KOR. JOUR. MICROBIOL. Vol. 19, No. 3, 137~141, 1981

Relative Level of Sucrose Metabolizing Enzymes in Oral Streptococci

CHOIH, Sun-Jin

(Dept. of Microbiology, Dental College, Seoul Nat'l University)

구강 Streptococci가 가진 Sucrose 대사 효소의 활성도의 비교

崔 宣 鎭

(서울대학교 치과대학 미생물학 교실)

ABSTRACT

Occurrence and distribution of sucrose metabolizing enzymes in oral streptococci had been studied. In these studies, the carbohydrate component of the culture medium had been glucose. I have extended these studies by analyzing bacterial culture supernatants for the relative content of hexosyltransferases,namely glucosyl and fructosyltransferase. As a carbohydrate, fructose was used. The growth measured for nine oral streptococci (*Streptococcus mutans* strains BHT, ING, AHT, 6715, LM-7, and SL-1; *Streptococcus sanguis* 903, 9811, and M-5) varied. The level of glucosyltansferase activity also varied among *S. mutans* strains, and its level in *S. sanguis* was relatively low. Fructosyltansferase activity of the various strains fluctuated more than that of glucosyltransferase. *S.mutans* strain LM-7 had significantly higher level of both enzymes. As a whole, fructose-grown cultures had generally an agreeable trend of enzyme activity to those from glucose-grown cultures.

INTRODUCTION

Dental caries has long been recognized as an infectious disease related to the presence of dense, adhesive, microbial deposits (dental plaque) on the surfaces of teeth (Newbrun, 1978). Demonstration of the etiological role of certain plaque forming streptococci, namely *S. mutans* (of which there are seven recognized serotypes) as infectious agents in multisurface dental caries in rodents (Fitzgerald, 1974) and primates (Bowen, 1969) has focused attention on a similar role of these microorganisms in humans. Their role in causation of dental caries in humans is now strongly established (Gibbons *et al.*, 1974) with *S. mutans* serotype c the most prevalent serotype associated with human dise ase (Bratthall, 1970).

The direct correlation of dietary sucrose, specifically the frequency of consumption, with the incidence of dental caries associated with *S. mutans* in humans and in experimental animal model systems is striking and well established (Hoover *et al.*, 1980). The biochemistry and physiology of *S. mutans* with respect to sucrose metabolism is essential to the caus-

This study was supported, in part, by the 1980 clinical research grant from the Seoul National University Hospital.

ation and, therefore, the prevention of dental caries (Tanzer and Freedman, 1978).

Sucrose dissimilation by *S. mutans* and other related streptococci involved i) hexosyltransferase activity, or ii) transport of the carbohydrate to intracellular sites for subsequent catabolism (Brown, 1974). *S. mutans* can hydrolyze a small portion of available sucrose by the action of hexosyltransferases, namely glucosyltransferase (GTF) and fructosyltransferase (FTF). The remainder of the sucrose has been thought to be transported into the cytoplasm.

The synthesis of high molecular weight water-insoluble dextran and levan polymers by hexosyltransferases is considered to be primarily responsible for the ability of these microorganisms to colonize and develop plaques on the smooth surfaces of teeth(Newbrun, 1978). The cohesive character of these polymers in plaque very likely contributes to the adherence of the cells. These polymers as components of the plaque matrix, also function as a diffusion barrier for the acids formed within the plaque by the acidogenic oral bacteria(Newman, 1980). Clearly, the metabolism of sugars is an important determnant in the ecology of oral streptococci.

Glucose-grown cultures contain GTF less dependent on dextran primer, possibly because small amount of dextran is synthesized from the contaminating source of the medium components, during early culture growth; and it promotes the formation of high molecular weight enzyme aggregates (Germaine *et al.*, 1974). On the other hand, the enzyme from fructose-grown culture is primer-dependent and produces greater quantities of GTF(up to four-fold), possiblely due to fructose-inhibition of dextran synthesis during culture grown (Germaine and Schachtele, 1976). Therefore, a non-aggregated primer-dependent from of GTF may be obtained from fructose-grown culture of *S. mutans*.

