1H nuclear magnetic resonance spectroscopy-based studies of the metabolism of food-borne carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline by human intestinal microbiota

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Evidence is accumulating that heterocyclic aromatic amines (HAs), which are food-borne carcinogens formed from amino acids in meats during cooking, may be involved in the etiology of colon cancer (10). The genotoxic/carcinogenic effect of HAs is closely related to a highly complex metabolism involving xenobiotic-metabolizing enzymes that generate very reactive metabolites as well as detoxified derivatives (17, 18). The xenobiotic-metabolizing enzymes that generate very reactive metabolites as well as detoxified derivatives (17, 18). The xenobiotic-metabolizing enzymes that generate very reactive metabolites as well as detoxified derivatives (17, 18).

On the one hand, the involvement of the intestinal microbiota in the digestive fate of HAs remains underinvestigated (19), although around 10% of the ingested HAs may reach the colon in their native form and thus come into contact with the resident microbiota (21, 34). Direct binding of HAs to the cell walls of intestinal bacteria has been reported and is currently considered a detoxification mechanism, since it prevents absorption of HAs through the intestinal mucosa (19, 23, 41). On the other hand, results of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced genotoxic assays in germfree and conventional rodents show that the presence of intestinal microbiota is essential to the induction of DNA damage in the colon and liver cells (13, 16). These findings suggest that intestinal microbiota take part in the biocconversion of HAs into harmful metabolites. Indications exist that hydrolysis of HA-glucuronides by bacterial β-glucuronidase activity is naturally higher in human-microbiota-associated rats gavaged with IQ on a regular basis. HPLC and 1H-NMR analyses showed that the three human microbiota degraded IQ with different efficiencies (range, 50 to 91% after 72 h of incubation) and converted it into a unique derivative, namely, 7-hydroxy-IQ. We found 10 bacterial strains that were able to perform this reaction: Bacteroides thetaotaomicron (n = 2), Clostridium clostridiiforme (n = 3), Clostridium perfringens (n = 1), and Escherichia coli (n = 4). On the whole, our results indicate that bacteria belonging to the predominant communities of the human intestine are able to produce 7-hydroxy-IQ from IQ. They also suggest interindividual differences in the ability to perform this reaction. Whether it is a metabolic activation is still a matter of debate, since 7-hydroxy-IQ has been shown to be a direct-acting mutagen in the Ames assay but not carcinogenic in laboratory rodents.
metabolite was 7-OH-IQ (Fig. 1), the identity of which was ascertained by \(^1\)H nuclear magnetic resonance (\(^1\)H-NMR) spectroscopy and mass spectrometry (MS) (1). This compound was subsequently detected in human feces following consumption of fried meat (35).

In the present study, we investigated the metabolism of IQ by human intestinal microbiota by using \(^1\)H-NMR spectroscopic analysis of unprocessed incubation media to detect potential metabolites in an exhaustive fashion. Indeed, \(^1\)H-NMR spectroscopy of crude biological samples allows measurement of a wide range of molecules simultaneously and without a priori hypotheses concerning their chemical structure; it has proved, on many occasions, to be a powerful tool to analyze the microbial degradation of xenobiotics (7, 8). Due to the detection limit of this analytical method, around 1 \(\mu\)M, we used a relatively high concentration of IQ, i.e., 100 to 200 \(\mu\)M, to ensure identification and quantification of IQ metabolites. Interindividual differences occur with regard to the species composition (31) and the metabolic activities (11, 20) of the human intestinal microbiota. Therefore, we examined the bioconversion potentials of fecal samples collected from different subjects. Eventually, we looked for individual bacteria able to metabolize IQ by screening representatives of the gut microbiota belonging to culture collections and by isolating new strains. On the assumption that IQ-degrading microbial populations may be favored by a continuous exposure to the substrate, the isolation procedure was performed on feces of HMA rats consuming IQ on a regular basis.

