Restricted distribution of *Streptococcus milleri* carbohydrate type antigens amongst other viridans streptococci

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Summary. The distribution of oral *Streptococcus milleri* carbohydrate type antigens in other viridans streptococcus species was examined. Rantz-Randall extracts of cells of the test strains grown in broth containing glucose were allowed to react with typing or grouping antisera for *S. milleri* serotypes *a–k*, or Lancefield groups A–G and K. Of 93 strains comprising more than 12 streptococcal species that included *S. mutans* and *S. sanguis* complexes, only 15 *S. salivarius* strains and one *S. mitis* strain were immunologically related to *S. milleri* serotype *f*. Unlike *S. milleri* strains, *S. salivarius* type *f* strains belonged to Lancefield group K, whereas the *S. mitis* strain was closely related to *S. milleri* serotype *f* but did not react with any of the Lancefield grouping antisera tested. Results suggest that oral *S. milleri* strains can be distinguished serologically from other oral viridans streptococci and that the typing antisera used in our researches might differentiate *S. milleri* isolates from the mouth from those associated with systemic purulent infections.

Introduction

Viridans streptococci designated *Streptococcus milleri* are commensal organisms on mucosal surfaces in the oral cavity, nasopharynx and gastrointestinal tract. Pathogenic strains are frequently isolated from purulent lesions in various systemic infections.^{1–3} However, these clinically important streptococci comprise a biochemically and genetically heterogeneous collection of strains referred to variously as *Streptococcus* MG, *S. intermedius*, *S. anginosus* or *S. constellatus*.^{3–10} Their taxonomic status is still unclear.

S. milleri strains are also serologically heterogeneous; many investigators have demonstrated that they frequently carry Lancefield group antigens F, A, C or G,^{11,12} or Ottens type antigens I to V,¹³ or both. Our recent studies divided oral *S. milleri* isolates into at least 11 serotypes, *a–k*.^{14,15} Approximately 80 % of oral *S. milleri* isolates^{14,15} and 40 % of isolates from systemic purulent infections¹⁶ have been identified to one of the serotypes. In this regard, it is of interest to know the distribution of *S. milleri* type antigens in the many other species of viridans streptococci indigenous to the oral cavity. The present study examined the possibility of distinguishing serologically *S. milleri* from other viridans streptococci and assessed the usefulness of typing antisera for identifying oral *S. milleri* in systemic foci. In this study, the term *S. milleri* is used as recommended.¹⁷

Materials and methods

Streptococcal strains

Ninety-three strains of more than 12 viridans streptococcal species were examined (table). The reference strain of *S. milleri* serotype *f*, ATCC9895 (Lancefield group F), was also used as required, as in previous studies of serotypes.^{14,15}

Streptococcal strains were grown in Brain Heart Infusion (BHI) Broth (Difco) anaerobically at 37°C overnight.

Immunological methods

Cell-surface carbohydrate antigens were extracted from cells grown in broth containing glucose by the method of Rantz and Randall¹⁸ as described previously.¹⁴ The 11 *S. milleri* type-specific antisera, *a–k*, were prepared as before.^{14,15} Lancefield group antisera (A–G, K) were purchased from Difco Laboratories.

Reactivity of antigen extracts with the typing antisera was first examined in a capillary precipitation test at room temperature for up to 30 min. To confirm the specificity of the precipitin reaction for *S. milleri* type antigens, double immunodiffusion analysis was performed in a Noble Agar (Difco) 1 % plate.¹⁴ The antigen preparations that reacted with the typing sera were examined further for reactivity with the Lancefield grouping antisera in similar tests.

Results

Of 93 streptococcal strains tested with the 11 typing

Table. Reactivity of viridans streptococcal strains with *S. milleri* typing antisera and Lancefield grouping sera

Streptococcal species	Strain nos.	Number of strains	Serological reactivity	
			Serotype	Group
<i>S. salivarius</i>	ATCC7073†, ATCC9222†, ATCC9759†, ATCC27945†, ATCC31067†, HHTR, HT9R*, HT9R-4r-1*, M1, M3, M6, Y1, Y2, S1, S2	15	<i>f</i>	K
	ATCC9758†, ATCC13419†, ATCC25975†, HT3R-3r-5*, M2, M4, M5, Y3, Y4, Y5, Y6, S3, S4, S5, S6	15	—	—
<i>S. mutans</i>	HS6(<i>S. cricetus</i> ATCC19462†), HS1, FIL, E49	4	—	nd
serotype <i>a</i>				
<i>b</i>	FA1(<i>S. rattus</i> ATCC194645†), BHT	2	—	nd
<i>c</i>	OMZ70(<i>S. mutans</i> ATCC33535†), GS5, P2, PK1, Ingbritt, C67-1, C67-25, MT8148*	8	—	nd
<i>e</i>	P4, B14, LM7, MT703R*	4	—	nd
<i>f</i>	OMZ175, MT3940*	2	—	nd
<i>d</i>	B13, P1	2	—	nd
<i>g</i>	K1R, OMZ65, MT3791§, 6715, 6715-11, 6715-DN*, 6715-DP*, AHT, AHT-M1, AHT-M2, AHT-M9, AHT-M13, AHT-M35	13	—	nd
<i>S. sanguis</i>	ATCC10556†*, OMZ9*, ST102*, ST107*, ST120*, ST129*, 9H	7	—	nd
<i>S. gordonii</i>	ATCC10558†*, M-5*, DL1	3	—	nd
<i>S. mitis</i>	ATCC33399†§, ATCC6249*	2	—	nd
	ATCC903*	1	<i>f</i> -	—
<i>S. oralis</i>	ATCC35037†§, ATCC10557*, ATCC9811§, H1	4	—	nd
<i>S. parasanguis</i>	ATCC15912†, ATCC15911†, ATCC15909†	3	—	nd
<i>S. intermedius</i>	31, 32, 306	3	—	—
<i>S. MG</i>	ATCC15910†, ATCC15913†	2	—	nd
Others	<i>S. faecalis</i> SS499*, <i>S. pyogenes</i> SV*, <i>S. bovis</i> DS96*	3	—	nd

ATCC, American Type Culture Collection; †, type strain.

