Degradation of Guar Gum by Intestinal Bacteria

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Guar gum is widely used in the food industry as a thickening agent. Guar and other galactomannans are ingested as a normal part of the human diet. Guar is completely degraded in the large intestine. Often large amounts of gas are produced. The objective of the study was to determine which species are responsible for the degradation of guar in the GI tract. It was observed that only a limited number of species is able to degrade and ferment guar. Guar degrading strains could be isolated from faecal samples of all volunteers and in 90% of the saliva of volunteers. The main species isolated from humans were Bifidobacterium dentium and Clostridium butyricum. From several samples of animal faeces Streptococcus bovis could be isolated. In addition some strains of Bacteroides ovatus were able to degrade guar to a limited extent. Fermentation resulted in the production of short-chain fatty acids and, when Cl. butyricum was present, in a large gas production. Competition experiments showed that Cl. butyricum degrades guar faster than both other species under simulated physiological conditions. It was concluded that Cl. butyricum is the main guar degrading species and the causative agent of the gas formation after guar intake.

Key words: guar; bifidobacteria; Clostridium butyricum; Streptococcus bovis; galactomannan

INTRODUCTION

Guar gum consists of a \( (1 \rightarrow 4)-\beta \)-linked \( \alpha \)-mannan backbone which carries single unit \( (1 \rightarrow 6)-\alpha \)-linked \( \alpha \)-galactose-residues with a mannose-galactose ratio of 1:2 (Fig. 1). It is obtained from the seeds of the guar plant or cluster bean (Cyamopsis tetragonoloba), a leguminous tree. Guar gum is widely used in many food applications (Table 1) (24). The annual world production of guar exceeds 100,000 tonnes, mainly produced in India, Pakistan and the USA (12). It can thus be stated that guar, together with other naturally occurring galactomannans, is often ingested as part of a Western-style diet. It forms part of the dietary fiber fraction of the diet.

Guar gum can not be degraded by the human small intestinal enzymes and thus reaches the colon unaltered (30). Although it has been widely investigated that guar is degraded completely by the intestinal microflora (5, 21, 22, 31), very little is known which species are involved in degradation and fermentation of guar and related galactomannans.

Fermentation of guar has been described for Bacteroides ovatus (about 25% of the strains tested), Bifidobacterium adolescentis (1 strain), Bif. dentium (all strains), an unidentified Bacteroides species and Ruminococcus albus (all strains) (7-10). Only for B. ovatus the fermentation has been characterised in more detail. In addition to this, fermentation of partially hydrolyzed guar gum (PHGG) has been tested by Japanese researchers (23). They observed a bifidogenic effect of the PHGG in humans, but none of the 16 bifidobacterial strains tested, was able to ferment PHGG. They did not, however, test Bif. dentium, which is mainly an oral species, although some strains have been isolated from intestinal contents. In addition, they tested 67 other intestinal bacteria. Of these only Clostridium butyricum, Cl. coccoides, B. ovatus and Ruminococcus productus were able to ferment the PHGG to some extent (23).

Fermentation of guar results, as is the case with most undigestible carbohydrates, in the production of short-chain fatty acids and gases. It has been noted in several studies that the fermentation of guar results in relatively large amounts of butyrate (5, 31). However, none of the species known to ferment guar produces butyrate as a major fermentation end product. B. ovatus produces mainly acetate and propionate, bifidobacteria produce only acetate and lactate. Only R. albus produces small amounts of butyrate, but mainly acetate (16). It is not likely that butyrate is formed from either lactate, propionate or acetate. Of the PHGG fermenting strains, only Cl. butyricum produces butyrate, Cl. coccoides and R. productus produce succinate and acetate as main end products, respectively (16, 17).

Fermentation of guar often, but not always, results in gas production. Trials using human volunteers have shown increased intestinal pressure and flatulence, as well as hydrogen and methane excretion in the breath (8, 25, 30, 32). As with the formation of butyrate, no...
hydrogen is produced in large amounts by the strains known to ferment guar. Some B. ovatus strains produce large amounts of carbon dioxide, only the three PHGG fermenting species may produce hydrogen (16).

