CHAPTER II

MATERIAL AND METHODS

1. Materials

1.1 Microorganisms

1.1.1 *Streptococcus mutans* 1B-1600 was obtained from Dr. Stig Edwardson ACADEMIA REGIA ODONTOLOGICA, MALMO, SWEDEN. (This strain can find in Thailand)

1.1.2 *Streptococcus sanguis* was obtained from the department of Microbiology, Faculty of Dentistry, Chulalongkorn University.

1.2 Culture media and chemicals

1.2.1 Bacto Peptone (Difco Laboratories, U.S.A.)

1.2.2 Bacto Tryptone (Difco Laboratories, U.S.A.)

1.2.3 Calcium hydroxide (RIEDEL-DEHAEN, AG, SEELZE-HANNOVER, GERMAN)

1.2.4 Cupric sulphate (M&B Laboratory chemicals, ENGLAND)

1.2.5 Dipotassium hydrogen phosphate (M&B Laboratory chemicals, ENGLAND)

1.2.6 Glucose (Difco Laboratories, U.S.A.)

1.2.7 Hydrochloric acid conc. 37.6% (Merck, GERMANY)

1.2.8 Lactose (Difco Laboratories, U.S.A.)

1.2.9 Mannitol (Difco Laboratories, U.S.A.)

1.2.10 P-hydroxy diphenyl (SIGMA chemical company, U.S.A.)

1.2.11 Sodium chloride (Mallinckodt chemical works, ST. LOUIS, NEWYORK, MONTREAL)

1.2.12 Sodium hydroxide (RIEDEL-DEHAEN, SEELZE-HANNOVER, GERMANY)

1.2.13 Sucrose (Difco Laboratories, U.S.A.)

1.2.14 Sulfuric acid conc. (Merck, GERMANY)

1.2.15 Tryptic soy broth (Difco Laboratories, U.S.A.)

1.2.16 Xylitol (SIGMA chemical company, U.S.A.)

1.3 Equipments and glasswares

1.3.1 Anaerobic incubator (NATIONAL APPLIANCE, U.S.A.)

1.3.2 Autoclave (A.H.T. Co., PHILA.DA., U.S.A.)

1.3.3 Balance (Sartorius, GERMANY)

1.3.4 CYCLO-MIXER (Clay-Adamps. U.S.A.)

1.3.5 Cuvette (Bausch & Lomb, U.S.A.)

1.3.6 Incubator (ARTHUR H. THOMAS Co., U.S.A.)

1.3.7 Laboratories Equipments

1.3.8 Magnetic bar (CENCO INSTRUMENTEN MIJ. NV., NETHERLAND)

1.3.9 Magnetic stirror (CENCO INSTRUMENTEN MIJ. NV., NETHERLAND)

1.3.10 pH meter (Radiometer, DENMARK)

1.3.11 Safguard centrifuge (Clay-Adamps, U.S.A.)

1.3.12 Spectrophotometer (Bausch & Lomb, U.S.A.)

1.3.13 Water bath (PRECISION SCIENTIFIC Co., U.S.A.)

2. Methods

2.1 Determination of lactic acid by p-hydroxydiphenyl (Snell and Snell's method)

Biological sample. To remove proteins and other interfering substances treat 1 ml of the sample with 5 ml of 2% copper sulfate pentahydrate solution and add 600 mg of calcium hydroxide. Shake and let stand at room temperature for 30 minutes. Centrifuge for 1 minute. To 0.5 ml sample add 3 ml of sulfuric acid copper sulfate reagent. Mix and place in ice bath. Stopper and heat for 15 minutes at 100°C. Cool briefly in ice and centrifuge for 1 minute to force down any liquid adhering to the side. Add 0.1 ml of the p-hydroxydiphenyl reagent and shake to disperse the precipitated reagent. Place in ice and stopper. Maintain at 30°C for 30 minutes to develop the color and heat at 100°C for 1 minute to decompose excess reagent. Cool and read at 570 nm against water.

2.2 Studies on lactic acid production of *S. mutans* and *S. sanguis* after cultivation in various concentrations of sugars.

Each culture was cultivated in tryptic soy broth and incubated overnight at 37°C. Each of them was numerized by Spectrophotometer by dilute them until optical density of *S. mutans* was .07 and *S. sanguis* was .09 and then standardized with tryptic soy broth to contain 0.1 ml to use as standard inoculum.

Each standardized culture was separately inoculated into 0.01%, 0.02%, 0.03%, 0.05%, 0.1%, 1% and 3% concentrations of each kind of sugars, pH 7.2, namely Glucose, Sucrose, Lactose, Mannitol and Xylitol. The formula of each concentration of each sugar was shown in Appendex.

One set of sugars incubated in aerobic incubator, another set incubated in anaerobic incubator by using N_2 95% and CO_2 5%.

After incubated overnight at 37°C. Each of them was measured pH, optical density and lactic acid.

2.3 Studies on the effects of pH on lactic acid production of S. mutans and S. sanguis.

Prepare optimum concentration of each of five sugars which promote growth and vary pH to pH 5, pH 6, pH 7, pH 8, and pH 9, by use 1N HCl and 1N NaOH.

Each of five sugars was inoculated by standardized culture

Separated sugars to two sets and incubated as 2.2

After incubated 24 hrs. at 37°C. Determinated lactic acid each of them.