Taxonomy of the Bacteroides

II. Correlation of Phenotypic Characteristics with Deoxyribonucleic Acid Homology Groupings for *Bacteroides fragilis* and Other Saccharolytic *Bacteroides* Species

J. L. JOHNSON¹ AND D. A. AULT²

Anaerobe Laboratory¹ and Department of Computer Science,² Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Two hundred ninety strains of *Bacteroides*, each belonging to *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus*, *B. uniformis*, *B. vulgatus*, *B. distasonis*, or *B. eggerthii*, or to one of three unnamed taxa designated "*B. fragilis* subsp. a," "3452-A," and "T4-1," were used to compare the patterns of 34 phenotypic traits with deoxyribonucleic acid (DNA) homology groupings. The responses for all but 13 of the traits were very similar for all of the DNA homology groups. The responses for the 13 traits (the production of indole, catalase, and hydrogen, and acid production from arabinose, cellobiose, melibiose, melezitose, raffinose, rhamnose, ribose, salicin, sucrose, and trehalose) varied between the various homology groups. Similarity coefficients, for which all of the strains were compared with the DNA reference strains, were calculated. Similarity coefficient averages of strains within a given DNA homology group with the DNA reference strains of that group ranged from 83 to 94%. Organisms having about 60 to 65% DNA homology to each other were in most cases also phenotypically very similar. In a couple of instances, however, homology groups with strains having 72 to 95% intrastain homology were quite variable with respect to certain phenotypic traits. There was no general pattern between levels of intergroup homologies and phenotypic properties. For example, *B. eggerthii* and the "subsp. a" homology groups, having about 50% intergroup homology, had average intergroup phenotypic similarity values of 65 and 69%, whereas *B. ovatus* and *B. thetaiotaomicron* homology groups, having only about 35% intergroup DNA homology, had intergroup similarity values that ranged from 86 to 93%. Similarity coefficients between homology groups having low levels of DNA homology ranged from 38 to 78%. Response probability values for the phenotypic traits were estimated for strains belonging to each of the DNA homology groups. The probability values obtained with 20 to 40 strains were not altered significantly by including more strains. A test identification matrix was constructed using the probability values of the 13 phenotypic traits listed above. On the basis of these traits, 93% of the strains could be assigned to the correct homology group if groups having 60% or greater intragroup homology were considered to be phenotypically the same.

The organisms included in this study are saccharolytic bacteroides that have many phenotypic properties in common. They were identified by deoxyribonucleic acid (DNA) homology studies as members of *B. fragilis*, *B. thetaiotaomicron*, *B. ovatus*, *B. uniformis*, *B. vulgatus*, *B. distasonis*, or *B. eggerthii*, or of three unnamed taxa designated "subsp. a," "3452-A," and "T4-1." All were anaerobic, gram-negative, nonmotile, nonsporing rods that had a similar morphology and produced succinic acid as a major product from the fermentation of glucose. None produced pigmented colonies on blood agar. All grew well in media containing 20% bile and appeared to have a requirement for or were

greatly stimulated by heme. All but some of the *B. distasonis* strains grew well on a mineral salts-glucose medium that was supplemented with heme and vitamin B₁₂ or methionine (30). The cells of all of the organisms were readily lysed with 1% sodium lauryl sulfate, and procedures for the isolation of their DNA were not complicated by the presence of endogenous nucleases.

In recent years there have been many reports concerning the identification of bacteria by the use of the probability of occurrence of various phenotypic properties (2, 10, 12, 20, 25, 27). However, in the majority of these studies the organisms used in the data base were themselves

identified using phenotypic tests. Therefore it is difficult to know whether the reported variable characteristics represent variations among genetically similar organisms or represent traits by which genetically diverse organisms can be distinguished. One way to overcome this problem is to correlate the observed phenotypic properties with the results from DNA homology studies since the DNA homology results provide a measure of overall relatedness among organisms.

There have been only a few studies in which phenotypic properties have been numerically correlated with DNA homology results. Colwell (9) determined probability values for phenotypic traits among strains in the *Vibrio cholerae* and *Vibrio parahaemolyticus* homology groups (8) and found that phenotypic similarity values correlated with the DNA homology values. Brenner et al. (6) determined the correlation between phenotypic similarity values and DNA homology values for strains of *Escherichia coli* and for one strain each of *Shigella flexneri* and *Salmonella typhimurium*. Palleroni et al. (23) compared DNA homology results and similarity coefficients within and among species of *Pseudomonas*.

The purpose of the present investigation was to determine the correlations between phenotypic properties and DNA homology results among some of the saccharolytic bacteroides. The organisms used to establish the phenotypic data base have been grouped by DNA homology (18). Accepting these results as the correct classification of the organisms, we have: (i) determined similarity coefficients (S_j) of all of the organisms and the DNA reference strains; (ii) estimated probability values for 34 phenotypic traits at various levels of DNA homology; (iii) determined the number of strains required to stabilize probability values; (iv) calculated the joint probability values for the phenotypic traits; and (v) constructed an identification matrix from the probability values and developed a test identification program.

MATERIALS AND METHODS

Bacterial strains. The sources of the strains used in this study and their individual genetic relationships, as measured by DNA homology experiments, were given in detail in the first paper of this series (18).

Phenotypic tests. The 34 phenotypic traits used in this study are listed in Table 1. Gelatin liquefaction, meat digestion, milk fermentation and digestion, nitrate reduction, growth in bile, and the production of indole and hydrogen were all determined by W. E. C. Moore by using methods previously described (16, 21). The method of determining catalase production has also been previously described (T. D. Wilkins, B. J. Veltri, Jr., D. L. Wagner, and E. L. Gregory, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C173, p. 64).