Strains were obtained from Drs S. Hamada (Osaka University)*, T. Tsurumizu (Kitasato Institute)†, S. Shizukuishi (Osaka University)‡, and T. Koga (National Institute of Health, Tokyo)§, respectively; the others were stock cultures from our laboratory.

f, serotype *f*; K, Lancefield group K; *f*-, *f*-cross-reactive; —, untypable and ungroupable; nd, not determined.

sera, only 15 *S. salivarius* strains reacted with the *f* antiserum in both the capillary tube and agar gel (table). One strain of *S. mitis* was cross-reactive with *f* antiserum. The other 77 strains including the 35 “mutans” streptococci and the “sanguis-mitis” complex (20 strains) as well as *S. salivarius* (15 strains) and *S. mitis* (two strains), did not react with any of the typing antisera.

Figure. Immunodiffusion reactions of the Rantz-Randall extracts from the serotype *f* and group K *S. salivarius* strains with the anti-*f* and -group K sera. The antigen extract of *S. salivarius* M3 (well 2) produced a precipitin line with the anti-*f* serum (well A). This completely fused with the line that antigen extracts from the serotype *f* *S. milleri* reference strain ATCC9895 (well 1) formed with its homologous anti-*f* serum, but crossed with the line formed with the anti-group K serum (well B).

In the immunodiffusion analysis, the antigen extracts of 15 *S. salivarius* strains produced a precipitin line with type *f* antiserum which fused completely with that formed between the homologous antigen of the serotype *f* type strain ATCC9895 (figure). However, the precipitin lines crossed with the line formed with the group K antiserum. The antigen from one *S. mitis* strain made a precipitin line with *f* antiserum; this crossed with the line produced between the homologous antigen of the type *f* reference strain, but did not react with any grouping serum.

Discussion

Of the 93 strains of various viridans streptococci, mostly indigenous to the oral cavity, only 15 *S. salivarius* strains and one *S. mitis* strain were immunologically closely related to oral *S. milleri* (table). The *S. milleri* type *f* antigen was the only one detected in other viridans streptococci, mainly in *S. salivarius*. However, the type *f* *S. salivarius* strains carried simultaneously the Lancefield group K antigen, whereas the type *f* *S. milleri* strains, including the type *f* vaccine strain ATCC9895, generally carry the group F antigen.¹⁴⁻¹⁶ In addition, one *S. mitis* strain cross-reacted with type *f* antiserum, which was not observed with oral *S. milleri* strains.¹⁴⁻¹⁶ Strains of type *f* and

group K and type *f*-cross-reactive and ungroupable have not been identified in *S. milleri*. These results indicate that the 11 typing sera in the previously proposed typing system for oral *S. milleri* can be used, particularly in combination with some Lancefield grouping antisera, to distinguish serologically *S. milleri* from other viridans species.

Our previous studies have demonstrated that about 40% of *S. milleri* isolates from systemic purulent foci carry one of the type antigens found in oral *S. milleri* isolates; their physiological characteristics are also very similar to the oral strains.¹⁶ However, vaginal isolates of *S. milleri* did not react with any of our typing antisera (unpublished data), which is consistent with earlier findings that vaginal strains are physiologically different from those of oral origin.¹ This, together with the results presented above, would indicate that the typing antisera could also be used to characterise the oral or non-oral origin of clinical *S. milleri* isolates from systemic foci and might be useful in identifying oral *S. milleri* in systemic infections.

It is known that the strains of *S. salivarius* type I carry Lancefield group K antigen¹⁹⁻²² and are immunologically closely related to *S. MG* which carries the common "salivarius" antigen in addition to Lancefield group F antigen and Ottens type III antigen.²³

The *S. milleri* type *f* antigen, which the vaccine strain ATCC9895 (called *S. MG* or *S. intermedius*) carries,¹⁴ was detected in 15 *S. salivarius* strains (table) and its immunodeterminant was clearly different from that of the group K antigen (figure). These findings strongly suggest that the *S. milleri* type *f* antigen is identical to the "salivarius" antigen but not to the Ottens type III antigen.

It should be noted that c. 30% of *S. milleri* isolates hitherto examined¹⁴⁻¹⁶ and the five *S. intermedius* or *S. MG* strains tested in this study did not react with any typing antisera (table). Furthermore, recent studies have shown that *S. salivarius*²² and *S. intermedius*²⁴ can be divided into four and five serovars on the basis of their cell-surface carbohydrate type antigens. Conclusions regarding the immunochemical relationship between *S. salivarius* (particularly type I) and *S. milleri* (particularly *S. MG* or *S. intermedius*) depend upon further chemical and immunological studies on their cell-surface antigenic components.

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