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APPLICATION OF RAPID CACO-2 CELL CULTURE SYSTEM IN THE STUDIES ON THE BACTERIAL ADHESION AND TRANSEPITHELIAL TRANSPORT

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Key words: Caco-2, differentiation, probiotic adhesion, transepithelial transport

The aim of the presented work was the functional comparison of conventional 21-day-old and modified 6-day-old Caco-2 cell cultures for their application in studies on the bacterial adhesion and transepithelial transport. In the short-term culture system, the cells were grown on polycarbonate filters in serum reduced medium supplemented with a combination of growth factors. Morphological comparison of two Caco-2 monolayers showed that the short-term culture had the similar ultrastructural characteristics of the long-term well-developed culture. The transepithelial electrical resistance measurement confirmed the high Caco-2 monolayer integrity in both cell culture systems. As a result, the modified 6-day-old Caco-2 culture could be applied in permeability and adhesion investigations. In the transport study, atrazine was used as a model molecule. The transport of atrazine was symmetric between the apical to basolateral and the basolateral to apical directions and independent of its concentration. No significant difference was found in atrazine permeability through conventional and modified Caco-2 cultures. In order to investigate the role of Caco-2 culture protocol might play in the adhesion of probiotic bacteria, three *lactobacilli* strains were used. The effect of Caco-2 culture modification on adhesion capacity of all tested bacteria was not observed. The proposed system of Caco-2 cell culture can be a considerable alternative to the conventional 21-day system.

INTRODUCTION

A few defined cell culture systems are described which have been used for probiotic adhesion [Blum *et al*., 1999; Coconnier *et al*., 1992; Sarem *et al*., 1996] and transport [Meunier *et al*., 1995; Braun *et al*., 2000; Pontier *et al*., 2001] studies. The ability for spontaneous differentiation and morphological as well as functional similarities to intestinal enterocytes have resulted in Caco-2 becoming one of the most popular cell lines in these applications. The conventional Caco-2 cell culture takes 21 days to complete cell differentiation to an enterocyte-like phenotype. As a result of the long culture period and daily medium replacement, the Caco-2 cell culture is very expensive, laboratory intensive and time consuming. To shorten and simplify the Caco-2 cell culture method, rapid protocols have been developed [Lentz *et al*., 2000; Liang *et al*., 2000; Yamashita *et al*., 2002; Olejnik *et al*., 2003].

Based on previous studies on the maturation of epithelial cell culture [Jenkins, 1991; Lentz *et al*., 2000], we selected the key growth factors and inductors promoting cell differentiation. Caco-2 cells were grown on polycarbonate filters in a 1:1 mixture of DMEM/F-12, with 2% iron supplemented calf serum. The induction of cell differentiation was carried out by addition of the following components: bovine insulin, human transferrin, dexamethasone, cholera toxin, epidermal growth factor, bovine pituitary extract and ascorbic acid. It was found that the Caco-2 differentiation characteristics of 4-6-day-old short-term system can be comparable with the conventional 21-day-old system [Olejnik *et al*., 2003].

The aim of this work was to determine the functional characterization of conventional and novel Caco-2 cell culture systems for their application in adhesion and transepithelial transport studies.

MATERIALS AND METHODS

Traditional cell culture

The established Caco-2 cell line was obtained from the American Tissue Culture Collection (USA). Cells were maintained in Dulbecco's modified Eagle medium (DMEM, Sigma), supplemented with 20% heat-inactivated (56°C, 30 min) fetal bovine serum (FBS, Gibco BRL), 1% non-essential amino acids 100X (NEAA, Sigma) and 50 mg/L gentamycin (Gibco BRL). The culture medium was changed daily in all experiments. Caco-2 cells at passage number 30-40 were seeded onto Millicell PCF (0.4 μm pore size) membranes (Millipore) and twenty four well cell cluster dishes at a seeding density of 1.04×10^4 cells per cm^2. Cells were cultured using 1 mL of the medium, 0.6 mL on the basolateral side and 0.4 mL on the apical side. Caco-2 cells grew as monolayers for 21 days at 37°C, in a 5% CO_2/95% air atmosphere, then were used for permeability and adhesion studies.

