : 1 (Caco-2 : HT29-MTX) provides an advanced model, mimicking the real ratio of Goblet cells to absorbing epithelial cells in the healthy tract (Laparra, Sanz, 2009; Volstatova et al., 2015, 2017), and producing mucin. Presence of this glycoprotein results in spatially rich network of binding sites and substrate moieties for commensal microbiota (Carriere et al., 1995), similar to that in the gut.

In our study, both *L. gasseri* and *L. plantarum* showed high ability to adhere to an *in vitro* Caco-2/HT29-MTX co-culture. This is in agreement with numerous *in vivo* studies investigating the capabilities of *Lactobacillus* strains to colonize human intestinal mucosa after oral administration. Both strains, *L. gasseri* (Fujiwara et al., 2001) and *L. plantarum* (Johansson et al., 1993, 1998), were among the species with high colonising properties, and were consistently found in faeces after withdrawing the oral administration.

Previously, *L. gasseri* showed moderate adhesion in an *in vitro* model based on HT-29 cell line (12.05 ± 1.34%) (Wang et al., 2008). In the same model, *L. plantarum* and *L. brevis* showed poor adhesion ability of 6.14 ± 0.85% and 3.75 ± 0.50%, respectively. Adhesion of *L. plantarum* to Caco-2 cell line model has shown to be either 6.7 ± 1.4% (Tuomola, Salminen, 1998) or 8.51 ± 2.46% (Bianchi et al., 2004).

The adhesion of probiotic strains can be significantly affected by the presence of dietary polyphenols in their administration. Recent studies have suggested that the health benefits of polyphenols might arise from their interactions with the gut microbiota (Bustos et al., 2012; Cueva et al., 2017). It is well proven that some dietary polyphenols and/or their metabolites can stimulate the growth, proliferation, and adhesion ability of commensal bacteria and inhibit the growth and adhesion of gut pathogens (Parkar et al., 2008; Laparra, Sanz, 2010).

A study by Bustos et al. (2012) showed that the effect of polyphenols is inconsistent, thereby suggesting high specificities. Most of them do not show any effect, but some seem to stimulate adhesion of selected strains to certain types of cell lines. For example, epigallocatechin increased the adhesion of *L. casei* to Caco-2 cells while procyanidins B1 and B2 increased its adhesion to HT-29 cells. The adhesion of *L. acidophilus* to Caco-2 cells has shown to be increased by epigallocatechin gallate. This implies that the effect of dietary polyphenols on bacterial adhesion depends on the *Lactobacillus* strain and the cell line in consideration. The lack of resveratrol activity, seen in our study, might be valid for the strains used, and therefore, the effect on other strains, not included in our panel, cannot be ruled out. A recent study (Celebioglu et al., 2017) has investigated the effect of some common dietary polyphenols, including resveratrol, on adhesive capacity of *L. acidophilus* toward mucin and HT-29 cells. In their model, resveratrol significantly increased adhesion of this *Lactobacillus* sp. to both mucin and HT-29 cells at 100 μg ml^{-1}, and has been proven to be one of the most effective among all polyphenols tested, owing to the possible changes in surface protein expression. However, concerns remain about the physiological relevance of the concentrations used.

CONCLUSION

In conclusion, our results have shown significant differences in the adhesion ability between *Lactobacillus* strains, however the main hypothesis that resveratrol increases bacterial adhesion was not confirmed. Among the tested lactobacilli, the highest adherence was shown by *L. gasseri*, followed by *L. plantarum*, thereby suggesting their potential for use in probiotic supplements. Even though resveratrol has not shown any effect on adhesion of our tested bacteria, many *in vitro* and rodent studies from last years have already shown that resveratrol intake may provide some health benefits through modulating gut microbiota and consequently contribute to the prevention of selected non-communicable diseases. The future research should be aimed at exploring this effect.

REFERENCES

Anhe FF, Roy D, Pilon G, Dudonne S, Matamoros S, Varin TV, Garofalo C, Moine Q, Desjardins Y, Levy E (2015): A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased *Akkermansia* spp. population in the gut microbiota of mice. Gut, 64, 872–883. doi: 10.1136/gutjnl-2014-307142.

Biais B, Krisa S, Cluzet S, Da Costa G, Waffo-Teguo P, Merillon JM, Richard T (2017): antioxidant and cytoprotective activities of grapevine stilbenes. Journal of Agricultural and Food Chemistry, 65, 4952–4960. doi: 10.1021/acs.jafc.7b01254.

