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Biochemical and Molecular Characterization of *Obesumbacterium proteus*, a Common Contaminant of Brewing Yeasts

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We have evaluated the effectiveness of API 20E, Biolog testing, plasmid profiling, ribotyping, and enteric repetitive intergenic consensus (ERIC)-PCR to characterize, classify, and differentiate nine bacterial isolates of the common brewery contaminant *Obesumbacterium proteus*. Of the five typing techniques, Biolog testing, plasmid profiling, and ERIC-PCR provided the most differentiation, and API 20E testing and ribotyping were relatively indiscriminate. The molecular biology approach of ERIC-PCR offered the ideal combination of speed, simplicity, and discrimination in this study. Overall, the results are supportive of the view that *O. proteus* can be subdivided into two biogroups, biogroup 1, which has considerable biochemical and genetic homology to *Hafnia alvei*, and biogroup 2, which is relatively heterogeneous.

Strains of the species *Obesumbacterium proteus* are important beer spoilage organisms (BSO) that grow alongside pitching yeast and can catalyze the formation of N-nitroso compounds in brewery fermentations. They are typically gram-negative, short, fat, pleomorphic rods when grown in brewer's wort with live yeasts and to date have not been isolated outside a brewery environment (6, 20).

O. proteus was for a long time considered to be a normal and harmless component of the brewing process (3) when present at levels approximating 1% of yeast cell numbers. However, this opinion has been significantly altered in the past two decades. *O. proteus* alters the course of a brewery fermentation by directly competing with yeasts for nutrients, resulting in a slower fermentation rate and giving a beer an elevated pH and specific gravity (12). *O. proteus* cannot survive below pH 3.9 and is consequently never present in beer. However, its growth and metabolism within the fermentor result in the release of volatile components such as dimethyl sulfide (4, 28), which impart an undesirable parsnip odor and flavor to the finished product (12, 23). Interest in this relatively uncharacterized bacterium has accelerated over the last 5 years following the discovery of its involvement in the formation of apparent total N-nitroso compounds during fermentation. Consequently, it is now considered necessary for effective control measures to be taken against *O. proteus* contamination (2, 7, 25, 26). Traditionally, this has been achieved by acid washing the yeast prior to fermentation (25, 26). In order to develop effective strategies of control, it is first necessary to detect and characterize the contaminating *O. proteus* strains.

Since its isolation in pure culture in 1936 by Shimwell and Grimes (24), the taxonomic position of *O. proteus* has remained unclear (6). This species was originally termed *Flavobacterium proteus* on the rather spurious basis that it could not be placed in any other taxonomic group, but it later became apparent that *F. proteus* exhibited little homology to other members of the *Flavobacteriaceae*, and in 1964 the genus *Obesumbacterium* was proposed (23). Later studies (21) showed *O. proteus* to be a close relative of many enteric bacteria. Results obtained from numerical taxonomy, gas chromatography ratio analysis, DNA hybridization experiments, and phage typing indicated that *O. proteus* is a heterogeneous species, with most of the isolates falling into two distinct groups, biogroup 1 and biogroup 2. The same studies showed that *O. proteus* biogroup 1 was very closely related to *Hafnia alvei*, and it was concluded that this biogroup was simply a metabolically inactive biogroup of *H. alvei* that had become adapted to a brewery environment (4, 6, 21).

Attempts to adequately differentiate between *O. proteus* strains and between the two biogroups themselves have involved the use of end product gas chromatography (29), polyacrylamide gel electrophoresis (7, 29), API 20E (7, 29), and, more recently, the Biolog GN microplate (7). The results generally reinforce the observation that *O. proteus* is indeed a heterogeneous species. The ability to differentiate between strains within the same biogroup, however, differed greatly depending on the technique used; biochemical testing with Biolog microplates has been the most informative to date. Biolog typing has recently been reviewed by Klingler et al. (13). It is done with a 96-well microplate containing 95 different metabolic substrates and has already been used to successfully differentiate between *Legionella* spp. (18) and *Acinetobacter* spp. (14).

