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Short communication

Human gut microbiota does not ferment erythritol

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Erythritol, a naturally occurring polyol, is gaining attention as a bulk sweetener for human nutrition. Industrially, it is produced from glucose by fermentation. From various studies it is known to be non-cariogenic. Moreover, it is rapidly absorbed in the small intestine and quantitatively excreted in the urine. Only about 10 % enters the colon. Earlier *in vitro* experiments showed that erythritol remained unfermented for a fermentation period of 12 h. In order to investigate whether fresh human intestinal microbiota is able to adapt its enzyme activities to erythritol, a 24 h lasting fermentation was carried out under well-standardised *in vitro* conditions. For comparison maltitol, lactulose and blank (faecal inoculum only) were incubated as well. Fermentation patterns were established by following total gas production, hydrogen accumulation, changes in pH value, SCFA production and substrate degradation. Taking all fermentation parameters into account, erythritol turned out to be completely resistant to bacterial attack within 24 h, thus excluding an adaptation within that period. Since under *in vivo* conditions more easily fermentable substrates enter the colon continuously, it seems very unlikely that erythritol will be fermented *in vivo*.

Erythritol: Maltitol: Lactulose: *In vitro* fermentation

Polyols (sugar alcohols) are being used as bulk sweeteners in human nutrition. Maltitol, sorbitol, lactitol, mannitol, isomalt and xylitol have a long history of use. Until some time ago erythritol was only available in Japan. However, recently erythritol has been launched on the American market and it obtained a positive evaluation from the European Commission's Scientific Committee of Food (2003), opening the doors for future use in Europe. Erythritol, a four-carbon sugar alcohol, occurs widely in nature and also in foods like wine, beer, mushroom, pear, grape and soy sauce (Bernt *et al.* 1996). Industrially, it is produced from glucose by an osmophilic yeast (Goossens & Röper, 1994). Subsequent separation and purification yield a crystalline product with a purity of 99 %. Erythritol is not metabolised by oral micro-organisms. *In vitro* incubation with a range of *Streptococci* species showed that neither lactic acid nor other organic acids are produced. Additionally, *Streptococci* were unable to grow on erythritol as sole C-source (Kawanabe *et al.* 1992).

In man, up to 90 % of erythritol is rapidly absorbed in the small intestine by passive diffusion. It is distributed widely through tissues but its metabolism is minimal. Being poorly reabsorbed by the kidneys it is quantitatively excreted in the urine (Bernt *et al.* 1996). Neither plasma glucose nor insulin levels are affected by oral erythritol intake (Bornet *et al.* 1996; Ishikawa *et al.* 1996). Mean glycaemic and insulinaemic indices of 0 and 2, respectively, have been reported (Livesey, 2003). Moreover, no effect on NEFA homeostasis has been observed (Ishikawa *et al.* 1996). All these observations indicate that erythritol is neither entering carbohydrate- nor fat-metabolic pathways. Compared to other polyols ingested in comparable amounts, erythritol leads to clearly lower osmotic pressures and to rarely any gas production (Oku & Okazaki, 1996). This is due to the fact that only a small fraction of erythritol enters the colon. Theoretically, each gram of polyol being completely absorbed is fully available as energy, whereas total fermentation would result in an energy contribution of approximately 50 % via SCFA that are absorbed by the colonic epithelium (Livesey, 2003). Based on both the negligible metabolism and the small amount reaching the large intestine, the energy value of erythritol was confirmed to be less than 0·9 kJ/g by the European Commission's Scientific Committee on Food (2003).

The relatively small unabsorbed fraction of erythritol enters the colon, where it may be subject to fermentation by the colonic microbiota (Bernt *et al.* 1996). Early animal experiments (Noda & Oku, 1992) suggest that 10 % of supplied erythritol may be susceptible to fermentation in rats. Data from human faecal inoculate are scarce. Hiele *et al.* (1993) studied the metabolic fate of 25 g ^{13}C-labelled orally ingested erythritol in six healthy human volunteers. No increase in breath $^{13}CO_2$ and H_2 was observed, indicating that the polyol absorbed is neither metabolised endogenously nor by colonic bacteria. Indeed, a high proportion of erythritol

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(84·1 (SE 3·3) %) was recovered unchanged from urine, leaving the possibility that the remainder was either excreted with faeces or converted to non-oxidised compounds by colonic bacteria. In order to check the latter possibility, the authors performed an *in vitro* fermentation experiment under strict anaerobic conditions, using fresh faecal microbiota from six volunteers. After 6 h the H_2 concentration was measured in the headspace of the vials. No H_2 production from erythritol was observed. The authors concluded that erythritol is not metabolised by human faecal bacteria but raised the point that some metabolism may occur after a longer adaptation period. In another, non-published, experiment (Barry *et al.* 1992), erythritol was introduced in fermentation flasks placed in a water bath at 38°C. For this experiment a fresh human inoculate from three healthy volunteers was used. Gas and SCFA production as well as erythritol disappearance were monitored during the 12 h lasting fermentation period. Neither gas nor SCFA were produced and the polyol was recovered virtually completely after fermentation. Therefore, the authors concluded that erythritol is non-fermentable.

