Selective Medium for Isolation of *Eikenella corrodens* from Periodontal Lesions

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The addition of 5 μ g of clindamycin per ml to a modified Todd-Hewitt growth medium permitted the ready enumeration of *Eikenella corrodens* from deep periodontal lesions because it allowed differential growth amongst the periodontal pocket gram-negative microaerophilic-anaerobic flora, maximized the numbers of *E. corrodens* in such culture, and inhibited the growth of most of the other confounding microorganisms.

Different forms of periodontal disease may have rather specific microbial etiologies (2, 5, 8, 9, 11-18; J. M. Tanzer, *Proceedings of the International Conference on Research in Periodontal Disease*, in press). Microbiological examination of deep subgingival plaque from periodontal lesions of patients showing signs of rapidly advancing bone loss has revealed a predominance of gram-negative microaerophilic and anaerobic rods (2, 11, 16, 18; J. M. Tanzer, *Proceedings of the International Conference on Research in Periodontal Diseases*, in press). Unfortunately, the progress of enumeration and characterization of the complex microbiota associated with such periodontal lesions has been severely hampered by a number of technical microbiological problems among which is the lack of suitable differential, selective, or semi-selective media for many of the fastidious residents of this ecological niche.

Eikenella corrodens is prominently isolated from such periodontal sites in patients with aggressive or advanced periodontitis (16, 18), and monoinfection of gnotobiotic rats by *E. corrodens* isolates induces similar lesions to those observed in humans (A. Crawford, S. S. Socransky, E. Smith, and R. Phillips, Am. Assoc. Dent. Res. Abstr. no. 275, 5:B120, 1977). Thus, there is strong evidence of the importance of this microorganism etiologically in such disease in humans.

Routine antimicrobial sensitivity screening of periodontal pocket microflora from cases of aggressive periodontitis indicated that *E. corrodens* isolates are invariably resistant to the antibiotic clindamycin (J. M. Tanzer and R. Tilton, unpublished data). This report describes attempts to utilize this finding for the development of a selective growth medium for *E. corrodens* from periodontal lesions.

MATERIALS AND METHODS

The basal growth medium employed was Todd-Hewitt broth (Baltimore Biological Laboratory [BBL], Cockeysville, Md.) supplemented with 2.0 mg of potassium nitrate (J. T. Baker Chemical Co., Phillipsburg, N.J.) per ml and 5 μ g of hemin (Sigma Chemical Co., St. Louis, Mo.) per ml. Agar (BBL), when required, was added to a final concentration of 1.5% (wt/vol). This medium supported the growth of *E. corrodens* strains at 37°C for 2 to 3 days under either anaerobic (GasPak, BBL; Anaerobic Chamber, Coy Manufacturing, Ann Arbor, Mich.) or microaerophilic (candle extinction jar) environments and compares favorably with Trypticase soy 5% sheep blood agar (BBL).

The sensitivities of 18 *E. corrodens* strains (Table 1) to a series of 13 antimicrobial agents commonly employed clinically was examined by using antimicrobial disks placed on the surfaces of the inoculated basal medium under either anaerobic or microaerophilic conditions for 2 days at 37°C. The following commercial antimicrobial disks (BBL) were initially utilized: tetracycline, 30 mg; chloramphenicol, 30 mg; clindamycin, 2 mg; colistin, 10 mg; kanamycin, 30 mg; sulfonamide-trimethoprim, 250 mg; gentamycin, 10 mg; nitrofurantoin, 300 mg; cephaloridine, 30 mg; erythromycin, 15 mg; ampicillin, 10 mg; methicillin, 5 mg; and, penicillin, 10 U.

Clindamycin as the hydrochloride hydrate (The Upjohn Co., Kalamazoo, Mich.), the antibiotic exhibiting the best selectivity for all 18 *E. corrodens* isolates was incorporated into modified Todd-Hewitt agar and assessed for its ability to enhance selection and recovery of *E. corrodens* from samples from the depth of periodontal lesions.

