Effect of Caseinomacropeptide Concentrate Addition on the Growth of Bifidobacteria

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Abstract

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We investigated the effect of caseinomacropeptide concentrate (CMP) on the growth and metabolic activity of *Bifidobacterium bifidum* CCDM 94 and *Bifidobacterium lactis* BB12 in skim milk with the addition of glucose and of various combinations of yeast extract, L-cysteine-HCl, ascorbic acid, and CMP. The microorganisms grew well on the medium containing a combination of all the nutrients mentioned, and on that where only L-cysteine-HCl was missing. In the media enhanced with CMP a shorter lag-phase occurred than in those without CMP. The shortest lag-phase and the longest log-phase with a high growth rate were observed in media No. 2 (complete medium with CMP) and No. 8 (complete medium without L-cysteine-HCl).

Keywords: *Bifidobacterium lactis*; *Bifidobacterium bifidum*; caseinomacropeptide; impedance

Bifidobacteria and other lactic acid bacteria (LAB) present in the intestine may be associated with good health and longevity of humans (O'Sullivan & Kullen 1998). Many studies and experiments postulated that human-specific species of *Bifidobacterium* have many favourable effects on the host (Holzapfel *et al.* 1998; O'Sullivan & Kullen 1998; Brigidi *et al.* 2001; Lammers *et al.* 2003; Burns & Rowland 2004; Medici *et al.* 2004). Therefore, some species of bifidobacteria are widely used in the food industry as cultures enriching fermented products (Vincent *et al.* 1998) and as dietary supplements (Fasoli *et al.* 2003). The addition of these probiotic bacteria to milk or fermented dairy products ensures their presence in the gut microflora (Vinderola & Reinheimer 1999). *Bifidobacterium lactis* has been claimed to benefit health by the enhancement of the immune response (Chiang *et al.* 2000; Gill *et al.* 2001), reduction of diarrhoea associated with rotavirus and *Escherichia coli* (Shu *et al.* 2001), antagonistic activity against enterotoxigenic *E. coli* (Gopal *et al.* 2001) and *Salmonella typhimurium* (Shu *et al.* 2000). Moreover, *B. lactis* shows in-

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teresting technological characteristics, such as certain oxygen and acid tolerances that render these microorganisms adequate for industrial utilisation in fermented milks in comparison with other bifidobacteria species (Meile *et al.* 1997; Crittenden *et al.* 2001).

Bifidobacteria need free amino acids and peptides for their growth. Various demands on nitrogen sources have been found with various species of bifidobacteria (Klaver *et al.* 1993). Another growth factors are depolymerised alginates or soya hydrolysates (Gomes & Malcata 1999). Gibson and Roberfroid (1995) consider bifidogenic factors to be usually saccharidic compounds utilised by bifidobacteria as a source of energy. Gomes and Malcata (1999) divided bifidogenic factors into several groups: (1) components of breast and cow's milks (κ-casein, CMP, enzymatically degraded κ-casein), (2) oligosaccharides (e.g. raffinose, stachyose, lactulose), (3) polysaccharides (e.g. inulin), (4) alcoholic saccharides (e.g. lactitol), (5) chinones (e.g. 2-amino-3-carboxy-1,4-naftochinone). Bifidobacteria show poor growth in milk, therefore many studies are concerned with the aim of finding a suitable nutrient supplement to incorporate into the culture media (Idota *et al.* 1994; Proulx *et al.* 1994; O'Sullivan 1996; Gomes *et al.* 1998; Janer *et al.* 2004). Much attention has been paid to milk-derived components.

Caseinomacropeptide (CMP) is a hydrophilic glycopeptide derived from the action of chymosin on κ-casein during the milk-clotting process in cheese making. CMP contains not only nitrogen available for bacterial growth but also amino-sugars, such as sialic acid and *N*-acetylgalactosamine that could be fermented by bifidobacteria (Azuma *et al.* 1984). Poch and Bezkorovainy (1991) found no enhancing activity of bovine CMP on the growth of *Bifidobacterium bifidum* and *B. longum*. It has been proposed that the difference between the growth enhancing activities of various forms of CMP is due to their amino-sugar contents (Modler 1994; Janer *et al.* 2004).