![IQ](image1.png)

**FIG. 1.** Structures of IQ and its 7-hydroxy derivative.

<table>
<thead>
<tr>
<th>Bacterial species and strain</th>
<th>Origin</th>
<th>Source or reference</th>
<th>% of initial IQ degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>Human feces</td>
<td>This study⁴</td>
<td>74</td>
</tr>
<tr>
<td>ATCC 29148</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-3JE</td>
<td>HMA rat cecum²</td>
<td>This study⁴</td>
<td>52</td>
</tr>
<tr>
<td>Bacteroides vulgatus ATCC 8482</td>
<td>Human feces</td>
<td>This study⁴</td>
<td>0</td>
</tr>
<tr>
<td>Bifidobacterium bifidum B536</td>
<td>Infant feces</td>
<td>This study⁴</td>
<td>14</td>
</tr>
<tr>
<td>Bifidobacterium longum ATCC 15707</td>
<td>Adult intestine</td>
<td>This study⁴</td>
<td>0</td>
</tr>
<tr>
<td>Clostridium clostridiiforme 5-1JD1</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>76</td>
</tr>
<tr>
<td>9-2JB</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>60</td>
</tr>
<tr>
<td>10-2JD2</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>50</td>
</tr>
<tr>
<td>Clostridium nexile ATCC 27757</td>
<td>Human feces</td>
<td>F. Marcille, unpublished data</td>
<td>100</td>
</tr>
<tr>
<td>Clostridium perfringens G22</td>
<td>Human feces</td>
<td>F. Marcille, unpublished data</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli 2-1JC</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>61</td>
</tr>
<tr>
<td>8-1JA</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>80</td>
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<td>8-1JC</td>
<td>HMA rat cecum</td>
<td>This study⁴</td>
<td>61</td>
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<td>10-2JB</td>
<td>HMA rat cecum</td>
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<td>47</td>
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<td>Eubacterium rectale ATCC 33656</td>
<td>Human feces</td>
<td>This study⁴</td>
<td>0</td>
</tr>
<tr>
<td>Ruminococcus gravis FRE12</td>
<td>Human feces</td>
<td>This study⁴</td>
<td>12</td>
</tr>
</tbody>
</table>

⁴ For incubation, resting-cell suspensions in PYE buffer were supplemented with 100 \(\mu\)M IQ (anaerobic conditions, 37°C, 150 rpm).

² At the end of incubation (72 h), the IQ concentration was determined by HPLC analysis.

³ HMA, human microbiota associated.

⁴ Among the 135 strains isolated from the mixed cecal contents of HMA rats and assayed for IQ degradation, only the 8 biodegradative strains are indicated in this table.

**MATERIALS AND METHODS**

**Chemicals.** IQ was obtained from Research Chemicals Inc. (Toronto, Canada). For incubation purposes, it was dissolved in (methyl-sulfoxide)-d6 and subsequently diluted using phosphate buffer (0.2 M, pH 7.0) with added yeast extract (0.2 g/liter) (PYE buffer); this stock solution (10 mM) was filter sterilized (Millipore-LG 0.22-\(\mu\)m filter; Millipore, St-Quentin-en-Yvelines, France) before use. For gavage of HMA rats, IQ was suspended in corn oil (Sigma, St-Quentin-Fallavier, France) and sterilized by autoclaving (120°C, 20 min); this process does not affect the concentration of IQ (data not shown).

The constituents of the culture media, namely, tryptone, brain heart infusion (BHI), Bi Tek agar, and yeast extract, were obtained from Difco (Le Pont-de-ClAIR, France). Horse blood came from BioMérieux (Marcy-l’Etoile, France), and glycerol was purchased from VWR (Fontenay-sous-Bois, France). Acetonitrile for high-pressure liquid chromatography (HPLC) analyses came from Merck (Nogent-sur-Marne, France). Hemin and all other salts and amino acids came from Sigma. Tetradecanated sodium trimethylsilylpropionate and deuterium oxide used for NMR analyses were purchased from Eurisotop (Saint-Aubin, France).

**Collection and preparation of human fecal samples.** Fresh stools from three healthy volunteers between 26 and 44 years of age were used. Donors were on a Western-type diet, and none had history of digestive pathology or had received antibiotics for the last 3 months. Stools collected in sterile plastic boxes were kept at -20°C before use. For gavage of HMA rats, IQ was suspended in corn oil (Sigma, St-Quentin-De-ClAIR, France). Horse blood came from BioMérieux (Marcy-l’Etoile, France), and glycerol was purchased from VWR (Fontenay-sous-Bois, France). Acetonitrile for high-pressure liquid chromatography (HPLC) analyses came from Merck (Nogent-sur-Marne, France). Hemin and all other salts and amino acids came from Sigma. Tetradecanated sodium trimethylsilylpropionate and deuterium oxide used for NMR analyses were purchased from Eurisotop (Saint-Aubin, France).

