Slime Formation in Bacteria

An Honors Thesis

By

Vicki M. Taylor

Thesis Director

Donald A. Hendrickson

Ball State University

Muncie, Indiana

May, 1979

Spring Quarter

SpColl
Thesis
LD
2485
.
1979
.T29

OUTLINE

I. PURPOSE

II. OBJECTIVES

III. REVIEW OF RELATED RESEARCH

- A. HISTORY
- B. CHARACTERISTICS
- C. ADHESION
- D. SYNTHESIS OF POLYSACCHARIDE
- E. CONTROL OF SLIMES

IV. RESEARCH

- A. MATERIALS AND METHODS
  1. IDENTIFICATION OF ORGANISMS
  2. MINIMAL SALTS MEDIA
  3. METHOD
- B. DATA AND RESULTS
- C. CONCLUSIONS

V. LITERATURE CITED

VI. APPENDIX

I. PURPOSE

The purpose of this research is to find a minimal salts medium that will induce the organism to produce the thickest glycocalyx or slime layer attainable on laboratory media, and then to experiment using different compounds such as detergents, cationic and/or metallic salts, chlorinated species, etc. to see if any decrease in the amount of slime formation is noted.

II. OBJECTIVES

The main objectives of this research are: 1) To determine what nutrients are needed by the bacteria to form the most prolific slime layer (glycocalyx), 2) To determine the mechanism by which the bacteria utilize the capsule (glycocalyx) to "stick" together and to inert surfaces, and 3) To attempt to find a compound or compounds that will cause a decrease in the amount of glycocalyx produced or prevent attachment.

III. REVIEW OF RELATED RESEARCH

A. HISTCRY

The subject of this research was slime formation in bacteria. The word "slime" in this sense is intended to mean the extracellular tangled mass of fibers known commonly as "glycocalyx" or "sweet husk"(1,5,19). The nature of these fibers, which extend from the cell surface, has become a subject of increasing interest. It is well established that a carbohydrate-rich layer exists at the cell surface and

among the many functions it appears to play an important role in the cellular adhesion and other contact phenomena (6). The adhesion mediated by the glycocalyx determines particular locations of bacteria in most natural environments; more specifically, it is a major determinant in the initiation and progression of bacterial diseases ranging from dental caries to pneumonia (5).

Studies are widely diverse on the subject of cellular adhesion. Some of the current research in water pollution is attempting to understand functions in the control of pollution in natural streams (9). In polluted waters, events leading to primary film formation are due to the abundance of a great variety of soluble and particulate organic substrata which gives rise to very complex populations of bacteria, protozoa, and diatoms(12). Initially, the adhesion of bacteria and other microorganisms and their products to solid, inert surfaces may affect the colonization of marine animal larvae and other microorganisms, or the settling of residual organic matter, mineral matter, etc. and thereby serve as a precursor of heavy destructive biological fouling(12,2). A second example of current research is the study of bacterial populations attached to gut mucosa and food particles. It is believed that the ability of the bacterium (Ruminococcus albus) to adhere can influence its pathogenicity and can increase its access to potential food sources(14). Other bacteria which are of

pathological importance to humans are Streptococcus mutans and the ability to colonize the tooth, S. salvarius and gum colonization, Bacteroides fragilis with adhesion to the peritoneum (studies done on rats), Vibrio cholera which adheres to the "brush border" of the human intestine, and Neisseria gonorrhea which will adhere only to the lining of the urethre, though adhesiveness in some cases is due to contacts other than a glycocalyx(5). In another light, a number of cases have been reported in which water-carrying conduits have suffered from remarkable losses in delivery capacity within relatively short operation periods. The loss was due to a thin slime layer(2,20). This reduction in capacities is caused by frictional losses(2). The slime layer also causes other problems industrially such as the accumulation of slime in the machinery of newspaper processing plants(11) and in contamination of sugar refineries (17). If the bacteria native to a rushing stream were not adherent, the stream would be virtually sterile because the bacteria would be swept away faster than they could swim against the current. The adaptive value of adherence is not hard to understand. The bacteria live on the organic molecules they extract from the passing water. Life in a stationary location with a continuous supply of organic nutrients, and with vigorous aeration and excellent waste removal also provided by the stream clearly agrees with the bacteria(5). Most certainly it can be seen that the production of the

is illustrated in the concentric layering around the organisms, and also exhibited by spaces which are found between the cells. These spaces demonstrate that the slime is not composed of a gelatinous mat of uniform height, but rather that each cell is autonomous within its own matrix and this matrix is usually joined with a neighboring cell matrix(9). This is a very important aspect of the slime-forming organisms. If the glycocalyx did not originate from the bacterial wall, then the bacterium found in an aqueous suspension apart from the main cell mass (ie. in a stream, urinary system, blood,etc.) would not be able to adhere to surfaces to obtain the optimal nutrition, or establish and propagate a microenvironment.