Another nutrient supplement can be whey protein concentrate (WPC), which contains CMP as well as whey proteins. WPC is a cheap and readily available additive, for which the growth-enhancing activity on lactic acid bacteria has been described by Bury *et al.* (1998). Janer *et al.* (2004) compared the effect of bovine CMP and combined ovine and caprine CMP and the effect of WPC on the growth of *Bifidobacterium lactis* in skim milk. The growth-enhancing effects on *B. lactis* of bovine CMP and combined ovine and caprine CMP were intermediate, and no differences were found between them. Maximum specific growth rates for milk with CMP ranged between 75% and 80% of that obtained with WPC, and not exceeding 39% for unsupplemented milk. Thus, it may be accepted that CMP and WPC can stimulate the growth of bifidobacteria when added to milk as a supplement. Besides the CMP content, WPC includes whey proteins, mainly α-lactalbumin and β-lactoglobulin. These proteins have been shown to stimulate the growth of *Bifidobacterium* spp. This could explain the higher growth-promoting activity of WPC as compared to CMP (Petschow & Talbott 1991; Ibrahim & Bezkorovainy 1994).

In this study, we have analysed the effect of the addition of CMP on the growth and acid production of *B. bifidum* CCDM 94 and *B. lactis* BB12 in enriched skim milk. Skim milk was enriched with glucose and with combinations of yeast extract, ascorbic acid, and L-cysteine-HCl. We have subsequently substituted each nutrient with an addition of CMP to find out which of these nutrients could be replaced by CMP.

The growth of bifidobacteria strains and especially their metabolism were monitored by BacTrac analyser recording the relative changes of media impedance (*M*-values).

MATERIALS AND METHODS

Caseinomacropeptide concentrate (CMP). CMP was isolated at the Department of Dairy and Fat Technology of the Institute of Chemical Technology, Prague, from cow milk whey remaining after the production of ricotta cheese (Čurda *et al.* 2008). Coarse filtration of whey was followed using two microfiltrations steps (ceramic membranes 1.400 nm and 200 nm) on the TIA filtration unit (TIA, Bollene, France). CMP was concentrated by ultrafiltration and diafiltration using TIA unit and membranes 20 nm and 5 kDa (in parallel configuration). The obtained retentate was further concentrated and diafiltered on the ARNO 700 unit (Micropur, Hradec Králové, Czech Republic) by means of 50kDa ceramic membrane (Tami Industries, France). The CMP isolate contained 69.7 ± 0.04% proteins and 39.2% CMP after lyophilisation. HPLC analyses, showed that

the product contained 54% glycosylated CMP forms (gCMP). Among the non-glycosylated forms, the A variant (33%) prevailed. The concentrate contained neither β-lactoglobulin A and B, nor α-lactalbumin.

Cultivation media. *M.R.S. agar*. The M.R.S. agar (Oxoid, Hampshire, UK) pH 6.8 was used for the determination of the microorganisms counts (MO). It was prepared according to the producer's instructions. Prior to agar sterilisation, pH was adjusted to 7.0 by means of 1% or 10% NaOH solution. L-Cysteine-HCl (0.5 g/l) (Merck, Gibbstown, USA) and dicloxacillin (02 µl/ml) (Sigma, St. Louis, USA) were added after the agar sterilisation.

Basic medium. Basic cultivation medium (base) intended for the preparation of the media without CMP addition (media 1, 3, 5, 7, and 9) (Table 1) was based on the study of Janer *et al.* (2004) with small adjustments in ascorbic acid concentration. The concentration (1%) mentioned in the study substantially affects the pH value. This could negatively influence the growth of bifidobacteria, and due to the high initial media impedance and along with a possible neutralisation distort the impedance measurements. The base was made from skim UHT milk (Meggle, Prague, Czech Republic) containing 0.5% fat with the addition of 0.5% glucose (Lach-Ner, Neratovice, Czech Republic). 0.5% of yeast extract (YE) (Oxoid, Basingstoke, UK), 0.05% of L-cysteine-HCl (CYS) (Merck, Gibbstown, USA), and 0.05% of ascorbic acid (ASC) (Penta, Mainaschaff, Germany) were added to this base (Janer *et al.* 2004), in different combinations in order to improve the growth. Two per cent of CMP was added to the basic medium intended for the preparation of the media with CMP addition (media 2, 4, 6, 8, and 10) (Table 1). Due to their insufficient thermal stability, ascorbic acid and L-cysteine-HCl were added to the media under sterile conditions through a microfilter 0.22 µm (Sartorius, Goettingen, Germany) after the sterilisation of the basic medium and shortly before the inoculation itself. Prior to inoculation, blank samples of the individual media were taken, the counts of microorganisms on M.R.S. agar were determined, and pH was measured.