The use of molecular typing methods to characterize and categorize organisms has been commonplace in recent years; plasmid profiling has been used very effectively in many studies to differentiate between environmental isolates of organisms on the basis of the plasmids they contain (16, 17). More recently, with the recruitment of PCR and DNA labelling, novel techniques which enable typing at the level of chromosomal DNA have been developed. Ribotyping (9) makes use of multiple copies of highly conserved rRNA genes that are present in every microorganism; when total chromosomal DNA is cut with restriction enzymes, the pattern of bands hybridizing to probes for the 16S and 23S rRNA genes can be determined. This technique has been widely used in clinical studies to characterize strains of *Salmonella enteritidis* (17), *Enterobacter cloacae* (8), and aeromonads (19). The use of

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TABLE 1. Metabolic profiles of the nine *Obesumbacterium* brewery isolates obtained with the API 20E identification system[a]

Isolate[b]	Positive reactions[c]	Profile[d]	Identification	% Identity[e]
444 (1)	ONPG, ADH, LDC, ODC, VP, GLU, MAN, AMY, NO_2	7105101	*Hafnia alvei*	93.5
451 (2)	ONPG, LDC, VP, GLU	5005000	*Hafnia alvei*	70.8
453 (2), 454 (2), 462 (2)	LDC, ODC, GLU, NO_2	4104000	*Hafnia alvei*	96.2
457 (2)	ADH, GLU	2004000	*Chromobacterium violaceum*	46.0
458 (2)	LDC, VP, GLU, NO_2	4005000	*Hafnia alvei*	62.5
459 (2)	ONPG, LDC, GLU, NO_2	5004000	*Pseudomonas cepacia*	41.4
460 (2)	LDC, ODC, GLU, NO_2	4104000	*Hafnia alvei*	99.8

[a] Each isolate was assessed in duplicate. *H. alvei* gave positive reactions with LDC, ODC, VP, GLU, MAN, rhamnose, L-(+)-arabinose, and NO_2 (see footnote *c* for abbreviations).
[b] Parentheses indicate biogroup number. Strain numbers are all BSO (beer spoilage organism collection of BRF International).
[c] Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; VP, acetoin production; GLU, glucose; MAN, mannitol; AMY, amygdalin; NO_2, reduction of nitrate to nitrite.
[d] Number given by API identification system.
[e] From the API data base; indicates the percent probability that the isolate is that organism.

enteric repetitive intergenic consensus sequences (ERICs) for characterization studies has recently been described (5, 10, 11). The positions of the ERICs are thought to be highly conserved in the genomes of genetically related gram-negative bacteria (11, 30). By use of DNA primers complementary to these sequences, PCR strain-specific DNA fingerprint patterns are produced when the amplified fragments are separated on an agarose gel.

In this study, the ability and comparative effectiveness of five typing methods (API 20E, Biolog, plasmid profiling, ribotyping, and ERIC-PCR) to characterize and differentiate between nine *O. proteus* isolates were assessed. In particular, we were looking to establish routine analyses that, while retaining the taxonomic value of techniques such as Biolog testing, could be performed directly on isolated colonies within 1 day.

MATERIALS AND METHODS

Bacterial strains and growth media. Eight of the nine strains of *O. proteus* were isolated at BRF International from commercial yeast samples from six different United Kingdom breweries and were all of biogroup 2; each strain was assigned a BSO number (7). The ninth isolate was a biogroup 1 strain obtained from the National Collection of Industrial and Marine Bacteria, NCIMB 8771 (BSO 444). All nine strains were gram negative, oxidase positive, and catalase negative, typical of *O. proteus* strains. Allocation of strains to their respective biogroups was carried out on the basis of their colony morphologies; biogroup 2 strains formed small pinpoint colonies on WL (Wallerstein Laboratories) agar (Oxoid); while those of biogroup 1 were much larger. *H. alvei* was the type strain NCTC 6578 (ATCC 9760).

API 20E method. Colonies of the bacteria were grown on WL agar plates for 48 h at 30°C and were characterized with API 20E strips as described in the manufacturer's instructions (API bioMerieux S.A., Marcy l'Étoile, France). The strips were incubated at 30°C for 48 h. The reactions were analyzed with the API profile data base. Each strain was assessed in duplicate.

