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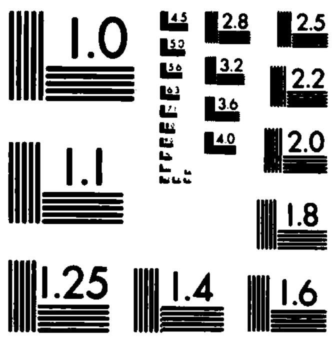

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Final Report

VIRULENCE FACTORS OF STREPTOCOCCUS MUTANS

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FINAL REPORT

VIRULENCE FACTORS OF STREPTOCOCCUS MUTANS

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INTRODUCTION

Streptococcus mutans is believed to be the prime etiologic agent of coronal caries in both humans (Gibbons, Van Houte 1975) and animals (Fitzgerald 1968). It has been suggested that the cariogenicity (virulence) of S. mutans is due to the ability of the organism to adhere to the tooth surface, then colonize or aggregate by synthesizing water insoluble glucans, and produce lactic acid by catabolizing fermentable carbohydrates to demineralize the enamel of teeth. The concept that the initiation of dental caries is associated with the development of sticky (insoluble) glucans has been proposed (de Stoppelaar, Konig, Plassecheart, Van der Hoeven 1971). They reported that a mutant of S. mutans, that was unable to synthesize insoluble glucans, was no longer cariogenic in germfree rats and that caries activity was greatly reduced in hamsters. The importance of the glucans in the etiology of dental caries has been reviewed by a number of authors. (Newbrun 1972; Gibbons, Van Houte 1975). There is little doubt that the insoluble glucan synthesized from sucrose by S. mutans plays a significant role in caries activity.

A second virulence factor characteristic of S. mutans is its ability to produce lactic acid. Some investigators (Jordan 1965; Drucker, Melville 1968) found no significant differences between cariogenic and non-cariogenic streptococci regarding either the amount of lactic acid or other types of fermentation acids produced. However, others (Hillman 1978; Mao, Rosen 1980) isolated several mutants of S. mutans that made less lactic acid than the wild type strains and lower caries activity in test animals. (Mao, Rosen 1980; Johnson, Gross, Hillman 1978). The above data supports the importance of lactic acid in the etiology of dental caries.

In previous studies dealing with the virulence of S. mutans either glucan synthesis or lactic acid production has been, evaluated alone relative to caries activity. The objective of this study was to determine the relationship between insoluble

METHODS

Quadruplicate cultures of each organism were grown for 48 hours in 5 ml of a chemically defined medium containing 5% sucrose (Osborne, Lamberts, Meyer, Roush 1976). Glucan was determined by using the total carbohydrate, phenol-sulfuric acid procedure. Lactic acid was assayed by using a gas chromatograph. DNA was determined by the diphenylamine procedure (Ashwell 1957). The amounts of lactic acid and insoluble glucan were expressed as moles of lactic acid per mg of DNA and mg of glucose equivalents per mg of DNA, respectively.

Strains of S. mutans used in this study were isolated from dental plaque of naval recruits. Half the isolates came from individuals with no caries and the remainder from individuals with rampant caries. Sterile dental floss was used to collect plaque from interproximal areas of posterior teeth. Serotypes of S. mutans were determined by the biotyping method of Shklair and Keene (Shklair, Keene 1974). The main criteria for selection of the isolates of S. mutans used in this investigation was that they produce varying amount of lactic acid and insoluble glucan. Distribution by serotypes was a secondary consideration.