**Strains from culture collections.** Nine strains were chosen from the collection of Unité d’Ecologie et de Physiologie du Système Digestif (INRA, Jouy-en-Josas, France). All of them originated from human feces or intestinal contents and had been purchased from the American Type Culture Collection or isolated locally.
suspension derived from the mixed cecal contents of HMA rats gavaged with IQ for the care and use of laboratory animals. For isolation of IQ-degrading bacterial strains as described in “Isolation and identification of bacterial strains” below, the other part was used for isolation of IQ-degrading bacterial strains as described in “Isolation and identification of IQ-degrading bacterial strains” below. All procedures were carried out in accordance with the European guidelines for care and use of laboratory animals.

Isolation and identification of IQ-degrading bacterial strains. The 100-fold suspension derived from the mixed cecal contents of HMA rats gavaged with IQ was diluted using serial 10-fold dilutions (10^{-2} to 10^{-19}) in modified TY broth supplemented with IQ (100 μM). Dilutions were incubated at 37°C under anaerobic conditions for 3 days and assayed at 24-hour intervals for residual IQ. At the same time intervals, samples from all dilutions were spread onto modified TY agar plates supplemented with horse blood (5%, vol/vol) and with IQ (100 μM) to maintain a continuous exposure of the bacteria to the substrate. Following incubation at 37°C under anaerobic conditions, five colonies per plate that differed, whenever possible, in size, shape, and color were picked, subcultured in modified TY broth supplemented with IQ (100 μM), and then stored as stock cultures at −80°C after addition of glycerol (40%, vol/vol). One hundred thirty-five viable strains were obtained using this process; those named after the dilution (10^{-2}, 10^{-3}, 10^{-4}, etc.) and the day of incubation (1, 2, or 3) from which they originated. Subcultures intended for strain storage were sampled at the initial and final times of incubation (3 days) for analysis of residual IQ in order to detect IQ-degrading strains. Further investigation of IQ metabolism by the biodegradative strains was performed on resting-cell suspensions prepared in PYE buffer and incubated with IQ (for details, see “Strains from culture collections” above and “Incubation conditions for bacterial resting-cell suspensions” below).

Identification of the biodegradative strains was performed phenotypically by microscopic examination, Gram staining, and determination of biochemical characteristics (API Systems, BioMérieux) and genetically by sequence comparison of the amplification products of the 16S rRNA genes. Total DNA was extracted from 48-hour cultures in modified TY broth by using the Wizard genomic DNA purification kit (Promega, Charbonnières, France). Primers W001 (3.6) and 23S1 (GenBank accession no. J01695) were used to amplify the 16S rRNA encoding genes, including the intergenic region located between the 16S rRNA and 235 rRNA genes. The PCR product (about 2.3 kb) was purified using the QIAGEN QIAquick PCR purification kit (QIAGEN, Courtabœuf, France). The sequence reaction was performed by using the primers SP3, SP4, and SP5 (6, 36) and the ABI Prism Big Dye Terminator version 2.0 kit (Applied, Courtabœuf, France). Sequences were analyzed using a 96-capillary 3700 sequencer (Perkin-Elmer, Courtabœuf, France). Each clone was identified by comparison with the Ribosomal Database Project II (http://rdp.cme.msu.edu).

Incubation conditions for bacterial resting-cell suspensions. Bacterial resting-cell suspensions in PYE buffer were supplemented with IQ and transferred into glass vials that were tightly closed with butyl rubber stoppers and sealed with aluminum caps to maintain an anoxic environment. The vials were subsequently incubated in a shaking water bath (150 rpm) at 37°C for 72 h. The IQ concentration was 200 μM for resting-cell suspensions derived from human feces and the cecal contents of HMA rats and 100 μM for resting-cell suspensions of individual strains. Incubation media were sampled at intervals by sterile puncture through the butyl rubber stoppers. Samples were centrifuged (8,000 × g, 10 min, 4°C), and the supernatants were kept at −20°C until HPLC and 1H-NMR analyses.

Three types of controls were used, namely, (i) sterile PYE buffer supplemented with IQ to check the stability of the substrate over time, (ii) resting-cell suspensions devoid of IQ to check the absence of coeluting peaks, and (iii) autoclaved resting-cell suspensions (120°C, 20 min) supplemented with IQ to ascertain that the disappearance of the substrate could be attributed to the metabolic activity of viable cells and not to a passive adsorption on bacterial cell walls (23, 41).