Table 1. Composition of cultivation media

Medium	Media composition	Note
1	base + YE + CYS + ASC	complete medium without CMP
2	medium 1 + 2% CMP	complete medium with CMP
3	base + YE + CYS	without ASC
4	medium 3 + 2% CMP	
5	base + CYS + ASC	without YE
6	medium 5 + 2% CMP	
7	base + YE + ASC	without CYS
8	medium 7 + 2% CMP	
9	base	without YE, CYS, and ASC
10	medium 9 + 2% CMP	

YE – yeast extract; CYS – L-cystein-HCl; ASC – ascorbic acid; CMP – caseinomacropeptide

Microorganisms. Lyophilised *Bifidobacterium bifidum* CCDM 94 (Milcom, Prague, Czech Republic) (2.7 g/100 g milk) and *Bifidobacterium lactis* (*Bifidobacterium animalis* subsp. *lactis*) BB12 (Chr. Hansen, Hørsholm, Denmark) (1.22 g/100 g milk) cultures were used. They were precultivated at 4°C for 2 hours. Two inocula were prepared from both cultures – high (V) and low (N). The high inoculum of *B. bifidum* CCDM 94 was prepared by adding 2.7 g of the culture into 100 g of skim milk, the high inoculum of *B. lactis* BB12 was prepared by adding 1.22 g of the culture into 100 g of skim milk followed by hundredfold dilution. The low inocula were prepared from the high inocula by hundredfold dilution for both strains. The media were inoculated with 2% inoculum. The counts of microorganisms were determined by the plate count method.

Cultivation and measurement of impedance changes by means of BacTrac 4100 analyzer (Sy-Lab, Austria). The cultures were cultivated in BacTrac cells (approx. volume 10 ml) at 37°C. The medium in the cells was poured over with sterile paraffin in order to ensure the anaerobic environment during the fermentation. Three parallel samples of the same medium and the same inoculum were always measured. At the same time, blank samples of non-inoculated media were cultivated. The cultivation was stopped (cells were removed from the BacTrac) either when the cultures reached the stationary phase or after 72 h of cultivation, whichever came first. After the fermentation, the counts of microorganisms were determined and pH was measured.

The advantages of the impedance system include the ease to automate the analysis of samples and rapid results. The BacTrac system measures electrical changes resulting from the microbial metabolic processes in both the growth medium

and on the electrode surfaces (impedance splitting) (Walker *et al.* 2005).

BacTrac evaluation. The principle of the impedance method resides in monitoring the changes of electrical properties of the growth medium that are caused by the metabolic activity of microorganisms. So-called *M*-value introduced expresses relative changes of impedance in percentage during the measurement. The record of *M*-value measurements is the impedance curve. Its course resembles the conventional growth curve of microorganisms and includes the following phases: system stabilisation (corresponding to lag-phase), linear course (corresponding to log-phase), inflection point (IT, maximum metabolic activity of microorganisms), stationary phase, and a moderate growth of impedance (increasing ion concentration in the media by releasing the cell content by autolysis) (Čurda 1999; Čurda & Holubová 2003).

With regard to the large amount of data and to the fact that the parallel measurements did not almost differ from one another, the arithmetic average was calculated from three parallel measurements. These were used for the calculations of further values such as the detection and generation times, the lengths of the lag and log-phases, growth rate, and change of *M*-value during the log-phase.

Detection time (*DT*) was recorded after exceeding the chosen constant value of media impedance (*M*-value) was exceeded; in our case this value was 5%. *DT* depends on the physical system conditions and microbial parameters, such as inoculum size, contamination level, generation time, and on the metabolism of microorganisms. DT is negatively correlated with the logarithm of the initial microorganism count (Čurda 1999).

The generation time (*GT*) of microorganisms can be calculated from the difference of the detection times of two culture dilutions (∂DT), between which is a difference of two orders of magnitude: $GT = (\partial DT \log 2)/2$ (Čurda 1999).