The hydrolysis and/or fermentation of glucose-1-phosphate and the fermentations of carbohydrates and sugar alcohols were determined by T. D. Wilkins by using a micromethod described previously (31, 34). The media used in the micromethod were modified in that they did not contain Tween 80. A fermentation was considered strongly positive if the final pH of the medium was 5.5 or below and weakly positive if the final pH was between 5.6 and 6.0. Reactions above pH 6.0 were considered negative.

Computer methods. Analysis of the data was carried out by means of a series of computer programs. The results of the laboratory tests were collected for each organism and entered into a record for that organism. Tabulated material for each organism included the strain number; codes to identify the organism by genus, species, and homology group; the moles percent guanine plus cytosine (mol% G+C); and a coded response level of either strong or weak fermentation, no fermentation, or no test results for each of the 34 phenotypic traits. Many characteristics, such as "anaerobic," "gram-negative," "nonmotile," "nonspor-ing," "nonpigmenting rod," "succinic acid production," etc., were not tabulated or coded because this group of strains had been preselected to contain organisms that had uniform responses for these tests.

The first computer program, written in the Statistical Analysis System (SAS) language (1), provided a preliminary testing for errors in the data transcription process. A check on the range of G+C values and the recorded result for each of the phenotypic traits provided for the detection of data-recording errors which showed up as symbols out of the range of acceptable values. Recording errors which were in the acceptable range but which were still incorrect were much harder to locate. A listing of the data in sorted order by homology group was used to locate these errors by looking for response codes that deviated from the majority of responses for a particular trait. The data for strains showing these deviations were first compared with the original data report to check for coding errors. If the coding was correct, the strains were then retested for those traits. These repeated tests usually confirmed that the original data were correct.

The second and third programs were written in programming language 1 (PL/1) (3). The second program calculated the percent of strongly positive responses and the percent of positive (strong plus weak positives) responses for each of the phenotypic tests in each homology group (see Table 1). Also estimated for each pair of phenotypic tests was the joint probability that an organism from a given homology group will show a strongly positive response for both tests.

To perform the two above-mentioned analyses, the data were sorted by homology group, and the data for each homology group were analyzed separately. For each homology group, the percent of a strongly positive (or positive) reaction for each test was calculated by making a count of the number of organisms with a strongly positive (or positive) reaction for this test and dividing this count by the total number of organisms in the homology group tested for this trait. As these counts were being determined, the number of strains needed to produce stabilized probability estimates, i.e., a probability estimate that will not change signifi-

cantly even if more organisms are examined, was estimated by printing the quotient of the two partial sums at regular intervals. This permitted determination of the number of strains needed by an examination of the rate at which the probability estimate for each trait approached a consistent value. To estimate the joint probability for each pair of phenotypic traits, the quotient of the number of pairs of phenotypic tests which showed both strong (positive) reactions and the number of strains that had been tested for both traits were calculated. Since the resulting matrix is symmetrical and its major diagonal contains estimates of the individual probabilities of a strongly positive (or positive) reaction, only the lower triangular matrix actually needed to be calculated.

The third program was designed to identify strains by homology group by means of their phenotypic traits. Two submatrices (see Tables 5 and 6) were prepared from the phenotypic responses (see Table 1). Traits that did not distinguish between homology groups were not included, i.e., the test showed the same reaction for all homology groups. The probability values represented the fraction of strains positive for a given trait: in the cases where all strains were positive or negative, values of 0.99 or 0.01, respectively, were used. The analysis followed the method described by Lapage et al. (20). This method can be described briefly as follows. To classify a given organism, the product of the probabilities or of their reciprocals (i.e., one minus the probability) was found for each homology group. The choice of using the probability or its reciprocal depended upon the nature of the response of the unknown strain to the particular test. If the test showed strongly positive (or positive), then the corresponding probability value from the table was used. If the response was not strongly positive (or positive), then the reciprocal was included in the product. Each of these products for the homology groups was normalized by following the method of Dybowski and Franklin (10). The highest possible product was calculated for each homology group, and the ratio of the first product to the corresponding highest product (likelihood value) was found for each homology group. The homology group chosen as the most likely one to which the unknown strain belonged was the group with the highest likelihood value. Once the most likely homology group was chosen, the traits of that homology group that did not agree with the test results for the sample strain were determined. The identification process on the organism was concluded by comparing the predicted homology group and the actual homology group of the organism as determined by DNA homology experiments. The frequency of correct identifications by homology group and for all homology groups together were tallied, and the percentage correct was calculated at the end of the program.

The fourth program determined the maximum, average, and minimum S_j values by comparing all of the strains within each homology group with each of the DNA reference strains. The S_j value between two strains was calculated as the ratio of the number of the 34 phenotypic traits for which both strains produced a strong (positive) reaction divided by the number of 34 phenotypic traits for which at least one of the strains produced a strong (positive) reaction (28).

RESULTS

The majority of the DNA homology groups used in this study have species rank and names (7, 16, 31). The characteristics of the organisms in the homology group here referred to as "subsp. a" were previously described by Moore and Holdeman (21) for a group they called "*B. fragilis* subsp. a." Two of the DNA homology groups that have not been phenotypically described previously are herein designated as homology groups "3452-A" and "T4-1." According to the criteria used for reinstating the species rank for *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*, and *B. distasonis* (7), these three groups also warrant species rank. However, because species descriptions and proposals of names are beyond the scope of this paper, the above-mentioned homology group designations will continue to be used here.