Modified cell culture

Caco-2 cells were grown on Millicell PCF (0.4 μm pore size) membranes (Millipore) in a 1:1 mixture of DMEM/F-12, with 2% iron-supplemented calf serum (sCs) and additional factors: bovine insulin (10 μg/mL), human transferrin (10 μg/mL),

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dexamethasone (10^{-8} mol/L), EGF (ng/mL), ascorbic acid (50 μg/mL), cholera toxin (25 ng/mL) and bovine pituitary extract (50 μg/mL). An initial cell seeding density of 2.12×10^5 cells per cm^2 was used. The medium was changed every 24 h (0.6 mL on the basolateral and 0.4 mL on the apical side) until the end of the culture period. Caco-2 cells were cultured for 6 days, at 37°C, and in a 5% CO_2/95% air atmosphere, then used for permeability and adhesion studies.

Determination of cell growth and differentiation

Cell proliferation was monitored by counting the number of cells using a Neubauer hemocytometer after trypsinization.

As a measure of cell differentiation alkaline phosphatase (ALP) activity was determined in cell lysates using Biochemtest No. 178152149 (POCh, Gliwice, Poland). After removal of the culture medium, the attached cells were removed by scraping and suspended in ice-cold PBS. The cells were centrifuged at 500×*g* and resuspended in PBS, and sonicated with an ultrasonic cell disrupter. Cellular debris was pelleted by centrifugation at 8000×*g*, at 0-4°C, and supernatants were stored at -70°C until assayed. The activities of ALP were expressed relative to cellular protein content which was determined with the Bradford's method [Bradford, 1976].

Determination of Caco-2 monolayer integrity

To monitor the evolution of confluency the transepithelial electrical resistance (TEER) of Caco-2 cell monolayer was measured daily. Monolayer integrity was determined by using the Millicell Electrical Resistance System (Millipore).

Cytotoxicity assay

Atrazine cytotoxicity was determined by the MTT reduction assay. Briefly, atrazine solutions were removed and the cell monolayers were washed with DMEM without phenol red. Then, the cells were incubated at 37°C for 3 h in DMEM without phenol red containing MTT (5 mg/mL). MTT solution was carefully aspirated and the formazan produced by the mitochondrial dehydrogenase was dissolved in isopropanol. Mitochondrial enzyme activity was measured as the difference in absorbance between 570 and 630 nm (baseline).

Transport study

Atrazine permeability studies were performed as to the method described by Tavelin *et al*. [2001]. Atrazine (Fluka) was first dissolved in DMSO and a 40×10^{-3} mol/L solution was prepared in a Hank's balanced salt solution (HBSS, Sigma). DMSO did not exceed 0.01% (v/v) of transport medium. This concentration of co-solvent did not affect the integrity of Caco-2 cell monolayers. Atrazine solution was filtered through a 0.22 μm filter (Millex GP, Millipore) and pre-heated at 37°C before permeability experiments.

After the development of cell monolayers in the traditional and modified Caco-2 cell culture system, atrazine permeability measurements were performed. Culture medium was removed from the cells, monolayers were washed with transport buffer and pre-incubated at 37°C for 30 min. Permeable supports were transferred to new wells. Atrazine solution and HBSS preheated to 37°C were added to apical – A (0.4 mL) and basolateral – B chambers (0.6 mL). The insert system was placed in an incubator (37°C) on a plate shaker. Samples (0.1 mL) were taken from a donor solution after 10, 20, 30, 40, 50 and 60 min. Each sample volume was replaced with fresh preheated HBSS.

Atrazine concentration was analysed with the HPLC method and the permeability coefficient across the Caco-2 cell monolayers was calculated.

Determination of atrazine was carried out on the MERCK-HITACHI system consisting of an autosampler (model L-7250), a pump (model L-7100) and DAD (model L-7455) set at 220 nm.

Analyses were performed isocratically at a flow rate of 0.60 mL/min, at 30°C on a Lichrospher®RP-18 column (Merck) using acetonitrile and sodium acetate (1 mmol/L) – 35:65 as an eluent. A standard was used to identify peak in chromatograms, and peak area was used to determine atrazine concentration. It was done by computer integration operated in the mode of external standard.

Permeability coefficients of atrazine across Caco-2 monolayer in both directions (A to B and B to A) were calculated according to basic protocols presented by Tavelin *et al*. [2001].

