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Short communication

Capillary zone electrophoresis as a new tool in the chemotaxonomy of oral treponemes

Ilia Brondz[a,b,*], Ulf R. Dahle[a,c], Tyge Greibrokk[b], Ingar Olsen[a]

[a]*Section of Microbiology, Institute of Oral Biology, University of Oslo, PB 1052 Blindern, 0316 Oslo, Norway*
[b]*Department of Chemistry, University of Oslo, Oslo, Norway*
[c]*Division of Endodontics, University of Oslo, Oslo, Norway*

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Abstract

The existing taxonomy of oral treponemes is not satisfactory. This is due to the fact that these strict anaerobic bacteria are not easily cultivated or differentiated. Therefore, new techniques that can contribute to improved cultivation, classification, and identification of these fastidious organisms should be welcomed. In the present study capillary zone electrophoresis (CZE) was used to distinguish oral treponemes by their metabolic product patterns generated in a liquid medium. To our knowledge this technique has not previously been used in bacterial chemotaxonomy. Reference strains of *Treponema denticola*, *Treponema pectinovorum*, *Treponema vincentii*, *Treponema socranskii* subspecies *buccale* and *Treponema socranskii* subspecies *socranskii* were cultured anaerobically in duplicate on different days in Pectin medium for 4 days at 37°C under nitrogen atmosphere. Treponemal cells were harvested by centrifugation. Thereafter, their supernatants were filtered through 0.22-μm Millipore filters and subjected to CZE. The resulting electropherograms clearly distinguished *T. denticola*, *T. pectinovorum* and *T. vincentii*. Minor differences were detected between *T. socranskii* subspecies *buccale* and *T. socranskii* subspecies *socranskii*. Subspecies were clearly different from species. It seems that CZE of culture metabolites, which showed high resolution and good reproducibility, may be a valuable tool in the chemotaxonomy of oral treponemes, even at the subspecies level.

1. Introduction

Capillary electrophoresis is a versatile microanalytical technique which in the last decade has been applied in almost every branch of medicine [1]. In bacterial chemotaxonomy (for a review, see Ref. [2]), this technique had not been reported until we recently presented studies on the application of capillary zone electrophoresis (CZE) in the distinction of oral treponemes [3,4]. Current taxonomy has assigned small-sized oral spirochetes, which are strictly anaerobic, to the genus *Treponema* [5]. Due to difficulties in culturing these organisms, they have been inadequately characterized [6] and the taxonomy of some species should be revised [7]. Proper classification and identification of treponemes is important because they may be major etiological agents in oral diseases such as marginal periodontitis and endodontic infection, where they are frequently seen (for a review, see Ref. [8]). Their pathogenicity is probably related to

* Corresponding author.

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the production of tissue-destroying enzymes such as trypsin and hyaluronidase and to their cytotoxic metabolic products, e.g. indole and hydrogen sulfide [6,8]. Furthermore, these organisms are able to evade normal host responsiveness by inhibiting polymorphonuclear leukocyte functions and fibroblast proliferation [8]. The present study was performed to introduce CZE in the chemotaxonomy of oral treponemes using metabolic products from liquid medium.

2. Experimental

2.1. Bacteria

Well characterized reference strains of *Treponema denticola* (strain Fm), *Treponema pectinovorum* (strain D36DR2), *Treponema vincentii* (strain N9), *Treponema socranskii* subspecies *buccale* (strain D2B8) and *Treponema socranskii* subspecies *socranskii* (strain DR56BRIII-6) were provided by Professors W.E.C. Moore and L.V.H. Moore, Department of Biochemistry and Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. The organisms were cultured anaerobically in duplicate on different days in prereduced anaerobically sterilized Pectin medium [9] for 4 days at 37°C under nitrogen atmosphere. The cultures were checked for contaminants by aerobic and anaerobic cultivation on blood agar plates and through dark-field and Gram-stain examination.

2.2. Samples for electrophoresis

The cultures were harvested by centrifugation and the supernatants filtered through 0.22-μm Millipore filters to prevent clogging of the capillary. Uninocculated Pectin medium, which was used as a control, was treated similarly. The supernatants were then diluted 1:20 with buffer. The buffer was 10 m*M* with respect to phosphate and 10 m*M* with respect to borate. The total molarity was 20 m*M* and the pH was adjusted to 9.5. Mesityl oxide (BioApplied, Foster City, CA, USA) dissolved in the buffer was used as a neutral marker (NM) during electrophoresis: 1 μl of mesityl oxide was diluted in 499 μl of separation buffer and thoroughly vortex-mixed. Due to rapid evaporation of mesityl oxide, this mixture was prepared fresh each day.

