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SHORT COMMUNICATIONS

EVALUATION OF KANAMYCIN AS AN AID IN THE ISOLATION OF *BACTEROIDES MELANINOGENICUS* FROM DENTAL PLAQUE

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Summary—Dental plaque known to harbour *Bacteroides melaninogenicus* was removed from the gingival half of selected buccal tooth surfaces. The plaque was dispersed, diluted and cultured on medium containing 0 per cent, 0·05, 0·1 and 0·5 per cent Kanamycin. When the medium contained 0·05 per cent Kanamycin, only *B. melaninogenicus* were found and the number recovered was about 20 per cent of the *B. melaninogenicus* colonies isolated on the antibiotic free medium. Apparently certain strains of *B. melaninogenicus* are resistant to this level of Kanamycin and the addition of this antibiotic to a culture medium would facilitate the primary isolation of this organism from source material.

Bacteroides melaninogenicus is indigenous to the oral cavity of man (SOCRANSKY *et al.*, 1963; GIBBONS *et al.*, 1963). This organism plays a prominent role in certain mixed anaerobic infections (SOCRANSKY and GIBBONS, 1965) and possesses attributes which would implicate it in human periodontal disease (MACDONALD, SOCRANSKY and GIBBONS, 1963). FINEGOLD (1965) has found stock strains of *B. melaninogenicus* to be uniquely resistant to Kanamycin and Vancomycin and has suggested that the isolation of *B. melaninogenicus* from heavily contaminated source material would be facilitated by the incorporation of these antibiotics in the media.

The purpose of the present investigation was to determine whether Kanamycin would be of value in the isolation of *B. melaninogenicus* from dental plaque. Inasmuch as *B. melaninogenicus*, by virtue of its black colony, is easy to recognize on blood media, the value of such a selection medium would be limited to those situations where the number of *B. melaninogenicus* in a sample are very low and when it is necessary to have completely separated colonies in order to make a pure culture isolation.

Dental plaque known to harbour *B. melaninogenicus* was removed from the gingival half of buccal tooth surfaces of individuals institutionalized at the Plymouth State Home and Training School, Plymouth, Michigan. The plaque was removed with periodontal scalers and collected in a shell vial containing 1 ml of a fluid (RDF) similar to that described by SPEARS and FRETER (1967) except that filter-sterilized 0·01 per cent dithiothreitol (Nutritional Biochemical Corp.) was substituted for cysteine, and $CaCl_2$ and resazurin were omitted. All subsequent manipulations were performed in a plastic anaerobic chamber (ARANKI *et al.*, 1969) in an atmosphere containing 85 per cent nitrogen, 10 per cent hydrogen and 5 per cent carbon dioxide.

The plaque was dispersed mechanically by means of a Waring Blender, serially diluted and plated on a medium containing 0·2 per cent trypticase (BBL Division of Bio-Quest) 0·05 per cent yeast extract, 0·1 mg per cent haemin, 0·05 per cent KNO_3, 0·4 per cent Na_2CO_3, 0·1 per cent glucose, 0·5 μg/ml menadione, 0·01 per cent dithiothreitol, 0·023 per cent K_2HPO_4, 0·023 per cent KH_2PO_4, 0·045 per cent NaCl, 0·045 per cent $(NH_4)_2SO_4$, 0·009 per cent $MgSO_4$, 0·0045 per cent $CaCl_2$ and 5 per cent sheep blood. Capsules of Kanatrex (Kanamycin sulphate capsules 0·5g, Bristol Lab, Syracuse) were added to give concentrations of 0·05, 0·1 and 0·5 per cent Kanamycin. *B. melaninogenicus* colonies were counted after 5–7 days anaerobic incubation, i.e. oxygen levels did not exceed 20 ppm.

Kanamycin at all levels tested inhibited *B. melaninogenicus* (Table 1). Only in one instance were the counts of a 0·05 per cent Kanamycin plate higher than those recovered in the absence of the antibiotic. The recovery of *B. melaninogenicus* on the 0·05 per cent Kanamycin plates averaged about 15–20 per cent of the count

TABLE 1. EFFECT OF KANAMYCIN ON RECOVERY OF *B. melaninogenicus* COLONIES FROM DENTAL PLAQUE

Subject	Kanamycin			
	None	0·05 %	0·1 %	0·5%
	B. melaninogenicus counts × 10^6 per sample			
M.D.	TMTC*	86	60	$<10^6$
P.H.	TMTC	340	142	62
W.L.	TMTC	16	16	$<10^6$
R.H.	TMTC	66	42	$<10^6$
W.L.	TMTC	76	42	$<10^6$
J.L.	20	6	—	$<10^6$
C.C.	5	12	—	$<10^6$
J.L.	570	10	—	$<10^6$
E.G.	50	21	—	$<10^6$
K.M.	200	95	—	$<10^6$
L.H.	60	5·5	—	$<10^6$
C.C.	105	3	—	$<10^6$

* TMTC = too many to count.

found on the control plates. The recovery of *B. melaninogenicus* declined as the antibiotic level was increased, so that growth was rarely observed with levels as high as 0·5 per cent Kanamycin. Antibiotic levels lower than 0·05 per cent permitted the recovery of more *B. melaninogenicus* strains but also allowed the growth of many other isolates. If the purpose of the primary medium is isolation of *B. melaninogenicus* from heavily contaminated material, then the addition of 0·05 per cent Kanamycin would seem to be optimal. At this level, the isolates observed were almost entirely *B. melaninogenicus*. If, however, a total count of *B. melaninogenicus* is desired, then an antibiotic-free medium should be employed. This fact is illustrated in Fig. 1 where recoveries of *B. melaninogenicus* in the presence of 0·05 per cent Kanamycin and its absence are shown.

