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Growth-promoting effects of caseinomacropeptide from cow and goat milk on probiotics

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Caseinomacropeptide (CMP), a 7-kDa phosphoglycopolypeptide fragment released from κ-casein during milk renneting, is heterogeneous with respect to post-translational glycosylation. Several studies have reported that CMP has growth-promoting activity on lactic acid bacteria belonging to the genera *Bifidobacterium*. The aim of this study was to evaluate the effect of glycosylation and sequence variations between bovine and caprine CMP on the growth of two probiotics: *Lactobacillus rhamnosus* RW-9595-M and *Bifidobacterium thermophilum* RBL67. The growth-promoting activities of CMP (mixture of glycosylated (gCMP) and non-glycosylated (aCMP) fractions), aCMP and gCMP were measured in a basal minimal culture medium using turbidimetric microplate assay at 37 °C. Supplementation of the culture media at 2 mg/ml significantly improved maximum growth by 1·5 to 1·8 times depending on the strain, the additive (CMP, aCMP, gCMP), and the bovine or caprine origin ($P<0·05$). CMP preparations also decreased the time needed to reach the inflexion point of the growth curve and increase the cell density at that time ($P<0·05$). The effects of CMP preparations were dose dependent and significantly superior to the effect of bovine β-lactoglobulin added to the culture media. As gCMP and aCMP were as efficient as bovine and caprine CMP ($P>0·1$), it was concluded that the presence of oligosaccharides linked to CMP was not essential for growth-promoting activity of CMP.

Keywords: Glycomacropeptide, κ-casein, growth factor, *Bifidobacterium*, *Lactobacillus*.

There is currently much interest in probiotics, live microorganisms which confer a health benefit on the host when administered in adequate amounts (Araya et al. 2002). The health benefits include establishment of an adequate microbiota in preterm infants, immunomodulation, cholesterol reduction, lactose tolerance, and prevention of infection and cancer (Shah, 2007). Several *Bifidobacteria* and *Lactobacilli* species are constituents of the human gastrointestinal tract microbiota and are recognized as efficient probiotics. These microorganisms are now added to many functional foods: yogurt, milk-based or non-milk-based beverages, dry food (Champagne et al. 2005).

To have a health effect, the amount of bacteria present in functional food must be high. For instance, CFIA (2009) recommends a minimum level of 10^9 viable cells per serving of the microorganism(s) subject to the health claim. This is a major concern for large-scale industrial production of functional foods. Moreover, the use of probiotics as starters in fermented milk is limited because of their slow growth rate mainly due to their low proteolytic activity. Hence, probiotics are added to functional foods as inoculate (Saxelin et al. 1999), lyophilized powder or after microencapsulation, which improves their survival and delivery (Champagne & Fustier, 2007). They must therefore be batch-cultured in milk-based or semi-synthetic rich media supplemented with protein hydrolysates (casein, whey proteins, yeast extract) because *Bifidobacteria* do not usually produce surface proteinases and grow poorly in media such as milk (Poch & Bezkorovainy, 1988; Petschow & Talbott, 1991; Klaver et al. 1993; Ibrahim & Bezkorovainy, 1994; Proulx et al. 1994; Dave & Shah, 1998; Gomes et al. 1998). The other possibility is to cocultivate probiotics with highly proteolytic bacterial strains (Yonezawa et al. 2010). There is a need for efficient and economic media that enable the production of high biomass.

Caseinomacropeptide (CMP) is a 7-kDa phosphoglycopeptide produced by the proteolysis of milk κ-casein (residues 106–169) in the stomach and is also released into cheese whey during chymosin-induced milk renneting. CMP is highly polymorphic because of post-translational glycosylation (Farrell et al. 2004). Bovine CMP is actually a mixture of a non-glycosylated form (aCMP), which

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corresponds to about 50% (g/g) of total CMP, and up to 14 glycovariants, the glycomacropeptide (gCMP), each differing by the amount and type of oligosaccharides linked to the polypeptide backbone (Mollé & Léonil, 2005). Caprine CMP differs from bovine CMP by 21 substitutions/deletions, mainly located in the C-terminal two-thirds of the polypeptide, the relative amount of gCMP, and the presence of N-glycolyl neuraminic acid a terminal sugar in addition to N-acetyl neuraminic acid (Moreno et al. 2001).