Adhesion study

In the adhesion study three probiotic strains were used: *Lactobacillus casei* Shirota (ATCC 39539), *Lactobacillus rhamnosus* GG (ATCC 53103) and *Lactobacillus acidophilus* LC1 (ATCC). Before adhesion experiments bacteria were cultured in the MRS broth at 37°C for 24 h, then harvested by centrifugation at 3000×*g* for 10 min, washed twice with phosphate buffered saline (PBS/pH 7.2) and resuspended in complete DMEM without gentamycin at a concentration of 1×10^6 CFU/mL. The number of CFU in each assay mixture was confirmed by plating appropriate dilutions of washed cells onto the MRS agar.

Traditional 21-day-old and modified 6-day-old Caco-2 cell cultures were washed twice with 8.5 mg/mL saline solution and transferred to new wells. For the adhesion assay, the bacterial suspension (0.2 mL) was added to apical chamber and complete DMEM without gentamycin (0.6 mL) to basolateral chamber of each insert system and incubated at 37°C. After incubation for 60 min free bacteria cells were removed by washing of the monolayers with PBS. The cell monolayers with attached bacteria were trypsinized and centrifuged at 3000×*g* for 10 min and treated with 1% Triton X-100 in PBS for 5 min at 37°C. The counts of adherent bacteria released from Caco-2 cells were determined by plating appropriate dilutions on the MRS agar (Merck). To express the results as a number of adherent bacteria per epithelial cell, Caco-2 cells from non-infected monolayers were collected by trypsinization and enumeration in parallel.

Statistical analysis

The results were expressed as mean values (±SEM) of triplicate replicates obtained in three independent experiments. The results were analysed by a one-way analysis of variance (ANOVA) to determine if data were significantly different and by the Tukey and Kruskal-Wallis tests to determine the statistical differences between groups using STATISTICA PL. 6.0. software. The level of significance was set at $p<0.05$.

RESULTS

Caco-2 cells growth

Caco-2 cells were grown in two culture systems: 21-days--old conventional culture using serum-containing medium (20% FBS) and 6-days-old modified culture utilizing serum-reduced medium (2% sCS) supplemented with growth and differentiation factors. As shown in Figure 1, the time courses of Caco-2 cells grown on the polycarbonate filters in different media are similar. It means that the modification of medium formulation had no significant influence on cells proliferation. However, modification of the traditional culture medium enabled rapid Caco-2 monolayer differentiation.

Scanning electron microscopic examination revealed the progressive formation of brush border with microvilli from 3 to 6-days-old Caco-2 cell culture grown in the modified medium. The brush border microvilli exhibited in two different patterns: as a thick carpet of brush border and as flower-like clusters which seem to join at their apical ends. As shown in Figure 2, the entire monolayer surface of the 6-days-old modified Caco-2 culture was covered with brush border microvilli. Whilst in the 6-days-old conventional culture, the brush border microvilli were present on 80% of the cells, which is typical of the early phase of cell proliferation under standard culture conditions in the absence of differentiation inducers [Pinto *et al*., 1983]. Morphological comparison of two culture methods shows that the new 6-days-old Caco-2 culture system has the similar ultrastructural characteristics to that of the conventional 21-old-day well-developed culture, as it can be seen in Figure 3.

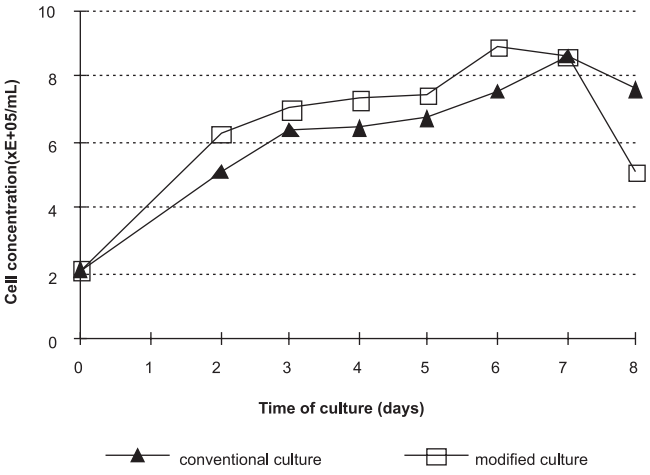

FIGURE 1. The time course of Caco-2 cells grown in modified and conventional medium.

Alkaline phosphatase is an enzyme that is present in the microvillus of enterocytes and commonly used as an indicator of enterocytic cell differentiation. Activity of alkaline phosphatase localised in the membrane fraction of Caco-2 cells grown in 6-days modified culture was 520 pmol per microgram total protein and was about 2-fold higher than that determined in 21-day conventional culture.