Biotyping with Biolog GN microplates. Bacterial cells were transferred from lawns and suspended in 20 ml of 0.85% (wt/vol) NaCl to an A_{590} between 0.345 and 0.425. Each of the 96 wells on the microplate (Biolog Inc., Hayward, Calif.) was inoculated with 150 μl of suspension. The plates were incubated in a water-saturated atmosphere at 30°C for 48 h. Positive reactions were recorded by eye. Each strain was assessed in duplicate.

Plasmid profiling. The plasmids were isolated from stationary-phase cultures grown in WL broth at 30°C with shaking (200 rpm) by the small-scale alkaline lysis procedure (22) with two phenol-chloroform steps included. Plasmids were detected by electrophoresis in a 0.8% agarose gel containing 0.5 μg of ethidium bromide per ml and photographed under a UV transilluminator.

Ribotyping. Total DNA was isolated by the cetyl tetraammonium bromide method (1) from cells in late-log phase grown at 30°C with shaking (200 rpm). DNA samples (2 μg) were digested with the restriction enzyme *Eco*RI (Boehringer Mannheim, Lewes, United Kingdom) as described by the manufacturer. The resulting fragments were separated by electrophoresis through a 0.8% agarose gel (containing 0.5 μg of ethidium bromide per ml) in TAE (80 mM Tris-acetate [pH 7.8], 19 mM EDTA) buffer. DNA fragments were transferred to a Hybond N^+ nylon membrane (Amersham International plc) by the alkaline Southern blotting method (22). 16S and 23S rRNA gene probes were produced by labelling 16S and 23S rRNA from *Escherichia coli* MRE 600 (Sigma, Poole, United Kingdom) with digoxigenin (Boehringer Mannheim) and avian myeloblastosis virus reverse transcriptase. Blots were hybridized at 68°C for 48 h, and washes in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate were carried out at 68°C. The hybrids were detected with anti-digoxigenin-alkaline phosphatase antibody conjugate as described in the manufacturer's instructions (Boehringer Mannheim).

ERIC-PCR method. Colony isolates were obtained from fresh WL agar plates incubated at 30°C. The 22-bp oligonucleotide primers used, ERIC 1R and ERIC 2 (30), were synthesized by the biopolymer section of the University of Nottingham.

A colony from an overnight streak plate was transferred into a micro-Eppendorf tube containing 47 μl of PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% [wt/vol] gelatin, 2.5 mM each deoxynucleoside triphosphate) and 1 μg each of the two primers. PCR was performed with a Techne PHC-3 thermal cycler. Following initial denaturation (94°C, 7 min) 1 U of *Taq* polymerase (Perkin-Elmer Cetus) was added, and extension was allowed at 52°C for 1 min. Subsequent cycles (30) were 72°C for 2 min, 94°C for 30 s, 45°C

TABLE 2. Substrate metabolism profiles of *Obesumbacterium* strains incubated at 30°C for at least 48 h[a]