Phenotypic properties. The results of the phenotypic tests used in this study are listed in Table 1 and are recorded as percent strongly positive (S) and percent positive (P = strong plus weak) for the strains in each DNA homology group. The strains in each of the different groups are given in reference 18.

Of the 34 phenotypic traits analyzed in this study (Table 1), 17 gave nearly identical results with organisms in all of the DNA homology groups, and an additional 5 traits differed in only one or two of the homology groups. Only 13 (about 38%) of the phenotypic traits varied significantly among the several DNA homology groups. The traits with the most variation among the groups were the production of indole, catalase, and hydrogen, and the production of acid from arabinose, cellobiose, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sucrose, and trehalose.

From results obtained in this study, it appears that organisms having 60% or greater DNA homology to a given reference strain are all very similar with respect to these phenotypic properties. This can most easily be seen by comparing the similarity coefficients within the subgroups of the various species (Table 2). For example, the phenotypic properties of strains belonging to the two *B. fragilis* DNA homology groups (groups I and II in Tables 2 and 4 of reference 18) are nearly the same. In the case of the *B. ovatus* strain (Table 6 of reference 18), 20 of the strains have high homology to the DNA reference strains 0038 and 3524 (homology group I, Table 2). The rest of the *B. ovatus* strains are rather heterogeneous; some had high homology to a third DNA reference organism (C11-2), but others had only 60 to 70% DNA homology to all three reference strains (homology group II) (Ta-

TABLE 1. The percentages of strongly positive and positive (strong and weak) reactions of bacteroides in various homology groups

Homology group	No. of strains	Type of reaction	Amygdalin 1 ^a	Arabinose 2	Cellobiose 3	Esculin hydrolysis 5	Fructose 6	Glycogen 8	Mannitol 12	Mannose 13	Melezitose 14
<i>B. fragilis</i> I	65 ^b	%S ^c %P	85 100	4 12	15 87	100 100	100 100	90 98	0 1	98 100	0 3
<i>B. fragilis</i> II	14	%S %P	79 100	0 8	0 50	93 93	100 100	93 100	7 7	93 93	7 14
<i>B. vulgatus</i> I	44	%S %P	85 88	100 100	6 10	36 63	98 100	85 98	0 4	100 100	4 8
<i>B. vulgatus</i> II	1	%S %P	100 100	100 100	0 0	100 100	100 100	100 100	0 0	100 100	0 0
<i>B. distasonis</i>	15	%S %P	93 100	14 28	100 100	93 93	87 100	0 0	0 0	100 100	100 100
'T4-1'	10	%S %P	60 100	10 10	0 20	40 100	100 100	0 11	0 0	90 100	10 80
'3452-A'	21	%S %P	91 100	100 100	32 86	91 95	100 100	4 4	0 0	100 100	91 95
<i>B. ovatus</i> I	24	%S %P	96 96	100 100	100 100	100 100	100 100	61 65	13 17	100 100	91 91
<i>B. ovatus</i> II	16	%S %P	94 100	94 100	94 100	100 100	100 100	94 100	13 31	100 100	94 100
<i>B. thetaiotaomicron</i> I	29	%S %P	100 100	100 100	90 97	100 100	100 100	100 100	3 3	100 100	90 93
<i>B. thetaiotaomicron</i> II	2	%S %P	100 100	100 100	100 100	100 100	100 100	100 100	0 0	100 100	100 100
<i>B. thetaiotaomicron</i> III	2	%S %P	100 100	100 100	100 100	100 100	100 100	50 50	0 0	100 100	100 100
<i>B. uniformis</i>	24	%S %P	100 100	100 100	96 96	96 96	100 100	91 100	4 4	100 100	4 34
'subsp. a'	17	%S %P	41 82	35 35	6 6	82 100	94 100	76 82	0 0	88 100	0 0
<i>B. eggerthii</i>	6	%S %P	14 57	100 100	16 83	100 100	0 83	100 100	0 0	100 100	0 0

ble 6 of reference 18). The phenotypic properties of these two groups were very similar (Table 2). Similar results were obtained within the species *B. vulgatus* and *B. thetaiotaomicron* (Table 2).

For groups of organisms having lower levels (generally lower than 60%) of intergroup DNA homology, one could not predict what the correlation between DNA homology and phenotypic similarity coefficients would be. Strains of "subsp. a" and *B. eggerthii* have average intergroup DNA homology levels of 54% (49 to 62% range) (Tables 9 and 10 of reference 18). Although the highest of these intergroup homology values were slightly above 60%, the intergroup average S_i values were only 65 and 69%, well within the range of others that have little or no DNA homology. Strains of *B. uniformis* averaged 36% intergroup homology with strains of

"subsp. a" and *B. eggerthii*, but the S_i values were higher with the reference strains of *B. ovatus* and *B. thetaiotaomicron* (Table 2). The *B. uniformis* strains have DNA with a G+C content that is 4% higher than those from *B. thetaiotaomicron* and *B. ovatus* and have only low levels of homology with them. Strains of *B. thetaiotaomicron* and *B. ovatus* have about 35% intergroup homology. In this case the similarity coefficients between the groups are nearly as high as those within the groups (Table 2).

