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Enzyme Profiles of Oral Spirochetes in RapID-ANA System

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Enzyme profiles of oral *Treponema* species were determined by using RapID-ANA (Innovative Diagnostic System, Atlanta, Ga.), a 4-h test system which detects 18 enzymatic reactions, including aminopeptidases and glycosidases. Seventy-two clinical isolates of *Treponema denticola*, four reference strains of *T. denticola* (ATCC 35404, ATCC 35405, ATCC 33520, and ATCC 33521), one strain of *T. vincentii* (ATCC 35580), and two strains of *T. socranskii* subspecies (*T. socranskii* subsp. *buccale* ATCC 35534 and *T. socranskii* subsp. *socranskii* ATCC 35536) were used in this study. All *T. denticola* strains produced indole and a variety of aminopeptidases and glycosidases. These organisms could be differentiated into two groups on the basis of tetrazolium reductase and serine, phenylalanine, and glycine aminopeptidase activities. *T. vincentii* produced *N*-acetylglucosaminidase and arginine aminopeptidase, which facilitated the differentiation of this organism from *T. socranskii* subspecies and the *T. denticola* group. *T. socranskii* subspecies gave positive reactions for alkaline phosphatase only. These findings suggest that the RapID-ANA system is useful for enzymatic characterization and differentiation of oral spirochetes.

Along with other bacterial species, spirochetes have been implicated as periodontopathic organisms involved in periodontal diseases (7, 8). Owing to their slow growth and difficulties in the isolation and maintenance of the viability of these organisms, they have not been extensively studied. Conventional methods for their characterization are time-consuming and costly.

In the past few years, commercially developed enzyme assays, such as API-ZYM, the Minitek system, and the RapID-ANA system (Innovative Diagnostic System, Atlanta, Ga.), have been used for the rapid characterization of clinically important and fastidious organisms (2, 3, 5, 6, 9, 12, 13). In the present study, the RapID-ANA system, a 4-h test system which detects 18 enzyme activities (Table 1), was used. The enzyme profiles of seven reference strains of *Treponema* spp. and 72 strains of *Treponema denticola* isolated from the subgingival plaques of periodontitis patients at the University of Michigan School of Dentistry were determined. The test results were screened for identification and differentiation of these organisms as reported previously (S. A. Syed, S. L. Salvador, and W. J. Loesche, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C318, p. 381).

Reference strains of spirochetes were obtained from the American Type Culture Collection, Rockville, Md., and included the following: *T. denticola* ATCC 35404, ATCC 35405, ATCC 33520, and ATCC 33521; *T. vincentii* ATCC 35580; and *T. socranskii* subsp. *buccale* ATCC 35534 and *T. socranskii* subsp. *socranskii* ATCC 35536. In addition, 72 strains of oral spirochetes from the subgingival plaques of periodontitis patients which were identified as *T. denticola* by the criteria recommended in the *Anaerobe Laboratory Manual* (4) were included in this investigation. All organisms were maintained in a modified spirochete broth of Laughon et al. (6) (MTYGVS [11]) which contained 5% heat-inactivated rabbit serum and Yeastolate (Difco Laboratories, Detroit, Mich.). The purity of the cultures was determined by phase-contrast microscopic examination.

For the RapID-ANA test, each organism was grown in 10 ml of MTYGVS broth in screw-cap tubes in an anaerobic glove box (85% N_2, 10% H_2, 5% CO_2) at 37°C for 6 to 8 days to maximum turbidity as determined by the optical density at 660 nm with Spectronic 21. After the purity was checked by phase-contrast microscopic examination, the cultures were centrifuged at 9,000 × *g* at room temperature for 10 min. The supernatant fluid was discarded, and the cells were washed once in a solution with the same composition as the Innovative Diagnostic System inoculating fluid (KCl, 7.5 g; $CaCl_2$, 0.5 g; distilled water, 1,000 ml [pH 7.5]) to remove the residual growth medium and suspended in 1 ml of the same fluid for use in the enzyme profile test. The turbidity of the suspensions varied between McFarland standards 3 and 4. The 18 enzymatic activities which can be tested in the RapID-ANA system are shown in Table 1. The use of the