HPLC analysis. IQ and its hydroyrivative, 7-OH-IQ, were analyzed by HPLC using the method described by Rafter and Gustafsson (24) with slight modifications. Briefly, culture and resting cell supernatants were injected with an "mixing period" (corresponding to several cycles of MLEV-17 spinlock sequence) of 20 to 90 ms. The responses of eight scans for each of 512 integrations were acquired. Zero-filling in 1T1 and sines window function in both dimensions were applied prior to 2D Fourier transformation.

MS analysis. MS analysis was performed using an HP 5989 B mass spectrometer equipped with an atmospheric pressure chemical ionization interface. Supernatants were diluted in methanol or acetonitrile. Sample injections were carried out by a Rheodyne valve type at 100 μl/min. Spectral widths were adjusted in both dimensions to encompass all 1H signals of interest. The “mixing period” (corresponding to several cycles of MLEV-17 spinlock sequence) was 20 s. The responses of six scans for each of 512 integrations were acquired. Zero-filling in 1T1 and sine window function in both dimensions were applied prior to 2D Fourier transformation.

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RESULTS

Metabolism of IQ by human feces and by the cecal contents of HMA rats (HPLC analysis). Figure 2 depicts the kinetics of IQ degradation and 7-OH-IQ formation measured by HPLC analysis in the resting-cell incubations prepared with the feces of the three human volunteers and with the mixed cecal contents of the two HMA rats. All three human feces degraded IQ, although with different efficiencies. Indeed, the amount of IQ degraded over 72 h ranged from 50 to 90% of the initial quantity. Furthermore, the $t_{50}$ (the times required for degradation of half of the initial substrate amount) were 39, 51, and 72 h for the high-, intermediate-, and low-degrading microbiota, respectively. HMA rats were inoculated with the fecal microbiota of the subject with high degrading capacity. In this regard, it must be emphasized that the 100-fold dilution of the microbiota of the subject with high degrading capacity. In this regard, it must be emphasized that the 100-fold dilution of the microbiota of the subject with high degrading capacity.

7-OH-IQ formation accompanied IQ degradation in each resting-cell incubation. Interindividual differences between the kinetics of 7-OH-IQ formation largely reflected those between the kinetics of IQ degradation. The low-degrading human microbiota produced the lowest quantity of 7-OH-IQ. Nevertheless, peak areas of the hydroxy derivative measured in the incubation media of the medium- and high-degrading human microbiota and of the HMA rats' mixed cecal contents all tended to be similar at the end of incubation. This phenomenon may be due to the loss of linearity between 7-OH-IQ amount and peak area at high concentrations.

Metabolism of IQ by individual bacterial strains (HPLC analysis). Among the nine collection strains that were assayed in the present experiment, only two strains were able to degrade IQ under resting-cell conditions as shown by HPLC analysis (Table 1). These strains were Bacteroides thetaiotaomicron ATCC 29148 and Clostridium perfringens G22, which consumed 74 and 100% of the initial amount of IQ (100 $\mu$M), respectively.

The serial 10-fold dilutions of the mixed cecal contents of HMA rats all degraded IQ to some extent (range, 50 to 100%) (data not shown). Among the 135 strains isolated from these dilutions, only eight strains were able to degrade IQ as measured by HPLC analysis of culture supernatants (data not shown). This ability was confirmed under resting-cell conditions similar to those applied to the collection strains; the amounts of IQ degraded by each strain after 72 h of incubation are presented in Table 1. The biodegradative strains were isolated mainly from the highest cecal dilutions, namely, $10^{-8}$ to $10^{-10}$, and hence belong to the predominant microbial communities. They were members of the species Bacteroides thetaiotaomicron (n = 1), Clostridium clostridiiforme (n = 3), and Escherichia coli (n = 4).

In situ $^1$H-NMR and MS analyses of human feces, HMA rats’ cecal contents, and individual bacterial strains incubated with IQ. Before interpretation of the kinetics monitored by in situ $^1$H-NMR, assignment of signals coming from the bacterial cells or from the PYE buffer used for resting-cell incubations (especially those from yeast extract) was performed. This was done by comparing control samples, namely, resting-cell suspensions devoid of IQ, sterile PYE buffer supplemented with IQ, and autoclaved resting-cell suspensions, with supernatants collected from experimental samples. Because IQ chemical shifts change with concentration, calibration curves of IQ chemical shifts versus concentration had to be prepared.