Probability values. The number of strains required for a stable probability value within a DNA homology group was estimated by calculating the percentage of positive reactions with five strains and then increasing this number by increments of five and recalculating until all of the strains within a homology group were in-

TABLE 1. *Continued*

Melibiose 15	Raffinose 16	Rhamnose 17	Ribose 18	Salicin 19	Sorbitol 20	Starch pH 21	Sucrose 22	Trehalose 23	Xylose 24	Gelatin 25	Indole 28	Catalase 30	Bile Growth 31	Glucose-1-PO ₄ 32	Glycerol 33	H ₂ Production 34
98	100	5	3	1	3	100	100	0	96	4	0	100	95	86	9	0
98	100	6	8	1	6	100	100	1	98	6	0	100	100	100	12	97
100	100	0	7	7	0	100	100	0	100	0	0	100	100	83	14	0
100	100	0	7	7	0	100	100	0	100	0	0	100	100	100	14	82
94	98	96	13	6	0	100	100	2	98	62	0	0	98	79	0	0
98	98	98	38	13	2	100	100	8	100	66	4	0	100	100	0	100
100	100	100	100	0	0	100	100	0	100	100	0	100	100	0	0	0
100	100	100	100	0	0	100	100	0	100	100	0	100	100	0	0	100
100	100	100	71	100	0	100	100	100	93	14	0	100	93	71	14	0
100	100	100	71	100	0	100	100	100	100	21	0	100	100	100	36	0
100	90	100	80	0	11	60	100	90	100	10	0	0	100	33	0	0
100	100	100	90	40	11	100	100	100	100	30	0	0	100	89	0	0
100	100	59	95	73	0	50	100	100	100	5	0	5	95	80	14	0
100	100	64	100	91	0	86	100	100	100	5	0	5	100	90	23	100
100	96	96	96	96	4	100	100	96	96	5	95	77	95	95	5	0
100	96	96	96	96	17	100	100	96	96	23	100	77	95	95	5	100
100	100	94	94	100	6	100	100	94	100	50	81	28	81	92	6	6
100	100	100	100	100	19	100	100	94	100	63	88	28	94	100	6	100
100	97	97	97	14	3	100	100	100	100	7	96	79	96	93	4	0
100	100	97	97	83	3	100	100	100	100	21	100	79	100	100	4	100
100	100	100	100	0	0	100	100	100	100	0	100	100	100	100	0	0
100	100	100	100	100	0	100	100	100	100	0	100	100	100	100	0	100
100	100	100	50	100	0	100	100	100	100	0	50	50	100	100	50	50
100	100	100	50	100	0	100	100	100	100	0	50	50	100	100	50	100
100	96	34	35	100	8	100	96	54	100	11	91	4	33	72	8	0
100	96	34	46	100	12	100	100	79	100	18	95	4	91	92	12	88
6	94	94	47	6	0	94	94	0	94	12	88	0	100	75	0	0
12	100	94	47	6	0	100	100	0	100	24	94	0	100	100	0	0
0	0	100	0	0	0	100	0	0	100	50	100	16	86	0	0	0
0	0	100	0	0	0	100	0	0	100	66	100	100	100	100	0	0

^aTest numbers.^bTo determine which strains are included in each group, see Tables 4–12, Ref. 18.^c%S = percent strongly positive; %P = percent positive.^dTests not included in the Table are: acid production from glucose, maltose and lactose which were nearly 100% strongly acid; acid production from erythritol and inositol which were nearly all negative; most of the strains produced an acid clot in milk and occasional strains weakly digested casein; nearly all of the strains did not digest meat or reduce nitrate.

cluded. The results for the *B. uniformis* homology group are shown in Table 3. Although this is not one of the larger homology groups, it was selected for this illustration because it has the greatest number of phenotypic traits that are not nearly all positive or all negative. By using the production of strong acid in ribose (Table 3) as an example, 60% of the first five strains gave a strong acid reaction, but when all 23 strains were included, the number of strains that gave a strong acid reaction stabilized near 34%. The

percentage of strains giving a strong positive reaction for fermentation of rhamnose decreased from 40 to 34 with an increasing number of strains. Most of the strains hydrolyzed esculin, and a strain negative for this trait appeared only among the first five strains. In similar analysis of species with more strains, such as *B. fragilis* group I (62 strains), it appeared that the results from testing 40 strains gave a good indication of the amount of variation for the species because the percentage of positive reactions observed did

TABLE 2. Average similarity coefficients between *Bacteroides* strains in each of the DNA homology groups and the DNA reference strains for all the DNA homology groups