TABLE 1. Enzyme profile comparison of reference and clinical strains of *T. denticola* in RapID-ANA system

Enzyme(s)	% of organisms giving positive reactions[a]	
	Reference strains	Clinical isolates
Alkaline phosphatase	75	1
β-Galactosidase	0	0
α-Glucosidase	100	100
β-Glucosidase	50	100
α-Galactosidase	50	100
α-Fucosidase	0	0
N-Acetylglucosaminidase	0	0
Tetrazolium reductase	100	4
Arginine dihydrolase	0	0
Trehalose fermenting	0	0
Leucylglycine aminopeptidase	75	0
Glycine aminopeptidase	100	4
Proline aminopeptidase	100	100
Phenylalanine aminopeptidase	100	50
Arginine aminopeptidase	100	100
Serine aminopeptidase	100	4
Pyrrolidine arylamidase	100	100
Tryptophanase	100	100

[a] The results are based on 4 reference strains and 72 clinical isolates. For details, see the text.

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TABLE 2. Enzymatic reactions for differentiation of oral treponemes in RapID-ANA system

Enzyme	Reaction[a]				
	T. denticola		*T. vincentii* ATCC 35580	*T. socranskii* subsp. *buccale* ATCC 35534	*T. socranskii* subsp. *socranskii* ATCC 35536
	Group 1[b]	Group 2			
Alkaline phosphatase	v	−	−	+	+
α-Glucosidase	+	+	−	−	−
α-Galactosidase	v	+	−	−	−
β-Glucosidase	v	+	−	−	−
N-Acetylglucosaminidase	−	−	+	−	−
Proline aminopeptidase	+	+	−	−	−
Tryptophanase	+	+	+	−	−
Phenylalanine aminopeptidase	+	−	−	−	−
Serine aminopeptidase	+	−	−	−	−
Glycine aminopeptidase	+	−	−	−	−
Tetrazolium reductase	+	−	−	−	−

[a] Key enzymatic reactions are in boldface. v, Variable reaction.
[b] Includes ATCC 35404, ATCC 35405, ATCC 33520, and ATCC 33521.

RapID-ANA panels, the reagents, the test procedure, and the interpretation of the results were according to the recommendations of the manufacturer. Briefly, spirochete suspensions in the Innovative Diagnostic System fluid were dispensed in the wells of RapID-ANA panels and incubated in room atmosphere at 37°C for 4 h. At the end of incubation, the enzyme reactions of self-color-developing substrates were recorded on the data sheet. Reactions for aminopeptidases and indole were recorded after the addition of RapID-ANA and spot indole reagents. The development of red to purple was considered positive for aminopeptidases. For indole, brown or black development after the addition of the reagent was considered positive.

Analysis of the data showed that the *T. socranskii* subspecies (ATCC 35534 and ATCC 35536) were least active in this test system and gave positive reactions for alkaline phosphatase only (Table 2); all other enzymatic reactions were negative for the two strains tested. *T. vincentii* ATCC 35580 gave positive reactions for arginine aminopeptidase, *N*-acetylglucosaminidase, and tryptophanase. All *T. denticola* strains were most active among the treponemes studied (Table 1). Comparison of enzyme profiles of reference and clinical strains of *T. denticola* revealed that most of the latter did not yield positive reactions for alkaline phosphatase, tetrazolium reductase, and glycine and serine aminopeptidases. Fifty percent of the clinical isolates were negative for phenylalanine aminopeptidase activity. None of the clinical strains of *T. denticola* showed leucylglycine aminopeptidase activity. Among the reference strains of *T. denticola*, 50% of the enzymatic reactions detectable in the RapID-ANA system were positive for all the strains. However, these strains differed in regard to alkaline phosphatase, β-glucosidase, α-galactosidase, and leucylglycine aminopeptidase activities.