Sometimes signals from “background” compounds overlapped signals of interest; in such cases, 2D $^1$H-TOCSY experiments were performed to increase the resolution and thus unambiguously assign signals belonging to IQ and IQ transformation products. This approach allowed us to observe solely one transformation product, whether the sample was human feces, cecal contents of HMA rats, or individual strains, and regardless of the initial concentration of IQ (100 $\mu$M versus 200 $\mu$M). This compound was identified as 7-OH-IQ from analysis of 2D TOCSY spectral patterns and from analysis of mass spectra of supernatants recorded for several samples without any purification. Such mass spectra showed two main peaks at $m/z$ 199 and $m/z$ 215, corresponding to the [M + H]$^+$ adducts of IQ and 7-OH-IQ, respectively. The complete assignment of $^1$H NMR spectra is shown in Fig. 3.

On this basis, and insofar as signals were well resolved, quantification of IQ and 7-OH-IQ could be achieved. This was the case for resting-cell incubations of individual bacterial strains and of the mixed cecal contents derived from HMA.
It was found that hydroxylation at position 7 was the sole transformation occurring under these conditions; 7-OH-IQ accumulated over time and accounted for all the IQ degraded (Fig. 4). It was not possible to precisely quantify IQ and 7-OH-IQ in resting-cell incubations of human feces since their signals were broadened, probably because of an interaction with unknown biomacromolecules.

DISCUSSION

By analyzing crude incubation media with 1H-NMR spectroscopy, we have shown that human fecal microbiota and individual bacteria isolated from them can transform IQ into a hydroxy derivative, namely, 7-OH-IQ. These findings agree with the original discovery by Bashir et al. (1), who found 7-OH-IQ as the major detectable metabolite following incubation of IQ with human fecal microbiota. However, since those authors analyzed the metabolite profile on a selective extract of the incubation medium, they could not rule out the possibility that other derivatives could have been released yet not recovered in the extract (1). In this connection, Carman et al. (4) hypothesized that a reductive deamination of IQ could occur, since removal of amino groups is a common reaction among the intestinal microbiota (28). Today, in situ 1H-NMR spectroscopy is one of the most powerful analytical methods for providing structural and quantitative information on the metabolic state of a biological medium. Therefore, we can assert here that 7-OH-IQ is unambiguously the unique metabolite produced by bacterial conversion of IQ, at least under our experimental conditions. According to Carman et al., the formation of 7-OH-IQ from IQ is a reversible reaction; indeed, incubation of IQ with the most active bacterial strain that they isolated, Eubacterium moniliforme VPI 13480, always led to an equilibrium in which IQ and its 7-hydroxy derivative
coexisted in equal amounts, regardless of the initial concentration of IQ (10 or 250 μM) (4). We did not confirm this phenomenon, since we found that 7-OH-IQ accumulated over time, whether the parent compound was incubated with a whole intestinal microbiota or with individual bacteria and regardless of its initial concentration (100 or 200 μM); in some instances, namely, upon incubation with the mixed cecal contents of HMA rats and with the strain Clostridium perfringens G22, IQ even totally disappeared from the incubation medium.

Strong interindividual variations occurred between the three human microorganisms with regard to their IQ-degrading capabilities. Indications of such variations have been provided by Hirayama et al., who observed that preincubation of IQ with human feces increased its direct mutagenicity in the Ames assay, although to different extents depending on the donor (13). They concluded that the ability of human microbiota to generate direct-acting mutagens from IQ varied with individuals. Rumney et al. also noticed variations in the extent of IQ degradation following incubation in phosphate buffer with feces from three different human donors (27). However, the differences were slight compared to those reported in the present experiment, perhaps because of a much lower (eightfold) concentration of IQ. Interindividual differences in microbial secondary metabolic activities are not uncommon. In another context, we have shown that the rate of conversion of sinigrin, a sulfurous compound commonly consumed via cruciferous vegetables, into its anticarcinogenic allyl isothiocyanate derivative depended on the origin of the fecal microbiota that was used to colonize a human large intestinal model (21). Another striking example is the bimodal distribution of the bacterially borne digestive ability to convert cholesterol into coprostanol among human populations; the U.S. population is thus divided into a vast majority of high converters and a minority of low to inefficient converters (38). Otherwise we found that the IQ-degrading ability of the human fecal microbiota was well preserved in the HMA rat model, as observed by Rumney et al. (27). However, unlike those authors, we observed that IQ was metabolized faster by the cecal contents of HMA rats than by the feces of the human donor. It is very likely that the repeated dosing of the HMA rats with IQ was responsible for this enhancement; indeed, such a metabolic adaptation has been reported previously with other xenobiotics, such as flavonoids or cyclamate (28). This alteration may result from quantitative shifts among bacterial groups, so that IQ-degrading populations become more abundant. Alternatively, there may be adaptive changes in the IQ-degrading groups which increase their metabolic capabilities. The fact that the yield of isolation of IQ-degrading bacteria from the mixed cecal contents of the HMA rats was rather low (8 out of 135) supports the latter hypothesis.