In vitro and in vivo studies suggest that CMP exhibits several biological activities associated with microbiota establishment and control within the gastro-intestinal tract (Thomä-Worringer et al. 2006). Studies using semi-synthetic media suggest that CMP from human (Azuma et al. 1984) and bovine milks (Idota et al. 1994) stimulate the growth of several *Bifidobacterium* strains. Azuma et al. (1984) concluded that oligosaccharide and polypeptide chains were important for the activity since promoting activity was lost after chemical and enzymatic treatment of CMP to remove sugar or after proteolysis of CMP. Janer et al. (2004) reported good growth-promoting activities with bovine CMP and a mixture of CMP from goat and ewe when added to skim milk, suggesting a possible generalization of growth-promoting activity among species. The effectiveness of CMP as a bifidogenic factor is not definitively established. In fact, Poch & Bezkorovainy (1991) concluded that intact and hydrolyzed glycosylated CMP were not efficient growth promoters in a culture media supplemented with peptone and yeast extract. Idota et al. (1993) showed bifidogenic activities of gCMP for some strains at 0·01 mg/ml, not at 1 mg/ml. Finally, Brück et al. (2006) did not observed significant bifidogenic effects of supplementation of infant milk formulas with CMP and β-lactoglobulin on faecal microbiota in infant. The reasons for these discrepancies can be the bacterial specie differences, the culture media, and the quality and purity of CMP. Actually, milk or semi-synthetic rich culture media that contain milk components other than CMP and/or peptides from other sources (yeast extract, peptone) are used in these studies. Moreover, CMP isolates were produced using acid treatments (Azuma et al. 1984) which can affect CMP quality, or by UF technology from cheese whey or Na-caseinate, which give partially purified CMP isolate. For instance, CMP content reached 58–80% in the study of Janer et al. (2004), Cicvárek et al. (2010) used a CMP isolate that contained 69% proteins and 39% CMP only, and CMP contents the supplements were less than 15% in in-vivo studies (Brück et al. 2002, 2006). It is possible that components of the culture media can hide or act synergistically with CMP, affecting the resulting growth promoting activity. It is then difficult to precisely evaluate the effectiveness of CMP and the contribution of oligosaccaridic side chains.

The aim of the project was to evaluate the effects of the addition of CMP (gCMP and aCMP in the ratio found in milk) or aCMP, and gCMP separately on the growth rates of two strains of probiotics: *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595 in a chemically-defined culture media. Moreover, CMP was prepared from bovine and goat milk to evaluate the influence of CMP primary sequence polymorphism on activity. We also compared the activity of CMP and bovine β-lactoglobulin (β-lg).

Materials and Methods

Bacterial strains

Bifido. thermophilum RBL67 (Von Ah et al. 2007) and *Lb. rhamnosus* RW-9595 were obtained from the culture collection of Food Research and Development Centre (Saint-Hyacinthe, QC, Canada). *Bifido. thermophilum* RBL67 (RBL-67) is a bacteriocin-producing strain (Touré et al. 2003) and *Lb. rhamnosus* RW-9595 is an exopolysaccharide-producing strain that shows potential for the enhancement of the immune system (Chabot et al. 2001). The bacterial strains were stored frozen at − 80 °C in milk-based medium made of 120 g/l reconstituted low-heat skim milk powder (Agropur, Granby, QC, Canada) in deionized water containing 50 g sucrose/l.