As a part of studies designed to analyze the plaque-fcrming capability of oral bacteria, we have compared the relative level of two sucrose metabolizing enzymes for nine representative oral streptococci grown in the presence of fructose.

MATERIALS AND METHODS

1. Bacterial strains and growth conditions

The strains of *S. mutans* utilized in this study were obtained from Dr. C.F. Schachtele and *S. sanguis* strains from Dr. B. Rosan. Stock bacterial strains were plated on Mitis-Salivarius (MS) agar and incubated anaerobically (Gas Pak system. BBL) at 37°C for 2 days. Single colonies from the MS plate were inoculated into 10ml of Trypticase soy broth (TSB) containing 0.4M fructose and $NaPO_4$ buffer (0.1M, pH 6.8) and incubated anaerobically overnight. This overnight culture was used to inoculate 500ml each of TSB supplemented with yeast extract (0.1%), fructose (0.4M), and $NaPO_4$ buffer(0.1M, pH 6.8). After overnight growth (approximately 0.3 absorbance units at 600nm), cultures were chilled on ice, and the bacteria were removed by centrifugation (10,000 x g, 10 min, 4°C). Residual cells and debris were removed by filtration(0.45μm membrane filter, Millipore Filter Corp.).

2. Enzyme preparation and assay

The procedures previously described by Chludzinski *et al.*, (1974) were utilized. Briefly, both hexosyltransferases were precipitated from culture supernatants at 4°C by adding solid ammonium sulfate(enzyme grade, Sigma), with stirring, to 60% of saturation. The precipitate was kept in refrigerator overnight, and then collected by centrifugation, dissolved in 0.01M sodium acetate buffer, pH5.5, and extensively dialyzed against the same buffer. Formation of a small amount of precipitate

was removed by centrifugation (10,000 x g, 20 min) prior to use.

1) Glucosyltransferase assay

The reaction tube received 75μl of the reaction mix, 25μl of 0.01M sodium acetate buffer, and 25μl of enzyme. The reaction mixture contained 40mM sodium actate buffer, pH 5.5, 1.7 mM NaF, 35mM total sucrose containing about 11.7 mM L[U-^{14}C] sucrose (3.35Ci/mole, New England Nuclear Corp.), 33.3mM dextran T_{10} (molecular weight 10,000, Pharmacia).

2) Fructosyltransferase assay

Total sucrose (35mM) containing about 11.7 mM [U-^{3}H-fructose] sucrose (275mCi/mmole) was used as substrate. The primer T_{10} was omitted. All other components of the reaction mixture was the same as for the GTF assay.

For the measurement of both enzyme activity, the reaction mixtures were incubated for 15 min and then 10μl of them was processed as described by Germaine *et al.*, (1974). Activity from the 15min incubation point was on linear portion of the time-course assay.

RESULTS AND DISCUSSION

One or two strains of each serotype (major 5 serotypes) of *S. mutans* and *S. sanguis* were studied. The final pH and relative absorbance of each 18hr culture were tabulated in Table 1. As can be seen, the growth varied among strains of *S. mutans*, and also variable was the final pH of the cultures. The strains of *S. mutans* grew generally faster than *S. sanguis*, with lower final pH;however, from the *S. sanguis* strain 903 were comparable to those of *S. mutans*. Of the taxa comprising the major streptococcal component of the oral cavity, *S. sanguis* appears homogenous (Gibbons, 1972), whereas *S. mutans* are more heterogeneous by virtue of their distinctive serotypes (Bratthal, 1970) and also by the existence of multiple deoxyribonucleic acid hybridization group (Coykendall, 1974) This fact probablely reflects the characteristics of the strains of *S. mutans* as illustrated by the variable growth and pH of the culture medium. Among oral Streptococci, *S. matans* has been known as one of the most acidogenic organisms and being relativly aciduric as well. In addition, this organism utilizes sucrose, when supplied with it, to form more latic acid and decrease the pH more rapidly than other streptococci. The data in Table 1 clearly show a facet of such a heterogeneity of *S. mutans* as described above.