Homology group	No. of strains	<i>B. fragilis</i>		<i>B. vulgatus</i>		<i>B. distasonis</i>	'T4-1' '3452-A'			<i>B. ovatus</i>		<i>B. thetaiotaomicron</i>		<i>B. uniformis</i>		'subsp. <i>B. eggerthii</i>	
		I	II	I	I		I	I	II	I	II						
		2553	2393	4245	B2-4	4243	T4-1	3452-A	0038	3524	C11-2	5482	2302	0061	C20-25	B5-21	B8-51
<i>B. fragilis</i> I	62	94 ^a	88	74	67	64	64	56	63	66	55	66	69	62	63	67	46
<i>B. fragilis</i> II	14	93	89	73	67	61	65	56	62	64	56	64	66	64	63	68	46
<i>B. vulgatus</i> I	46	73	69	84	83	56	72	64	62	65	63	69	67	65	71	69	55
<i>B. vulgatus</i> II	1	79	75	79	74	65	70	67	72	75	67	83	78	68	68	65	55
<i>B. distasonis</i>	15	65	62	66	56	90	71	69	80	83	75	77	79	63	65	53	38
'T4-1'	10	61	59	71	62	63	83	73	58	60	59	61	63	52	54	61	42
'3452-A'	21	60	58	72	60	74	73	84	74	77	76	72	75	67	64	52	41
<i>B. ovatus</i> I	23	63	61	66	59	81	66	70	90	93	87	86	89	76	76	58	48
<i>B. ovatus</i> II	16	61	59	66	61	76	65	69	87	90	89	86	86	78	78	60	49
<i>B. thetaiotaomicron</i> I	29	68	65	69	65	77	69	73	90	93	87	92	96	75	75	63	52
<i>B. thetaiotaomicron</i> II	2	70	67	70	65	79	70	74	92	96	88	96	100	75	75	64	52
<i>B. thetaiotaomicron</i> III	2	62	60	70	62	79	66	70	85	88	84	81	84	72	75	57	46
<i>B. uniformis</i>	24	65	62	69	63	63	60	58	76	79	83	72	75	87	87	62	51
'subsp. a'	17	65	62	70	64	52	64	59	60	62	61	63	65	62	66	84	65
<i>B. eggerthii</i>	6	48	46	54	57	41	40	43	49	51	49	54	53	49	56	69	92

^aSimilarity coefficients (SJ). Averages X 100.TABLE 3. Variations in the percent of *B. uniformis* organisms that are positive for the various phenotypic traits as the number of strains analyzed is increased by increments of five

Response	Cellobiose 5	Esculin hydrolysis 5	Glycogen 8	Mannitol 12	Melzeritose 14	Raffinose 16	Rhamnose 17	Ribose 18	Sorbitol 20	Sucrose 22	Trehalose 23	Celatin 25	Indole 28	Nitrate 29	Bile growth 31	Glucose-1-PQ ₄ 32	Glycerol 33	H ₂ Production 34
Frequency	5 ^a	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
% Strong	80	80	100	0	0	100	40	60	0	100	60	20	80	0	40	75	0	0
% Positive	80	80	100	0	0	100	40	60	0	100	60	40	100	20	100	100	0	80
Frequency	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10
% Strong	90	90	90	0	0	100	30	40	0	90	50	30	90	0	40	55	0	0
% Positive	90	90	100	0	0	100	30	40	0	100	80	40	100	10	100	100	0	90
Frequency	15	15	14	15	14	15	14	14	15	15	15	15	15	14	15	13	14	13
% Strong	93	93	92	0	7	93	35	42	7	93	53	20	93	0	40	69	0	0
% Positive	93	93	100	0	28	93	35	50	7	100	80	26	100	7	93	100	14	92
Frequency	20	20	19	20	19	20	19	19	19	20	20	20	20	19	20	18	18	18
% Strong	95	95	89	0	5	95	36	36	5	95	55	15	95	0	30	72	0	0
% Positive	95	95	100	0	36	95	36	47	10	100	80	20	100	5	90	94	11	88
Frequency	24	24	23	24	23	24	23	23	23	24	24	24	24	23	24	22	22	22
% Strong	95	95	91	4	4	95	34	34	8	95	54	12	91	0	33	72	4	0
% Positive	95	95	100	4	34	95	34	43	13	100	79	20	95	4	91	90	13	90

^aOccasional strains were not tested for a given trait and since there were a total of 24 strains in the homology group the frequency numbers were not always increments of 5.

not change appreciably when all of the strains were included.

Joint probability matrix. An assumption of probability theory is that the result of one test is independent of the result of another (10). This, however, need not be true; e.g., an organism that does not grow at a given temperature will also be negative for other traits (35). As another example, several substrates may be dependent upon a single transport system, and if the transport system is impaired, none of the substrates normally transported by it would be utilized. Also, particular traits in an organism may have

been established by genetic exchange, in which case the proximity of the genes on the DNA would influence whether one trait or two were introduced. If the DNA homology results are accepted as measurements of overall relatedness between bacteria, the independence or dependence for pairs of phenotypic traits can be calculated by a joint probability matrix. This was tested for in each DNA homology group. The joint probability matrix for the *B. uniformis* homology group is shown in Table 4. Those phenotypic traits that were either 100% positive or negative are not included in the matrix. The

numbers in the rows and columns represent the percentages of the strains that are positive for the tests indicated by the trait numbers at the top of the column and at the left of the rows. For example, 12 to 34% of the strains were positive for fermentation of inositol and rhamnose, respectively. Since none of the strains that fermented inositol fermented rhamnose, the traits would be considered unlinked. Fifty-four percent of the strains fermented trehalose, and 26% fermented both rhamnose and trehalose. Therefore, 76% (26/34) of the strains that fermented rhamnose also fermented trehalose, indicating linkage. To determine similar percentage values for any number in the table, the number was divided by the boldface numbers at the top of the column or at the right of the rows.

The fermentations of rhamnose, ribose, and trehalose are the most interesting in that the percent of strains positive for these reactions ranged from 34 to 54, and these are traits (of those tested here) which are useful in separating strains of *B. uniformis* from those of *B. thetaiotaomicron* and *B. ovatus*. The fermentations of ribose and rhamnose, although apparently independent of each other, appear to be linked to the fermentation of trehalose. Only 23% (8/34) of the strains that fermented ribose also fermented rhamnose, and vice versa. On the other hand, 62% of the strains that fermented ribose also fermented trehalose, and 76% of the strains that fermented rhamnose also fermented trehalose. The phenotypic traits that are positive or negative in only a small fraction of the strains were found to be quite independent of each other. This was also found to be true for the rest of the DNA homology groups.