Growth experiments

Frozen cultures were first transferred at 1% (v/v) to MRS broth (Difco Laboratories, Detroit, MI, USA) and grown overnight at 37 °C under anaerobic conditions. The overnight bacterial culture was used to inoculate at 1% (v/v) basal minimal media for *Lactobacilli* (BMM) containing cysteine-HCl (0·5 g/l) and ascorbic acid (1 g/l). BMM was prepared as described by Morishita et al. (1981) from concentrated solutions of vitamins, amino acids, nucleotide (Sigma Chemical Co., St Louis, MO, USA) and salts (Fisher Scientific, Ottawa, ON, Canada). The overnight cultures were subcultured for another 6 h, the optical density of the bacterial suspension was adjusted to about 0·2 $OD_{600\ nm}$, and bacterial cell suspensions were prepared by inoculating BMM at 1% (v/v). The experiments were carried out in a 96-well microplate using turbidimetric to follow growth. Briefly, 100 µl of the cell suspension was added to the 100 µl of the 0, 1 and 4 mg/ml of culture supplement: CMP, aCMP, gCMP, and β-lg (Sigma Chemical Co.), in the wells of a 96-well microplate. The concentration of the stock solutions of CMP and β-lg were determined using $OD^{10\ g/l}_{1\ cm\ \ 214\ nm} = 140$ (Coolbear et al. 1996) and $OD^{10g/l}_{1\ cm\ \ 280\ nm} = 9{\cdot}41$, respectively. The culture supplements were prepared in BMM and sterilized by filtration on 0·45-µM filters. The microplates were placed at 37 °C for 2 h in a Forma Scientific anaerobic chamber (Thermo Scientific, Nepean, ON, Canada), sealed under anaerobic condition using acetate plate sealers (ThermoLabsystems, Franklin, MA), and transferred to the temperature-controlled microplate reader (PowerWaveX, Bio-Tek Instruments Inc, Winooski, VT) maintained at 37 °C. The turbidity at 600 nm ($OD_{600\ nm}$) was recorded every 20 min; the plates were shaken for 4 s at intensity 2 before each reading. Preliminary experiments have been

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conducted to correct the $OD_{600\,nm}$ measurements obtained from the reader for the deviation from the response predicted by Beer's Law, which occurs at values $\geqslant 0{\cdot}3$ and results in falsely low estimates of cell density (Dalgaard, 1994). Briefly, we measured the observed $OD_{600\,nm}$ obtained by the microplate reader at various bacterial cell densities (20 measurements, three replicates) and the corresponding accurate $OD_{600\,nm}$ measurements taken with a Beckman DU800 spectrophotometer (optical path 1 cm). From the two sets of data, a correction polynomial function relating the observed and the accurate $OD_{600\,nm}$ was derived.

From the growth curves, four parameters were extracted to compare the effects of supplementation: the time needed to reach the inflexion point of the sigmoid curve (T_{inf}), the $OD_{600\,nm}$ at T_{inf} (OD_{inf}), the doubling time of the bacterial population at T_{inf} (G_{inf}), the $OD_{600\,nm}$ at the end of the incubation period (OD_{max}). All assays were conducted in triplicate and data from four independent replicate trials were analysed to evaluate the additives and concentrations as fixed effects on the bacterial growth using the GLM procedure (SAS Inst. Inc., Cary, NC).

Polypeptide preparation

Bovine milk was obtained from Dairy and Swine Research and Development Centre (Agriculture and Agri-Food Canada, Sherbrooke, Canada) and goat milk was obtained from Laiterie Tournevent (Drummondville, Qc, Canada). CMP, aCMP and gCMP were prepared as described previously (Robitaille et al. 2012). Briefly, sodium caseinate was treated with chymosin, and CMP was isolated by UF, purified and fractionated into aCMP and gCMP by anion-exchange membrane adsorption chromatography on a Mustang® Q cartridge (Pall (Canada) Ltd., Mississauga, Canada). The quality of aCMP and gCMP preparations was analysed by IEC-HPLC on a mono-Q column (GE-Healthcare Inc, Baie d'Urfé, QC) using a linear gradient of 0–0·5 M NaCl with a flow rate of 1 ml/min in 10 mM phosphate buffer, pH 7·5. The extent of gCMP glycosylation was determined spectrophotometrically by the acidic ninhydrin assay method, which evaluates N-acetylneuraminic acid content (Fukuda et al. 2004).

Results

The IEC-HPLC chromatograms of the analysis of aCMP and gCMP are presented in Fig. 1. A single peak is eluted at 21 min for aCMP, while several peaks are eluted later (>26 min) for gCMP fraction; this heterogeneity was due to the presence of N-acetylneuraminic as terminal charged amino sugar of oligosaccharides. Hence, aCMP was monomorphic and gCMP pool was highly glycosylated (Mean value 3·4 moles per mole of gCMP).

Figure 2 shows the growth curves of *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M in the BMM in the presence or absence of bovine CMP, aCMP gCMP, and β-lg.