The results of the present study clearly suggest that the *T. denticola* strains could be placed into two groups on the basis of the following enzymatic reactions, as shown in Table 2: tetrazolium reductase and phenylalanine, serine, and glycine aminopeptidases. The reference strains of *T. denticola* were included in group 1. The *T. vincentii* and *T. socranskii* reference cultures had enzyme activity profiles distinct from those of each other and from those of the *T. denticola* strains (Table 2). Therefore, the enzymatic reactions shown in Table 2 can serve as a practical enzyme profile scheme for the rapid screening and identification of the spirochetes to the species level in the laboratory. We found that the enzymatic reactions of these organisms were consistent and reproducible in this test system.

Knowledge of the enzymatic activities of oral spirochetes is useful since it facilitates the identification of these organisms. From the physiological and biochemical standpoints, it provides some insight as to the capabilities of the organisms to degrade various substrates which might serve as nutrients for themselves or other microbes found in the oral environment. Since spirochetes are associated with periodontal disease (7) and acute necrotizing ulcerative gingivitis (8) and invade the tissues (1, 10), the study of these enzymes in greater depth may open new avenues for understanding the virulence of these organisms in such infections.

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LITERATURE CITED

1. **Frank, R. M.** 1980. Bacterial penetration in the apical pocket wall of advanced human periodontitis. J. Periodontal Res. **15:** 563–573.
2. **Fukomoto, Y., K. Okuda, T. Tato, K. Ohta, and I. Takazoe.** 1987. Taxonomic study of spirochetes isolated from periodontal lesions. Oral Microbiol. Immunol. **2:**82–87.
3. **Hanson, C. W., R. Cassorla, and W. J. Martin.** 1979. API and Minitek systems in identification of clinical isolates of anaerobic gram-negative bacilli and *Clostridium* species. J. Clin. Microbiol. **10:**14–18.
4. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
5. **Kato, T., M. Ono, M. Ikawa, K. Okuda, and I. Takazoe.** 1984. Proteolytic activities of gram-negative bacteria isolated from human periodontal lesions. Jpn. J. Oral Biol. **26:**953–958.
6. **Laughon, B. E., S. A. Syed, and W. J. Loesche.** 1982. API ZYM system for identification of *Bacteroides* spp., *Capnocytophaga* spp., and spirochetes of oral origin. J. Clin. Microbiol. **15:**97–102.
7. **Loesche, W. J., and B. E. Laughon.** 1982. Role of spirochetes in periodontal disease, p. 62–75. *In* R. J. Genco and S. E. Mergenhagen (ed.), Host-parasite interactions in periodontal diseases. American Society for Microbiology, Washington, D.C.
8. **Loesche, W. J., S. A. Syed, B. E. Laughon, and J. Stoll.** 1982. The bacteriology of acute necrotizing ulcerative gingivitis. J. Periodontol. **53:**223–230.
9. **Marler, L., S. Allen, and J. Silders.** 1984. Rapid enzymatic characterization of clinically encountered anaerobic bacteria

with the API-ZYM system. Eur. J. Clin. Microbiol. **3**:294–300.
10. **Saglie, R., F. A. Carranza, M. G. Newman, L. Cheng, and K. J. Lewin.** 1982. Identification of tissue-invading bacteria in human periodontal disease. J. Periodontal Res. **17**:452–455.
11. **Salvador, S. L., S. A. Syed, and W. J. Loesche.** 1987. Comparison of three dispersion procedures for quantitative recovery of cultivable species of subgingival spirochetes. J. Clin. Microbiol. **25**:2230–2232.
12. **Sela, M. N., K. S. Kornman, J. L. Ebersole, and S. C. Holt.** 1987. Characterization of treponemes isolated from human and non-human primate periodontal pockets. Oral Microbiol. Immunol. **2**:21–29.
13. **Winkelhoff, A. J. V., T. J. M. Vansteenbergen, N. Kippuw, and J. de Graaff.** 1986. Enzymatic characterization of oral and non-oral black pigmented *Bacteroides* species. Antonie van Leeuwenhoek J. Microbiol. **52**:163–171.