To answer some of the discrepancies mentioned above, we decided to study the fermentation of guar by the intestinal flora in more detail.

**MATERIALS AND METHODS**

*Galactomannans*. The guar used was a commercial product, intended for use in foods (Vellinga Food Ingredients, Oudewater, The Netherlands). Other galactomannans, with a galactose-mannose ratio of 1:1, 1:3 and 1:4 were obtained from Diamalt (Diamalt, Munich, Germany). Pure mannans from yeasts was obtained from Sigma (M 7504, Sigma, Saint Louis, USA).

*Strains*. The strains used for the screening experiment were either type strains or isolates from human or swine faeces. Strains used for the initial screening included Actinomyces species (3 strains), Bacteroides fragilis group (49), bifidobacteria (17), clostridia (27), Eubacterium limosum, Fusobacterium necrophorum (2), lactobacilli (29), Megaphera elsdenii (5), anaerobic cocci (3), propionibacteria (3), enterobacteria (6), streptococci (3) and 22 unidentified faecal isolates.

*Liquefaction*. Degradation of guar was determined by liquefaction. A 1.5% guar solution was prepared in-carbohydrate free thioglycolate broth. This broth was prepared from the ingredients of thioglycolate broth (Oxoid CM 391). In addition 0.1% cysteine.HCl (Sigma, C 7880) was added. The pH was adjusted to 6.7 ± 0.1, unless otherwise stated. The guar was dissolved by mixing the guar slowly into the broth, while stirring rapidly. This highly viscous solution was rapidly poured into test tubes (10–15 ml/tube) and sterilized 15 min at 121°C. After sterilisation the tubes were placed in an anaerobic chamber for at least 16 hr before use. The anaerobic atmosphere consisted of: nitrogen 80%, carbon dioxide 10% and hydrogen 10% (SHK050H, HoekLoos, Rotterdam, The Netherlands). Tubes were inoculated with either culture medium (0.1–1 ml) or test material using sterile loops. The tubes were incubated up to 5 days anaerobically at 37°C. Guar degradation was considered positive when the tubes were completely liquefied. Liquefaction indicates that either the galactose backbones are removed (it was found to be impossible to make a gel of pure mnan, using the same concentrations) or that the backbone is degraded, resulting in the loss of the gelating properties. The actual degree of degradation was defined using a HPGPC technique.

**HPGPC**. In some samples checking for liquefac-
tion was combined with degradation measurements using High-Performance Gel Permeation Chromatography (HPGPC). The HPGPC system consisted of three combined (linear) Biogel TSK columns, 40XL, 30XL and 20XL, each column being 300 × 7.5 mm. The columns were eluted using 0.4 m sodium acetate buffer at pH 3.0 and a flow rate of 0.8 ml/min. Detection was by refraction index measurements, using a Shodex SE-61 detector. The column was kept at 30°C and the injection volume was 20 μl.

Isolation of guar degrading strains. Guar degrading strains were isolated from faeces or saliva of healthy human volunteers or faecal samples obtained from different animals. Guar tubes were inoculated with different serial dilutions of the samples. After liquefaction 100 μl of the liquid was transferred into new guar tubes. This procedure was repeated twice. After the third liquefaction 5 μl of the liquid was plated onto agar plates with Reinforced Clostridial Agar (RCA, Oxoid CM 151). These plates were incubated anaerobically and colonies were purified, using the same medium. Purified colonies were tested for guar liquefaction. Strains positive in the liquefaction test were characterised using morphology, spore formation, aerobic growth, catalase and oxidase tests, as well as a API 50CH test (BioMerieux, France). For identification the procedure described by Holdeman et al. was used (16).

As only a limited number of species could be isolated, the procedure was changed to improve isolation of other species. To improve isolation of lactic acid bacteria the pH of the guar tubes was decreased to 5.0. To isolate lactobacilli tubes with pH 5.0 and vancomycin (20 mg/l) were used (14). To isolate Gram-negative bacteria vancomycin was added. To isolate aerobes the whole procedure was carried out aerobically and cysteine was omitted from the guar tubes.