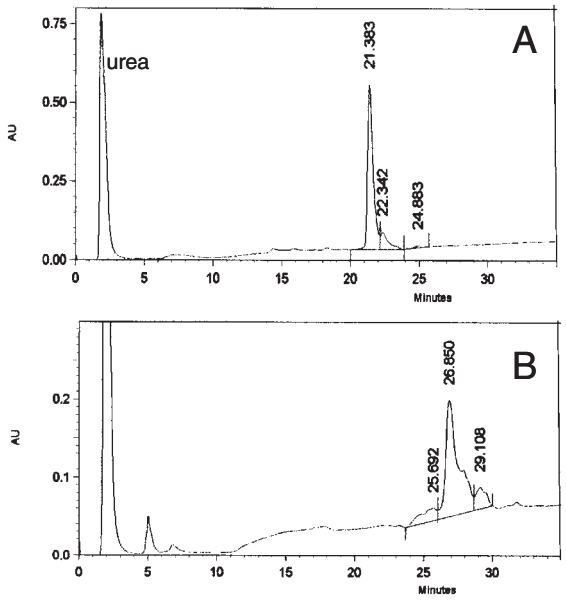

Fig. 1. Chromatogram of a CMP (a) and gCMP (b) fractionated by IEC-HPLC on mono-Q using a linear gradient of 0–0·5 M NaCl with a flow rate of 1 ml/min in 10 mM phosphate buffer, pH 7·5.

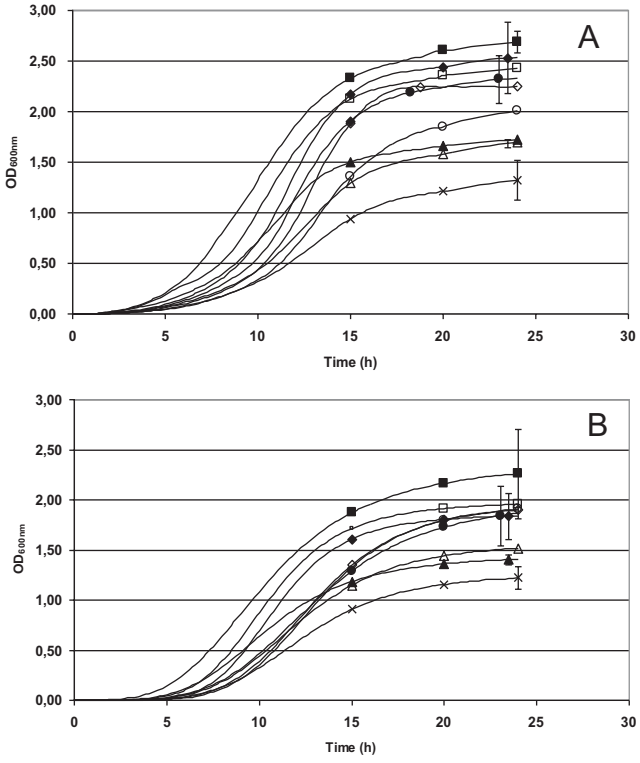

Fig. 2. Bacterial growth curves of *Bifido. thermophilum* RBL67 (a) and *Lb. rhamnosus* RW-9595M (b) in BMM (-X-) and in BMM supplemented at 2 mg/ml with CMP (-■-), aCMP (-◆-), gCMP (-●-) and β-lg (-▲-) and with 0·5 mg/ml CMP (-□-), aCMP (-◊-), gCMP (-o-) and β-lg (-△-). Supplements were prepared from bovine milk. (Average of replicates ± SE is given for BMM and BMM with additive at 2 mg/ml.)

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Table 1 Statistical analysis of the parameters extracted from bacterial growth curves of *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595M in BMM with or without supplementation with CMP preparations (CMP, aCMP and gCMP) from cow and goat milk or with bovine β-lactoglobulin (β-lg)

