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Note

Usefulness of bifidobacteria for the detection of faecal contamination in milk and cheese

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Abstract – Research of faecal contamination is most often based on the research of *Escherichia coli*, a rather unspecific marker showing both survival and multiplication outside the intestinal tract. When researching a more specific and sensitive marker, we examined human and cow's faeces, raw milk and dairy products for the presence of *E. coli*, but also bifidobacteria. In 146 human faecal samples, bifidobacteria were present at a mean level of 7.7 log cfu·g^{-1}, and the identified species were *B. bifidum, B. longum, B. adolescentis* and *B. infantis. E.coli* was present at a level of 6.9 log cfu·g^{-1}. In 30 samples of cow's faeces, the highest count of bifidobacteria was 8 log cfu·g^{-1}, while *E. coli* was present at a level of 6 log cfu·g^{-1}. Among 15 samples of cheese prepared with raw milk, bifidobacteria were found in 14 samples at levels comprising between 2 and 5 log cfu·g^{-1}. *E. coli* was never found (detection level 2 log cfu·g^{-1}). Analysis of 207 samples of raw cow's milk sampled on the teat allowed isolation of bifidobacteria in 90% and *E. coli* in 68% of samples. Among 66 samples of raw milk sampled in refrigerated tanks, bifidobacteria were isolated in 95% and *E. coli* in 86%. In all cow's samples (faeces, milk and cheese), one distinct species was identified: *Bifidobacterium pseudolongum* var *globosum*. Levels of bifidobacteria are higher than values of *E. coli* in both human and animal faeces and raw milk, underlining the choice of bifidobacteria as a valid marker for faecal contamination of dairy products. Identification of bifidobacterial species even allows the determination of the origin (animal or human) of contamination.

***Bifidobacterium* / *E. coli* / faecal contamination / raw milk**

Résumé – La recherche des bifidobactéries comme indicateur d'une contamination fécale dans les produits laitiers. La recherche d'une contamination fécale est le plus souvent basée sur la mise en évidence d'*Escherichia coli*, marqueur peu spécifique, car capable de survivre et de se multiplier en dehors du tractus digestif. À la recherche d'un marqueur plus spécifique et sensible, nous avons recherché dans des matières fécales humaines et bovines, du lait cru et des produits laitiers, la présence d'*E. coli* et de *Bifidobacterium*. Dans 146 matières fécales humaines, les bifides sont présents à un taux moyen de 7,7 log ufc·g^{-1}, les espèces identifiées sont *B. bifidum, B. longum, B. adolescentis* et *B. infantis*. *E. coli* est présent à un taux de 6,9 log ufc·g^{-1}. Dans 30 échantillons de bouses, le taux le plus élevé de *Bifidobacterium* est de 8 log ufc·g^{-1} alors qu'*E. coli* est présent à un taux de 6 log ufc·g^{-1}. Parmi 15 échantillons de fromage au lait cru, les bifides sont retrouvés dans 14 échantillons à des taux entre 2 et 5 log ufc·g^{-1}. *E. coli* est toujours en dessous du seuil de détection (< 2 log ufc·g^{-1}). L'analyse de 207 échantillons de lait cru pris à la mamelle a permis l'isolement des bifides dans 90 % et d'*E. coli* dans 68 % des échantillons. L'analyse de 66 échantillons de lait cru prélevés dans les tanks réfrigérés montre l'isolement des bifides dans 95 % et d'*E. coli* dans 86 % des échantillons. Dans tous les échantillons d'origine bovine (bouses, lait et produits laitiers),

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une espèce distincte est retrouvée : *Bifidobacterium pseudolongum* var *globosum.* Les taux des bifides sont supérieurs aux taux d'*E. coli* aussi bien dans les matières fécales humaines et bovines que dans le lait cru, indiquant les bifides comme marqueurs fiables pour une contamination fécale des produits laitiers. L'identification des espèces de *Bifidobacterium* permet même de déterminer l'origine (animale ou humaine) de la contamination.

***Bifidobacterium* / *E. coli* / contamination fécale / lait cru**

1. INTRODUCTION

Faecal contamination is an important marker in establishing the microbiological quality of food. *Escherichia coli* is considered as a specific species for indicating the faecal origin of the contamination [8]. But *E. coli* is not specific for human faecal contamination, it is also encountered in animal faeces (mammals and birds) [13]. So even though *E. coli* is a good predictor of faecal contamination, it cannot be used as a target for bacterial source tracking [20]. Early phenotypic methods [18] attempted to differentiate *E. coli* strains from different animals but they are not satisfactory for predicting the host source and the virulence of *E. coli* isolates. Amplified fragment length polymorphism methods have been used recently with more success [10]. However, another inconvenience in using *E. coli* is its ability to multiply outside the intestine [1].