Therefore, the suitability of the short-term culture method for use in compound permeability screening and bacteria adhesion studies was investigated.

Permeability studies

Caco-2 cells require 21 days to differentiate using the well--established conventional culture protocol. Therefore transport studies are usually done after 21 days of culture, when the expression of transporters reaches its maximum [Anderle *et al*., 1998; Braun *et al*., 2000]. Prior to permeability experiments, the Caco-2 cell culture confluency was examined by measuring transepithelial electrical resistance values. Measurement of TEER is believed to be a good indicator of tightness of the junctions between cells [Tavelin *et al*., 2001]. TEER value was observed to changed in dependence of the growth phase. In Caco-2 cell culture, the TEER values increased during the exponential phase and stabilised in the stationary growth phase. Monitoring of monolayer development as a function of the culture period (Figure 4) showed that TEER values increased rapidly in both traditional and modified cell culture reaching a maximum value respectively on the 9th and 6th day. After 9 days of culture, the TEER of Caco-2 monolayers in traditional medium was relatively constant and reached 463 ($\pm$33) Ω x cm^2. As shown in Figure 4, the TEER values examined directly before transport were similar in both cul-

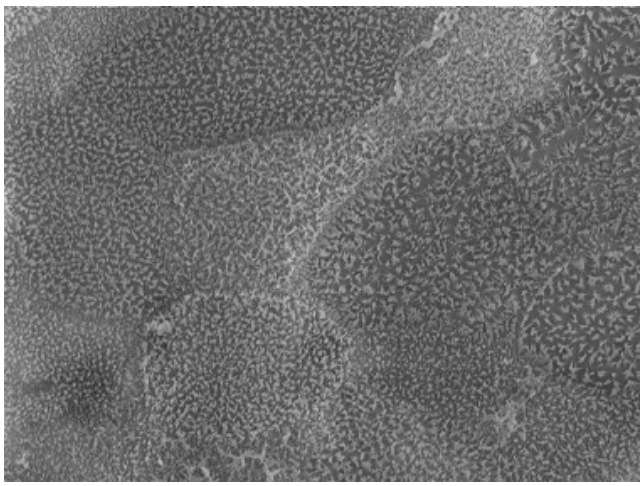

FIGURE 2. The 6-day-old Caco-2 cell monolayer developed in a modified medium. Scanning electron microscopy examination (4980x).

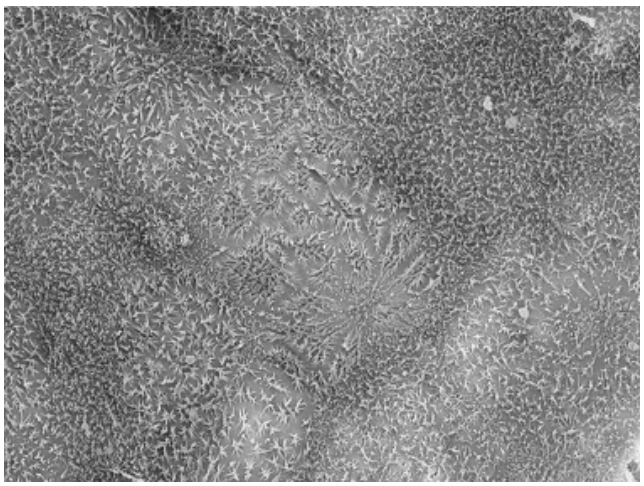

FIGURE 3. The 21-day-old well-developed Caco-2 cell culture grown in a conventional medium. Scanning electron microscopy examination (4240x).

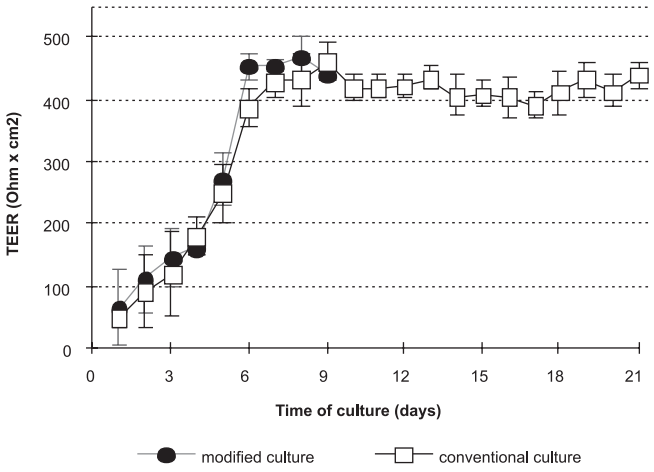

FIGURE 4. Transepithelial electrical resistance of Caco-2 monolayer as a function of culture time.