Identification matrix. A 13-trait identification matrix was constructed to determine how useful these phenotypic trait probability values were for placing the organisms used in this study in the correct DNA homology group. The matrix was used with two sets of probability values. The first set (Table 5) contained strong reactions only (except for hydrogen production), and the second (Table 6) contained both strongly and weakly positive reactions. Only small (but definite) amounts of hydrogen were ever produced; therefore, the positive values (weak or small amount) were used in both tables.

The results obtained by using these matrices in the identification program (see computer methods) are shown in Table 7. The strains in this data base were most accurately identified when only the strong reactions were considered as positive. Perhaps many of the weak reactions are due to variations in the initial pH of the medium. Using the strong reaction matrix (Table 5), the percent of organisms in each homology group that were correctly identified ranged from 80 to 100 when the small homology groups (i.e., *B. vulgatus* II and *B. thetaiotaomicron* II and III) were not considered. Ninety-three percent of all of the strains were correctly identified. As one would expect, the homology groups most accurately identified were those having the greatest number of phenotypic differences from the other groups (Table 7). For example, 100% of the *B. eggerthii* strains, which have five to nine phenotypic differences with strains in most of the other homology groups, were correctly identified. On the other hand, only 81, 87, and 89% of the strains in the *B. ovatus* I, *B. thetaiotaomicron* I, and *B. uniformis* homology groups,

TABLE 4. *B. uniformis* homology group joint probability matrix for strong responses

Test	Test no.	Test number															
		3	5	8	12	14	16	17	18	20	22	23	25	28	31	32	33
Cellobiose	3	95															
Esculin hydrolysis	5	95	95														
Glycogen	8	86	86	91													
Mannitol	12	4	4	4	4												
Melezitose	14	4	4	4	0	4											
Raffinose	16	91	91	91	4	4	95										
Rhamnose	17	34	34	30	0	4	34	34									
Ribose	18	34	34	30	4	4	34	8	34								
Sorbitol	20	8	8	8	0	4	8	4	4	8							
Sucrose	22	91	91	86	4	4	91	34	34	8	95						
Trehalose	23	50	50	47	4	4	54	26	21	4	54	54					
Inositol	25	12	12	13	0	0	12	0	4	0	8	0	12				
Indole	28	87	87	82	4	4	87	26	30	8	87	50	12	91			
Growth in bile	31	33	33	26	4	4	29	8	17	4	33	16	4	33	33		
Glycerol-1-PO ₄	32	72	72	72	4	4	72	27	31	4	68	40	9	63	18	72	
Glycerol	33	4	8	4	4	0	4	0	4	0	4	4	0	4	4	4	4

TABLE 5. Probability matrix for strong reactions in the *Bacteroides* homology groups

Substrate or product	Probability of strong reaction in:											
	<i>B. fragilis</i> I 62 ^a	<i>B. fragilis</i> II 14	<i>B. vulgatus</i> I 48	<i>B. distasonis</i> 15	'T4-1' 10	'3452-A' 22	<i>B. ovatus</i> I 23	<i>B. ovatus</i> II 16	<i>B. thetaiotaomicron</i> I 29	<i>B. uniformis</i> 27	'subsp. a' 17	<i>B. eggertii</i> 7
Indole	.01	.01	.01	.01	.01	.01	.95	.81	.96	.91	.89	.99
Catalase	.99	.99	.01	.99	.01	.05	.77	.28	.79	.04	.01	.16
H ₂ (weak prod.)	.97	.82	.99	.01	.01	.99	.99	.99	.99	.88	.01	.01
Arabinose	.04	.01	.99	.14	.10	.99	.99	.94	.99	.99	.35	.99
Ribose	.03	.07	.13	.71	.80	.95	.96	.94	.97	.35	.47	.01
Salicin	.01	.07	.06	.99	.01	.73	.96	.99	.14	.99	.06	.01
Cellobiose	.15	.01	.06	.99	.01	.32	.99	.94	.90	.96	.06	.16
Melibiose	.98	.99	.94	.99	.99	.99	.99	.99	.99	.99	.06	.01
Sucrose	.99	.99	.99	.99	.99	.99	.99	.99	.99	.96	.94	.01
Melezitose	.01	.07	.04	.99	.10	.91	.91	.94	.90	.04	.01	.01
Raffinose	.99	.99	.98	.99	.90	.99	.96	.99	.97	.96	.94	.01
Rhamnose	.05	.01	.96	.99	.99	.59	.96	.94	.97	.34	.94	.99
Trehalose	.01	.01	.02	.99	.90	.99	.96	.94	.99	.54	.01	.01

^aNumber of strains.TABLE 6. Probability matrix for positive (strong plus weak) reactions in the *Bacteroides* homology groups