Isolate	Substrates metabolized[b]	% Similarity to *H. alvei*[c]
444	dex, gly, *N*-gal, *N*-glu, L-arab, D-fru, L-fuc, D-gal, α-D-glu, malt, D-mann, D-man, β-met-glu, psi, D-tre, met-pyruv, mono met-succ, acet, form, D-gal-lac, D-gal acid, D-glu acid, D-gluc acid, α-hyd-but, DL-lac, succ acid, bro-succ acid, ala-amide, D-Ala, L-Ala, L-ala-Gly, L-Asp, L-Asn, L-Glu, gly-Glu, L-His, L-Orn, L-Phe, L-Pro, D-Ser, L-Ser, L-Thr, GABA, uro, ino, urid, thymid, put, glycerol, DL-α-gly-phos, g-1-P, g-6-P	65.0
451	D-fru, L-fuc, D-gal, α-D-glu, D-mann, β-met-glu, D-tre, gluc acid, urid, phen-ethyl, g-1-P, g-6-P	17.6
453	dex, *N*-glu, L-arab, D-fru, D-gal, α-D-glu, malt, D-mann, β-met-glu, psi, D-tre, met-pyruv, form, D-gal-lac, D-glu acid, DL-lac, L-Asp, ino, urid, thymid, g-1-P, g-6-P	42.8
454	dex, *N*-glu, L-arab, D-fru, D-gal, α-D-glu, malt, D-mann, β-met-glu, psi, D-tre, met-pyruv, form, D-gluc acid, DL-lac, L-Asp, ino, urid, thymid, g-1-p, g-6-p	40.4
457	dex, *N*-glu, D-fur, D-gal, α-D-glu, D-mann, β-met-glu, D-tre, D-glu acid, gluc-amide, succ acid, ino, thymid, g-1-P, g-6-P	23.0
458	*N*-glu, L-arab, α-D-glu, malt, D-mann, D-tre, D-gluc acid, g-1-P, g-6-P	12.6
459	*N*-glu, L-arab, D-fru, L-fuc, D-gal, α-D-glu, D-mann, β-met-glu, psi, D-tre, form, acet, D-gluc acid, succ acid, ino, thymid, g-1-P, g-6-P	29.0
460	dex, gly, *N*-glu, D-fru, D-gal, α-D-glu, malt, D-mann, β-met-glu, D-tre, met-pyruv, form, D-glu acid, gluc-amide, ino, urid, thymid, g-1-P, g-6-P	31.1
462	*N*-glu, D-fru, D-gal, α-D-glu, malt, D-mann, β-met-glu, D-tre, form, D-gluc acid, thymid, g-1-6, g-6-P	19.4

[a] Each strain was assessed in duplicate.

[b] Abbreviations: dex, dextrin; gly, glycogen; *N*-gal, *N*-acetyl-D-galactosamine; *N*-glu, *N*-acetyl-D-glucosamine; L-arab, L-arabinose; D-fru, D-fructose; L-fuc, L-fucose; D-gal, D-galactose; α-D-glu, α-D-glucose; malt, maltose; D-mann, D-mannose; D-man, D-mannitol; β-met-glu, β-methylglucoside; psi, psicose; D-tre, D-trehalose; met-pyruv, methyl pyruvate; mono met-succ, monomethyl succinate; acet, acetic acid; form, formic acid; D-gal-lac, D-galactonic acid lactone; D-gal acid, D-galacturonic acid; D-glu acid, D-gluconic acid; D-gluc acid, D-glucuronic acid; α-hyd-but, α-hydroxybutyric acid, DL-lac, DL-lactic acid; succ acid, succinic acid; bro-succ acid, bromosuccinic acid; gluc-amide, glucuronamide; ala-amide, alanilamide; D-Ala, D-alanine, L-Ala, L-alanine; L-ala-Gly, L-alanyl-glycine; L-Asp, L-aspartine; L-Asn, L-asparagine; L-Glu, L-glutamic acid; gly-Glu, glycyl-L-glutamic acid; L-His, L-histidine; L-Orn, L-ornithine; L-Phe, L-phenylalanine; L-Pro, L-proline; D-Ser, D-serine; L-Ser, L-serine; L-Thr, L-threonine; GABA, γ-aminobutyric acid; uro, urocanic acid; ino, inosine; urid, uridine; thymid, thymidine; phen-ethyl, phenylethylamine; put, putrescine; DL-α-gly-phos, DL-α-glycerol phosphate; g-1-P, glucose-1-phosphate; g-6-P, glucose-6-phosphate. The *H. alvei* strain metabolized all but 16 of the substrates. The substrates which were not metabolized were itaconic acid, α-ketobutyric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, L-aspartic acid, hydroxy-L-proline, L-leucine, L-pyroglutamic acid, DL-carnitine, phenylethylamine, 2-aminoethanol, and 2,3-butanediol.

[c] Percent similarity to *H. alvei* = [number of positive reactions in common with *H. alvei*/(total number of positive reactions − number of reactions in common)] × 100.

for 1 min, and a final extension cycle at 72°C for 4 min. The amplified products (20 μl) were then separated in a 4% Nusieve 3:1 agarose gel (FMC Bioproducts, Sittingbourne, United Kingdom) containing 0.5 μg of ethidium bromide per ml and photographed under a UV transilluminator.

The statistical relationship between the results obtained from Biolog and ERIC-PCR analyses was determined with the StatWorks software package for the Apple Macintosh.