Alternative faecal source identifiers are being researched. *Bacteroides* have been used in several studies to differentiate between human and animal sources [5, 9, 21]. Another candidate is the genus *Bifidobacterium* [14, 15]. This genus has been suggested as being among the most promising alternative indicators [17, 19]. It has already been widely studied in samples such as water [11, 12]. Bifidobacteria are present at higher levels in the human intestine when compared with *E. coli*. Furthermore, bifidobacteria will not multiply outside the intestinal tract. They seem more sensitive and specific as human faecal indicators [6] because the species distribution is not the same in humans and animals. In order to elaborate a new standardised test for the research of faecal contamination in milk products, we compared levels of *E. coli* and bifidobacteria in human and cow's faeces and raw milk and cheese.

2. MATERIALS AND METHODS

2.1. Samples

2.1.1. Faecal samples

A total of 176 faecal samples were analysed.

– Human faecal samples: freshly voided faeces were obtained from 146 healthy volunteers (19 to 46 years old) without medical treatment and mostly no antibiotics used for at least 2 months.

– Cow's faeces: 30 fresh samples were taken on 10 different farms.

All samples were transported under anaerobic conditions and analysed within four hours.

2.1.2. Dairy products

A total of 288 milk and cheese samples were obtained: 207 samples of raw milk were taken on the teat when starting milking; 66 samples of raw milk were taken in the refrigerated tanks of 10 farms; plus 15 samples of cheese made from raw milk.

All samples were analysed within 4 h.

2.2. Methods

2.2.1. Treatment of human faecal samples

Bifidobacteria: 0.1 mL of the tubes –2 to –8 from the tenfold dilution series were plated on Béerens' agar [2] and incubated for 5 to 7 d under anaerobic conditions. Colonies were counted and subcultured for identification.

E. coli: 0.1 mL of the tubes –2 to –8 of the tenfold dilution series were plated on McConkey's agar (BioMérieux, Marcy

Table I. Distribution of bifidobacterial and *E. coli* counts in human faecal samples.

	Number of samples positive at values		
	>8 log cfu·g^{-1}	>9 log cfu·g^{-1}	Total
Bifidobacterium	64	34	98 (67%)
Escherichia coli	27	0	27 (18%)

l'Étoile, France) and incubated for 48 h under aerobic conditions. Colonies were enumerated and subcultured for identification (API 20 E system).

The results are expressed as log cfu·g^{-1}.

2.2.2. Treatment of cow's faeces

Bifidobacteria: 0.1 mL of the tubes –1 to –7 were plated on Béerens' agar [2] and incubated for 5 d at 37 °C under anaerobic conditions.

E. coli were enumerated using ID medium (BioMérieux).

2.2.3. Milk and cheese samples

Bifidobacteria: 1 mL of each of the dilutions –1 to –9 were inoculated into 10 mL of Béerens' broth [3] and incubated for 48 h at 37 °C. Each subculture was isolated on Columbia agar plates incubated under anaerobic conditions. Colonies were subcultured for identification.

E. coli were enumerated on ID medium (BioMérieux).

2.2.4. Identification of Bifidobacterium

The different isolates were assigned to the genus *Bifidobacterium* by the presence of the enzyme F6PPK [16]. Species determination was based on Scardovi's tests [16].

3. RESULTS

3.1. Human faecal samples

Mean bifidobacterial counts were 7.7 log cfu·g^{-1}, while *E. coli* counts were 6.9 log cfu·g^{-1}. Bifidobacterial counts were between 8 and 9 log cfu·g^{-1} in 64 of 146 samples and above 9 log cfu·g^{-1} in 34 samples (Tab. I). For *E. coli* only 27 samples (18%) showed counts higher than 8 log cfu·g^{-1}. The bifidobacterial species identified were *B. bifidum, B. longum, B. adolescentis* and *B. infantis.*

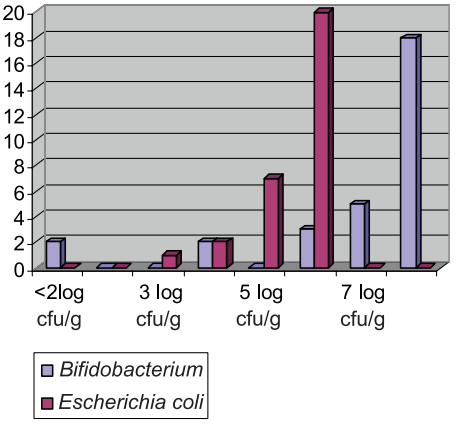

Figure 1. Distribution of bifidobacteria and *E. coli* in cow's faeces.

3.2. Cow's faeces, milk and cheese

The results concerning the two markers are shown in Figures 1 to 3.