Substrate or Product	Probability of positive reaction in:											
	<i>B. fragilis</i> I 62 ^a	<i>B. fragilis</i> II 14	<i>B. vulgatus</i> I 48	<i>B. distasonis</i> 15	'T4-1' 10	'3452-A' 22	<i>B. ovatus</i> I 23	<i>B. ovatus</i> II 16	<i>B. thetaiotaomicron</i> I 29	<i>B. uniformis</i> 25	'subsp. a' 17	<i>B. eggertii</i> 7
Indole	.01	.01	.04	.01	.01	.01	.99	.88	.99	.95	.94	.99
Catalase	.99	.99	.01	.99	.01	.05	.77	.28	.79	.04	.01	.99
H ₂ (weak prod.)	.97	.82	.99	.01	.01	.99	.99	.99	.99	.88	.01	.01
Arabinose	.12	.08	.99	.28	.10	.99	.99	.99	.99	.99	.35	.99
Ribose	.08	.07	.38	.71	.90	.99	.96	.99	.97	.46	.47	.01
Salicin	.01	.07	.13	.99	.40	.91	.96	.99	.83	.99	.06	.01
Cellobiose	.87	.50	.10	.99	.20	.86	.99	.99	.97	.96	.06	.83
Melibiose	.98	.99	.98	.99	.99	.99	.99	.99	.99	.99	.12	.01
Sucrose	.99	.99	.99	.99	.99	.99	.99	.99	.99	.99	.99	.01
Melezitose	.03	.14	.08	.99	.80	.95	.91	.99	.93	.34	.01	.01
Raffinose	.99	.99	.98	.99	.99	.99	.96	.99	.99	.96	.99	.01
Rhamnose	.06	.01	.98	.99	.99	.64	.96	.99	.97	.34	.94	.99
Trehalose	.02	.01	.08	.99	.99	.99	.96	.94	.99	.79	.01	.01

^aNumber of strains.

respectively, were correctly identified. Only one or two traits differentiate between organisms belonging to these latter groups and between these and the "3452-A" homology group (Table 7).

DISCUSSION

Where possible, the organisms selected for this investigation were from widely distributed hospital laboratories and from specimens of hu-

man feces obtained from diverse geographical areas (18). This was done so that the organisms in each DNA homology group, at least the larger ones, would more likely represent a genetic and phenotypic cross section of that group.

The phenotypic variabilities of the tests used in this study do not appear to be correlated with the heterogeneity of the nucleotide sequences down to the level of about 60 to 70% homology. For example, strains of *B. fragilis* DNA homol-

TABLE 7. Phenotypic identification, by the use of probability matrices, of *Bacteroides* strains in each DNA homology group

Homology group	No. of strains	Matrix	Percent of strains not identified	Percent of strains identified as belonging to homology group									
				<i>B. fragilis</i> I	<i>B. vulgatus</i> I	<i>B. distasonis</i>	'T4-1'	'3452-A'	<i>B. ovatus</i> I	<i>B. thetaiotaomicron</i> I	<i>B. uniformis</i>	'subsp. a'	<i>B. eggerthii</i>
<i>B. fragilis</i> I	65	S ^a P	1 1	97 97	1			1					
<i>B. fragilis</i> II	14	S P		100 100									
<i>B. vulgatus</i> I	46	S P			93 89			2 9			2 2	2	
<i>B. vulgatus</i> II	1	S P			100 100								
<i>B. distasonis</i>	15	S P				100 100							
'T4-1'	10	S P					100 100						
'3452-A'	21	S P						95 5	5 95				
<i>B. ovatus</i> I	22	S P	4		4				92 92		4 4		
<i>B. ovatus</i> II	16	S P	6					12 12	75 88		6		
<i>B. thetaiotaomicron</i> I	29	S P	3 3						7 83	86 14	3		
<i>B. thetaiotaomicron</i> II	2	S P								100			
<i>B. thetaiotaomicron</i> III	2	S P						50 50	50 50		50		
'0061'	24	S P							4 8		96 92		
'subsp. a'	17	S P	6 6									94 94	
<i>B. eggerthii</i>	6	S P											100 100

^aS = strong reaction matrix (Table 5), P = positive (strong plus weak reactions) reaction matrix (Table 6).

ogy subgroups I and II, which have intergroup homology values that range from 61 to 78% and 64 to 72%, respectively (18), are phenotypically indistinguishable (Table 1). Very similar results were found for strains of the *B. ovatus* and *B. thetaiotaomicron* homology groups. On the other hand, the phenotypic results for strains in some of the more homogeneous homology groups are quite variable with respect to certain traits. Only 71% of the *B. distasonis* strains strongly produced acid in ribose medium, and of the *B. uniformis* strains, only 35, 34, and 54% strongly produced acid in ribose, rhamnose, and trehalose, respectively (Table 1). Similar results were found among strains of the "3452-A" and "subsp. a" homology groups. Perhaps some of

these properties are associated with DNA that has been moved between strains via plasmids or phages or by transformation. Many of these organisms have plasmids (13, 29), and phages that can infect organisms belonging to most of the homology groups can be readily isolated (22, 24).

The average S_j values (using positive similarities only) between the strains in each DNA homology group and the reference strains of all the DNA homology groups did not correlate with the intergroup DNA homology levels when they were below about 60%. *B. eggerthii* and "subsp. a" are the two most closely related homology groups that have previously been phenotypically delineated (15, 21). They had an

intergroup DNA homology range of 49 to 62% (18). The average S_J value between strains in one group and the DNA reference strain of the other was as low as or was lower than those between groups having little or no DNA homology (Table 2). *B. uniformis* had an intergroup DNA homology range of 29 to 38% with both *B. eggerthii* and "subsp. a." However, *B. uniformis* strains had average S_J values with the reference strains of the other DNA homology groups that were equal to or higher than the values with the *B. eggerthii* and "subsp. a." reference strains. On the other hand, the *B. thetaiotaomicron* and *B. ovatus* homology groups, with intergroup DNA homology values ranging from 32 to 42%, had nearly identical intergroup S_J values.