RESULTS AND DISCUSSION

API 20E. The results of the API tests (Table 1) generally indicate that the nine organisms tested are related to *H. alvei*. Four of the eight biogroup 2 isolates appear more closely related to *H. alvei* than does the biogroup 1 isolate (BSO 444); biogroup 2 isolates 453, 454, and 462 produce identical profiles. All the isolates were capable of metabolizing glucose and, with the exception of BSO 457, tested lysine decarboxylase and NO_2 positive. These results, however, conflict with previous experimental conclusions regarding the taxonomic relationship of the two biogroups to *H. alvei* (4, 6, 21); this, together with the limited number of positive reactions registered, emphasizes the observation that API 20E strips should not be used as a method of classifying *O. proteus*. This is in broad agreement with the conclusions reached by Fernandez et al. (7).

Biolog. The results from the Biolog analysis are shown in Table 2. For a positive reaction, sufficient reducing power must be generated to reduce the colorless tetrazolium violet present in each well to a purple formazan. The results show that, as with the API experiments, the one biogroup 1 strain tested utilized a much wider range of substrates than the biogroup 2 isolates, indicating fundamental differences in cell biochemistry between the two biogroups. The positive reactions obtained from BSO 444 (biogroup 1) were more intense (a deeper purple coloration) than those of biogroup 2, which were often difficult to see, with biogroup 2 isolates having to be incubated for longer periods of time (up to 72 h). *H. alvei* gave positive reactions with 80 of the 95 substrates, 52 of which were also shared by biogroup 1 (BSO 444), giving an overall similarity of 65%. A much lower level of similarity was found with the biogroup 2 isolates, which metabolized between 9 and 22 of the 95 substrates. Some substrates were found to be utilized by all of the isolates (notably D-trehalose, D-fructose, D-galactose, α-D-glucose, mannose, and D-gluconic acid), which is consistent with the isolation of the strains from a largely carbohydrate-rich environment. Table 3 shows the percentage similarity of the isolates to each other on the basis of their metabolic profiles and emphasizes the high degree of differentiation among the nine isolates achieved by the Biolog microplates.

TABLE 3. Comparison of the metabolic profiles of the nine brewery isolates by Biolog microplates

Species or isolate	% Similarity to isolate[a]:								
	444	451	453	454	457	458	459	460	462
H. alvei	65.0	17.6	42.8	40.4	23.0	12.6	29.0	31.1	19.4
444		18.2	42.5	40.7	25.9	17.0	31.5	33.3	24.5
451			33.3	34.6	50.0	31.3	55.6	47.6	47.1
453				95.8	50.0	37.5	50.0	72.0	54.1
454					52.0	39.0	51.8	75.0	56.5
457						41.1	57.1	70.0	64.7
458							35.0	42.1	57.1
459								54.1	55.0
460									68.4

[a] Percent similarity = [number of reactions in common/(total number of reactions − number of reactions in common)] × 100.

These results are consistent with those obtained by Fernandez et al. (7).

Plasmid profiles. Figure 1 shows that the isolates can be discriminated from each other on the basis of their individual plasmid profiles. No distinctive grouping pattern is obvious, however, and none of the isolates resembled the profile obtained from the *H. alvei* type strain (lane 2), which produced only one plasmid, with a mobility of around 3 kb. Large plasmids with a size greater than the 23.1-kb λ *Hin*dIII marker were extracted from all of the isolates. Very similar profiles were obtained from isolates 453 and 454 (lanes 5 and 6), and a high degree of similarity to these was demonstrated by isolate 460, which shared plasmids of sizes 7 and 3.5 kb, and by isolates 451, 459, and 462, which shared a plasmid of around 15 kb. Taken as a whole, plasmid profiling indicates a high degree of heterogeneity among the nine strains, which could be helpful for tracking individual strains in a particular brewery environment. As a marker for the classification of *O. proteus*, however, it is unhelpful.