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Résumé—Des plaques dentaires, contenant du *Bacteroides melaninogenicus* ont été prélevées au niveau de la moitié gingivale de certaines surfaces dentaires vestibulaires. La plaque est dispersée, diluée et cultivée sur un milieu contenant 0%, 0,05, 0,1 et 0,5% de Kanamycine. Lorsque le milieu contient 0,05% de Kanamycine, on ne trouve que du *B. melaninogenicus* et leur nombre s'élève à environ 20% des colonies de *B. melaninogenicus* isolées sur un milieu ne contenant pas d'antibiotique. Apparemment certaines espèces de *B. melaninogenicus* sont résistantes à cette concentration de Kanamycine et l'adjonction d'antibiotique au milieu de culture peut faciliter l'isolement primaire de l'organisme.

Zusammenfassung—Zahnplaque, von der bekannt war, daß darin *Bacteroides melaninogenicus* vorhanden war, wurde von der gingivalen Hälfte ausgewählter bukkaler Zahnoberflächen entfernt. Die Plaque wurde aufgeschwemmt, verdünnt und auf ein Medium kultiviert, das 0, 0,05, 0,1 bzw. 0,5% Kanamycin enthielt. Bei einer Konzentration von 0,05% Kanamycin wurde ausschließlich *B. melaninogenicus* gefunden; die so erhaltene Anzahl entsprach etwa 20% der *B. melaninogenicus*-Kolonien, die auf antibiotikafreiem Nährboden isoliert wurden. Offensichtlich sind gewisse Stämme von *B. melaninogenicus* gegenüber dieser Kanamycin-Konzentration resistent. Der Zusatz dieses Antibiotikums zu einem Kulturmedium dürfte daher die erste Isolation dieses Organismus aus dem Ursprungsmaterial erleichtern.

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PLATE 1 OVERLEAF

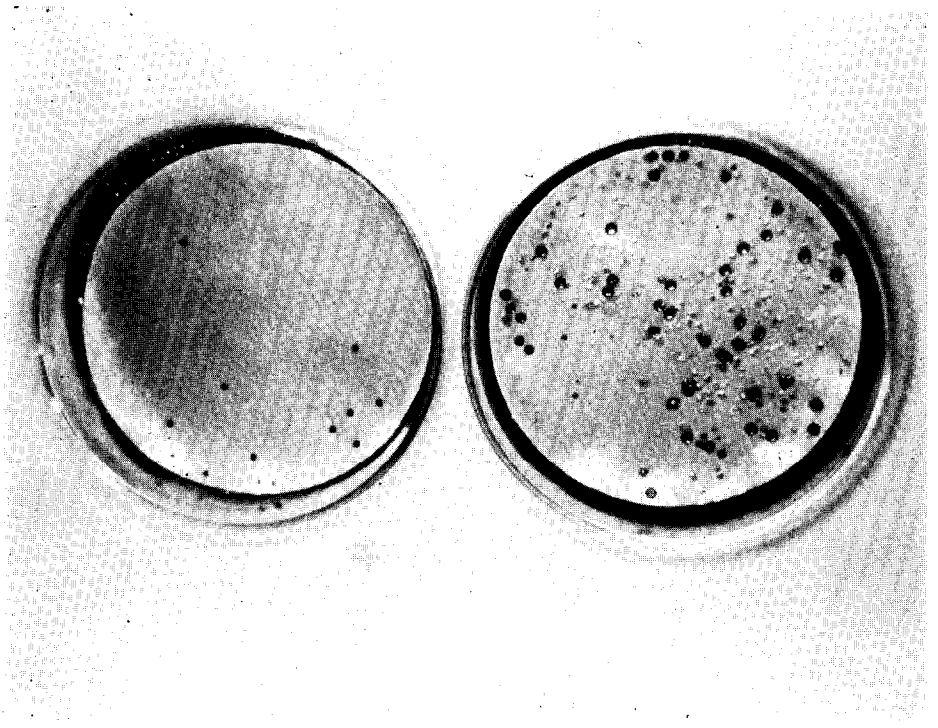

FIG. 1. A 10^{-7} dilution of dental plaque was plated in the presence and absence of Kanamycin. The plate on the left which contained Kanamycin shows only black *B. melaninogenicus* colonies, whereas the antibiotic-free medium on the right shows many more *B. melaninogenicus* colonies as well as other bacterial colonies.

PLATE 1

A.O.B. f.p. 816