Since both known *in vitro* experiments using fresh human faecal microbiota showed no degradation of erythritol within 12 h, the aim of the present study was to prolong the fermentation period to 24 h and thus to check whether colonic bacteria are able to adapt their enzyme activities within this period. For comparison, maltitol was chosen as a second substrate. This disaccharide-type polyol is characterised by a lower absorption, but a higher fermentation rate (approximately 40 and 60 %, respectively) compared to erythritol (Livesey, 2003), thus being expected to be better fermentable. In addition, lactulose as a very easily fermentable substrate and a blank (faecal inoculum only) were included to standardise the experiment.

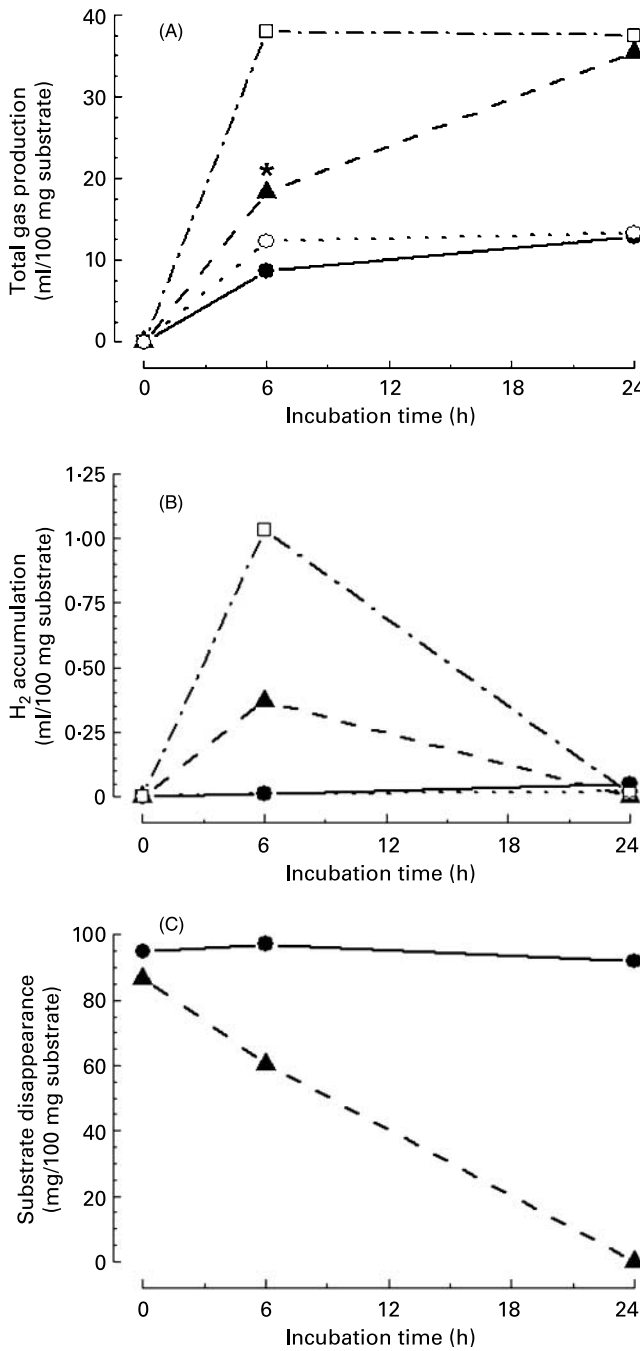

Fig. 1. Total gas production (A), hydrogen accumulation (B) and substrate disappearance (C) during *in vitro* fermentation of erythritol (●) compared to maltitol (▲), lactulose (□) and blank (○). For details of procedures, see p. 644. Values are means of duplicates with standard deviation <5 % (except where indicated: *10 %).

Materials and methods

Substrates

Erythritol and maltitol (purity on DM basis: ≥99·8 % and ≥99·0 %, respectively) were obtained from Cerestar-Cargill R&D Center (Vilvoorde, Belgium). Lactulose was purchased from Fluka Chemie (Buchs, Switzerland) and was ≥98·0 % pure.

Methodology

In vitro fermentation was carried out by applying a well-standardised batch technique (Lebet *et al.* 1998). Incubations were performed under strictly anaerobic conditions with a mixture of freshly collected human faeces of four non-methanogenic individuals. Duplicate samples were taken at 0, 6 and 24 h. $HgCl_2$ was used to stop fermentation. pH, total gas, H_2 and SCFA production in an acidified aliquot of the supernatant were determined as described in the standard procedure. The residues were freeze-dried and ground in a ball mill. To determine substrate disappearance residual polyols were quantified by HPLC, based on ISO International Standard (1998) method 10504.