2.3. Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) was performed with a Model BA 270A capillary electrophoresis apparatus (BioApplied). The quartz capillary column used was 72 cm long with 50 μm I.D. The distance to the detector was 50 cm. The analytical conditions for the CZE were as follows: field −347 V/cm; current 8 μA; vacuum application 1.5 s; temperature during analysis: 25°C; electrolyte: 20 m*M* phosphate–borate buffer, pH 9.5; detection UV: 200 nm. External standards were N-Acetyl-Met-Asp-Arg-Val-Leu-Ser-Arg-Tyr peptide (Sigma, St. Louis, MO, USA) and chicken egg albumin (Sigma) dissolved in the phosphate–borate buffer. Their migration times on the electropherograms were 7.6 min and 26.8 min, respectively. All reagents used were of p. a. quality. The water used was double-distilled, and the electrolytes were filtered through 0.22-μm Millipore filters. All CZE analyses were performed in triplicate. By dividing the peak heights on the electropherograms by the peak height of the NM (internal standard) numerical data were obtained for peaks comparison (see Table 1).

3. Results and discussion

Reproducibility of the electropherograms obtained was good. All peaks were detected between the two external standards used. Fig. 1 shows the electropherograms after CZE analysis of the metabolic products from the culture medium of *T. denticola*, *T. pectinovorum* and *T. vincentii*. There were clear differences in the metabolite patterns from these species. Characteristic peaks in the electropherograms used for comparison were numbered from 1 to 6. For *T. vincentii*, peaks 1–6 were all conspicuous. In *T. pectinovorum*, peaks no. 3 and 6 could hardly be

Table 1
Ratio of peak heights to the height of the neutral marker (NM) mesityl oxide in electropherograms obtained from CZE of bacterial metabolites

Organism	Peak no.					
	1	2	3	4	5	6
Trepomena denticola	1.080	1.500	–[a]	0.267	0.642	0.225
Treponema pectinovorum	1.200	1.460	0.700	0.350	0.600	0.040
Treponoma vincentii	1.384	1.869	1.010	0.465	0.495	0.303
Treponema scoranskii ss. *socranskii*	1.552	1.322	0.690	0.460	0.632	0.000
Treponema socranskii ss. *buccale*	1.442	1.345	0.814	0.540	0.575	0.000

[a] No peak detected.
Mean values from three experiments, expressed in parts of the NM, are given.

seen, and peak no. 4 (0.350, Table 1) was considerably lower than peak no. 5 (0.600). In *T. denticola*, peak no. 3 was absent. Peaks no. 4 and 6 were minor. Peak no. 4 (0.267) in *T. denticola* was comparable in size to peak 4 (0.350) in *T. pectinovorum*, whereas peak no. 6 (0.225) in *T. denticola* was considerably higher than peak 6 (0.040) in *T. pectinovorum*. It should be realized, though, that it may be difficult to decide exactly which peak is no. 6 in the electropherogram of *T. pectinovorum*.

Fig. 2 shows the electropherograms after CZE analysis of the metabolic products from *T. socranskii* subspecies *buccale* and *T. socranskii* subspecies *socranskii*. The patterns of the metabolites from the subspecies showed minor differences. In *T. socranskii* subspecies *buccale* peaks no. 1 (1.442, Table 1) and 2 (1.345) were high and well resolved. In *T. socranskii* subspecies *socranskii* peaks no. 1 and 2 originated from a broad base consisting of more than two peaks. Whereas peaks no. 4 (0.540) and 5 (0.575) were almost similar in *T. socranskii* subspecies *buccale*, peak no. 5 (0.632) was higher than no. 4