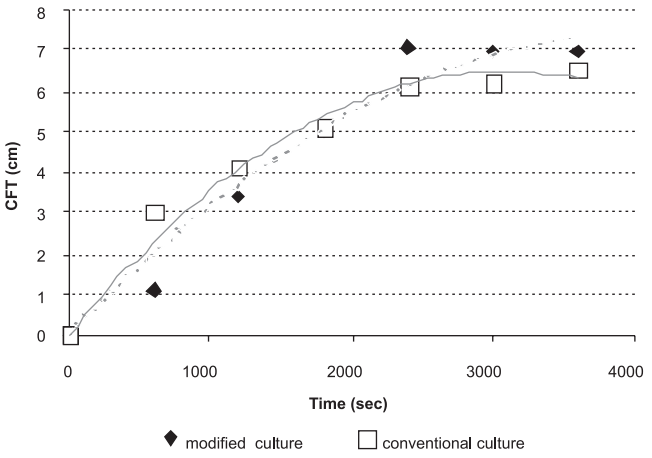

FIGURE 6. Atrazine transport kinetics of basolateral to apical direction monitored by collecting the receiver solutions at 10-min intervals.

ture protocols. The statistical analysis confirmed an insignificant difference in TEER between 6-old-day modified and 21-old-day conventional cultures (p=0.341). The transepithelial electrical resistance measurement indicated high integrity of the Caco-2 cell monolayer in both cell culture systems.

Two independent Caco-2 cell culture protocols were used to determine the atrazine permeability across epithelial barrier. For atrazine at concentrations ranging from 5 to 250 μmol/L, the apparent permeability coefficients (Papp) were not found to be concentration-dependent (data not shown). In order to compare permeabilities through 6-day-old short-term and 21-day-old traditional Caco-2 cultures transport of atrazine at concentration of 40 μmol/L was studied in apical to basolateral and in basolateral to apical directions. Prior to carrying out transport study, the cytotoxicity of atrazine was examined. For toxicity examination, measurement of intracellular dehydrogenase activity with the MTT assay was used. No cytotoxicity of 40 μmol/L atrazine dissolved in 0.01% DMSO and in the transporting medium was observed.

Figure 5 and 6 depict the effect of Caco-2 culture method on atrazine transport kinetics. The graphs showed deviations between experimental and theoretical data of the cumulative fraction transported (CFT). The atrazine cumulative fraction transported across Caco-2 cell monolayers increased non-linearly with time. Because of the atrazine concentration in the receiver chamber exceeded 10% of the concentration in the donor chamber, the apparent permeability coefficients (Papp) were calculated using non-sink conditions analysis. Determination of Papp in the apical to basolateral and the basolateral to apical side showed that the permeability coefficient was independent of transport direction. Table 1 summarizes the Papp values obtained from the different culture conditions.

No significant difference in atrazine permeability was observed between the conventional culture at 21 day and the modified culture at 6 day (p=0.984). The transport of atrazine was symmetric between the apical to basolateral and the basolateral to apical directions and independent of its concentration (data not shown, manuscript in preparation).

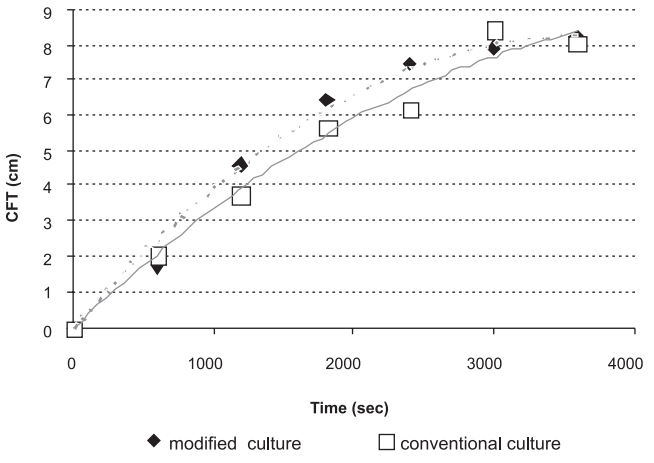

FIGURE 5. Atrazine transport kinetics of apical to basolateral direction monitored by collecting the receiver solutions at 10-min intervals.

