Comparison of the Superoxide Dismutases of *Branhamella catarrhalis*, *Neisseria ovis*, and *Neisseria caviae*

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We characterized the superoxide dismutases of *Neisseria ovis*, *Neisseria caviae*, and *Branhamella catarrhalis* after electrophoresis. Comparisons of the metal contents (as determined by sensitivity to hydrogen peroxide) and the relative mobilities of the dismutases showed that the dismutases of all six isolates of *B. catarrhalis* were identical to each other and to the dismutase of *N. ovis* strain T2B. The dismutase of *N. caviae* was similar to the dismutases of the *B. catarrhalis* strains in that it also contained manganese. However, the dismutase of *N. ovis* strain NRL 30014 was more similar to the dismutases of 17 other *Neisseria* strains previously tested in that it appeared to contain iron instead of manganese.

The classification of *Neisseria ovis* and *Neisseria caviae* is uncertain. In *Bergey's Manual of Determinative Bacteriology*, 8th ed., these bacteria are listed as species incertae sedis (25). Proposals to place *N. ovis* and *N. caviae* in the same genus as *Branhamella catarrhalis* have been based upon several characteristics of *N. ovis* and *N. caviae* which are shared with *B. catarrhalis* but not with other species of *Neisseria*. These characteristics include the guanine-plus-cytosine content of the deoxyribonucleic acid (2), electrophoretograms of esterases (10), and gas chromatograms of cellular carbohydrates (3, 13), cellular fatty acids (3, 12, 13, 16), and metabolic products (20). Recently, we reported that *N. ovis* was the only 1 of 17 *Neisseria* strains tested which had a manganese-containing superoxide dismutase (22). This led us to compare the superoxide dismutases of *N. ovis*, *N. caviae*, and *B. catarrhalis*.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The strains used in this study are listed in Table 1. All strains were grown in a defined liquid medium (21), as described previously (22).

Assays for superoxide dismutases. Cell-free extracts were prepared as described previously (22). Spectrophotometric assays for superoxide dismutases were based on the inhibition of cytochrome *c* reduction (18). After electrophoresis (6) on polyacrylamide gels containing 10% acrylamide and 0.03% *N,N'*-methylene bisacrylamide, the gels were stained for superoxide dismutase activity (1). Before staining, duplicate gels were treated with 5 mM hydrogen peroxide (5) or buffer; iron-containing superoxide dismutases were inhibited markedly, whereas manganese-containing superoxide dismutases were not affected.

Test for DNase activity. Bacteria were grown on

dioxyribonuclease (DNase) test agar (Difco Laboratories, Detroit, Mich.) at 37°C in 4% CO₂ for 24 h. Deoxyribonucleic acid hydrolysis was detected by adding 0.1% toluidine blue in deionized water (26).

RESULTS

Table 2 shows that the superoxide dismutases of *N. ovis* T2B (the strain of *N. ovis* previously tested [22]), six isolates of *B. catarrhalis* (including ATCC 25238), and *N. caviae* ATCC 14659 shared the following characteristics: (i) superoxide dismutase activity in gels after electrophoresis was confined to a single band; (ii) the relative mobilities of the enzymes from all isolates of *B. catarrhalis* and *N. ovis* T2B were identical, whereas the relative mobility of the *N. caviae* enzyme was similar, although slightly less; and (iii) superoxide dismutase activity was not inhibited by dilute hydrogen peroxide. This lack of inhibition strongly suggested that these enzymes contain manganese (5), whereas the superoxide dismutases of all other *Neisseria* strains that have been tested contain iron (22).

The superoxide dismutase of *N. ovis* NCTC 11227 seemed more similar to the superoxide dismutases of other *Neisseria* species than to the dismutases of the other strains of *N. ovis*, *N. caviae*, and *B. catarrhalis* in that it was inhibited by hydrogen peroxide. The difference between the superoxide dismutases of the two strains of *N. ovis* was surprising and raised the question of whether *N. ovis* T2B might share other characteristics with *B. catarrhalis*. The DNase activity of *B. catarrhalis* may be helpful in distinguishing *B. catarrhalis* from other *Neisseria* strains (19, 26). We tested all of the bacteria listed in Table 2 for DNase activity and

TABLE 1. Production of DNase and beta-lactamase by *B. catarrhalis* and *Neisseria* strains

Species	Strain	Production of DNase	Production of beta-lactamase ^a	Source of strain ^b	Reference
<i>B. catarrhalis</i>	W-786	+	+	Doern and Morse	7
	ATCC 8176	+	—	ATCC	
	324	+	+	Doern and Morse	7
	634	+	+	Doern and Morse	7
	318	+	+	Doern and Morse	7
	NRL 30018 (ATCC 25238) ^c	+	—	NRL (ATCC)	7
	6D2	+	+	UOHSC	
	7C10	+	+	UOHSC	
	7H9	+	—	UOHSC	
	1165	+	—	UOHSC	
	4120	+	+	Doern and Morse	
	NRL A1262	+	+	NRL	
	NRL A1246	+	+	NRL	
	NRL A1649	+	+	NRL	
	NRL A1650	+	+	NRL	
	NRL A1656	+	+	NRL	
	1526	+	—	OSPHL	7
	2233	+	+	Doern and Morse	
	T2B	—	—	CDC	
<i>N. ovis</i>	NRL 30014 (NCTC 11227) ^c	—	—	NRL (NCTC)	7
<i>N. caviae</i>	NRL 30002 (ATCC 14659) ^c	—	—	NRL (ATCC)	
<i>N. perflava</i>	ATCC 10555 ^c	—	—	ATCC	7
	4326	—	—	WRAIR	
<i>N. subflava</i>	B886	—	—	CDC	
<i>N. flava</i>	4322	—	—	WRAIR	7
<i>N. flavescens</i>	ATCC 13120 ^c	—	—	ATCC	
<i>N. mucosa</i>	A7895	—	—	CDC	