In addition it was tried to isolate guar degrading strains using solid media. The media used were MRS, Raffinose Bifidobacterium Agar (RB) (15), Kanamycin Azide Agar (KAA, Oxoid CM 591), Bacteroides Bile Esculin Agar (BBE) (29), which were prepared from the ingredients, replacing glucose by 0.25% guar. Samples were plated on these media and colonies were purified and tested for guar degradation using the liquefaction procedure.

Gas measurements. Gas produced by fermentation of guar was determined with a pressure meter. Strains or faecal samples were grown in 50 ml screw-capped glass bottles, with a butyl-rubber gas-tight septum. A maximum of 30 ml culture medium was added to the bottles. Gas was measured using a needle attached to a three-way valve, which was connected to the pressure meter and a syringe. The needle was pierced through the septum and the pressure was read. The pressure was reduced to zero by pulling the syringe. The volume of gas produced was read from the syringe.

Faecal inocula. Fermentation of guar was determined using faecal inocula. Faecal inocula were prepared from fresh faeces in buffered peptone water with cysteine.HCl (0.5 g/l) in approximately 10-fold dilution. In an anaerobic chamber a 1 ml sample of this dilution was transferred into a screw-capped bottle with 25 ml of the following medium (Medium 1): yeast extract 5 g/l (Oxoid L21), hemin solution 5 ml/l (Sigma H 2250), salis solution 40 ml/l (15, 16), LabLemco powder 5 g/l (Oxoid L29), potassium phosphate buffer 25 mmol/l (Merck 1.05104), sodium thioglycolate 0.5 g/l (Sigma T 0632), guar 5 g/l and cysteine.HCl 0.5 g/l. The pH was adjusted to 6.7 ± 0.1 using a 6 N NaOH solution. The bottles were sterilized by autoclaving 15 min at 121°C. After inoculation the bottles were incubated at 37°C for 36 hr. The gas production was measured as described above at three different time intervals. Final pH was measured after 36 hr. Some samples were frozen at −80°C and stored for later analysis of short-chain fatty acid profile (acetate, propionate, butyrate) and lactate concentrations using HPLC (18).

Faecal inocula were prepared from 10 volunteers. Use was made of faecal samples obtained in a large nutrition trial. During this trial, which was not related to our experiments, the volunteers received a completely controlled diet. The diet was essentially free of guar and other galactomannans. All volunteers received the same diet. Faeces was sampled twice in the third week as well as twice in the sixth week of the trial, resulting in 40 faecal inocula. All volunteers were of good health. The bacterial counts of all the samples were within a 7% range for the following bacterial groups: total anaerobes, total aerobes, bifidobacteria, Escherichia coli, clostridia and lactobacilli (unpublished data).

Competition experiments. Strains used for the competition experiments were Cl. butyricum G-13 (isolated from human faeces), Bif. dentium DSM 20484T and St. bovis G-2 (obtained from ID-DLO, Lelystad, The Netherlands). Clostridium butyricum G-13 was chosen as it produced the highest amount of gas, compared to the other Clostridium isolates (data not shown). The other two strains were chosen as these also liquefied guar rapidly, and were well characterised strains.

Competition was determined using gas measurements as described above. The strains were pre-cultured overnight in carbohydrate free RCM broth (made from the
ingredients according to the RCM broth (Oxoid CM 149), with 0.25% glucose or guar added. In addition the pH of the RCM medium was adjusted to pH 5.5, 6.0, 6.5 or 7.0 using 1 M HCl and 1 M NaOH.

After incubation 1 ml of the overnight culture, or appropriate dilution in reduced physiological salt solution, was added to 25 ml of the same medium in a screw-capped glass bottle. Either single cultures or combinations of the test strains were added. All dilutions and inoculations were performed in an anaerobic chamber to maintain anaerobic conditions.