Lag and log-phases (see above) of the bacterial growth were calculated from the impedance (growth) curves (Figure 1). By extrapolating the graph by a tangent (tangent 2) to the growth curve in the lag-phase (tangent parameters calculated by means of linear regression in MS Excel from the data whose derivation did not have absolute values higher than 0.05) and a tangent to the inflection point (tangent 1) in log-phase, there ensues a point of intersection of both tangents (intersection 1). The length of lag-phase is then equal to the value of the *x*-coordinate of this intersection point (x_1). By affixing the tangent to the stationary growth phase (tangent 3) (absolute derivation value max. 0.05), a further intersection point ensues with the tangent of the log-phase (intersection 2, value x_2). The difference between the values of *x*-coordinates

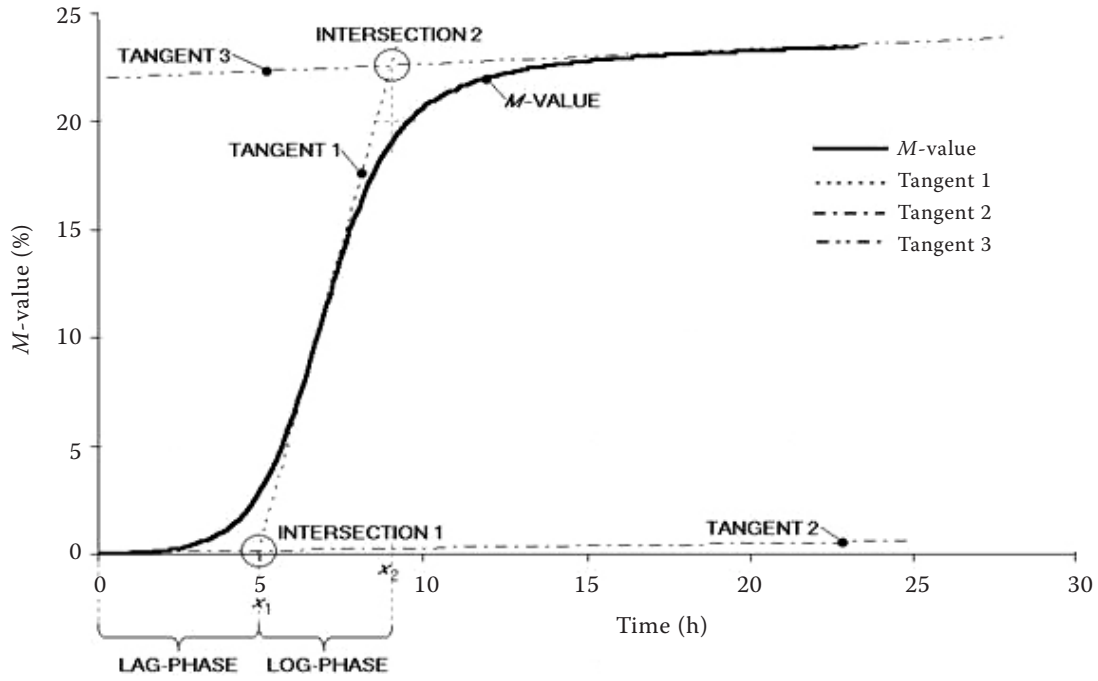


Figure 1. Principle of calculation of lag and log phases from impedance (growth) curves

of both intersection points then denotes the length of the log-phase, thus the log-phase = $x_2 - x_1$.

Further, the highest growth rate was estimated which was equal to the tangent gradient of the log-phase of the growth curve (tangent 1).

From these data, the change of *M*-value during the log-phase was calculated which was equal to the product of the highest growth rate and the duration of the log-phase, and, for an overall comparison of the metabolic activity of the strains studied, the ratio was calculated between the change of *M*-value during log-phase and the length of lag-phase.

Determination of MO counts. Microorganism count was determined by the plate count method on the modified M.R.S. agar (composition see above). The incubation proceeded for 48 h at 37°C under anaerobic conditions (Anaerogen; Oxoid, Basingstoke, UK).

RESULTS AND DISCUSSION

pH values

Shortly after the inoculation, pH of all media was approximately 6.6. From pH values after fermentation it ensues that the *B. lactis* BB12 culture was able to grow in all media studied, of course in the dependence on the inoculum size (Table 2). The low inoculum was not able to ferment the medium No. 3 (missing ASC and CMP), medium No. 9 (only base), and medium No. 10 (base + CMP). The lowest pH for the low inoculum was reached in the media No. 2 (complete medium with CMP), 4 (missing ASC), 7 (missing CYS and CMP), and No. 8 (missing CYS), whose pH was 4.1, and in the medium No. 1 (complete medium without CMP) with pH value of 4.2. The lowest pH for the high inoculum was reached in the media Nos 3 and 7 (pH 4.0), and in the medium No. 2 (pH 4.1) (Table 2). The growth of the BB12 strain in the medium No. 3 was determined by the inoculum size as well. The highest pH values remained in the media Nos 9 and 10, but in comparison with the low inoculum they were considerably lower. It is possible to compare roughly the results obtained with the media No. 2 and No. 9 with the study of Janer *et al.* (2004). pH value in the medium No. 2 was 4.1, while in Janer *et al.* (2004) the pH value ranged from 5.0 to 5.1. In the medium No. 9 the difference was smaller. Janer *et al.* (2004) stated 5.4, while we found 4.8. But Janer *et al.* (2004) used, on the contrary to us, skim milk without glucose addition as control.