In general, the above observations are similar to those of Brenner et al. (6), who found that typical *E. coli* strains, with phenotypic similarity values of 81 to 95%, had DNA homology values to a reference strain that ranged from 73 to 100%. Two atypical *E. coli* and *S. typhimurium* strains, with phenotypic similarity values ranging from 78 to 71%, had only 12 to 17% DNA homology to the same reference *E. coli* strain. Other atypical *E. coli* strains and a strain of *S. flexneri* had low phenotypic similarity values (67 and 59%, respectively) but high levels of DNA homology with reference strains. Therefore, it does not appear that any general correlations between homology and phenotype can be predicted since all combinations have been observed, i.e., high homology—high phenotypic similarity, high homology—low phenotypic similarity, low homology—high phenotypic similarity, and low homology—low phenotypic similarity. Many of the phenotypic traits that are being considered in these studies are enzyme activities. Except for probably the active sites, the amino acid arrangements in enzymes with similar activities need not be the same and consequently could be coded for by DNAs having differences in their nucleotide sequences. Therefore, as far as fermentation tests are concerned, genetically diverse organisms could have many phenotypic expressions in common. The natural habitats of *E. coli* and of the organisms that we studied are the intestinal tracts of man and other animals. This provides a very restrictive and stable environment, in terms of moisture, temperature, and substrates, which could allow for converging evolution of many phenotypic expressions. It does not appear that the utilization of specific carbohydrates can always be considered as a measurement of taxonomic relationships. They often provide, however, useful markers for identification.

The S_J values reported in this study are based

on 34 traits. This is a somewhat lower number than has been used in most other phenotypic studies. However, this was a preselected group of organisms, and a number of similar characteristics, such as cell shape, Gram reaction, and oxygen relationship (see Materials and Methods), were not included. Had these been included, the S_J values would have been somewhat higher, but the overall results would not have been altered. Salyers et al. (26) recently tested some of these organisms on 22 additional carbohydrates, including many complex polysaccharides. For some homology groups, their results were similar to those obtained in this study, but for others there were differences. For example, both *B. thetaiotaomicron* and *B. ovatus* fermented more of the substrates (16 out of 22) than strains from the other DNA homology groups, and both fermented 14 of them. On the other hand, strains of *B. eggerthii* or of "subsp. a." fermented nine of the substrates, and strains of both groups fermented seven of them. Therefore, had these additional substrates been included in this study, the S_J values between these latter two species would have been somewhat higher.

The tests used in the identification matrix were essentially the same ones that have been used by other investigators (4, 11, 14, 16, 27, 31, 32) in identification schemes for this group of organisms. Ninety-three percent of the strains studied here were identified to the correct homology groups when only strong reactions were considered. Using positive reactions (strong plus weak), 85% of the strains were correctly identified. The lower level of success using the positive reactions was due, for the most part, to the inability to differentiate between *B. ovatus* and *B. thetaiotaomicron*. *B. ovatus* produced a strong acid reaction from salicin, and the majority of the *B. thetaiotaomicron* strains produced only a weak acid reaction. Therefore, on the basis of positive reactions, most of the strains in both groups had the same likelihood values, and because *B. ovatus* was listed first in the program, strains of both groups were always identified as *B. ovatus*. If the identification program were to be rerun now with incorporation of the test for xylan (26), these two groups would be separated. The identification success in some of the other homology groups was also a little lower, but none higher, when positive reactions were used. Perhaps the use of an acid end point of pH 5.6 or 5.7 would improve the identification percentage for that matrix because most of the strongly acid pH values are between 5.0 and 5.5. Raising the pH end point by 0.1 to 0.2 units would also include those cultures that may not have grown

quite as well. Most of the misidentifications, however, involved the small proportion of strains that gave a reaction unequivocally different from that of the rest of their group. On the other hand, those traits that were positive for 40 to 60% of the strains in a particular homology group had little positive or negative effect on the identification of organisms belonging to that group.

Another feature of the identification matrix that can increase the probability of an incorrect identification is that an unexpected result for a key differentiating test has a greater effect than an unexpected result from other tests. For example, suppose one is attempting to determine by means of the probability matrix (Table 5) whether an unidentified strain of *Bacteroides* belongs to *B. fragilis* or to *B. vulgatus*. Although these two homology groups differ by two traits, a positive response for either arabinose or rhamnose would result in very nearly the same probability values for both groups. On the other hand, a positive result for ribose fermentation would lower the probability products for both groups. These types of results, however, would tend to have the same effect on any identification system.

Since the identification matrices (Tables 5 and 6) contain only the traits that are useful for phenotypically delineating these organisms, they could be used as a basis for constructing noncomputerized identification schemata.

The results of this study demonstrate that the reliability of routine identification by phenotypic tests can be increased by including DNA homology results in the data base. Firstly, one is able to accurately determine the degree of variability of the various phenotypic traits. The "T4-1" and the "3452-A" groups had, for the most part, been phenotypically identified as belonging to *B. distasonis*. When the DNA results were also considered, it was noted that several of the traits that had been considered variable for *B. distasonis* were specific for distinct homology groups. Secondly, the traits that really are variable among closely related organisms can be demonstrated. Finally, if one begins with field strains, DNA homology experiments can also provide a means of evaluating identification matrices by verifying the accuracy of the phenotypic identification of these organisms.

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REPRINT REQUESTS

Address reprint requests to: Dr. John L. Johnson, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

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