Bianchi MA, Del Rio D, Pellegrini N, Sansebastiano G, Neviani E, Brighenti F (2004): A fluorescence-based method for the detection of adhesive properties of lactic acid bacteria to

Caco-2 cells. Letters in Applied Microbiology, 39, 301–305. doi: 10.1111/j.1472-765X.2004.01589.x.

Bird JK, Raederstorff D, Weber P, Steinert RE (2017): Cardiovascular and antiobesity effects of resveratrol mediated through the gut microbiota. Advances in Nutrition, 8, 839–849. doi: 10.3945/an.117.016568.

Bustos I, Garcia-Cayuela T, Hernandez-Ledesma B, Pelaez C, Requena T, Martinez-Cuesta MC (2012): Effect of flavan-3-ols on the adhesion of potential probiotic lactobacilli to intestinal cells. Journal of Agricultural and Food Chemistry, 60, 9082–9088. doi: 10.1021/jf301133g.

Calamini B, Ratia K, Malkowski MG, Cuendet M, Pezzuto JM, Santarsiero BD, Mesecar AD (2010): Pleiotropic mechanisms facilitated by resveratrol and its metabolites. Biochemical Journal, 429, 273–282. doi: 10.1042/BJ20091857.

Carriere V, Lesuffleur T, Barbat A, Rousset M, Dussaulx E, Costet P, de Waziers I, Beaune P, Zweibaum A (1995): Expression of cytochrome P-450 3A in HT29-MTX cells and caco-2 clone TC7. FEBS Letters, 362, 98–99. doi: 10.1016/0014-5793(94)01199-0.

Celebioglu HU, Delsoglio M, Brix S, Pessione E, Svensson B (2017): Plant polyphenols stimulate adhesion to intestinal mucosa and induce proteome changes in the probiotic *Lactobacillus acidophilus* NCFM. Molecular Nutrition and Food Research, 62: 1700638. doi: 10.1002/mnfr.201700638.

Cueva C, Gil-Sanchez I, Ayuda-Duran B, Gonzalez-Manzano S, Gonzalez-Paramas AM, Santos-Buelga C, Bartolome B, Moreno-Arribas MV (2017): An integrated view of the effects of wine polyphenols and their relevant metabolites on gut and host health. Molecules, 22: E99. doi: 10.3390/molecules22010099.

Fremont L (2000): Biological effects of resveratrol. Life Sciences, 66, 663–673. doi: 10.1016/S0024-3205(99)00410-5.

Fresco P, Borges F, Diniz C, Marques MPM (2006): New insights on the anticancer properties of dietary polyphenols. Medicinal Research Reviews, 26, 747–766. doi: 10.1002/med.20060.

Fujiwara S, Seto Y, Kimura A, Hashiba H (2001): Establishment of orally-administered *Lactobacillus gasseri* SBT2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. Journal of Applied Microbiology, 90, 343–352. doi: 10.1046/j.1365-2672.2001.01251.x.

Han X, Shen T, Lou H (2007): Dietary polyphenols and their biological significance. International Journal of Molecular Sciences, 8, 950–988. doi: 10.3390/i8090950.

Johansson ML, Molin G, Jeppsson B, Nobaek S, Ahrne S, Bengmark S (1993): Administration of different *Lactobacillus* strains in fermented oatmeal soup: *in vivo* colonization of human intestinal mucosa and effect on the indigenous flora. Applied and Environmental Microbiology, 59, 15–20.

Johansson ML, Nobaek S, Berggren A, Nyman M, Bjorck I, Ahrne S, Jeppsson B, Molin G (1998): Survival of *Lactobacillus plantarum* DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. International Journal of Food Microbiology, 42, 29–38. doi: 10.1016/S0168-1605(98)00055-5.

Laparra JM, Sanz Y (2009): Comparison of *in vitro* models to study bacterial adhesion to the intestinal epithelium. Letters in Applied Microbiology, 49, 695–701. doi: 10.1111/j.1472-765X.2009.02729.x.

Laparra JM, Sanz Y (2010): Interactions of gut microbiota with functional food components and nutraceuticals. Pharmacological Research, 61, 219–225. doi: 10.1016/j.phrs.2009.11.001.