In one journal article reviewed, Fletcher and Floodgate (8) claim that actually there are two types or stages of development of the glycocalyx which were identified while using a Ruthenium Red preparation(a stain specific for acidic polysaccharides). The first substance, designated as primary acidic polysaccharide, was an electron-dense layer on the wall surface of both suspended and attached bacteria. A secondary acidic polysaccharide was found predominantly in the preparations of attached bacteria and was usually associated with groups of organisms. It was a fibrous, reticular substance which stretched between and around adjacent bacteria. Interestingly, further observation and research seemed to indicate to them that the secondary acidic poly-

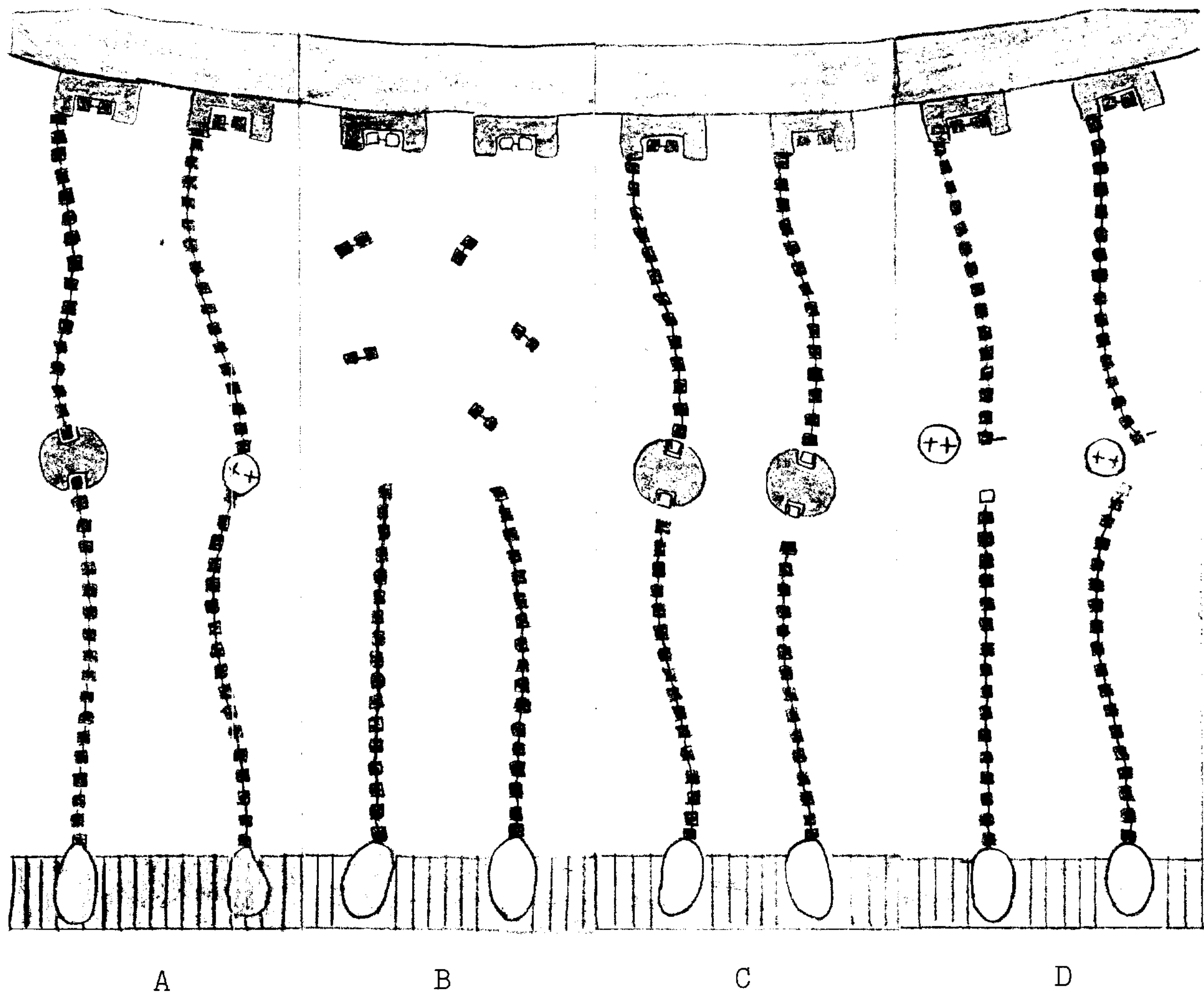
saccharide probably evolved from the primary acidic polysaccharide. After attachment the primary acidic polysaccharide became stretched and fibrous in areas where bacteria were adjacent to each other or to the (filter) surface, and assumed the appearance of the secondary acidic polysaccharide. In microcolonies, however, the secondary polysaccharide completely replaced the primary polysaccharide, indicating that the production of the secondary polysaccharide is a time-dependent process. The thin, electron-dense line is usually present on the bacterial surface, irrespective of the type of polysaccharide surrounding it, and this may indicate that it is the area of synthesis.