B. bifidum CCDM 94 culture was able to reduce pH value in almost all the media investigated considerably more than *B. lactis* BB12 (Table 2),

Table 2. Counts of microorganisms and pH of media after cultivation

Medium	Low inoculum (N)				High inoculum (V)			
	CCDM 94		BB12		CCDM 94		BB12	
	pH	MO count log (CFU/ml)	pH	MO count log (CFU/ml)	pH	MO count log (CFU/ml)	pH	MO count log (CFU/ml)
1	3.9	8.5	4.2	9.5	3.9	8.9	4.4	8.6
2	3.9	8.9	4.1	8.6	4.0	8.7	4.1	8.6
3	5.7	9.0	6.4	4.8	3.9	8.4	4.0	9.4
4	4.0	8.6	4.1	9.5	4.0	9.1	4.2	8.9
5	4.7	6.7	4.6	9.1	4.8	4.7	4.5	8.9
6	4.4	8.0	4.9	7.3	4.4	7.9	4.7	7.8
7	3.9	9.0	4.1	9.1	4.7	9.0	4.0	9.5
8	3.9	9.3	4.1	9.3	4.2	8.8	4.2	9.1
9	4.5	8.8	6.6	7.0	4.4	8.6	4.8	8.9
10	5.9	0.9	6.4	8.5	6.0	5.5	4.9	8.5

MO – microorganism; CFU – colony forming units; CCDM 94 – *Bifidobacterium bifidum* CCDM 94; BB12 – *Bifidobacterium lactis* BB12

even with the low inoculum. The lowest pH values were achieved in media Nos 1, 2, 4, 7, and 8 (pH 3.9–4.0). The media fermented at the least were No. 3 (pH 5.7), and No. 10 (5.9), but on the contrary to *B. lactis* BB12 the pH value was lower by 0.5–0.7. The most fermented media for the high inoculum were the media Nos 1–4 (pH 3.9–4.0), the least one medium No. 10 (pH 6.0).

Idota *et al.* (1994) compared the growth of *B. bifidum* in Pepton Yeast Extract Fildes Solution broth with the additions of glucose, *N*-acetylneuraminic acid, sialyl-lactose, or CMP and reported that there was only a little use of CMP by *B. bifidum* compared to glucose or *N*-acetylneuraminic acid. Our media contained glucose in the base in the skim milk, the other nutrients were added. Azuma *et al.* (1984) suggested that not only the carbohydrate portion but also the polypeptide portion of CMP might play a significant role as a bifidus growth promoter.

Microorganism counts

The microorganism count was assessed in every medium after finishing the cultivation (Table 2). For the low inoculum of *B. lactis* BB12, the lowest count of microorganisms was reached in the media No. 3 (4.8 log (CFU/ml)) and No. 9 (7.0 log (CFU/ml)) which corresponds with the high pH values in these media. A relatively interesting ratio between pH and microorganisms count could be found in the medium No. 10. The value of pH in this medium did not show any drop, whereas the count of microorganisms was relatively high (8.5 log (CFU/ml)). The highest counts of microorganisms were reached in the media Nos 1 and 4 (9.5 log (CFU/ml)), which corresponds again with the lowest pH values in these media.

The counts of *B. lactis* BB12 for the high inoculum were relatively high in all media (Table 2), the highest ones having been found in the media No. 7 (9.5 log (CFU/ml)), and No. 3 (9.4 log (CFU/ml)). The lowest one was found in the medium No. 6 (7.8 log (CFU/ml)). In the study of Janer *et al.* (2004), the count of microorganisms in the medium enriched with CMP after 24 h was equal to the microorganism count in our medium No. 2. The only difference was found with the medium No. 9 where we reached the count of microorganisms 8.9 log (CFU/ml) while Janer *et al.* (2004) achieved the count of 8.0 log (CFU/ml). The difference was probably caused by glucose addition into the medium No. 9.