Ribotyping. The rRNA gene hybridization patterns of the isolates are shown in Fig. 2. The results unequivocally demonstrate that the isolates are split into two groupings. All of the biogroup 2 isolates (lanes 3 to 9) produced identical ribotyping patterns, with the single biogroup 1 strain (lane 2) showing a typing profile closely similar to that of *H. alvei*. Although the overall number of isolates tested is small and contains only a single representative of biogroup 1, it appears that ribotyping could offer an unequivocal molecular discrimination for biogroup 1 and biogroup 2, a process which is currently based on colony morphology. In any event, the consistent profile among the eight biogroup 2 isolates offers potential for strain classification into biogroup 2.

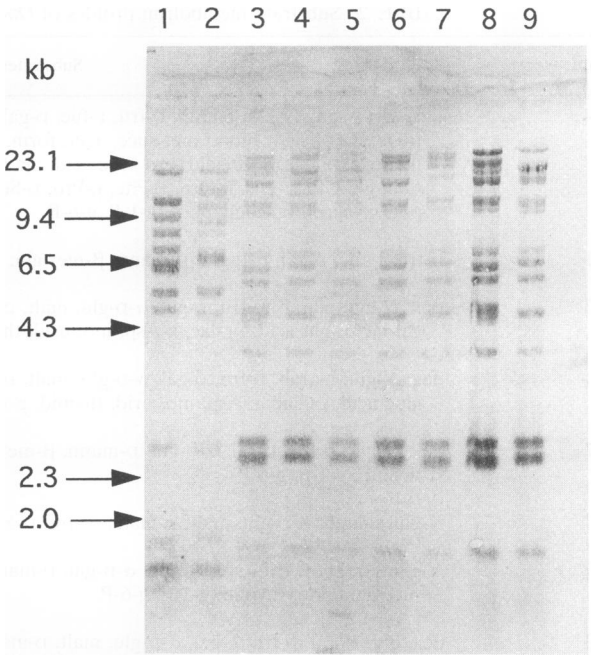

FIG. 2. Southern hybridization of *E. coli* MRE 600 rRNA probe to *Eco*RI-digested genomic DNA from *H. alvei* and nine *O. proteus* strains. Lanes: 1, *H. alvei*; 2, *O. proteus* biogroup 1 (BSO 444); 3 to 9, *O. proteus* biogroup 2 strains (3, BSO 451; 4, BSO 453; 5, BSO 454; 6, BSO 457; 7, BSO 458; 8, BSO 459; 9, BSO 460).

ERIC-PCR. The results of PCR with the ERIC primers are shown in Fig. 3. The isolates were classified according to the

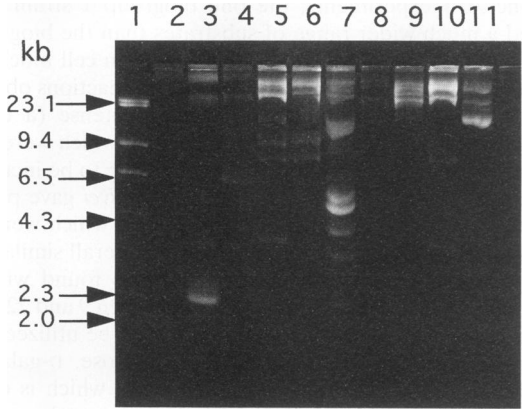

FIG. 1. Plasmid profiles of *H. alvei* and of *O. proteus* strains. Lanes: 1, molecular size marker (λ *Hin*dIII DNA); 2, *H. alvei*; 3, *O. proteus* biogroup 1 (BSO 444); 4 to 11, *O. proteus* biogroup 2 strains (4, BSO 451; 5, BSO 453; 6, BSO 454; 7, BSO 457; 8, BSO 458; 9, BSO 459; 10, BSO 460; 11, BSO 462).

