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SHORT COMMUNICATIONS

BUOYANT DENSITIES OF DNA FROM VARIOUS STRAINS OF *STREPTOCOCCUS MUTANS*

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Summary—The buoyant densities of eight strains of *Streptococcus mutans* were determined by analytical ultracentrifugation in caesium chloride. The results were as follows (in g/cm^3): K-1-R(1·7044); SL-1(1·7040); E-49(1·7010); BHT(1·7009); FA-1(1·7000); Ingbritt(1·6967); NCTC-10449(1·6964); GS-5(1·6962). These data add to the mounting evidence for genetic heterogeneity in *Strept. mutans*.

CARIOGENESIS has been correlated with the presence of certain streptococci in the oral cavity and on tooth surfaces. Various studies dealing with the serological relationships of cariogenic strains resembling *Streptococcus mutans* (CLARKE, 1924; EDWARDSSON, 1968) have implied a certain amount of heterogeneity within this group (TOW and SHKLAIR, 1967; ZINNER and JABLON, 1968; BRATTHALL, 1970). Recent studies by COYKENDALL (1970; 1971), involving melting point determinations and homology studies on deoxyribonucleic acid extracted from different strains, indicate that these microorganisms vary widely in their genetic material and should probably be classified into several groups.

In this communication, we present data dealing with CsCl buoyant density determinations on DNA isolated from various strains of *Strept. mutans* and essentially confirm, using a different method of analysis, the results of COYKENDALL.

The strains used in this study were obtained from Dr. COYKENDALL and have been described previously (COYKENDALL, 1970). Stock cultures were maintained in Todd-Hewitt Broth (Difco) with $CaCO_3$ added. They were periodically recultured and checked for purity. Growth, lysis and extraction was as follows: 2 ml of the stock culture was inoculated into 30 ml of Todd–Hewitt Broth and incubated at 37°C overnight. This entire culture was then added to 500 ml of Todd–Hewitt Broth plus (if the DNA was to be isotopically labelled) 0·5 mCi of thymidine–methyl–[^{3}H] [6·7 Ci/mM (New England Nuclear)] and incubated at 37°C with occasional swirling. Growth was measured turbidimetrically until it approached late log phase (5–7 hr). The cells were then placed in an ice bath to stop growth, harvested and washed with 100 ml of distilled water. They were resuspended in 7·5 ml of 25 per cent sucrose to which 1·25 ml of 0·25 M EDTA (pH 8·0) was added. Then 2·5 ml of a 5 mg/ml solution of lysozyme (Calbiochem) in H_2O was added and the mixture (at pH 8·0) incubated 2–2·5 hrs at 37°C. To this 2·5 ml of a 5 mg/ml pronase (Calbiochem) solution was added and the mixture incubated another 30 min. Cells were lysed by adding 5 ml of a 2 per cent

solution of sodium lauryl sulphate in 0·01 M tris buffer at pH 8·0. Key factors in obtaining optimal lysis were: (1) stopping the growth of cells while still in log phase and (2) the long incubation in lysozyme. At this point, if the DNA was radioactively labelled, 0·1 ml of the lysate was counted using a Beckman L.S. 250 liquid scintillation counter (CLEWELL and HELINSKI, 1970). The DNA was purified from the lysate using 2 or 3 deproteinization steps with phenol-choloroform and precipitations with ethanol and isopropanol (MARMUR, 1961). The purified DNA was redissolved in 0·015 M NaCl and 0·0015 M Na citrate, pH 7·3. A u.v. spectrum of each DNA preparation was measured on a Bausch & Lomb Spectronic 505 spectrophotometer to check purity. Also a 0·1 ml sample of purified DNA solution was counted so that the percentage of DNA recovered from the cells could be calculated. Recovery ranged from 5 to 30 per cent, depending on the bacterial strain.

Analytical CsCl-equilibrium centrifugation was carried out on a portion of the purified samples in a Beckman model E ultracentrifuge by the method of MANDEL, SCHILDKRAUT and MARMUR (1968). *Micrococcus lysodeikticus* DNA (buoyant density = 1·731 gm/cm^3) was used as a marker. The percentage of guanine plus cytosine was calculated from the buoyant density by the formula of SCHILDKRAUT, MARMUR and DOTY (1962): % G+C = $(\rho - 1{\cdot}660)/0{\cdot}00098$ where ρ = buoyant density of DNA in CsCl.

The buoyant densities and corresponding G+C values of the DNA of the various strains are shown in Table 1. There are marked differences between certain strains with as much as a 0·0082 gm/cm^3 difference between the highest and lowest density DNAs. Corresponding values of G+C content agree reasonably well with values obtained by COYKENDALL (1970) and appear to reflect a considerable amount of genetic diversity among these organisms. A group consisting of strains GS-5, Ingbritt, and 10449 had a relatively low G+C content of about 37 per cent while strains K-1-R

TABLE 1. BUOYANT DENSITY ANALYSES OF DNA FROM STRAINS OF *Streptococcus mutans*

Strain	Buoyant density of each DNA (Prep. No.)	(gm/cm^3)	Mean buoyant density	Mean % G+C	COYKENDALL (1970) Mean % G+C
K-1-R	1	1·7041	1·7044	45·3	45·2
	2	1·7046			
SL-1	1	1·7037	1·7040	44·9	45·1
	2	1·7043			
E-49	1	1·7015	1·7010	41·8	43·7
	2	1·7005			
BHT	1	1·7006	1·7009	41·8	43·4
	2	1·7012			
	3	1·7010			
FA-1	1	1·6995	1·7000	40·8	42·2
	2	1·7014			
Ingbritt	1	1·6965	1·6967	37·5	37·1
	2	1·6969			
NCTC 10449	1	1·6967	1·6964	37·1	37·9
	2	1·6961			
GS-5	1	1·6962	1·6962	36·9	37·7
	2	1·6961			

and SL-1 had relatively high values of about 45 per cent. Strains with intermediate values include FA-1, BHT, and E-49. The results presented here, supporting those of COYKENDALL (1970), combined with serological relationships obtained by BRATTHALL (1970), strongly suggest the necessity for dividing these cariogenic streptococci into several groups.

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Résumé—Les densités flottantes de huit races de *Streptococcus mutans* ont été déterminées par ultracentrifugation analytique dans de la chlorure de cesium. Les résultats ont été comme suit (en g/cm^3): K-1-R(1,7044); SL-1(1,7040); E-49(1,7010); BHT (1,7009); FA-1(1,7000); Ingbritt (1,6967); NCTC-10449(1,6964); GS-5(1,6962); Ces dates s'ajoutent à l'évidence croissante de l'hétérogénéité génétique du *Streptococcus mutans*.

Zusammenfassung—Die schwimmenden Konzentrationen von acht Arten des *Streptococcus mutans* wurden durch analytische Schleuderung mit der Ultrazentrifuge in Cäsiumchlorid ermittelt. Die Ergebnisse waren, wie folgt, (in g/cm^3): K-1-R(1,7044). SL-1(1,7040). BHT(1,7009). FA-1(1,7000). Ingbritt (1,6967). NCTC-10449(1,6964). GS-5(1,6962). Diese Daten tragen zu dem Wachsen der Anzeichen der genetischen Verschiedenartigkeit bei dem *Strept. mutans* bei.

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