Table 1. Growth expressed as absorbance and final pH of cultures of oral streptococci

Organisms		Serotype	Absorbance*	Final pH
S. mutans	BHT	b	100	5.5
	ING	c	96	4.4
	AHT	a	64	5.0
	6715	d	60	5.8
	SL-1	d	88	4.7
	LM-7	e	80	4.7
S.sanguis	903	I	84	5.0
	M-5	II	42	6.4
	9811	Heterogeneous	35	6.3

* Expressed as percentage of that from *S.mutans* BHT strain.

For the preparation of the two hexosyltransferases, culture media, filtered and neutralized, were precipitated with ammonium sulfate at 60% of saturation. The ammiuonm sulfate concentration used was increased from 40% (Mukasa and Slade, 1973) to 60% in order to recover most of the activities (95%) present in the filtered medium (Chludzinski et al., 1974) Since the activity of both GTF and FTF is extracellular (Chassy et al., 1976), this method of enzyme preparation could account for most of the activity present in the cells.

The relative activity of both GTF and FTF is shown in Fig. 1. The relative GTF activity varied among streptococci studied. Within the strains of *S. mutans*, the enzyme activity ranged

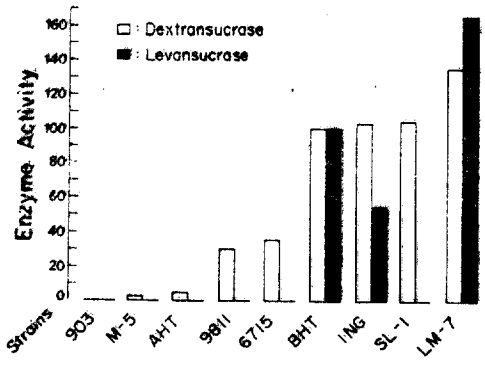

Fig. 1. Glucosyltransferase (dextransucase) and fructosyltransferase (levansucrase) activity from the strains of *S.mutans* and *S. sanguis*. Results are expressed as percentage of *S.mutans* strain BHT activity.

from 5.2% to 135% The strains of *S. sanguis* were relatively low in their activity as compared to those of *S. mutans*. Relative activity of the FTF fluctuated much more than that of GTF did. Among *S. mutans*, the strains LM-7, BHT, and ING were producers of higher activity, whilst this enzyme was not measureable in three other strains. No FTF was detected in *S. sanguis* as was reported previously by other investigators (Chassy *et al.*, 1976)

Of the *S. mutans* strains, LM-7 had significantly higher levels of both enzymes. This is in agreement with the investigation by Chassy *et al.* (1976), although the carbohydrate of their medium is different from the carbohydrate utilized in the present study. However, since GTF and FTF are constitutive enzymes (Wenham *et al.*, 1979) the rate of their synthesis would be expected to be similar under the conditions of various substrates being added to the medium. This may explain why our fructose-grown cultures had generally an agreeable trend of enzyme activity to those from the glucose-grown cultures (Chassy *et al.*, 1976)

Because *S. mutans* strain SL-1 and *S.sanguis* strain 9811 produce no detectable amount of FTF, but are fairly good in GTF production, they would be the strains of choice when one studies a mechanism of in vitro plaque-formation where GTF or its catalytic product glucans are involved, because the interference of FTF or its product levan could be easily eliminated.

요 약

구강세균 streptococci에 들어있는 설탕(sucrose) 대사효소 활성도를 비교 검토하였다. 두개의 hexosyltransferase 즉 glucosyltransferase와 fructosyltransferase를 세균배양 상층액을 써서 측정하였다.

과거의 연구에서는, 배지의 구성 탄수화물로 glucose를 사용하였으나 본 실험에서는 fructose를 썼다. 아홉균주의 구강 streptococci (*S. mutans* 여섯주, *S. sanguis* 석주)로 부터 측정한 성장값은 균주에 따라서 차이가 많았다.

Glucosyltransferase의 효소활성은 *S. mutans* 균주사이에서 차이가 역시 있었고, 이 효소의 활성이 *S. sanguis*에서는 비교적 낮았다.