After inoculation the bottles were incubated at 37°C for 120 hr. Gas was measured at regular time intervals and after 120 hr the final pH was determined.

RESULTS

Screening and Isolation

None of the identified strains tested in the initial screening experiment was able to liquefy guar, with the exception of the Bif. dentium type strain and one strain of Bacteroides ovatus (WAU B-203). Samples of human or animal faeces as well as saliva liquefied guar within 24 hr, most often even within 12 hr.

From human faecal samples only two types of guar-degrading bacteria could be isolated. These were identified as described above as strains of Clostridium butyricum and Bifidobacterium dentium/adolescents. The latter were identified as Bif. dentium on their ability to degrade guar (7), carbohydrate fermentation pattern (16) and presence of β-glucuronidase (26). Identification was further confirmed, as known strains of these species from culture collections were also capable of degrading guar.

Fermentation of guar by strains of Bif. dentium resulted in a final pH of 4.2–4.6, whereas the final pH of the Clostridium butyricum strains was between 5.0 and 5.6. All Clostridium butyricum strains fermented guar with the production of large amounts of gas, none of the bifidobacteria produced any gas.

In addition to the human strains some guar-degrading strains were isolated from animal faeces. Strains were isolated from the following species: pig (Sus scrofa), pekari (Tayassu tajacu), kudu (Tragelaphus strepsiceros), wallaby (Wallabia sp.), watussi (Bos taurus), giraffe (Giraffa camelopardalis), cheetah (Acinonyx jubatus), camel (Camelus bactrianus), water antelope (Kobus ellipsiprymnus), yak (Bos grunniens), elephant (Elephant maximus), oryx (Taurotragus oryx), lama (Lama glama) and bison (Bison bison). With the exception of three strains, all strains were streptococci. The strains were identified as Strep. bovis using the API-strep system (BioMérieux, France). The identity was again further confirmed by testing known strains of Strep. bovis (type strain and strains obtained from pigs and cows at the ID-DLO institute, Lelystad, The Netherlands) for guar degradation. No gas was produced by any of the strains, the final pH was between 4.5 and 5.0. The three non-streptococci, isolated from pigs and cheetah, were identified as Cl. butyricum. These strains produced large amounts of gas.

Selective isolation from faecal samples using specific media, selective agents, aeration or antibiotics did not result in any additional species, neither from human nor from animal samples. In addition to the faecal isolates we could isolate Bif. dentium from the saliva of 37 out of 40 volunteers as well as from 10 out of 10 samples of dental plaque (obtained using dental floss). No other guar degrading strains could be isolated from the oral cavity.

Faecal inocula

Fermentation of guar by 40 faecal inocula resulted in the production of gas and short-chain fatty acids. Gas production was between 7 and 50 ml, with an average of 26 ml. The final pH was between 4.4 and 6.6 with an average of 5.25 (Fig. 2). Only 8 samples had a pH below 5.0. Incubation of the same faecal inocula in the same medium without carbohydrate resulted in a gas formation of less than 5 ml and a pH of 6.5 ± 0.1.

Short chain fatty acids and lactate were determined in 12 samples. The total acid concentration was between 24.5 and 69.8 mmol/l, with an average of 54.9 mmol/l (Table 2). The major acid produced was acetate, followed by butyrate, propionate and lactate. The average percentage of butyrate, expressed as percentage of all
Table 2. Final pH, gas, lactate and short-chain fatty acids after batch fermentation of guar with faecal inocula from 12 healthy human volunteers.