For the low inoculum of *B. bifidum* CCDM 94, the highest counts were reached in the media No. 8 – 9.3 log (CFU/ml)), No. 7 (9.0 log (CFU/ml)), and No. 3 (9.0 log (CFU/ml). The lowest count of microorganisms was reached in the medium No. 10 (0.9 log (CFU/ml)).

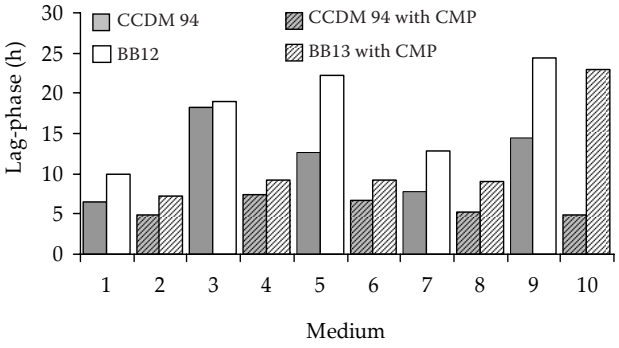
For the high inoculum of *B. bifidum* CCDM 94 the highest counts were reached in the media No. 4 – 9.1 log (CFU/ml)) and No. 7 (9.0 log (CFU/ml). The lowest count of microorganisms was obtained in the medium No. 5 (4.7 log (CFU/ml)).

Correlation coefficients between pH values and microorganisms counts were –0.75 for both the low inocula, and –0.78 and –0.74 for the high inocula of *B. lactis* BB12 and *B. bifidum* CCDM 94, respectively.

Length of lag-phase

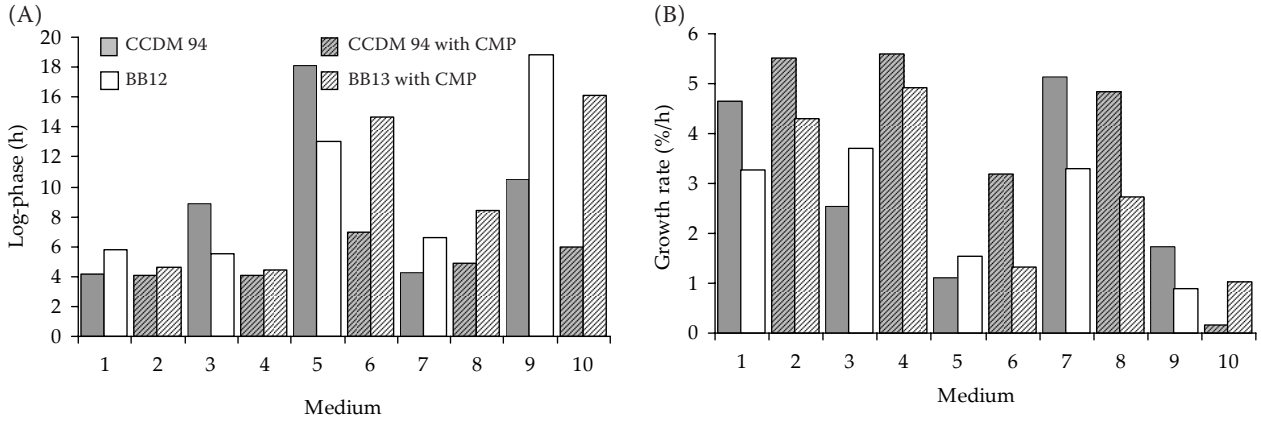
During the lag-phase, the cell count remains constant but their volume increases and the enzymes are being activated (Čurda 2003). The high inoculum corresponds best to the industrial needs, so we are presenting first of all the results obtained with the high inoculum.

Generally, the addition of CMP helped to reduce the lag-phase (Figure 2). The greatest difference between the medium without and with CMP for *B. bifidum* CCDM 94 was found in the media Nos 3 and 4 (high inoculum) where the lag-phase was reduced by less than 11 h, for *B. lactis* BB12 in the media Nos 5 and 6 (high inoculum). For



CCDM 94 – *Bifidobacterium bifidum* CCDM 94; BB12 – *Bifidobacterium lactis* BB12; CMP – caseinomacropeptide

Figure 2. Comparison of lag-phases in the studied media for high inoculum



CCDM 94 – *Bifidobacterium bifidum* CCDM 94; BB12 – *Bifidobacterium lactis* BB12; CMP – caseinomacropeptide