Further morphological evidence illustrating the fibrous network of polysaccharide chains is given by electron microscopy. In electron micrographs of sectioned material, the capsule appeared to be composed of a tightly packed mass of electron-dense fibers which extended from the outer surface of the cell wall to an irregular outer boundary from which individual fibers protruded and often established contact with the capsules of the other cells(3). Also, freeze-etching preparations were found to be consistent with the current knowledge in that they contained a very extensive mass of long intercellular strands which could form either a tangled or a very highly ordered pattern(3).

Compositionally, the slime fibers are, as previously mentioned, polysaccharide, acidic (negatively charged), for

the most part(5,8), and can form a polar bond with higher cell polysaccharides by divalent positive ions in the medium (5)(See figure 1a and accompanying caption). They may be simple homopolymers(12) such as short B 1-4 glucan or dextran chains(10,3),or they may be very complex heteropolymers containing glucose and amino sugars(12,14). One source states (in regard to sponges) that a large protein-polysaccharide complex had been isolated-the proteins containing the average amino acids and sugars being mostly galactose, glucosamine, and uronic acid. Further, there were a few other neutral sugars isolated which were minor components. The result of this protein-polysaccharide complex was a very assymetric molecule (18). Presumably, the carbohydrate residues are attached to a protein backbone(18,5) (See figure 2 and accompanying caption) which can be attacked by proteolytic enzymes. Glycoproteins arrayed in the membrane of animal cells have been isolated and identified, and it has been shown that the polysaccharide fibers they bear extend outward from the membrane to form glycocalyx(5).

A mclecular weight of greater than 10,000 indicates that there are at least 60 sugar residues (10), these sugar residues being held most responsible for the physical characteristics of the glycocalyx. The presence of a surface glycocalyx-like coat rich in both acidic and neutral carbohydrates and the presence of a negative surface potential were discovered by utilizing Alcian Blue staining,



Pathogenic Adhesion might be blocked, in order to prevent or treat infection, by a new kind of antibiotic, The adhesion of a bacterium (top) to an animal cell(bottom) by means of a polar bond or a lectin (a) might be disrupted in one of three ways. An analogue (White squares) of the units that are polymerized to form the bacterial glycocalyx might be supplied, occupying the active sites of the polymerizing enzyme and preventing the synthesis of a polysaccharide fiber(b). The active sites of the lectin might be blocked by a similar analogue(c), or a blocking agent that mimics the glycocalyx material could be supplied to block the animal-cell glycoprotein receptors(d).