In 2 faecal samples, bifidobacteria were below the detection level, and in 23 samples (77%) their level exceeded 7 log cfu·g^{-1}. This level was never reached for *E. coli.*

90% of the samples of raw milk (teat samples) evidenced bifidobacteria with a mean count of 2.35 log cfu·mL^{-1}. *E. coli* was evidenced in 68% of samples with a mean count of 1.64 log cfu·mL^{-1}. Counts of bifidobacteria reached values of

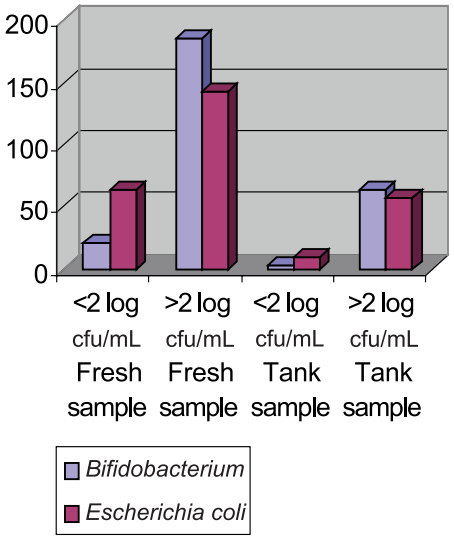

Figure 2. Distribution of bifidobacteria and *E. coli* in milk.

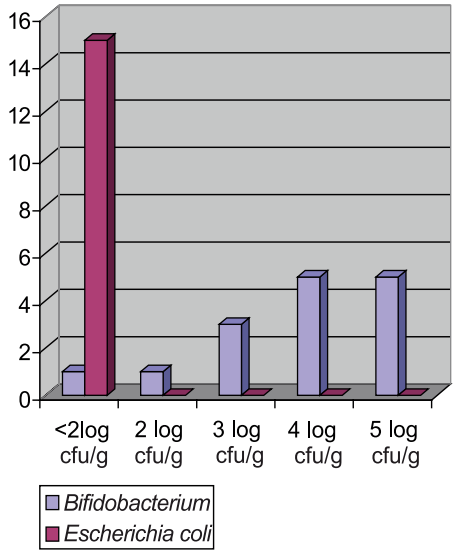

Figure 3. Distribution of bifidobacteria and *E. coli* in cheese samples.

3 log cfu·mL^{-1} while *E. coli* counts never exceeded 2 log cfu·mL^{-1}.

94% of raw milk (tank samples) were positive for bifidobacteria (mean count 3.09 log cfu·mL^{-1}), and 86% for *E. coli* (mean count 2.21 log cfu·mL^{-1}). 93% of cheese samples were positive for bifidobacteria, while *E. coli* was never found.

In all animal samples, the bifidobacterial strains isolated belong to the same species: *Bifidobacterium pseudolongum* var *globosum.*

4. DISCUSSION

After analysis of human faecal samples, bifidobacteria seem indeed good candidates for the research of human faecal contamination in milk and milk products because they are usually present at higher levels than enterobacteria [19]. High counts of bifidobacteria are also present in cow's faeces, and all strains isolated from cow's faeces belong to the same, unique species, *Bifidobacterium pseudolongum* var *globosum.* This species is never encountered in human samples. Differences in species distribution in humans and animals has already been stated [7]. This species seems to survive well in air [3]. Distribution of bifidobacterial species in the intestinal tract of ruminants is not well understood; their presence seems, however, to be linked to feeding patterns [4]. Indeed, cows fed only with grass provided the two negative samples. Research of bifidobacteria in raw milk constitutes a valuable test for faecal contamination, identifying concomitantly the source of contamination, which was here always of animal origin.

When comparing bifidobacteria and *E. coli* counts in raw milk sampled directly or after storage in refrigerated tanks, the frequency of isolation and counts of *E. coli* was higher in tank samples. This might be explained by the fact that bifidobacteria do not grow at a temperature below 30 °C, while *E. coli* can readily grow when the temperature of the tank is not well controlled. In contrast, bifidobacteria will die after contact with air. So levels of bifidobacteria

reveal levels of a recent faecal contamination. *E. coli* levels after storage may not be related to the initial contamination level.

In samples such as cheese, *E. coli* cannot be used as an indicator for faecal contamination because in these products, pH decreases during maturation. When pH drops below 5.0, *E. coli* no longer remains viable. Bifidobacteria, in contrast, survive even at this low pH.

Research in bifidobacteria as a faecal contaminant seems well adapted to naturally acidified samples such as cheese, and they can be also tested for in fermented milk products. This work reveals a new research field for defined species of bifidobacteria in dairy products where, up to now, bifidobacteria were mainly used as probiotics.

In conclusion, bifidobacterial levels are superior to *E. coli* levels in faeces from both humans and cows, indicating a good specificity for bifidobacteria as indicators of faecal contamination. Application to raw milk and cheese confirms the higher sensitivity of this method for detecting faecal contamination. Identification of bifidobacteria to the species level can allow the determination of the origin of the contamination: species encountered in faeces are not the same in humans and ruminants.

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