			CMP from cow milk				CMP from goat milk
Strain	Additive	Concentration (mg/ml)	T_{inf}(h) value ± SD	OD_{inf} value ± SD	OD_{max} value ± SD	G_{inf}(h) value ± SD	OD_{max} value ± SD
RBL 67	–	0	$12{\cdot}17 \pm 0{\cdot}31^{ab}$	$0{\cdot}57 \pm 0{\cdot}08^{d}$	$1{\cdot}30 \pm 0{\cdot}16^{d}$	$2{\cdot}79 \pm 0{\cdot}13^{a}$	$1{\cdot}69 \pm 0{\cdot}11^{f}$
	CMP	2	$9{\cdot}69^{e}$	$0{\cdot}96^{bc}$	$2{\cdot}38^{ab}$	$2{\cdot}63^{ab}$	$2{\cdot}65^{bc}$
		0·5	$10{\cdot}53^{de}$	$0{\cdot}92^{bc}$	$2{\cdot}13^{bc}$	$2{\cdot}45^{abc}$	$2{\cdot}51^{cd}$
	aCMP	2	$10{\cdot}93^{cd}$	$1{\cdot}08^{ab}$	$2{\cdot}45^{ab}$	$2{\cdot}15^{cd}$	$3{\cdot}03^{a}$
		0·5	$12{\cdot}19^{ab}$	$1{\cdot}04^{ab}$	$2{\cdot}26^{b}$	$1{\cdot}88^{d}$	$2{\cdot}51^{cd}$
	gCMP	2	$11{\cdot}48^{bcd}$	$1{\cdot}24^{a}$	$2{\cdot}81^{a}$	$2{\cdot}50^{abc}$	$2{\cdot}96^{ab}$
		0·5	$12{\cdot}46^{a}$	$1{\cdot}04^{ab}$	$2{\cdot}34^{ab}$	$2{\cdot}47^{abc}$	$2{\cdot}37^{cd}$
	β-LG	2	$9{\cdot}89^{e}$	$0{\cdot}74^{c}$	$1{\cdot}76^{cd}$	$2{\cdot}26^{bcd}$	$2{\cdot}30^{de}$
		0·5	$11{\cdot}58^{abc}$	$0{\cdot}73^{d}$	$1{\cdot}69^{d}$	$2{\cdot}44^{abc}$	$1{\cdot}99^{ef}$
9595	–	0	$12{\cdot}57 \pm 0{\cdot}35^{ab}$	$0{\cdot}68 \pm 0{\cdot}07^{c}$	$1{\cdot}54 \pm 0{\cdot}15^{c}$	$3{\cdot}42 \pm 0{\cdot}21$	$2{\cdot}04 \pm 0{\cdot}10^{c}$
	CMP	2	$10{\cdot}07^{d}$	$0{\cdot}96^{ab}$	$2{\cdot}36^{a}$	$2{\cdot}75$	$3{\cdot}14^{a}$
		0·5	$10{\cdot}90^{cd}$	$0{\cdot}92^{ab}$	$2{\cdot}06^{ab}$	$2{\cdot}39$	$2{\cdot}75^{b}$
	aCMP	2	$10{\cdot}24^{d}$	$0{\cdot}98^{a}$	$2{\cdot}44^{a}$	$2{\cdot}79$	$3{\cdot}22^{a}$
		0·5	$11{\cdot}56^{bc}$	$0{\cdot}90^{bc}$	$2{\cdot}15^{ab}$	$2{\cdot}66$	$2{\cdot}69^{b}$
	gCMP	2	$11{\cdot}79^{bc}$	$0{\cdot}93^{ab}$	$2{\cdot}13^{ab}$	$2{\cdot}70$	$3{\cdot}05^{ab}$
		0·5	$12{\cdot}87^{a}$	$1{\cdot}04^{a}$	$2{\cdot}32^{a}$	$2{\cdot}80$	$2{\cdot}63^{b}$
	β-LG	2	$9{\cdot}99^{d}$	$0{\cdot}73^{c}$	$1{\cdot}76^{c}$	$2{\cdot}87$	$1{\cdot}74^{d}$
		0·5	$11{\cdot}70^{bc}$	$0{\cdot}79^{bc}$	$1{\cdot}78^{bc}$	$2{\cdot}93$	$2{\cdot}13^{c}$

BMM as growth medium supported the growth of the two strains to about 1·3 $OD_{600\,nm}$ after 24 h of incubation at 37 °C. The addition of 2 mg/ml CMP to BMM increased bacterial growth to values $\geqslant 2$ $OD_{600\,nm}$. The kinetics of bacterial growth was also enhanced with addition of CMP as the time needed to reach the inflexion point of the growth curve (T_{inf}) decreased in conjunction with an increase of $OD_{600\,nm}$ at T_{inf}. These effects were dose dependent. For comparison, addition of β-lg to BMM improved bacterial growth to a much lesser extent. The growth curve patterns of *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M in BMM containing β-lg were intermediate between those obtained in BMM without supplementation and in BMM containing CMP preparations.