Adhesion studies

In order to investigate the role of Caco-2 cell culture method might play in the adhesion of probiotic bacteria, three well-characterised lactobacilli strains were used. The adhesion capacity was expressed as the number of bacteria adhered to 1000 Caco-2 cells and as the number of bacteria bound to one cm^2 of cell monolayer. As shown in Figure 7, *L. rhamnosus* GG and *L. acidophilus* LC1 were the most adhesive strains with 19(±2) and 16(±3) bacteria cells adhered to 1000 Caco-2 cells grown in conventional 21-days-old culture, respectively. The adherence of these strains was significantly better than that of *L. casei* Shirota.

TABLE 1. Bidirectional (A → B – from apical to basolateral side, B → A – from basolateral to apical side) mean apparent permeability coefficients of atrazine across Caco-2 cells grown in different cultures.

Transport direction	Apparent permeability coefficients Papp (x 10^{-5} cm/s) ±SD	
	traditional culture	modified culture
A → B	8.80±1.04	8.97±1.15
B → A	8.78±1.10	9.03±0.24
AB/BA	1.00±0.00	0.99±0.05

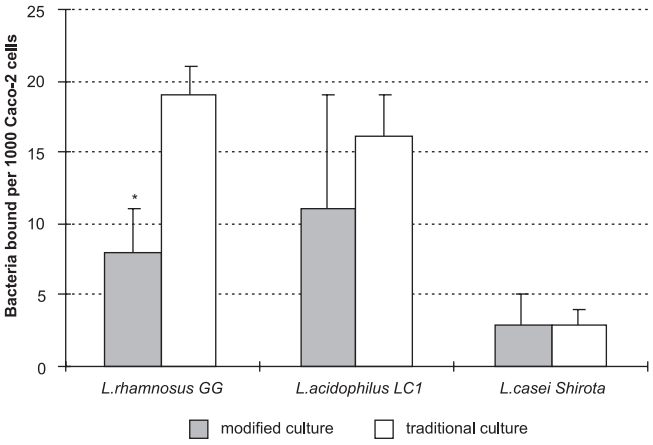

FIGURE 7. Effect of Caco-2 cell culture modification on probiotic adhesion expressed as the number of bacteria adhered to 1000 Caco-2 cells.

The ability of probiotic bacteria strains to adhere to the Caco-2 cell monolayers obtained in conventional 21-days-old and modified 6-days-old culture was compared. Based on the concentration of bacteria adhered to one cm^2 of cell monolayer, the effect of Caco-2 culture protocol on adhesion capacity of all tested bacteria was not observed (Figure 8). However, significant differences ($p=0.010$) between the number of *L. rhamnosus* GG bacteria adhered to 1000 Caco-2 cells grown in two independent culture methods were determined (Figure 7). *L. rhamnosus* GG adhesion ability measured in 6-days-old modified culture was 2.4-fold lower than that obtained using 21-days-old traditional culture. The different Caco-2 cell densities determined in conventional and modified cultures at 21st and 6th day, respectively, can be expected to cause a reduction in the number of bacteria adhered to 1000 Caco-2 cells. The Caco-2 cell concentration in modified medium was 3-fold higher than that obtained in traditional medium at the infection moment. The adhesion of *L. rhamnosus* GG to 1000 Caco-2 cells grown in 6-days-old modified culture was comparable with their colonization on the 14-day-old cell monolayer obtained using the conventional culture protocol.

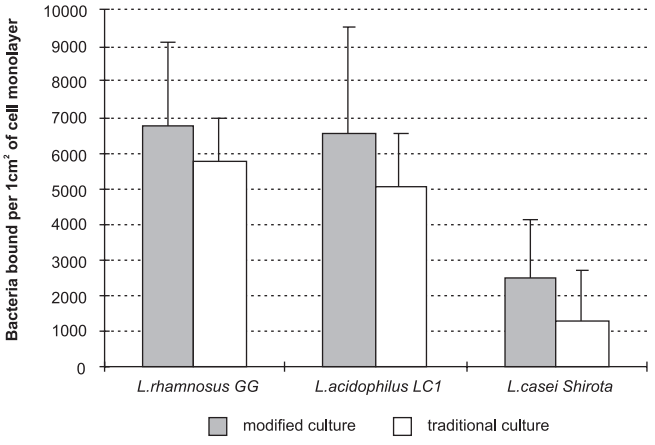

FIGURE 8. Effect of Caco-2 cell culture modification on probiotic adhesion expressed as the number of bacteria adhered to one cm^2 surface of Caco-2 monolayer.