Results

Total gas production and H_2 accumulation can be followed in Fig. 1(A and B). Values obtained for lactulose (easily fermentable substrate as positive control) and blanks (endogenous fermentability of inoculum as negative control) were found to be in agreement with overall mean values obtained with the same fermentation system. Compared to the blank, virtually no additional gas was produced when fermenting erythritol. Moreover, no H_2 accumulation could be observed. Maltitol showed a clearly lower total gas production during the first 6 h as well as an intermediate H_2 accumulation compared to lactulose. By the end of the incubation, however, almost identical amounts in total gas were produced.

Table 1. Changes in pH values and SCFA production during *in vitro* fermentation of erythritol compared to maltitol, lactulose and blank*
(Values are means of duplicate samples; standard deviation < 5 % except where indicated)

			SCFA production (μmol/100 mg substrate)			
	Sample time (h)	pH	Total	Acetate	Propionate	Butyrate
Blank	0	6·75	65·3	35·6	7·2	9·3
	6†	6·85	122·7	73·9	13·2	19·0
	24	6·83	163·2	94·0	27·5‡	26·5
Erythritol	0	6·64	70·1	41·4	8·3	11·6
	6	6·83	122·9§	70·4§	17·0§	20·7‡
	24	6·83	165·7	89·3	21·6§	38·5‡
Maltitol	0	6·62	66·4	33·2	9·9	11·3
	6	6·68	369·7	256·3	27·5	68·9
	24	5·95	1196·0	828·6	115·3	238·0
Lactulose	0†	6·72	79·0	42·5	7·6	10·8
	6†	6·15	1012·5	778·9	50·7	167·6
	24	6·05	1113·0	852·9	64·7	173·8

*For details of procedures, see p. 644.
†Single values.
‡Standard deviation < 10 %
§Standard deviation > 10 %.

When looking at changes in pH values and SCFA production (Table 1), similar observations were made. Erythritol and blanks did not differ in any parameter, whereas maltitol and lactulose reached similar end values. As also seen for gas production, SCFA formation and thus pH drop was found to be considerably slower for maltitol compared to lactulose within the first 6 h of fermentation. Although the total SCFA production reached comparable end values for both substrates, it has to be mentioned that qualitative differences were observed. Significantly higher amounts of propionate and butyrate were produced from maltitol.

Substrate disappearance can be followed in Fig. 1(C). Erythritol turned out to be completely resistant to the attack by the colonic microbiota during the 24 h of fermentation. In contrast, maltitol degradation was found to be linear but complete within that period. Lactulose disappearance was not determined in the present study. However, its complete degradation within 6 h was shown in earlier experiments (Arrigoni *et al.* 2004).

Discussion

Gas production from erythritol turned out to be negligible. Similar *in vitro* results were also observed by Hiele *et al.* (1993), who showed that H_2 formation by faecal bacteria after 6 h of incubation with erythritol was not higher than after the blank incubation. Moreover, Barry *et al.* (1992) reported neither total gas nor H_2 production over a fermentation period of 12 h. The fact that gas production from maltitol is retarded compared to that from lactulose is in accordance with *in vivo* results by Storey *et al.* (1998). They found significantly lower breath H_2 values (cumulated over a period of 6 h after ingestion) from maltitol. SCFA production as well as the concomitant pH drop reflects the observations made for gas production. No published data are available for total SCFA production from erythritol. However, the changes in pH values observed in the present study confirm observations made by Barry *et al.* (1992) for both erythritol and maltitol. As can be seen from substrate disappearance data, erythritol turned out to be completely resistant to the attack by faecal bacteria, a fact which has already been reported for a period of 12 h (Barry *et al.* 1992). However, the present experiment showed that erythritol is even not susceptible to the colonic microbiota within 24 h. The nearly linear degradation of maltitol is in accordance with results obtained by Barry *et al.* (1992), who also found a retarded metabolisation of the substrate.

Taking all fermentation parameters into account, it can be stated that erythritol as a sole substrate is completely non-fermentable by freshly collected human faecal microbiota within a period of 24 h. Based on the currently obtained data, the contribution to daily energy intake by gut fermentation of erythritol after oral intake in man is considered to be nil. For verification *in vivo*, a complete balance study would be required. Erythritol determination in faeces is rather simple, but a quantification of the absolute amount entering the colon is very complex. Although its total resistance to faecal bacterial fermentation *in vivo* is not definitively proven, it seems very likely that erythritol is excreted unchanged in man. The latter is supported by the fact that undigested dietary substrates as well as endogenous sources enter the colon continuously in considerable amounts (Gibson *et al.* 1996), most of them being more easily fermentable.

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