Enterococcus hirae, a New Species That Includes Amino Acid Assay Strain NCDO 1258 and Strains Causing Growth Depression in Young Chickens

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Deoxyribonucleic acid base composition, deoxyribonucleic acid-deoxyribonucleic acid hybridization, lipid, and biochemical studies were performed with *Enterococcus faecium* NCDO 1258 (= Snell strain R) and other atypical *Enterococcus faecium* strains from pigs and chickens in an attempt to clarify their taxonomy. Our results indicate that these strains constitute a new species, for which the name *Enterococcus hirae* sp. nov. is proposed. The type strain of *Enterococcus hirae* is strain NCDO 1258.

Until recently, *Enterococcus* (*Streptococcus*) *faecium* was considered to represent a well-defined taxon (10) and encompassed strains previously designated "*Streptococcus durans*" (14). However, recent deoxyribonucleic acid (DNA)-DNA hybridization studies (4, 11) have shown that *Enterococcus faecium* and "*S. durans*" are distinct entities, and the latter taxon has been designated *Enterococcus durans* (2). There are a number of atypical "*Enterococcus faecium*" strains which cannot be allocated unequivocally to either of these taxa (4, 11, 12). Schleifer and Kilpper-Bälz (12) reported that three strains designated *Enterococcus faecium* shared a relatively low level of DNA homology (ca. 40%) with the type strain of *Enterococcus faecium* and suggested that these strains may represent a distinct species. However, an examination of the histories of the strains (strains DSM 20160, CCM 2423, and CCM 2424) studied by these workers revealed that all were derived from a common strain (Snell strain R^T [T = type strain]). Snell strain R^T (= NCDO 1258^T) also was shown to be genotypically distinct from the type strains of *Enterococcus faecium* and *Enterococcus durans* by Farrow et al. (4). The taxonomic position of some enterococci which cause growth depression in young chickens (5, 9) is also equivocal. Although these strains are referred to in the literature as *Streptococcus faecium* (5, 9), their biochemical properties are intermediate between those of *Enterococcus faecium* and *Enterococcus durans*. Thus, there is considerable interest to determine whether these chicken isolates along with Snell strain R^T constitute the nucleus of a new species.

In the present study DNA base composition, DNA-DNA homology, lipid, and biochemical studies were performed with the atypical *Enterococcus faecium* strains described above and with some unclassified group D streptococci (Sharpe-Fewins serological types 26C and 39) (13). Our results indicate that these strains represent a new species, for which the name *Enterococcus hirae* sp. nov. is proposed.

MATERIALS AND METHODS

Strains. The test strains shown in Table 1 were obtained from the National Collection of Dairy Organisms, Shinfield, Reading, United Kingdom.

DNA base composition and DNA-DNA hybridization. To prepare DNA, we used a modification (4) of the method of Garvie (6). DNA base composition was estimated by thermal denaturation in standard saline citrate, as described by

Garvie (7), using DNAs from *Leuconostoc mesenteroides* NCDO 768 and *Escherichia coli* K-12 strain NCDO 1984 as standards. DNA-DNA hybridizations were performed under optimum conditions (25°C below the melting temperature) and stringent conditions (10° below the melting temperature) by using the membrane filter technique described previously (8).

Fatty acid analyses. Cells for fatty acid analyses were grown in yeast extract-phosphate broth (7) supplemented with 0.5% (wt/vol) glucose at 30°C for 2 days. Fatty acid methyl esters were prepared from dry cells (50 mg) by acid methanolysis and were analyzed by gas-liquid chromatography as described previously (3).

Biochemical tests. The tests were performed by using the API 20 STREP and API 50CH systems (API Products, Basingstoke, United Kingdom) according to the instructions of the manufacturer. Tests were incubated at 37°C, and readings were made at 4, 24, and 48 h.

RESULTS AND DISCUSSION

The DNA base compositions of the test strains are shown in Table 1. *Enterococcus hirae* NCDO 1258^T, NCDO 1631, NCDO 1648, NCDO 2683, and NCDO 2708 had a guanine-plus-cytosine content range of 37 to 38 mol%. These data are within the range of 37 to 45 mol% guanine plus cytosine reported for the genus *Enterococcus* and similar to the values reported for *Enterococcus faecium* and *Enterococcus durans* (2, 4, 12).