Fructosyltransferase의 활성은, 균주에 따라서, glucosyltransferase의 그것보다 차이가 더 있었다. *S. mutans* LM-7 균주는 두 효소활성도에 있어서, 다른 균주에 비하여, 훨씬 높았다. 요컨대, fructose의 존재에서 자란 세균이 생성한 효소활성도는 glucose를 써서 성장한 세균의 것과 유사하였다.

REFERENCES

1. Bowen, W.H. 1969. Induction of rampant dencaries in monkeys (Macaca irus). *Caries Res.* 227—237
2. Bratthall, D. 1970. Demonstration of five serological groups of streptococcal strains resembling *Streptococcus mutans*. *Odontol. Revy* 21, 143—152
3. Brown, A.T. 1974. Carbohydrate metabolism in caries-conducive oral streptococci, p. 689—719 In H.L. Sipple and K.W. NcNutt (ed.), Sugars in nutrition, vol. 33. Academic Press Inc., New

York.

4. Chassy ,B.M., J.R. Beall, R.M. Bielawask, E.V. Porter, and J.A. Donkersloot. 1976. Occurrence and distribution of sucrose-metabolizing enzymes in oral streptococci. *Infect. Immnn.* **14** 408—415
5. Chlud zinski, A.W., G.R. Germaine, and C.F. Schachtele. 1974. Purification and properiies of dextransucrase from *Streptococcus mutans. J. Bacteriol.* **117**, 1—7
6. Coykendall, A.L. 1974. Four types of *Streptococcus mutans* based on their genetic and biochemical characteristics. *J. Gen. Microbiol.* **83.** 327—338
7. Fitzgerald, R.J. (ed.). 1974. *Streptococcus mutans* and dental caries. U.S. Department of Health, Education and Welfare Publication no. NIH 74—286. U.S. Government Printing Office, Washington, D.C.
8. Germaine, G.R., A.M. Chludzinski, and C.F. Schachtelt. 1974. *Streptococcusmutans* dextransucrase: requirement for primer dextran. *J. Bacteriol.* **120**, 287—294
9. Germaine, G.R., C.F. Schachtele. 1976. *Streptococcus mutans* dextransucrase: Mode of interaction with high-molecular-weight dextran and role in cellular aggregation. *Infect. Imm un.* **13**, 365—372
10. Germaine, G.R., C.F. Schachtele, and A.W. Chludzinski. 1974. Rapid filter paper assay for the dextransucrase activity from *Streptococcus mutans. J. Dental Res.* **53**, 1355—1360
11. Gibbons, R.J. 1972. Ecology and cariogenic potential of oral streptococci, p. 371—385. In L.W. Wanamaker and J.M. Matsen (ed.), Streptococci and streptococcal diseases. Academic Press Inc., New York.
12. Gibbons,R.J., P.F. Depaola, D.M. Spinell, and Z. Skobe. 1974. Interdental localization of *Str eptococcus mutans* as related to dental caries experience. *Infect. Immun.* **9**, 481—488
13. Hoover, C.I., E. Newbrun, G. Mettraux, and H. Graf. 1980. Microflora and hemical compcosition of dental plaque from subjects with hereditary fructose intolerance. *Infect. Immun.* **28**, 853—859
14. Mukasa, H., and H.D. Slade. 1973. Mechanism of adherence of *Streptococcus mutans* to smoo th surface. *Infect. Immun.* **8**, 555—562
15. Newbrun, E. 1978. Cariology. The Williams Company, Baltimore.
16. Newman, H.N. 1980. Dental plaque: The ecolo gy of the flora on human teeth. Charles C. Thomas Ppblisher. Springfield, Illinois.
17. Tanzer, J.M., and M.L. Freedman. 1978. Genetic alterations of *Sterptococcus mutans* virulence. *Adv. Exp. Med. Biol.* **107**, 661—672
18. Wenham, D.G., T. D. Hennessey, and J.A. Cole. 1979. Regulation of glucosyl-and fructosyltransferase synthesis by continuous cultures oe *Streptococcus mutans. J. Gen. Microbiol.* **114**, 117—127