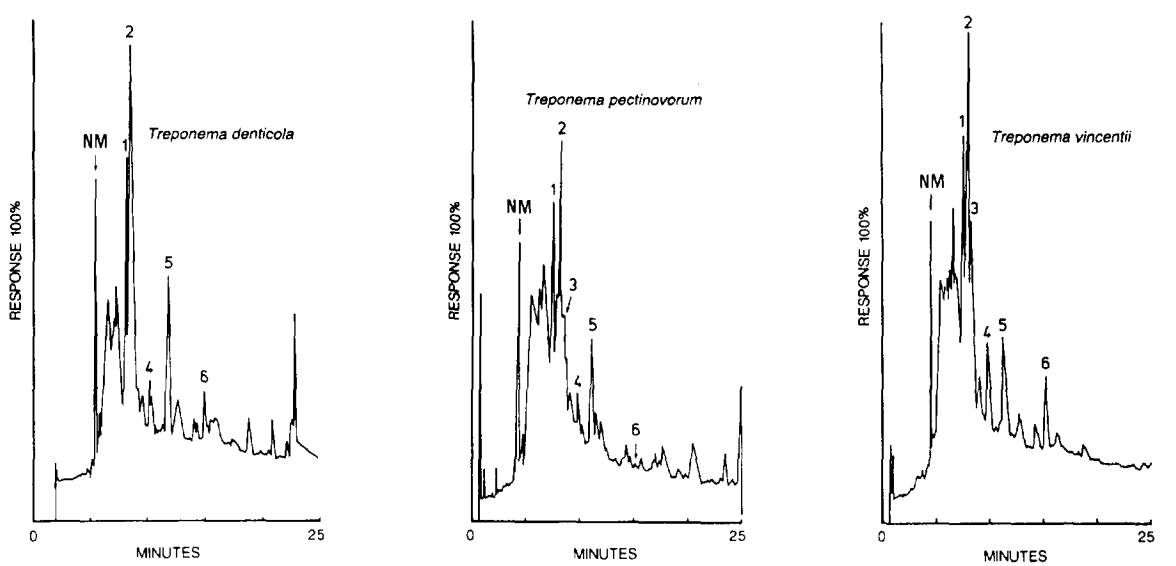

Fig. 1. Electropherograms from three different oral treponemal species after CZE of metabolic products from their culture medium. For experimental conditions, see Section 2.2. Mesityl oxide was used as a neutral marker (NM).

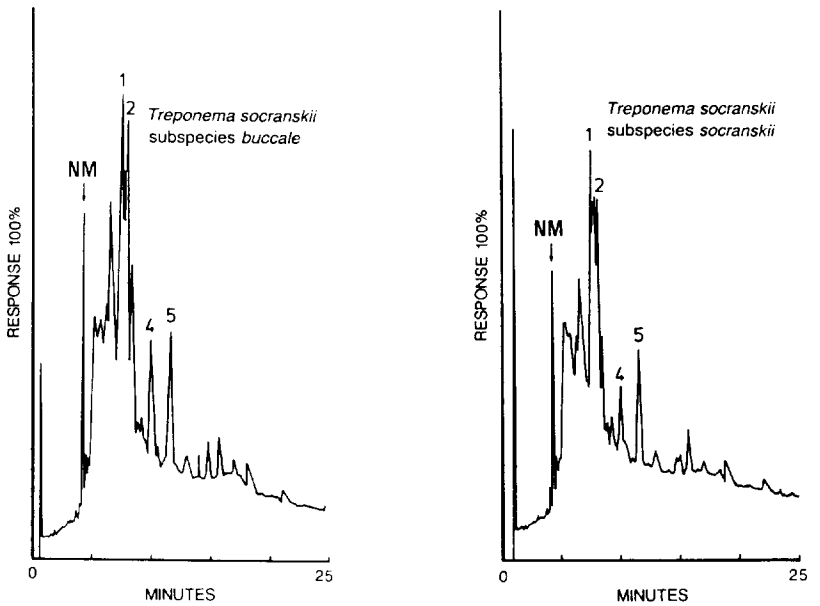

Fig. 2. Electropherograms from two different oral treponemal subspecies after CZE of metabolic products from their culture medium. For experimental conditions, see section 2.2. Mesityl oxide was used as a neutral marker (NM).

(0.460) in *T. socranskii* subspecies *socranskii*. In addition there were differences in the patterns of the minor peaks of *T. socranskii* subspecies *buccale* and *T. socranskii* subspecies *socranskii*.

There were marked differences in the patterns of the metabolites from treponemal species and subspecies (Fig. 1 and 2, Table 1). In *T. vincentii*, peaks no. 4 and 5 were quite similar to the corresponding peaks in *T. socranskii* subspecies *buccale*. However, peak no. 6 (0.303) was considerably higher in *T. vincentii* than in the two subspecies of *T. socranskii* (0.000). While peak no. 1 dominated in both subspecies (Fig. 2, Table 1), peak no. 2 was dominant in all the species.

Among the metabolic products generated from bacteria in liquid medium, only short-chain fatty acids have been used to any extent in the classification and identification of anaerobic bacteria [1]. The metabolic products generated in the present study were not identified. However, it was possible from pattern recognition of the electropherograms not only to distinguish between treponemal species, but even between treponemal subspecies. Since the present study examined only 5 different organisms, all of which though were well characterized species and subspecies of oral treponemes, an extended study with a larger number of strains should be encouraged. We think that CZE, with its unprecedented sensitivity and ability for automated separation of a number of low-volume samples in a reproducible manner with a relatively short analysis time, has appealing characteristics compared to similar chemotaxonomic techniques in current use.

4. Conclusions

CZE, using metabolic products from a liquid medium, could be used to distinguish treponemal species such as *T. denticola*, *T. pectinovorum* and *T. vincentii* and subspecies such as *T. socranskii* subspecies *buccale* and *T. socranskii* subspecies *socranskii*. This technique, which showed high resolution and good reproducibility, may serve as a valuable tool in the chemotaxonomy of oral treponemes.

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