Statistical analysis to quantify the growth-promoting effects of supplements is presented in Table 1. CMP at 2 mg/ml significantly affected bacterial growth of *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M in terms of OD_{max}, T_{inf}, OD_{inf} ($P<0{\cdot}05$). The OD_{max} increased 1·8 and 1·5 times for *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M, respectively, compared with OD_{max} reached in BMM alone. β-Lg slightly increased OD_{max} by 1·3 and 1·1 times for *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M, respectively ($P>0{\cdot}1$), regardless of the dose. CMP was more efficient than β-lg as BMM supplement ($P<0{\cdot}05$). The OD_{max} reached by bacteria in the media supplemented with both aCMP and gCMP at 2 and 0·5 mg/ml was similar ($P>0{\cdot}1$) to the value obtained with CMP at the same concentration. T_{inf} was reached earlier in the presence of CMP, aCMP and gCMP at 2 mg/ml in BMM, in conjunction with a higher OD_{inf} for the two strains, compared with the values in BMM alone ($P<0{\cdot}05$). All these effects were dose-dependent for the three CMP preparations. β-Lg as additive also decreased T_{inf}, but its effect on OD_{inf} was low, with an increase of 1·3 and 1·1 times only for *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M respectively. G_{inf} tended to be lower in presence of additive in the culture media for the two strains.

In a second set of experiments, we tested the effect of caprine CMP on the two strains (Fig. 3). The growth curve shows that CMP, aCMP and gCMP added at 0·5 and at 2 mg/ml in BMM allowed better bacterial growth for the two strains: higher OD reached at the end of incubation period, and faster growth kinetic, compared with BMM alone or BMM containing β-lg. The statistical analysis for OD_{max} presented in Table 1 supports that conclusion. OD_{max} was increased 1·6 to 1·8 times and 1·5 to 1·6 times with CMP, aCMP, and gCMP at 2 mg/ml in the culture media of *Bifido. thermophilum* RBL67 and *Lb. rhamnosus* RW-9595-M, respectively, compared with BMM alone ($P<0{\cdot}05$). A significant dose effect was observed in the majority of cases. As for bovine, the extent of glycosylation of caprine CMP did not significantly affect the growth-promoting activity, the bacterial cultures reaching similar OD_{max} values in presence of CMP, aCMP and gCMP at 2 mg/ml and at 0·5 mg/ml ($P>0{\cdot}1$), for *Bifido. thermophilum* RBL67 and for *Lb. rhamnosus* RW-9595-M. It was not possible to precisely locate T_{inf} on the growth curves as the stationary growth phase was not reached at the end of the incubation period for low growing cultures. Consequently, it was not possible to precisely evaluate and compare T_{inf} and the corresponding OD_{inf} and G_{inf} values. β-Lg had a small effect on OD_{max} for

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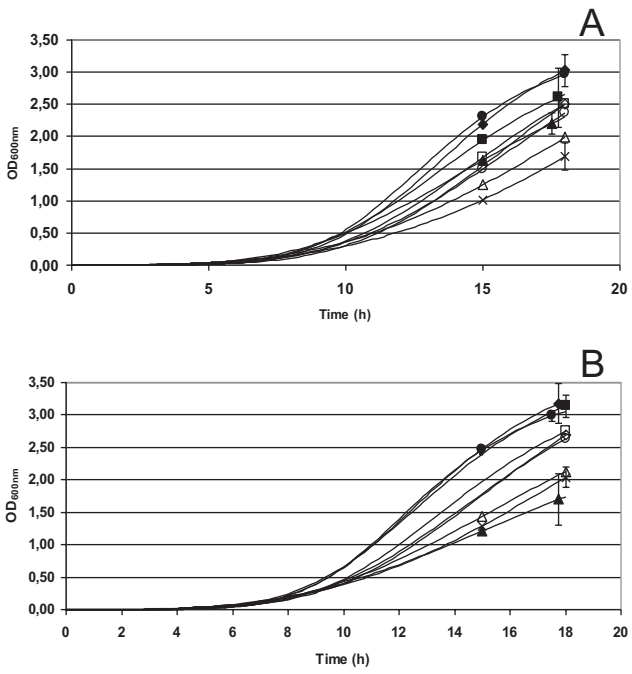

Fig. 3. Bacterial growth curves of *Bifido thermophilum* RBL67 (a) and *Lb. rhamnosus* RW-9595M (b) in BMM (-X-) and in BMM supplemented at 2 mg/ml with CMP (-■-), aCMP (-◆-), GCMP (-●-) and β-lg (-▲-) and with 0·5 mg/ml CMP (-□-), aCMP (-◇-), GCMP (-o-) and β-lg (-Δ-). CMP preparations were isolated from goat milk. (Average of replicates ± SE is given for BMM and BMM with additive at 2 mg/ml.)