DISCUSSION

Differentiated Caco-2 cells provide an *in vitro* model of intestinal barrier for the transport study and of intestinal surface for bacteria adhesion examination. There may be some differences between differentiated Caco-2 cell monolayers grown under various culture conditions. The importance of cell culture standardisation in view of their use as transport models has already been documented [Braun *et al*., 2000]. Standardisation of Caco-2 cell culture includes the number of cell passage, culture medium with serum or serum replacement and defined experimental conditions such as seeding density, reproducible cell numbers per area in stationary growth phase, reproducible TEER values, characteristic morphology, characteristic tight junction formation and characteristic transporters expression [Braun *et al*., 2000; Wunderli-Allenspach, 2000].

Traditional serum-containing Caco-2 cell culture should be performed for 3 weeks before permeability studies and 2-3 weeks before adhesion investigation. As a result of the long culture period and daily medium replacement, the conventional Caco-2 cell culture is very expensive, laboratory intensive and time consuming. To shorten and simplify the culture method, rapid culture protocols have been developed [Lentz *et al.*, 2000; Liang, 2000; Yamashita *et al*., 2002; Olejnik *et al*., 2003; Korjamo *et al*., 2005]. In the presented studies, we propose a rapid Caco-2 cell culture system, described in details in previous work, for transport and adhesion studies [Olejnik *et al*., 2003]. In the modified Caco-2 culture method, cells grown on polycarbonate filters in serum-reduced medium containing 2% iron-saturated calf serum and supplemented with a combination of growth and differentiation factors. Using this system, the differentiated enterocyte-like monolayer was established within 6 days. Scanning electron microscopic examination revealed that the differentiation characteristic of modified 6-day-old culture is comparable with 21-day-old traditional culture. The activity of alkaline phosphatase considered as an indicator of cellular differentiation, was 2-fold higher in Caco-2 cells grown in modified culture than in the conventional culture. An increase of alkaline phosphatase activity in modified Caco-2 cell culture is in agreement with previous results [Korjamo *et al*., 2005]. Differentiation includes also the formation of a tight junction between the cells. The transepithelial electrical resistance measurement confirmed high integrity of the Caco-2 cell monolayer in the new culture system. As a result, the modified 6-day-old Caco-2 culture would be applied in permeability and probiotic adhesion studies.

Atrazine, 2-chloro-4-ethylamino-6-izopropylamino-*s*-triazine as a model substance was used in permeability studies for two reasons: (i) small size of molecule with good water solubility and (ii) environmental contamination with atrazine and its high toxity. Among trazine herbicides, atrazine is most commonly used, with hundred of milions of tons being used yearly worldwide. It is included in 10 most frequently found pesticides in ground and superficial water and food. A lot of reports confirmed a relationships between environmental contamination with atrazine and increased incidence of many human diseases [van Leeuven *et al*., 1999; Mills, 1998; Kettles, *et al*., 1997]. Due to an increased incidence of cancer diseases,

atrazine was classified as "possibly carcinogenic to humans" (Group C) by the International Agency for Research on Cancer [International Agency for Research on Cancer, 1999].

To quantify atrazine transport across the Caco-2 monolayer grown in two different culture systems, the apparent permeability coefficient was used. The transport of atrazine was symmetric between the apical to basolateral and the basolateral to apical directions and independent of its concentration ranging from 5 to 250 μmol/L, which suggests a transcellular or paracellular process (data not shown, manuscript in preparation). As a consequence, we used one atrazine concentration of 40 μmol/L to screen its permeability across the 6-day-old modified Caco-2 culture. No significant differences between apical to basal and basal to apical permeation were found for atrazine tested in traditional 21-day-old and modified 6-day-old cultures. The results obtained in this work indicated that the new rapid Caco-2 culture method could be a considerable alternative to long-term conventional culture system usually applied in transport study.