<table>
<thead>
<tr>
<th>Sample</th>
<th>mL gas</th>
<th>Final pH</th>
<th>Concentration of (mmol/L)*</th>
<th>Total (mmol/L)</th>
<th>Relative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>A</td>
<td>P</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>4.9</td>
<td>12.55</td>
<td>29.55</td>
<td>9.37</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>5.2</td>
<td>4.73</td>
<td>18.56</td>
<td>3.27</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>5.4</td>
<td>2.58</td>
<td>20.94</td>
<td>10.74</td>
</tr>
<tr>
<td>4</td>
<td>49.5</td>
<td>5.0</td>
<td>11.40</td>
<td>21.07</td>
<td>8.06</td>
</tr>
<tr>
<td>5</td>
<td>34.5</td>
<td>5.4</td>
<td>4.06</td>
<td>18.15</td>
<td>6.93</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>6.6</td>
<td>10.26</td>
<td>7.94</td>
<td>2.72</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>5.3</td>
<td>1.59</td>
<td>24.05</td>
<td>17.74</td>
</tr>
<tr>
<td>8</td>
<td>35.5</td>
<td>5.2</td>
<td>5.29</td>
<td>20.04</td>
<td>8.82</td>
</tr>
<tr>
<td>9</td>
<td>7.5</td>
<td>5.6</td>
<td>5.45</td>
<td>22.78</td>
<td>12.36</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>5.6</td>
<td>2.14</td>
<td>27.75</td>
<td>12.28</td>
</tr>
<tr>
<td>11</td>
<td>11.5</td>
<td>5.7</td>
<td>2.66</td>
<td>29.76</td>
<td>25.07</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>5.2</td>
<td>4.02</td>
<td>33.83</td>
<td>14.63</td>
</tr>
</tbody>
</table>

Average: 25.29 5.43 5.56 22.87 11.00 15.47 54.90 11.48 41.20 19.35 27.97
SD: 13.86 0.42 3.60 6.56 5.92 6.59 11.68 10.33 6.59 8.60 10.79

* L, lactate; A, acetate; B, butyrate; P, propionate. Values are averages of duplicate analyses.

**HPGPC**

The degradation of guar was determined by HPGPC for seven strains, one B. ovatus (WAU B-203), three strains of Strep. bovis, one strain of Cl. butyricum and two strains of Bif. dentium. After 24 hr incubation all guar gels were completely liquefied. Gas was produced by the Cl. butyricum strain. The HPGPC patterns showed that the degradation was very limited for the B. ovatus strain (Fig. 3). Both the Strep. bovis and Bif. dentium strains degraded the guar to smaller compounds and monomers. It was impossible to obtain a degradation pattern from the Cl. butyricum strain, the reason for this is unknown.

All strains tested also liquefied gels made with galactomannans with other mannose-galactose ratios. Final pH values were similar as obtained with guar. Final pH on pure mannan generally was higher, indicating less fermentation (data not shown).

**Competition Experiments**

The competition experiments between Cl. butyricum G-13 and the Bif. dentium (type) showed that guar is fermented faster by Bif. dentium than by the Clostridium strain tested (Table 3). Gas production was around 27 ml for the Cl. butyricum strain alone. Addition of Bif. dentium reduced the gas production, indicating fermentation by Bif. dentium. In a ratio 1:100 (Bif. dentium vs. Cl. butyricum) Bif. dentium reduced the overall gas production by 20–40%, the strongest at the lower pH values. When equal amounts of both strains were added, gas production was reduced by half for pH 6.5 and 7.0 and by 80% for the two lower pH values. In a ratio of 10:1 (Bif. dentium vs. Cl. butyricum) gas production was practically zero at the lower pH values and reduced by 70–80% at pH 6.5 and 7.0.

Final pH values also showed a similar trend, indicating fermentation of guar by Bif. dentium.

Gas production was relatively slow, probably due to the low concentration of guar (Fig. 4). The maximum amount of gas produced was not reached within 3 days of incubation. The influence of Bif. dentium became
Table 3. Competition experiments between \textit{Cl. butyricum} G-13 (C) and \textit{Bif. dentium} type (B). Total gas production and final pH after 120 hr incubation at 37°C at four different initial pH values. Guar concentration 0.25%. Initial cell counts were $3 \times 10^5$/ml ± $2 \times 10^4$/ml. Data are mean of three different bottles.