Germfree rats of known age were purchased from Charles River Breeding Laboratories, Wilmington, MA., or from the University of Wisconsin, Madison, Wi., At 22 days of age they were placed in sterile flexible plastic isolators. The rats were distributed into 4 isolators, 10 rats per isolator. They were given drinking water (double distilled), sterilized by autoclave. Diet 2000, fortified with 1% Gustafsson's vitamin mix (Teklad Mills, Madison, Wisc.), was sterilized by exposure to 5 M rads of Cobalt 60 irradiation (Neutron Products, Dickerson, Md.). Water and diet were given ad libitum. The rats were infected by swabbing the molar teeth with a fresh culture and placing the remainder of the culture into the drinking water. This was done for two consecutive days. Sterility checks were performed on blood agar incubated at 37^{0}C in anaerobic and aerobic atmospheres every two weeks. Rats were removed at the end of 5 weeks and caries scores were determined according to the method of Keyes (Keyes 1958).

RESULTS

A total of 16 strains of S. mutans were evaluated in two experiments. The results are given in Table 1.

All 16 strains of S. mutans caused caries in gnotobiotic rats.

There was no significant correlation between buccal-lingual and total severity caries scores and levels of glucan, lactate, or levels of S. mutans. Figure I shows a significant positive correlation between lactate production and proximal caries ($p < 0.001$)

DISCUSSION

Possible explanations for lack of correlation between levels of insoluble glucan and buccal-lingual caries are as follows: (1) not enough insoluble glucan was produced; (2) tenacity of organisms to the teeth could have been weak; (3) in vitro may not parallel in vivo conditions; (4) the rat may not be a suitable model. (Other animal model systems should be evaluated to determine the correlation of insoluble glucan with caries.); (5) numbers of microorganisms may not have reached a critical level on the buccal-lingual surfaces of the teeth; and (6) adherence factors may be more significant than aggregating factors.

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TABLE 1

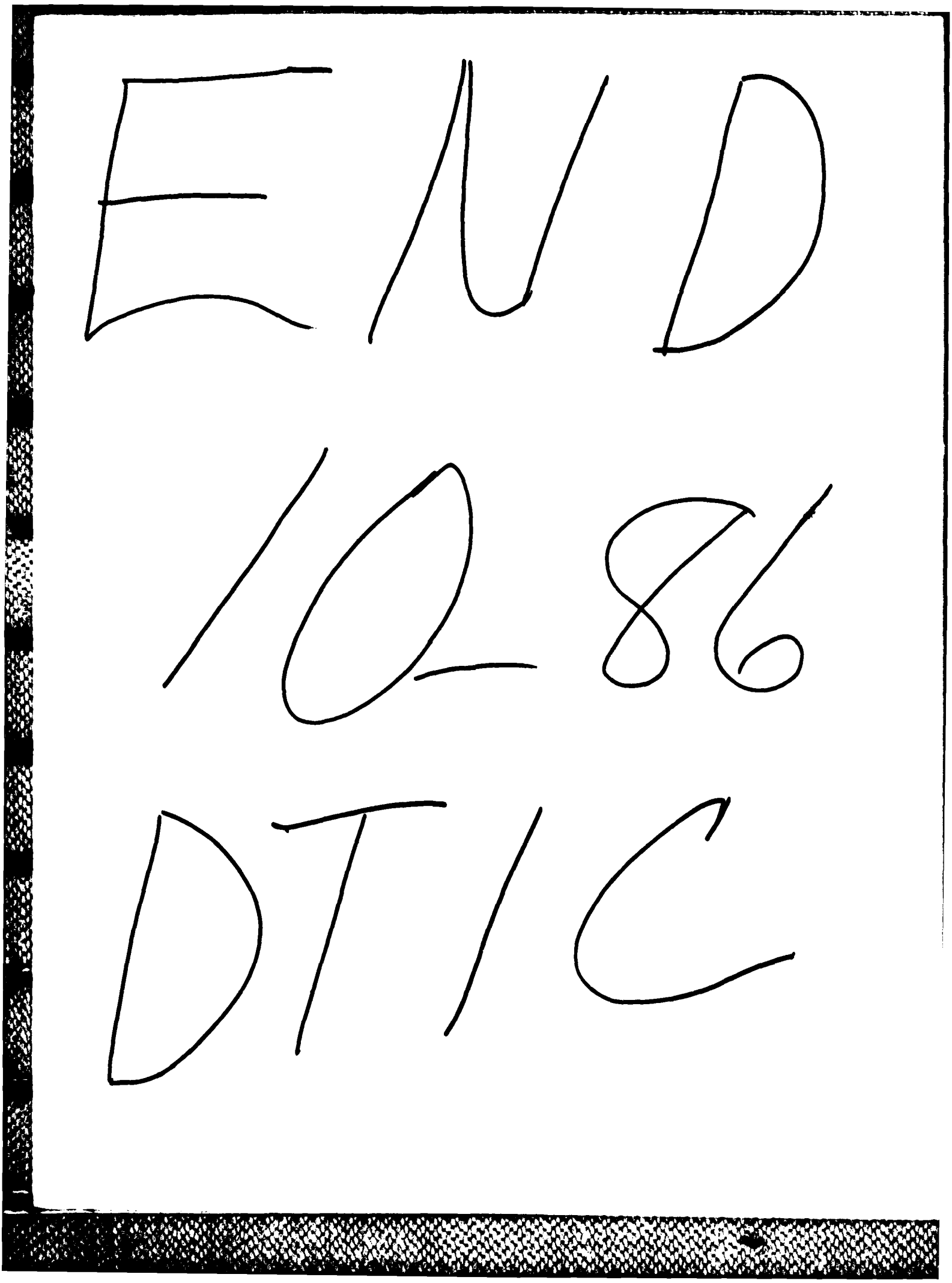
GLUCAN, LACTIC ACID AND CARIES ACTIVITY OF SELECTED STRAINS OF STREPTOCOCCUS MUTANS