^a Beta-lactamase activity was detected with the chromogenic substrate Nitrocefin (Galaxo).^b ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, England; NRL, *Neisseria* Reference Laboratory, U.S. Public Health Service Hospital, Seattle, Wash.; UOHSC, Clinical Pathology Laboratory, University of Oregon Health Sciences Center, Portland; OSPHL, Oregon State Public Health Laboratory, Portland; CDC, Centers for Disease Control, Atlanta, Ga.; WRAIR, Walter Reed Army Hospital Institute for Research, Bethesda, Md.^c Type strain (27).TABLE 2. Comparison of superoxide dismutases of *B. catarrhalis* and *Neisseria* strains

Organism	Strain	Superoxide dismutase activity (U/mg of protein)	No. of bands positive for dismutase activity per strain	Relative mobility	
				Bands with dismutase activity	Bands inhibited by H ₂ O ₂ ^a
<i>B. catarrhalis</i>	W-786	40.9	1	0.66	
<i>B. catarrhalis</i>	ATCC 8176	19.1	1	0.66	
<i>B. catarrhalis</i>	324	10.8	1	0.66	
<i>B. catarrhalis</i>	634	21.8	1	0.66	
<i>B. catarrhalis</i>	318	7.4	1	0.66	
<i>B. catarrhalis</i>	ATCC 25238	18.1	1	0.66	
<i>N. ovis</i>	T2B	32.0	1	0.66	
<i>N. ovis</i>	NCTC 11227	6.8	1	0.55	0.55
<i>N. caviae</i>	ATCC 14659	4.9	1	0.61	
<i>N. gonorrhoeae</i>	Seven strains ^b	0–1.1	0–1	0.38–0.45	All
<i>N. meningitidis</i>	Three strains ^b	6.3–14.4	1	0.42	All
Nonpathogenic <i>Neisseria</i>	Six strains ^b	1.9–22.3	1–4	0.45–0.59	All

^a Manganese-containing superoxide dismutases are not inhibited by 5 mM H₂O₂, whereas iron-containing dismutases are inhibited (5).^b See reference 22.

found that all isolates of *B. catarrhalis* were positive, whereas *N. caviae* and both strains of *N. ovis* were negative. For further evidence that DNase activity distinguished *B. catarrhalis* from other *Neisseria* strains, we tested the *Neisseria* strains listed in Table 1 and found that all were negative for DNase activity.

DISCUSSION

Superoxide dismutase, which catalyzes the dismutation of the superoxide radical to hydrogen peroxide and oxygen, is probably important in preventing oxygen toxicity (11, 18). Whereas eucaryotic dismutases contain either copper and zinc or manganese (9, 17, 18, 28), all bacterial dismutases thus far discovered contain either manganese or iron (4, 5, 23), with the exception of a copper-containing dismutase from a fish symbiont (24). Some bacteria, such as *Escherichia coli*, possess both iron-containing superoxide dismutases (4, 29) and manganese-containing superoxide dismutases (4, 14), whereas other bacteria possess one or the other (5, 23). Identification of the metal in a superoxide dismutase is possible without purification of the enzyme. After electrophoresis, iron-containing enzymes are sensitive to hydrogen peroxide, whereas manganese-containing dismutases are not (5). The reliability of this method has been shown by reconstitution of the apoenzymes with the appropriate metals (15).

Our results show that the superoxide dismutases of the six isolates of *B. catarrhalis* examined in detail are identical in that upon electrophoresis the relative mobilities are the same and the single dismutase of each isolate contains manganese. The various sources of these strains and the occurrence of β -lactamase activity which is not due to the presence of a plasmid (8) indicate that the identical dismutases of the *B. catarrhalis* isolates are not the result of dissemination of a single clone. Since the superoxide dismutases of *N. ovis* T2B and *N. caviae* ATCC 14659 appeared to be similar to the superoxide dismutase of *B. catarrhalis*, we initially thought that this provided additional evidence that *N. ovis* and *N. caviae* belong in the genus *Branhamella*, especially since, of the 17 other *Neisseria* strains previously examined, only *N. ovis* T2B had a manganese-containing superoxide dismutase. These bacteria are related on the basis of other characteristics. The detection of an apparent iron-containing dismutase in *N. ovis* NCTC 11227 may invalidate classification on the basis of the metal content of the superoxide dismutase. However, the identity of *N. ovis* NCTC 11227 should be established before final conclusions are reached. Even though the metal

contents of the superoxide dismutases in the strains currently identified as *N. ovis* may vary, our results show that some isolates (such as *B. catarrhalis* isolates) characterized on the basis of other criteria are strikingly similar in DNase activity, electrophoretic characteristics of their superoxide dismutases, and metal contents of their superoxide dismutases.

REPRINT REQUESTS

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