Several strategies were used to improve the Caco-2 cell culture method [Lentz *et al*., 2000; Liang, 2000; Yamashita *et al*., 2002; Olejnik *et al*., 2003; Korjamo *et al*., 2005]. The novel cell culture protocols described by Lentz *et al*. [2000] and Korjamo *et al*. [2005] were similar with that proposed in the presented work. Caco-2 cells were grown in serum-reduced medium supplemented with growth factors and hormones. Transport of three compounds (metoprolol, mannitol and taurocholate) through 4-day-old modified culture and traditional 21-day-old, 10% FBS system was compared [Lentz *et al*., 2000]. No difference in permeability was observed for metoprolol transport between the two culture methods. Permeability of mannitol was about 2-fold higher and taurocholate was 6-fold lower from novel culture, as compared to the 10% FBS system. The results show that the Lentz's culture method appears to be applicable in passive permeability determination. However, low taurocholate permeability across the modified monolayer indicated the lack of active transporter capability [Lentz *et al*., 2000]. Liang *et al*. [2000] have developed a traditional Caco-2 culture method for accelerated 3-7-day culture in which some special supplements, including cholera toxin, bovine pituitary extract, mouse epidermal growth factor, insulin, dexamethasone and other, were added to the medium to promote cell differentiation. Caco-2 monolayers obtained according this culture protocol were less confluent and differentiated, with lower expression levels of several transporters [Liang *et al*., 2000].

It should be noted that a commercially available 3-day system for rapid differentiation of Caco-2 cells (BD BioCoat™ HTS Caco-2 Assay System, Becton, Dickinson and Company) can be purchased. One of the most unique aspects of this system is the use of butyric acid as a differentiating agent. As described in the literature, the barrier function of the monolayer grown in short-term BD system is low compared with that obtained in the traditional 21-day culture [Liang *et al*., 2000]. Besides, BD system is significantly more expensive than that elaborated in our study.

Yamashita *et al*. [2002] have demonstrated that by a simple modification of the original 3-day culture BD protocol it is possible to obtain Caco-2 monolayer with better barrier properties than that typical of the conventional 21-day Caco-2 system. The best improved 3-day protocol, which included 10% FBS in the culture medium during the first day of culture, markedly enhanced TEER and lowered mannitol permeability of the monolayer. The prolongation of culture period to 5 days induced the tighter monolayer and the highest activity of transporters.

The second aspect of the presented work is the application of a modified Caco-2 cell culture in probiotic adhesion. The adhesive ability of bacteria to intestinal cells has been considered as one of the selection criteria for probiotic strains [Salminen *et al*., 1996]. Human intestinal epithelial cell lines, like Caco-2 or HT-29, have been extensively used to select for adhesive strains *in vitro*. For bacteria adhesion examination, traditional Caco-2 culture requires a 3-2-week period [Braun *et at*., 2000]. The aim of this study was to determine the differences in adhesion of three commonly used probiotic bacteria to the 21-day-old traditional and modified 6-day-old cultures. Independently of Caco-2 culture method, *L. rhamnosus* GG and *L acidophilus* LC1 were the most adhesive strains. Their adherence was significantly better than that of *L. casei* Shirota. Based on the number of bacteria adhered to one cm^2 of cell monolayer, the effect of Caco-2 culture modification on adhesion capacity of all tested bacteria was not observed.

The results obtained in different laboratories using identical probiotic strains are dramatically differed. It is a consequence of various adhesion conditions including differences in cell models, growth phase of bacteria, buffers and pH applied in the adhesion assays [Blum *et al*., 1999]. This lack of method standardization complicates the interpretation of the data and effective adhesion estimation. Moreover, in the current literature, there is no information about determination of bacteria adhesion using Caco-2 monolayer obtained according to short-term modified cell culture protocol.

CONCLUSIONS

1. Morphological comparison of two cultures shows that the new 6-days-old Caco-2 culture system has similar ultrastructural characteristic to that of the conventional 21-old--day well-developed culture.

2. The transport of atrazine was symmetric between the apical to basolateral and the basolateral to apical directions and independent of its concentration. Moreover, no significant difference was observed in atrazine permeability between the conventional 21-day-old and the modified 6-day-old cultures.

3. The effect of Caco-2 culture modification on adhesion capacity of all tested bacteria was not observed.

4. The proposed system of Caco-2 cell culture can be an considerable alternative to the conventional 21 days system with the application of 20% FBS. The new Caco-2 cell culture system allows reducing the serum concentration from 20 to 2% and the time of culturing from 21 to 6 days.

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