Samples were obtained from the apical front of periodontal lesions of ten patients diagnosed radiographically to have rapidly advancing periodontitis. Initially, supragingival plaque was removed carefully by dental scaler from the site to be sampled. Then a fine curette was placed to the depth of the lesion, and subgingival plaque was removed. The curette was immediately wiped with the swab of the Becton, Dickinson & Co. anaerobic transport system and sealed in

TABLE 1. *Designations and sources of E. corrodens strains*

Strain designation	Source
UHC 2852	Clinical isolate, J. Tanzer
UHC 3321	Clinical isolate, J. Tanzer
UHC 3322	Clinical isolate, J. Tanzer
UHC 2963	Clinical isolate, J. Tanzer
UHC 1081	Clinical isolate, J. Tanzer
UHC 4430	Clinical isolate, J. Tanzer
UHC 2539	Clinical isolate, J. Tanzer
UHC 2540	Clinical isolate, J. Tanzer
3342	V. Sutter, Veterans Administration, Los Angeles, Calif.
3370	
3347	
D 8324	R. Weaver, Center for Disease Control, Atlanta, Ga.
D 8239	
D 8524	
D 8598	
373	S. Socransky, Forsyth Dental Center, Boston, Mass.
374	
384	

the anaerobic atmosphere therein. All samples were quickly transported to and placed in an anaerobic chamber. The plaque material was placed into modified Todd-Hewitt broth without antibiotic and agitated with a Vortex mixer to disrupt bacterial clumps, and 10-fold dilutions were plated onto modified Todd-Hewitt agar with or without clindamycin and onto Trypticase soy 5% sheep blood agar. All plates were incubated anaerobically at 37°C for 3 days.

After incubation, the plates were examined for total colony counts and counts of *E. corrodens*. Presumptive *E. corrodens* isolates on blood and modified Todd-Hewitt agars, along with all colonies from Todd-Hewitt plus clindamycin, were picked, and their identities as *E. corrodens* were confirmed by using morphological and biochemical criteria (7).

RESULTS

Eighteen isolates of *E. corrodens* were sensitive to all 13 commercial antimicrobial agents tested, with the exception of clindamycin.

All 18 test isolates grew, albeit sparsely and only after 5 days of anaerobic incubation, on modified Todd-Hewitt agar containing clindamycin at concentrations of up to 80 µg/ml. A 5-µg amount of clindamycin per ml in modified Todd-Hewitt agar permitted growth similar to that observed with Trypticase soy 5% sheep blood agar and modified Todd-Hewitt agar without clindamycin (Table 2). Concentrations of clindamycin greater than 5 µg/ml apparently partially inhibited the growth of *E. corrodens* (Table 2).

Parallel inoculations of 20 plaque samples from periodontal lesions indicated that modified Todd-Hewitt agar plus clindamycin permitted the ready isolation and enumeration of *E. corrodens* (Table 3). The addition of 5 µg of clindamycin per ml to modified Todd-Hewitt agar

TABLE 2. *Recoverability of pure cultures of E. corrodens on nonselective and selective media*

Medium	Total colony count	% Recovery
Blood agar (BBL)	1.84×10^4	100
Todd-Hewitt agar	1.83×10^4	100
Todd-Hewitt + 5 µg of clindamycin per ml	1.64×10^4	90
Todd-Hewitt + 10 µg of clindamycin per ml	1.20×10^4	65
Todd-Hewitt + 25 µg of clindamycin per ml	1.00×10^4	55

TABLE 3. *Recoverability of total flora and E. corrodens from periodontal lesion after plating on selective and nonselective media*

Medium	Total colony count	<i>E. corrodens</i> colony count
Blood agar (BBL)	4.0×10^6	3.0×10^2
Todd-Hewitt agar	6.0×10^5	2.0×10^2
Todd-Hewitt + 5 µg of clindamycin per ml	6.0×10^3	5.0×10^3
Todd-Hewitt + 10 µg of clindamycin per ml	4.5×10^3	4.2×10^3
Todd-Hewitt + 25 µg of clindamycin per ml	3.0×10^3	3.0×10^3