<table>
<thead>
<tr>
<th>Inoculation level ml C ml B</th>
<th>Initial pH of media 5.5 6.0 6.5 7.0</th>
<th>Total gas produced 26.7 27.3 27.3 27.3</th>
<th>Initial pH of media 5.5 6.0 6.5 7.0</th>
<th>Final pH 5.9 5.8 6.0 5.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>15.3 14.3 23.7 20.7</td>
<td>17.7 15.7 12.3 8.0</td>
<td>5.1 5.1 5.4 5.8</td>
</tr>
<tr>
<td>1</td>
<td>0.01</td>
<td>4.3 5.7 17.3 12.3</td>
<td>4.9 4.9 5.1 5.1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.7 1.7 5.7 8.0</td>
<td>4.9 4.8 5.0 4.9</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>1</td>
<td>0.7 n.d. 0.7 n.d.</td>
<td>4.9 4.8 5.0 4.9</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0.7 n.d. 0.7 n.d.</td>
<td>4.9 4.8 5.0 4.9</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Effect of initial inoculum of \textit{Bif. dentium} on the gas production resulting from the fermentation of guar in the presence of a fixed inoculum of 10 ml \textit{Cl. butyricum} at pH 5.5. C only is no \textit{Bif. dentium} added, 1 0.01 ml \textit{Bif. dentium} added, 1 1 ml \textit{Bif. dentium} added. Concentration of \textit{Bif. dentium} was 3,10^9/ml.

Fig. 5. Effect of initial inoculum of \textit{Cl. butyricum} on the gas production resulting from the fermentation of guar in the presence of a fixed inoculum (1 ml) of \textit{Strep. bovis} at different pH values: 5.5, 6.0, 6.5 and 7.0. Concentration of \textit{Cl. butyricum} was 2.5 x 10^9/ml, that of \textit{Strep. bovis} was 7 x 10^9/ml.

noticable directly when equal amounts of both strains were added, but only after 40 hr when added in the ratio 100:1 (\textit{Bif. dentium} vs. \textit{Cl. butyricum}). At higher pH values growth and gas production was faster, reaching a maximum already after 50 hr at pH 6.5.

The competition between \textit{Cl. butyricum} G-13 and \textit{Strep. bovis} G-2 showed a different pattern (Fig. 5). No effect of \textit{Strep. bovis} on the gas formation could be observed at pH 6.5 or 7.0, not even at the initial ratio of 100:1 (\textit{Strep. bovis} vs. \textit{Cl. butyricum}). At pH 6.0 the gas production was reduced by 20% at a ratio of 1:1 or 10:1 and by 75% at a ratio 100:1. At pH 5.5 gas production was clearly reduced, even at a 1:1 ratio, final gas production was reduced by 75% under these conditions.

**DISCUSSION**

The results show that guar can only be degraded by a limited number of individual bacterial species from the intestinal contents. We have only been able to isolate strains from three species, \textit{Bif. dentium}, \textit{Strep. bovis} and \textit{Cl. butyricum} that are able to degrade and ferment guar. In addition, some strains of \textit{B. ovatus} were able to liquefy guar, but probably did not completely ferment the mannose chain. Besides \textit{B. ovatus} and \textit{Bif. dentium}, only \textit{R. albus} has been isolated previously. In addition to this, \textit{Cl. coccoides} and \textit{R. productus} have been found to be able to degrade PHGG. None of the latter two strains, however has been isolated previously as a guar fermenting species from human or animal faeces. Even though we have found only a limited number of guar degrading strains, it is not unlikely that a combination of strains from different species are able to degrade and ferment guar and galactomannans in vivo.

The strain most frequently isolated from human faecal samples was \textit{Cl. butyricum}. The numbers of \textit{Cl. butyricum} were found to be around or over 10^9/g faeces
in most samples. Although this species has been isolated previously from human faeces in similar numbers, we have isolated this species from most humans. Previous results did only show a low frequency of isolation, probably due to the presence of other clostridial species. *Clostridium butyricum* has not been isolated previously as a guar degrading strain, but it was found to be able to degrade PHGG (23) and glucomannan (20).