Figure 1

Redrawn from Costerton, et al, Scientific American 238(1):86-95

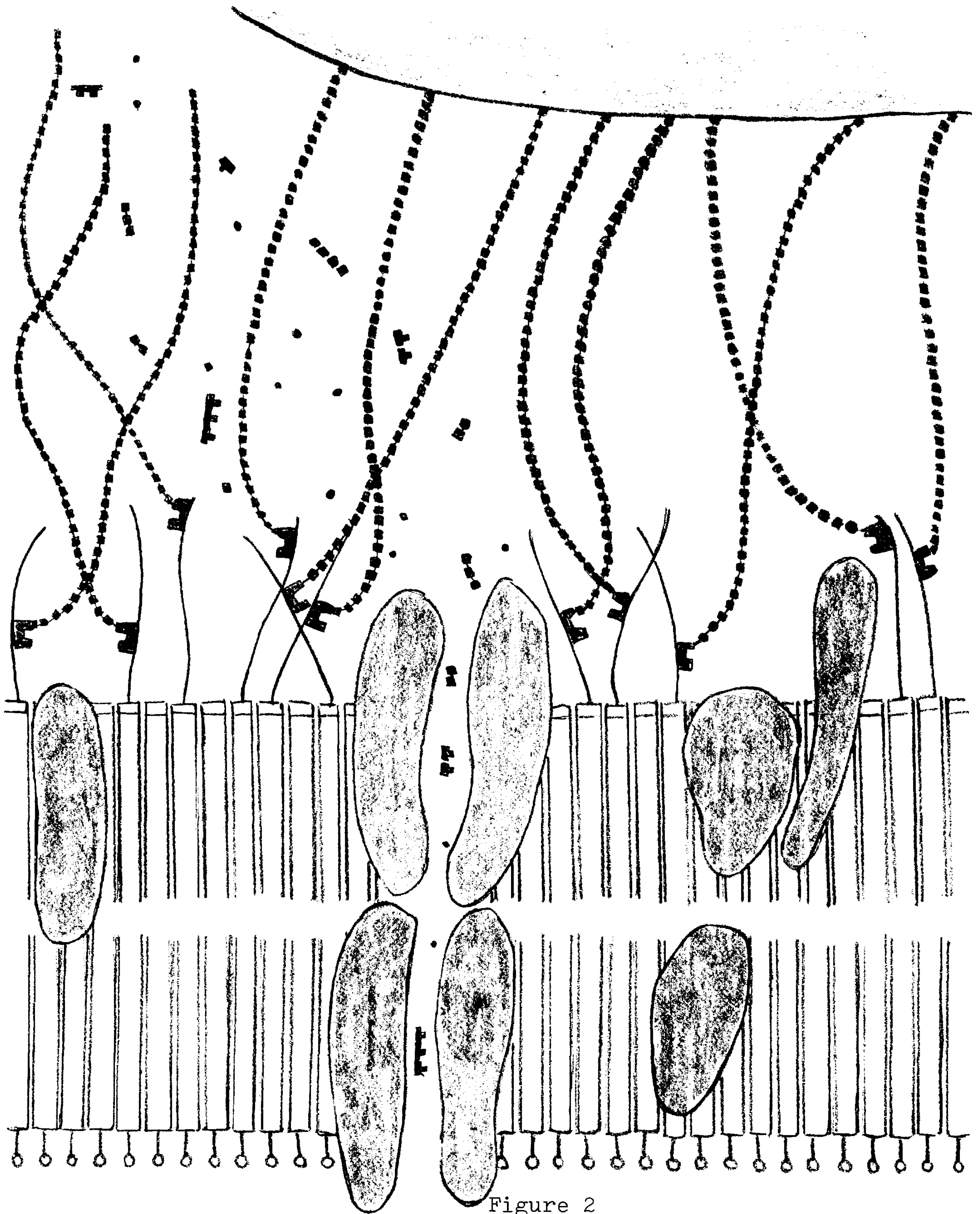


Figure 2

Redrawn from Costerton, et al, Scientific American 238(1):86-95

Glycocalyx extends from the outer membrane of a bacterium as is indicated in the generalized and highly schematic diagram. The membrane is a bilayer of lipid moledules (forked structures) in which protein molecules (gray shapes) are embedded. Lipopolysaccharide molecules (black hairlike structures) extend from the membrane. The glycocalyx is a mass of long polysaccharide fibers (chains of colored squares). The fibers are chains of sugar molecules that are generated by bacterial enzymes called polymerases (C-shaped structures) affixed to the lipopolysaccharides. The glycocalyx fibers adhere to nearby surfaces, in the case an inert surface (top right). In addition to mediating bacterial adhesion the fibers channel toward the bacterium various nutrients such as sugars(rectangles), amino acids (Tshaped objects) and inorganic ions (dots), which enter the cell through channels in the membrane formed by arrays of proteins.

---

concanavalin A, and iron hydroxide labeling techniques(19). Moreover, a viscous or slimy character is imparted to fluids in which large numbers of organisms synthesizing (polysaccharide) are growing(3,7). Viscous environments have interesting properties such as high degree of resistance to diffusion of materials, susceptibility to sudden reductions of viscosity because of enzymatic hydrolysis, the ability to trap and accumulate particulate elements(7), and decreased electrical resistance and capacitance.