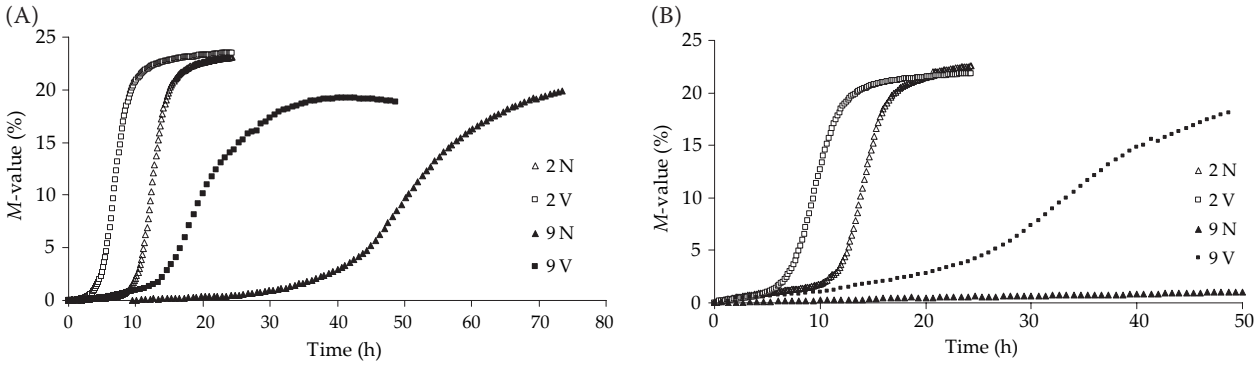
Figure 3. Comparison of log phases (A) and growth rates (B) in studied media for high inoculum

B. bifidum CCDM 94, this difference was caused by a very long lag-phase in the medium No. 3 from which ascorbic acid was omitted. In the medium No. 4, where ascorbic acid was replaced by CMP, the lag-phase returned to the original value (thus approaching the lag-phase in the medium No 1). A similar situation was found for *B. lactis* BB12. On the contrary, the smallest reduction occurred just in media 1 and 2 (high inoculum) where the difference made less than 100 minutes. For both strains studied, the shortest lag-phase was recorded in the medium No. 2, and further in the medium No. 8 (L-cysteine-HCl replaced by CMP). However, for *B. lactis* BB12 the lag-phases were similar in media 4, 6, and 8. The course of the impedance (growth) curve in the medium No. 10 was very unbalanced and, with the given methodology, it was not possible to determine precisely either the lag-phase or other growth phases. On the other hand, Gomes *et al.* (1998) reported that the growth of *B. lactis* on unsupplemented milk was poor. When supplemented with milk hydrolysate, the growth improved ($P < 0.05$); the exponential phase of the growth occurred during the first 8 h to 10 h following the inoculation.

Length of log-phase and growth rate

Unlike the lag-phase, the log-phase is desirable to be extended as much as possible, nevertheless with the highest growth rate and metabolic activity of microorganisms feasible. The longest log-phase for *B. bifidum* CCDM 94 was found in the medium No. 5 (approx 18 h), for *B. lactis* BB12 in the medium No. 9 (approx 19 h) (Figure 3A), but the growth rates were very low in these media (1.12%/h and 0.89%/h, respectively) (Figure 3B). The highest growth rates for both strains were found again in the medium No. 4 (CMP replaces ASC) but the log-phase belonged to the shorter ones in these media. Anyhow, the optimum connection of the length of the lag-phase and growth (change of *M*-value during log-phase), that could be identified from the total change of *M*-value during log-phase, was found with the medium No 8 (CMP replaces CYS) for both strains. The change of *M*-value during the log-phase (except for media Nos 9 and 10) ranged from 19% to almost 24% in all media, but in the medium No. 8 the values reached their maximum. If we consider all these criteria, i.e. the lengths of the lag- and log-phases and the growth rate, we find that the best results were achieved in the media Nos 2 and 8. It follows that CMP in the medium No 8 could replace not only the missing L-cysteine-HCl, whose deficiency caused the drop of the metabolic activity in the medium No. 7 as compared with medium No. 1 by 5% and 11% in *B. bifidum* CCDM 94 and *B. lactis* BB12, respectively, but it was able to support the metabolic activity of the studied strains by almost 52%, as compared with the base in *B. bifidum* CCDM 94, and by less than 33% in *B. lactis* BB12.