The presence of *Bifidobacterium dentium* in faeces has been described previously (7). Literature results indicate that *Bifidobacterium dentium* is present in relatively small numbers compared to other bifidobacterial species. The isolation of *Bifidobacterium dentium* from faeces therefore requires selective techniques. Also, *Bifidobacterium dentium* is difficult to differentiate from *Bifidobacterium adolescentis* and *Bifidobacterium catenulatum*, which are both more common in faeces (16). It is thus likely that some *Bifidobacterium dentium* isolates have previously been misidentified as *Bifidobacterium adolescentis* or *Bifidobacterium catenulatum*. The two selective tests to differentiate between the species are the presence of β-glucuronidase and guar fermentation, both of which are positive for *Bifidobacterium dentium* (7, 26).

*Bifidobacterium dentium* is normally associated with dental caries. We have shown, using guar degradation, that this species was present in saliva in higher numbers than observed previously (28).

*Streptococcus bovis* has not previously been isolated as a guar degrading species. We isolated strains of *Streptococcus bovis* from most samples of all faecal faeces. *Streptococcus bovis* has been isolated previously from many animals, but also from human faeces (13). As all our isolates grew aerobically, they could not be misidentified with *Ruminococcus* or *Ruminobacter*, two anaerobic species that were associated with guar fermentation in previous studies. Also, the type strain of *Streptococcus bovis*, as well as strains that we obtained from other institutes, were capable of degrading and fermenting guar.

In contrast to previous research we failed to isolate guar degrading *Bacteroides ovatus* strains. The reason for this is unknown. It was shown in previous studies that about 25% of all strains of *Bacteroides ovatus* were capable of degrading and fermenting guar (27). The average log counts of *Bacteroides fragilis* group organisms in the tested faecal inocula was generally above 10^9/g, indicating that the conditions were not inhibiting to *Bacteroides ovatus*. Even with the addition of vancomycin to suppress the Gram-positive species, no liquefaction of guar gels was observed. In a similar technique as Bayliss and Houston (2), using *Bacteroides* Bile Esculin Agar with guar as the only carbohydrate source, we isolated many Gram-negative rods, none of which liquefied guar in subsequent tests. *Bacteroides ovatus* is not present in faeces of all humans. Finegold et al. isolated *Bacteroides ovatus* only in 8 out of 18 persons on a Western style diet (9). Similarly *Bacteroides ovatus* was isolated only in 9 out of 70 samples of infant faeces (4), 13 out of 30 samples of older persons (3), 4 out of 13 samples of healthy vegetarian adults and 3 out of 14 non-vegetarian adults (10). It is unknown whether *Bacteroides ovatus* is common in healthy Dutch adults and/or our donors. *Bacteroides ovatus* is also rarely isolated from intestinal or faecal samples in general hospitals in the Netherlands (pers comm).

Guar fermentation by faecal inocula resulted in the production of gas and butyrate as a major short-chain fatty acid. Some lactate was produced as well. This is in agreement with other studies (3, 31). The data presented in Table 2 include SCFA and lactate produced from the medium components. However, the total concentration of acids produced from the medium without carbohydrate source was less than 15 mmol/l, indicating that most acids were produced from the carbohydrate (unpublished data). Butyrate is formed by direct fermentation of carbohydrates, it is not formed from other acids, such as acetate or lactate by faecal bacteria. Of all guar fermenting species isolated in our or previous studies, only *Clostridium butyricum* produces butyrate.

Gas production varied between the samples (Fig. 2), but was always present. In vivo studies have also shown production of considerable amounts of gases, resulting in intestinal bloating, flatus and hydrogen excretion (8, 25, 30, 32). Of all species isolated in our or previous studies only *Clostridium butyricum, Clostridium cocoides* and *Ruminococcus* produce hydrogen, some of the *Bacteroides ovatus* strains are reported to produce carbon dioxide (16). As we failed to isolate the latter three species, it can be concluded that *Clostridium butyricum* is the species responsible for the gas production. Gas may also be produced as a secondary metabolite from lactate by *Megasphaera elsdenii* or *Veillonella* species (16). This would result in high concentrations of acetate and propionate. Similarly, the *Bacteroides ovatus* strains do not produce hydrogen, nor butyrate (16). The presence of large concentrations of butyrate, therefore, emphasizes the role of *Clostridium butyricum*. Some secondary fermentation yielding butyrate may, however, not be excluded, neither in vivo nor in vitro.