The specific activity of [³H]DNA from strain NCDO 2708 was approximately 2,600 cpm/μg. Snell strain R^T (= NCDO 1258^T), the chicken isolates (strains NCDO 2683 and NCDO 2708), and representative strains of Sharpe-Fewins serotypes 26C and 39 (13) formed a single DNA homology group that was approximately 70 to 100% related to strain NCDO 2708 (Table 2). These strains shared relatively high levels of homologies (58%) with *Enterococcus faecium* NCDO 942^T and *Enterococcus durans* NCDO 596^T under optimum hybridization conditions; these values decreased to <30% under stringent conditions, indicating that the former strains are genetically distinct. It is worth noting that under stringent hybridization conditions four *Enterococcus hirae* strains (strains NCDO 1258^T, NCDO 1648, NCDO 2683, and NCDO 2708) were approximately 85 to 100% related to strain NCDO 2708, but *Enterococcus hirae* NCDO 1631 (Sharpe-Fewins serotype 26C) was only 43% related to the latter. Therefore, strain NCDO 1631 is best considered a peripheral member of this homology group.

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TABLE 1. Strain details and DNA base compositions

Species	Strain	Other designation(s) and comments	Guanine-plus-cytosine content (mol%)
<i>Enterococcus hirae</i>	NCDO 1258 ^T	Snell strain R ^T ; ATCC 8043 ^T , ATCC 9790 ^T ; NCIB 6459 ^T , NCIB 8123 ^T , NCIB 8191 ^T , CCM 2423 ^T , CCM 2424 ^T ; DSM 20160 ^T	38 ^a
	NCDO 1631	PG39; Sharpe-Fewins serotype 26C; from pig intestine	38
	NCDO 1648	PG48; Sharpe-Fewins serotype 39; from pig intestine	37
	NCDO 2683	2SY3; Fuller isolate from chicken crop	37
	NCDO 2708	SY1; Fuller isolate from chicken crop	37
<i>Enterococcus avium</i>	NCDO 2369 ^T		40 ^a
<i>Enterococcus casseliflavus</i>	NCDO 2372 ^T		43 ^a
<i>Enterococcus durans</i>	NCDO 596 ^T		38 ^a
<i>Enterococcus faecalis</i>	NCDO 581 ^T		39 ^a
<i>Enterococcus faecium</i>	NCDO 942 ^T		38 ^a
	NCDO 1632	CH12; Sharpe-Fewins serotype 27; from canned ham	39
<i>Enterococcus gallinarum</i>	NCDO 2313 ^T		40 ^a
<i>Enterococcus malodoratus</i>	NCDO 846 ^T		40 ^a
<i>Enterococcus</i> sp.	NCDO 2138	Roguinsky strain 47-16	40

^a Data from reference 4.

The results of our study confirm the results of studies done by Schleifer et al. (11, 12) and Farrow et al. (4), who demonstrated that Snell strain R^T (= NCDO 1258^T = CCM 2423^T) is genotypically distinct from *Enterococcus faecium* and *Enterococcus durans*. The data further suggest that the chicken isolates of Fuller and colleagues (5, 9) and unclassified group D streptococci of Sharpe and Fewins (13) are closely related to Snell strain R^T. Taking 70% homology under optimum hybridization conditions as the border line for species differentiation, Snell strain R^T, the chicken isolates, and the group D streptococci of Sharpe and Fewins are members of a single genetic species. The name *Enterococcus hirae* is proposed for these strains.

Description of *Enterococcus hirae* sp. nov. *Enterococcus hirae* (hir.ae. L.gen.s.n. *hirae* of the intestine or gut) cells are ovoid and occur mostly in pairs or short chains. The cells are gram positive and nonmotile. Surface colonies on blood or nutrient agar are circular, smooth, and entire. Nonhemolytic. Catalase negative. Growth occurs at 10 and 45°C, in 6.5% NaCl, and at pH 9.6. Facultatively anaerobic. L-Lactic

acid is the major end product of glucose fermentation. All strains hydrolyze esculin, are Voges-Proskauer, arginine dehydrolase, β-galactosidase, leucine arylamidase, and pyrrolidonylarylamidase positive, and produce acid from amygdalin, arbutin, N-acetylglucosamine, cellobiose, fructose, galactose, glucose, β-gentiobiose, lactose, maltose, mannose, melibiose, ribose, salicin, sucrose, and trehalose. All strains fail to produce acid from adonitol, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, glycogen, gluconate, inositol, inulin, 2-keto-gluconate, 5-keto-gluconate, D-lyxose, mannitol, α-methyl-D-glucoside, α-methyl-D-mannoside, α-methyl-xyloside, rhamnose, sorbose, sorbitol, xylitol, D-xylose, and L-xylose, do not hydrolyze hippurate, and are alkaline phosphatase and β-glucuronidase negative. Variable results are obtained for glycerol (one strain positive), melezitose (one strain positive), raffinose (two strains positive), starch (three strains positive), D-tagatose (one strain positive), and D-turanose (one strain positive). Reacts with Lancefield group D antisera. Group A type peptidoglycan based upon