	Glucan (mg/mg DNA)				Lactic Acid (μ moles/μg DNA)		Mean Caries Scores Gnotobiotic Rats *			S. mutans	
	Soluble		Insoluble								
	Pre	Post	Pre	Post	Pre	Post	Proximal	Buccal-Lingual	Total Severity	Serotype	No. x 10 / Quadrant
DOWD	1.00	13.0	70	160	3.0	3.0	1.9	8.1	104	d/g	-
STACEY	89.0	138	12	8	2.7	2.2	2.4	7.6	107	c	-
WARD	98.0	107	19	34	2.0	1.8	1.7	1.2	96	c	-
GUNN	77.4	113	4	19	2.9	2.2	2.6	2.6	104	c	-
107 B	143	35.5	5	3	1.7	1.1	0.3**	8.8	77	b	-
OMB 175	24.9	10.5	15	26	1.2	2.3	2.6	7.0	94	e	-
THRESHER	39.4	25.8	7	3	3.8	2.2	2.3	12.3	98	c	-
HS-6	48.9	51.4	25	26	2.0	1.6	1.8	11.4	103	c	-
PARKER	91	-	18	-	4.0	4.1	6.6	15.6	77	c	110
SILVER	85	96	12	7	3.1	3.5	6.4	16.4	71	c	130
VON WICK	122	108	26	46	3.8	3.9	6.6	17.2	71	c	130
CLARK	63	59	13	12	2.8	3.7	7.0	11.6	68	c	198
130 P	86.7	12.0	0.79	6.2	2.7	4.9	13.8	4.2	105	b	221
TEA	71.0	5.7	78.3	46.7	3.1	5.4	25.6	8.2	124	d/g	124
SINK	1.4	5.7	29.8	43.5	3.4	3.7	26.0	7.8	118	d/g	239
FORD	4.3	5.1	50.5	42.8	3.3	4.7	27.0	1.8	112	d/g	167

*Values within lines are not significanlty different. **107 B significanlty different from OMZ 175 and Thresher. Each block of four lines represents a separate experiment. There were 10 rats/organism in the first 3 block and 8 rats/organism in the fourth block.

CORRELATION OF PROXIMAL
CARIES WITH LACTIC ACID
r=0.78
p<0.01
Proximal Caries Score
Lactic Acid (u moles/ug DNA)

FIGURE 1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