The final pH of guar fermentation by faecal inocula was relatively high as compared with other substrates (unpublished data). With a few exceptions, the final pH was above 5.0. This further emphasises the role of *Clostridium butyricum* in many samples, as this strain does not acidify below 5.0, whereas the two other species can
acidify below 5.0.

The B. ovatus strain tested did not completely degrade guar, but probably only removed the galactose units. The molecular mass was slightly less than that of the original guar, but the sample was completely liquefied. Removing of the galactose results in liquefaction of the gel, as mannan does not form gels. This B. ovatus strain thus was positive in the liquefaction test, but does not completely ferment guar. The two necessary enzymes for guar degradation (α-galactosidase and mannanase), have been described for B. ovatus. Two different α-galactosidases have been described, both of which are not active on intact guar gum. α-Galactosidase I is only active on partially degraded guar gum (11). In another study it was shown that α-galactosidase activity is induced by guar gum and that these enzymes are acting towards guar (19). A mannanase has been described, but not purified (1). Whether both enzymes are present in all strains is not known.

Repeated incubation and storage (over 1 year) in non-guar containing media (cooked meat medium) or at –80°C with subsequent inoculation of guar tubes, did not result in the loss of the guar degrading capacity for the isolated strains (data not shown). Similarly, the culture collection strains, which were most likely never tested on guar degradation, as it is normally not included in identification schemes, did liquefy guar rapidly. These data indicate that the enzymes are constitutive and not, or very rapidly, induced by guar.

The competition experiments showed that Bif. dentium was capable of influencing the gas production by Cl. butyricum at all pH values tested. The strongest inhibition was observed at the lower initial pH values. This influence was also reflected in lower final pH values. As Bif. dentium grows faster than Cl. butyricum at physiological conditions (pH 6.5–6.8), isolation of Bif. dentium using guar degradation should occur, when both species are initially present in equal amounts, or even when Cl. butyricum outnumbers Bif. dentium by a factor 10–100. However, we could not detect Bif. dentium in liquefied guar gels using facial samples. This indicates that Bif. dentium is present in much lower numbers than Cl. butyricum. It is therefore unlikely that Bif. dentium is responsible for the bifidogenic effect of PHGG as has been reported previously (23) as the addition of PHGG to the diet of volunteers increased the numbers of bifidobacteria from (log counts) 9.9 to 10.3 and Bif. dentium would thus outnumber any clostridia present. It is not expected that the Japanese population tested has a much higher faecal count of Bif. dentium than our volunteers, as the presence of this species has not been reported previously by Japanese researchers in high numbers in faeces (3, 4). Therefore the bifidogenic effect of PHGG as described by Okubo et al. (23) can not readily be explained.

Competition experiments between Strept. bovis and Cl. butyricum indicated that Strept. bovis has no influence on the gas production by Cl. butyricum under physiological conditions, not even when Strept. bovis outnumbers Cl. butyricum with a factor 100. Only at pH 6.0 gas production was reduced. It can thus be concluded that in human faeces it is not likely to isolate Strept. bovis using guar gel liquefaction when Cl. butyricum is present. As we isolated all our strains from animals it can be concluded that, in these samples, Cl. butyricum was present in much lower numbers than Strept. bovis.

The fermentation of guar results in the formation of relatively large concentrations of butyrate (5, 31). Formation of butyrate is considered to have a beneficial effect in the intestine (6). Guar does not directly stimulate the growth of beneficial bacteria such as bifidobacteria and lactobacilli, with the exception of Bif. dentium. On the contrary, guar seems to stimulate mainly Cl. butyricum, and the stimulation of clostridia is not considered beneficial. The effects of guar on intestinal health are thus difficult to determine.

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