TABLE 2. Levels of DNA homology of enterococci under optimum and stringent conditions

Species	Strain	% homology with [³ H]DNA from:			
		<i>Enterococcus hirae</i> NCDO 2708		<i>Enterococcus faecium</i> NCDO 1632	
		Optimum conditions	Stringent conditions	Optimum conditions	Stringent conditions
<i>Enterococcus hirae</i>	NCDO 2708	100	100	53	16
	NCDO 1258 ^T	95	85	53	21
	NCDO 1631	70	43	66	19
	NCDO 1648	85	88	61	20
	NCDO 2683	99	105	55	22
<i>Enterococcus faecalis</i>	NCDO 581 ^T	42	18	45	15
<i>Enterococcus faecium</i>	NCDO 942 ^T	58	27	110	65
<i>Enterococcus avium</i>	NCDO 2369 ^T	40	13	24	8
<i>Enterococcus casseliflavus</i>	NCDO 2372 ^T	43	15	57	18
<i>Enterococcus durans</i>	NCDO 596 ^T	58	28	42	28
<i>Enterococcus gallinarum</i>	NCDO 2313 ^T	46	19	49	11
<i>Enterococcus malodoratus</i>	NCDO 846 ^T	39	15	51	13
<i>Enterococcus</i> sp.	NCDO 2138	35	17	41	12

TABLE 3. Percent long-chain fatty acid composition of *Enterococcus hirae* strains

Strain	Fatty acid composition (%) ^a							
	C _{14:0}	C _{15:0}	C _{16:0}	C _{16:1}	C _{17:0}	C _{18:0}	C _{18:1} ^b	ΔC ₁₉
NCDO 1258 ^T	4.4	1.5	30.4	9.1	0.5	1.5	36.6	0.1
NCDO 1631	5.6	1.6	35.0	11.4	0.1	1.7	28.1	0.2
NCDO 1648	3.4	1.2	30.1	7.2	0.5	2.7	38.8	0.4
NCDO 2683	3.1	1.8	27.7	8.1	1.0	2.2	41.2	0.2
NCDO 2708	3.6	2.0	28.4	7.3	0.2	1.0	39.4	0.1

^a Abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, straight-chain saturated hexadecanoic acid; C_{18:1}, monounsaturated octadecenoic acid; ΔC₁₉, *cis*-11,12-methylenoctadecanoic acid.

^b *cis*-11,12-Octadecenoic acid.

TABLE 4. Differential characteristics of *Enterococcus hirae*, *Enterococcus casseliflavus*, *Enterococcus durans*, and *Enterococcus faecium*

Characteristic	<i>Enterococcus hirae</i>	<i>Enterococcus casseliflavus</i>	<i>Enterococcus durans</i>	<i>Enterococcus faecium</i>
Acid produced from: ^a				
L-Arabinose	— ^b	+	—	+
Glycerol	v	—	—	+
Inulin	—	+	—	—
Melibiose	+	+	—	v
α-Methyl-D-glucoside	—	+	—	—
Sucrose	+	+	—	v
Hydrolysis of hippurate ^c	—	—	v	+
β-hemolytic	—	—	v	—
Motile	—	+	—	—
Menaquinone composition	—	MK-8, MK-7	—	—

^a Tests performed with API 20 STREP and API 50CH systems.

^b +, Positive; —, negative; v, variable.

^c Test performed with API 20 STREP system.

lysine (type Lys-D-Asp). Menaquinones are absent. The long-chain fatty acids are predominantly of the straight-chain saturated, monounsaturated, and cyclopropane ring types. The major fatty acids are hexadecanoic, octadecenoic, and *cis*-11,12-methylenoctadecanoic acids (Table 3). The guanine-plus-cytosine content of the DNA ranges from 37 to 38 mol%, as determined by melting temperature. The strains form a distinct homology group, as determined by DNA-DNA hybridization. Isolated from chicken crops and pig intestines. The type strain is strain NCDO 1258 (= E.E. Snell strain R = ATCC 8043 = CCM 2423 = DSM 20160).

Description of the type strain. In most respects the description of the type strain resembles the description of the species. The type strain is α-galactosidase negative. Acid is produced from starch and D-tagatose. Acid is not produced from glycerol, melezitose, raffinose, or D-turanose. The type strain has complex nutritional requirements and is of industrial importance in the assay of a plethora of amino acids, vitamins, and other compounds (1). *Enterococcus hirae* is distinguished from *Enterococcus casseliflavus*, *Enterococcus durans*, and *Enterococcus faecium* by the tests shown in Table 4.

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