The results achieved with BacTrac corresponded more or less with those obtained from the pH determination and microorganism counts, however, small differences existed, e.g. the medium No. 8 showed excellent results both in pH values and in microorganism counts but the medium

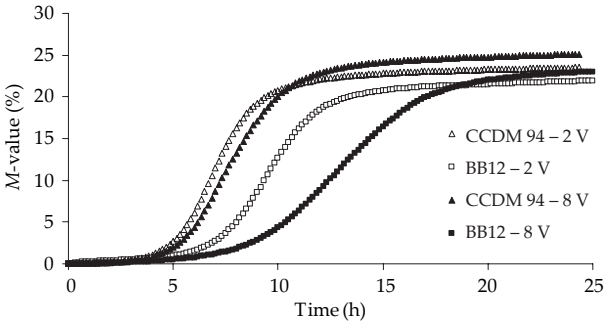


2 N – medium No. 2 low inoculum; 2 V – medium No. 2 high inoculum; 9 N – medium No. 9 low inoculum; 9 V – medium No. 9 high inoculum

Figure 4. Comparison of media No. 2 (base with yeast extract, L-cystein-HCl, ascorbic acid and caseinomacropeptide) and No. 9 (only the base) for *Bifidobacterium bifidum* CCDM 94 (A) and *Bifidobacterium lactis* BB12 (B)

No. 2 had, despite its low pH value, relatively low microorganism counts. This could be caused by the fact that BacTrac can primarily display the metabolic activity of microorganisms and is not fully dependent on their counts. Further, it is necessary to take into consideration that both pH and electrical properties of the media are affected by their composition.

To compare the impedance (growth) curves, we present three figures showing the growth of *B. bifidum* CCDM 94 (Figure 4A), and *B. lactis* BB12 (Figure 4B) in the media Nos 2 and 9. In Figure 5, the impedance (growth) curves of both strains in media Nos 2 and 8 are presented.

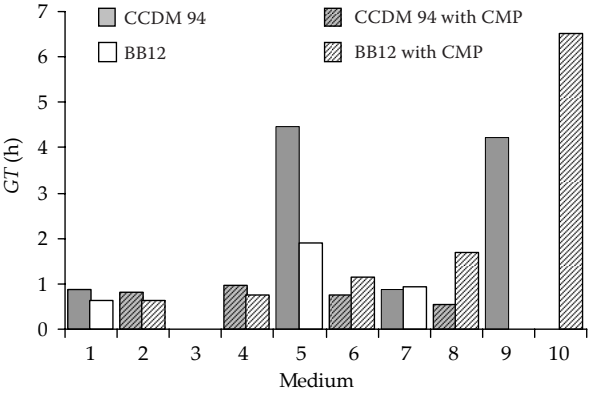


2 V – medium No. 2 (high inoculum); 8 V – medium No. 8 (high inoculum)

Figure 5. Comparison of the growth of *Bifidobacterium bifidum* CCDM 94 and *Bifidobacterium lactis* BB12 in media No. 2 (base with yeast extract, L-cystein-HCl, ascorbic acid, and caseinomacropeptide) and No. 8 (base with yeast extract, ascorbic acid and caseinomacropeptide)

Detection and generation times

The generation time (*GT*) is the time during which the cells double their count in a given volume of the cultivation media. *GT* indicates the extent of cell viability under the given conditions. With bacteria, it ranges from 20 min to 30 min under optimum conditions (Kodíček 2007). The shortest generation time demonstrated the medium No. 8 with *B. bifidum* CCDM 94 (ca 32 min). Next to the medium No. 8 followed the medium No. 2 (50 min) for this strain. The shortest generation time for *B. lactis* BB12 was assessed in the medium No. 2. (38 min) (Figure 6). Both cultures in the medium No. 3 and the *B.*



CCDM 94 – *Bifidobacterium bifidum* CCDM 94; BB12 – *Bifidobacterium lactis* BB12; CMP – caseinomacropeptide

Figure 6. Generation times of *B. bifidum* CCDM 94 and *B. lactis* BB12 in all media; **M*-value limit (5%) was not reached before 72 h

bifidum CCDM 94 culture in the medium No. 10 did not reach the *M*-value 5% in low inoculum.

CONCLUSION

CMP was able to support the growth and metabolic activity of the tested strains of bifidobacteria, especially in the complete medium with CMP and in the medium with CMP and without L-cysteine-HCl where the lag-phase of the microorganism growth was curtailed and where a higher final microorganism count was achieved.

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