Bifido. thermophilum RBL67 and did not significantly increase OD_{max} for *Lb. rhamnosus* RW-9595-M.

Discussion

Growth-promoting activity of human and bovine CMP has been reported for several *Bifidobacteria* species in rich media (Thomä-Worringer et al. 2006) but not yet fully established. In that context, this study is interesting because it analyses and compares the growth promoting activity of purified CMP, aCMP and highly glycosylated CMP. These preparations were all produced by ion exchange chromatography, an efficient and non-deleterious method of purification. Moreover, all tests were carried out in a chemically-defined media (BMM) which is rich enough to supply bacteria with carbon, nitrogen, vitamins and minerals, insuring good bacterial growths for *Bifidobacteria* as well as *Lactobacillus,* species. We also extent the analysis to the glycosylated and non-glycosylated isoforms of CMP isolated from cow and goat milk, which differ in terms of amino acid sequence and glycosylation.

The results presented in Fig. 2 and in Table 1 clearly demonstrated that, in a fully synthetic medium, bovine CMP was an efficient growth promoter for *Bifido. thermophilum* RBL 67 and for *Lb. rhamnosus* RW-9595-M. We also showed that glycosylation of CMP did not alter the growth-promoting activity, as aCMP was as efficient as gCMP or CMP in improving bacterial growth. To substantiate the results obtained with bovine CMP, we analysed CMP, aCMP and gCMP purified from goat milk, the rationale being that species differences at the molecular level can be highly informative in the analysis of the functional properties of CMP. As shown by the growth curves in BMM with or without supplementation (Fig. 3) and by OD_{max} reached by the bacterial cultures in supplemented media, caprine CMP, aCMP and gCMP, were also growth promoters. As for bovine counterpart, the activity of caprine gCMP does not differ from caprine aCMP. We can conclude that glycosylation is not a contributing factor of the growth-promoting activity.

Previous reports demonstrate that individual whey proteins can be growth promoters for *Bifidobacterium* species such as β-lg (Petschow & Talbott, 1991; Ibrahim & Bezkorovainy, 1994). In this study, we included β-lg as a positive marker for growth-promoting activity. Our results showed that CMP preparations (CMP, aCMP, gCMP) from cow and goat milk were consistently better growth promoters than β-lg. CMP should be an important contributor of the growth-promoting activity of whey proteins from cheese whey.

In batch culture condition, the maximum growth is limited either by the availability of essential nutrients and/or the accumulation of inhibitory metabolic products, such as lactic acid during fermentation. It is unlikely here that growth promotion was due to extra nutrients supplied by CMP. In fact, the strains are not proteolytic and BBM broth supplies all essential amino acids, vitamins and salt needed. Moreover, neutral and amino sugar coming from linked oligosaccharides, are not directly involved in the growth-promoting activity. We suggest that CMP acts by triggering metabolic adaptations associated with acid tolerance response, allowing a better growth in acidic media during fermentation. Incidentally, the survival of *Lb. rhamnosus* RW-9595-M was improved by CMP during acid stress (Robitaille et al. 2012). The bioactive domain of the polypeptide would be located within the N-terminal portion, as glycosylation and the vast majority of single nucleotide polymorphisms between bovine and caprine CMP are located in the second C-terminal part of the polypeptide. In this context, it would be interesting to analyse the growth-promoting activity of peptide fragments from the N-terminal portion of the polypeptide.

In conclusion, the use of probiotics as starter culture for fermented products is limited because of limited growth and organoleptic problems (aroma and acidification extent). As a result, they are usually produced separately and added to the functional food at a concentration needed for health benefits. There is therefore a need for an economical rich media allowing the production of high biomass to deal with the demand for probiotics as an ingredient. This study reinforced the position of CMP as an efficient additive for probiotic growth. Moreover, it showed that CMP does not require fractionation into aCMP and gCMP to be fully active.

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Interestingly, CMP is available in a large amount as it represents up to 0·2 g/g of cheese whey proteins and can be prepared by UF (Thomä-Worringer et al. 2006). Finally, the use CMP as a supplement in culture media is interesting in the context of the valorization of cheese whey by-products.

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