Lin HY, Tang HY, Davis FB, Davis PJ (2011): Resveratrol and apoptosis. Annals of the New York Academy of Sciences, 1215, 79–88. doi: 10.1111/j.1749-6632.2010.05846.x.

Makinen K, Berger B, Bel-Rhlid R, Ananta E (2012): Science and technology for the mastership of probiotic applications in food products. Journal of Biotechnology, 162, 356–365. doi: 10.1016/j.jbiotec.2012.07.006.

Park JW, Choi YJ, Jang MA, Lee YS, Jun DY, Suh SI, Baek WK, Suh MH, Jin IN, Kwon TK (2001): Chemopreventive agent resveratrol, a natural product derived from grapes, reversibly inhibits progression through S and G2 phases of the cell cycle in U937 cells. Cancer Letters, 163, 43–49. doi: 10.1016/S0304-3835(00)00658-3.

Parkar SG, Stevenson DE, Skinner MA (2008): The potential influence of fruit polyphenols on colonic microflora and human gut health. International Journal of Food Microbiology, 124, 295–298. doi: 10.1016/j.ijfoodmicro.2008.03.017.

Piotrowska H, Kucinska M, Murias M (2012): Biological activity of piceatannol: leaving the shadow of resveratrol. Mutation Research, 750, 60–82. doi: 10.1016/j.mrrev.2011.11.001.

Qiao Y, Sun J, Xia S, Tang X, Shi Y, Le G (2014): Effects of resveratrol on gut microbiota and fat storage in a mouse model with high-fat-induced obesity. Food and Function, 5: 1241. doi: 10.1039/c3fo60630a.

Queipo-Ortuno MI, Boto-Ordonez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, Cardona Diaz F, Andres-Lacueva C, Tinahones FJ (2012): Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. American Journal of Clinical Nutrition, 95, 1323–1334. doi: 10.3945/ajcn.111.027847.

Renaud S, de Lorgeril M (1992): Wine, alcohol, platelets, and the French paradox for coronary heart disease. The Lancet, 339, 1523–1526. doi: 10.1016/0140-6736(92)91277-F.

Ritchie LE, Sturino JM, Carroll RJ, Rooney LW, Azcarate-Peril MA, Turner ND (2015): Polyphenol-rich sorghum brans alter colon microbiota and impact species diversity and species richness after multiple bouts of dextran sodium sulfate-induced colitis. FEMS Microbiology Ecology, 91: fiv008. doi: 10.1093/femsec/fiv008.

Riviere C, Richard T, Quentin L, Krisa S, Merillon JM, Monti JP (2007): Inhibitory activity of stilbenes on Alzheimer's β-amyloid fibrils *in vitro*. Bioorganic and Medicinal Chemistry, 15, 1160–1167. doi: 10.1016/j.bmc.2006.09.069.

Tuomola EM, Salminen SJ (1998): Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. International Journal of Food Microbiology, 41, 45–51. doi: 10.1016/S0168-1605(98)00033-6.

Valdes L, Cuervo A, Salazar N, Ruas-Madiedo P, Gueimonde M, Gonzalez S (2015): Relationship between phenolic compounds from diet and microbiota: impact on human health. Food and Function, 6, 2424–2439. doi: 10.1039/C5FO00322A.

Volststova T, Havlik J, Doskocil I, Geigerova M, Rada V (2015): Effect of hydrolyzed milk on the adhesion of lactobacilli to intestinal cells. Scientia Agriculturae Bohemica, 46, 21–25. doi: 10.1515/sab-2015-0012.

Volstatova T, Marsik P, Rada V, Geigerova M, Havlik J (2017): Effect of apple extracts and selective polyphenols on the adhesion of potential probiotic strains of *Lactobacillus gasseri* R and *Lactobacillus casei* FMP. Journal of Functional Foods, 35, 391–397. doi: 10.1016/j.jff.2017.06.005.

Wang B, Wei H, Yuan J, Li Q, Li Y, Li N, Li J (2008): Identification of a surface protein from *Lactobacillus reuteri* JCM1081 that adheres to porcine gastric mucin and human enterocyte-like HT-29 cells. Current Microbiology, 57, 33–38. doi: 10.1007/s00284-008-9148-2.

Corresponding Author:

doc. Ing. Jaroslav H a v l i k , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Food Quality and Safety, Kamýcká 129, 165 00 Prague 6-Suchdol, Czech Republic, phone: +420 224 382 965, e-mail: havlik@af.czu.cz