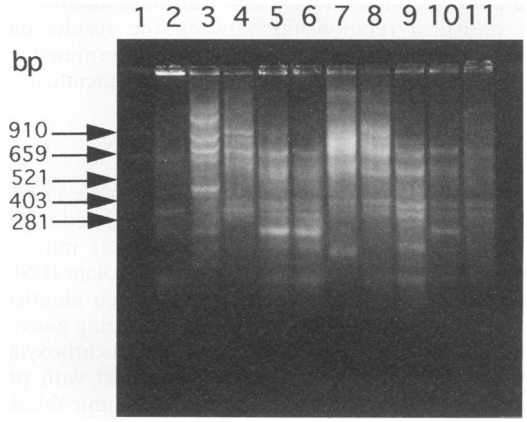

FIG. 3. ERIC-PCR fingerprint patterns of *H. alvei* and of *O. proteus* strains. Lanes: 1, molecular size marker (pBR322 digested with *Alu*I); 2, *H. alvei*; 3, *O. proteus* biogroup 1 (BSO 444); 4 to 11, *O. proteus* biogroup 2 strains (4, BSO 451; 5, BSO 453; 6, BSO 454; 7, BSO 457; 8, BSO 458, 9, BSO 459; 10, BSO 460; 11, BSO 462).

TABLE 4. Comparison of ERIC-PCR profiles obtained from *H. alvei* and the nine brewery isolates

Species or isolate	% Similarity to isolate[a]:								
	444	451	453	454	457	458	459	460	462
H. alvei	46.1	50.0	60.0	60.0	30.7	50.0	54.5	60.0	54.5
444		46.1	41.6	41.6	28.5	46.1	28.5	41.6	50.0
451			60.0	60.0	70.0	100	54.5	54.5	88.8
453				100	50.0	60.0	66.6	100	66.6
454					50.0	60.0	66.6	100	66.6
457						70.0	45.0	50.0	60.0
458							54.5	60.0	88.8
459								66.6	60.0
460									66.6

[a] Percent similarity = [number of PCR fragments in common/(total number of PCR fragments − number of PCR fragments in common)] × 100.

method of de Bruijn (5). The results from the agarose gel were converted into a binary matrix; isolates which had a PCR product of a particular size were given a score of 1, and those that did not were scored 0. The profiles shown in Fig. 3 and the derived results shown in Table 4 indicate that a high degree of similarity exists among the biogroup 2 isolates, with 6 discrete fragments being present in every profile. The profiles of isolates 453, 454, and 460 were identical, as were those of isolates 451 and 458.

Collectively, the five typing methods used to characterize nine strains of *O. proteus* confirm the existence of two distinct biogroups. Consistent with previous studies, the biogroup 1 isolate showed a closer relationship to *H. alvei* than did the eight biogroup 2 isolates. The ability to discriminate between isolates beyond that of the biogroup varied depending on the typing method used. For the biogroup 2 isolates, ribotyping failed to show any diversity but emphasized an underlying homogeneity. Similarly, the technique emphasized the genetic similarity of biogroup 1 and *H. alvei.*

API 20E strips provided very poor discrimination among biogroup 2 isolates; differences within biogroup 2 were evident, however, from plasmid profiling, Biolog, and ERIC-PCR. All three techniques highlighted a close relationship among isolates 453, 454, 460, and 462. Interestingly, there is no statistical relationship between the percentage similarities of the nine isolates obtained by Biolog and those obtained by ERIC-PCR. Simple regression analysis of the data presented in Tables 3 and 4 gave an r^2 of 0.28. Thus despite the high degree of differentiation achieved by both techniques, no concordance per se between gene expression (Biolog) and DNA (ERIC-PCR) was apparent.

Although the discriminatory power of ribotyping is likely to increase if the number of restriction enzymes used is increased, it is a poor candidate for the identification of *O. proteus* strains in a brewery environment, which demands a simple, easy to use, cost-effective approach; ribotyping is none of these. Both plasmid profiling and Biolog procedures are very effective in differentiating between isolates but require an initial purification step from mixed culture which is labor- intensive and time-consuming. There is no doubt that ERIC-PCR is by far the preferred analysis method; it shares the discriminatory powers of Biolog and plasmid profiling but does not require a 2-day incubation and does not present the difficulties encountered by plasmid extraction. By use of a microtiter format, 96 isolates could be analyzed in 4 to 5 hours.

Overall, this study extends the characterization of *O. proteus* by detailed genetic fingerprinting and compares these data with those obtained by existing biochemical techniques. The conclusions are highly supportive of the existing classification of the genus into two biogroups and add new genetic data to the assignment of biogroup 1 to a subspecies of *H. alvei* (6). In addition, the technique of ERIC-PCR is shown to be an important tool for the rapid classification of new isolates of *O. proteus.*

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