The 10 IQ-degrading bacterial strains that we discovered in culture collections (n = 2) and in the mixed cecal contents of the HMA rats (n = 8) belong to four different species, namely, Bacteroides thetaiotaomicron, Clostridium clostridioforme, Clostridium perfringens, and Escherichia coli. All of them converted IQ solely into 7-OH-IQ, regardless of the extent of substrate consumption (range, 47 to 100%). Bacteroides thetaiotaomicron is well known for its diverse and adaptive metabolic capabilities (9, 40). As for the genus Clostridium and the species Escherichia coli, they have been shown to be able to metabolize other polycyclic compounds such as flavonoids (2, 39).

In their investigations on the microbial metabolism of IQ, Carman et al. have focused their research on bioactive bacteria on individual representatives of the genus Eubacterium belonging to various environments, e.g., blood, mouth, and feces (4). They found that 14 strains out of 60 were able to perform the conversion of IQ into 7-OH-IQ. We have tested the only strain that is still available in culture collections, i.e., Eubacterium saburreum ATCC 33271 (VPI 11763), which was isolated from human dental plaque. However, we failed to reproduce its IQ-converting capability. This may be due to the difference in incubation conditions, i.e., resting cells in phosphate yeast buffer in the present experiment versus growing cells in peptone yeast broth in the pioneering work by Carman et al. (4).

On the whole, the present study shows that the conversion of IQ to 7-OH-IQ is performed by bacteria belonging to the predominant communities of the human colon (Bacteroides and Clostridium) as well as by minor populations (Escherichia coli).

So far, the metabolic pathway leading from IQ to 7-OH-IQ remains unknown. Liver cytochrome P450 in humans and rats is able to realize several hydroxylations of the IQ molecule, but never at position 7 (33). Furthermore, Rumney et al. have clearly shown that this metabolite cannot be produced by feces of germ-free rats, while it is always produced by feces of conventional or HMA rats (27). Consequently, the metabolic reaction leading to the addition of a hydroxyl group at position 7 seems to be carried out exclusively by bacteria. Among the bioactive strains that we have identified, Bacteroides thetaiotaomicron ATCC 29148 (type strain of the species) is of particular interest since its genome has been recently sequenced (GenBank accession no. NC004663). Of the 4,779 predicted proteins in its proteome, 1,997 (42%) have homology to proteins with no known function or no appreciable homology to entries in public databases (40). Proteins involved in the conversion of IQ to its 7-hydroxy derivative are likely to belong to this group of proteins having unknown functions. An interesting feature is that the highest number of best hits between protein sequences from Bacteroides thetaiotaomicron and from other members of the human intestinal microbiota is with Clostridium perfringens, the most efficient IQ converter identified in the present study. We can thus reasonably assume that a thorough analysis of proteins common to both species may help to characterize the metabolic pathway leading from IQ to 7-OH-IQ.

7-OH-IQ has been investigated in the past for its potential mutagenic/genotoxic activity. It is a direct-acting mutagen in the Ames assay, and some reports suggest that addition of S9 liver fraction may increase its mutagenicity (4, 37). Nevertheless, when administered by intrarectal infusion to adult rats or by oral gavage and intraperitoneal injections to newborn mice, 7-OH-IQ does not induce cancer, either in the colon or in sites remote from the digestive tract (37). It therefore seems that, in marked contrast with IQ, 7-OH-IQ would not be genotoxic or carcinogenic in laboratory rodents and would hence be considered a benign metabolite. On the other hand, it has been shown that the intestinal microbiota is essential to the induction of DNA damage by IQ (13, 16), and recent investigations (S. Rabot, F. Kassie, F. Ferk, et al., submitted for publication)
indicate that Bacteroides thetaiotaomicron ATCC 29148 activates an HA mix representative of fried meat to DNA-damaging metabolites in a gnotobiotic rat model. Such contrasting data highlight the necessity of identifying the metabolites produced by bacteria from the main food-borne HAJs in situ in the human colon and of further evaluating their genotoxic/carcinogenic activity.

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