suppressed the growth of other microorganisms by a factor of 2 to 3 logs and increased the recoverable number of *E. corrodens* by over a factor of 1 log compared to the nonselective blood and modified Todd-Hewitt medium without clindamycin. Higher concentrations of clindamycin reduced further the number of other subgingival plaque microorganisms; however, this was accompanied by a concomitant decrease in the recoverable number of *E. corrodens* isolates. With the addition of 10 µg of clindamycin per ml, *E. corrodens* and only one or two other species of bacteria grew, both of which were uncharacterized gram-negative rods. The latter microorganisms presented colonial morphologies and pigmentation which were distinct from *E. corrodens* isolates. The addition of 25 µg of clindamycin per ml to modified Todd-Hewitt agar permitted only the growth of *E. corrodens*. Although some isolates of *E. corrodens* may be expected to be lost at this concentration (Table 2), the apparent total colony count for *E. corrodens* was still higher than that observed on blood agar or modified Todd-Hewitt agar.

In all trials with clindamycin, both corroding and noncorroding isolates were readily observed and distinguished from other microorganisms. Other corroding oral microorganisms failed to grow on modified Todd-Hewitt agar plus clindamycin, although they were recoverable on blood agar. Colonies of *E. corrodens* on modified

Todd-Hewitt agar plus clindamycin were yellow, slightly speckled, with some showing varying degrees of corrosion or pitting of the agar surface (Fig. 1 to 3) and clearly distinguishable from other microorganisms. All presumptive *E. corrodens* isolates picked from Todd-Hewitt agar plus clindamycin were gram negative; asaccharolytic, lysine, and ornithine decarboxylase positive; urease negative; and nitrate and oxidase positive.

DISCUSSION

Like a number of other microorganisms apparently resident in periodontal pockets, *E. corrodens*, a fastidious microaerophilic gram-negative rod, is comparatively slow growing, particularly on primary isolation, and probably for a variety of reasons may thus be missed or its numbers underestimated. In addition, although the trait of corroding or pitting of an agar growth medium does allow for an easy enumeration on nonselective growth media, not all isolates of *E. corrodens* consistently present this characteristic, and other species found in periodontal pockets also corrode agar (6, 7, 10, Slee and Tanzer, unpublished data).

The present study demonstrates that *E. corrodens* is resistant to clindamycin, which is normally effective against anaerobic bacteria (4, 19). Addition of a 5- μ g/ml concentration to modified Todd-Hewitt agar permits the ready isolation and easy enumeration of *E. corrodens* from

subgingival plaque from periodontal lesions. It is of interest that Brooks et al. (1) have previously reported that the addition of 5 μ g of clindamycin per ml to Muller-Hinton agar facilitated the isolation of *E. corrodens* from mixed cultures of *Streptococcus* sp. and *Staphylococcus* sp. in samples obtained from a number of nonoral abscesses.

The total colony count with respect to *E. corrodens* is higher on clindamycin medium than on blood agar. This is, in part, probably due to the suppression of microorganisms which swarm or glide over the surface of the agar, which outgrow and thus obscure the slower growing *E. corrodens*, and which produce metabolic products antagonistic to *E. corrodens*. Furthermore, both corroding and noncorroding *E. corrodens* isolates are readily identified, a task difficult to accomplish on nonselective media. All presumptive *E. corrodens* colonies picked were later identified to be *E. corrodens*, with the exception of approximately 0.1%, which are termed by Socransky *Eikenella* sp. I (S. S. Socransky, personal communication) because they do not possess all of the traits common to typical *Eikenella corrodens* (7); namely, they were lysine and ornithine decarboxylase negative.

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FIG. 1. A noncorroding isolate of *E. corrodens* isolated from a deep periodontal lesion growing on modified Todd-Hewitt agar plus 10 μ g of clindamycin per ml. $\times 40$.

FIG. 2. A deep periodontal lesion isolate of *E. corrodens* demonstrating slight corrosion and peripheral spreading after growth on modified Todd-Hewitt agar plus 10 μ g of clindamycin per ml. $\times 40$.

FIG. 3. A deep periodontal lesion isolate of *E. corrodens* demonstrating marked corrosion of the agar surface and concomitant peripheral spreading. Colony grown on modified Todd-Hewitt agar plus 10 μ g of clindamycin per ml. $\times 40$.

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