The slime or glycocalyx produced by the bacterial cell has many,many functions besides its adherent property(5,15, 14,10,18,7). Adhesiveness is important in the linkage between cells(18), in binding to surfaces(5,7), in bacterial resistance to removal(5), and so that adherent bacteria may play a role in the digestion and degradation of sloughed off surface cells (bovine intestinal studies)(14). Other characteristics include: acting as a filter for particles,

molecules, and ions (1), creation of a microenvironment surrounding cells, with the polysaccharide strands acting as a gradient through which nutrients pass to the void area around each cell (9), acting as a buffer zone(9), trapping essential ions, influencing electrokinetic characteristics of the cell and stability of suspensions, aiding bacteriophage adsorption, preventing death because of hydrophilic nature (7), and protecting from phagocytosis (7) by ciliate protozoans (14). In addition it is a physical barrier against predatory bacteria (5), it guards against harmful molecules and ions by decreasing the already limited penetrability (15), offers protection against stress (5), is a food reservoir, and is used to concentrate digestive enzymes and direct them toward the host cell (5).

C. ADHESION

Although the function of adhesion is of prime importance, the mechanism is not known. Many studies are being conducted which indicate that the polysaccharides on the cell surface are involved in the adhesion process (14, 6) and other contact phenomena (6). Evidently, the same forces which hold other substances together-chemical, electrostatic, covalent, and hydrogen-bonds, and vander Waals forces--are the forces responsible for this adherence (12). A. Cecil Taylor reports that before the chemical bonds (forces) can be established, physical attraction forces must operate directly between molecules of the cell surface and

the substrate (12). It has been proposed that a possible role of calcium, at least in short-term contact phenomena, may be to raise the cohesive strength of the perifery of individual cells through tangentially oriented "bridges." This rise in cohesive strength hinders the separation of one cell from another, as distinct from promoting the adhesion of one cell to another at the intercellular interface (12).

Of the few suggestions concerning the mechanism of intercellular adhesion, one idea was offered by Tyler and Weiss (from 16). This hypothesis may be designated the antigen-antibody theory, and is based on the assumption that cell surfaces contain antigen-like and antibody-like substances which interact in the usual manner, resulting in intercellular adhesion.

Another mechanism involves the complex carbohydrates in that the interaction is with other carbohydrates on a neighboring cell surface,with the interaction based on the formation of hydrogen bonds between glycose units on the two surfaces. To obtain stable intercellular adhesions it would be necessary to form a large number of hydrogen bonds though.

The simplest and most flexible mechanism is the enzyme-substrate hypothesis. The suggestion is that cell surfaces contain both substrates and enzymes, and that the binding of one to the other results in adhesion. These

enzymes can be the proposed polymerases (15) or glycosyltransferases, which penetrate the lipid layers of the membrane, a concept in full accord with recent ideas on the enzymes which transfer carbohydrates across bacterial membranes. If glycosyltransferases and their acceptor molecules are responsible for intercellular adhesion, simple extensions of the theory can be used to explain several biological phenomena which involve changes in intercellular adhesion (16).

The interaction between an enzyme and its substrate is subject to a wide variety of controls, one of which in the case of glycosyltransferases, is the requirement of divalent cations for activity. Many enzymes require Mn^{++} or Mg^{++}, while ions such as Ca^{++} are highly inhibitory. So a major mode for regulation would be via flucuations in divalent ions.

The proposed mechanisms may work beautifully for the cell-cell interactions, but what about inert surfaces? They obviously do not have a glycocalyx with which to interact, so how does the adhesion take place? Unfortunately the mechanism in this case is not known and is wide open to speculation.

D. SYNTHESIS OF THE POLYSACCHARIDE

"Protein intermediates have been found during the synthesis of starch in potato tissue and in glycogen formation in E. coli (10). The synthesis of the slime polysaccharide

is more complex than that of a homopolysaccharide such as starch, since several sugars are present in the polymers. Bowles and Northcote showed that synthesis of low molecular weight polysaccharides occured in a membrane fraction. These polysaccharides were probably attached to protein and could be intermediates of biosynthesis of high molecular-weight slime and wall polysaccharides.

"It is possible that the glycoprotein produced within the cell membrane is attacked by transglycosidases or proteinases as it lies between the plasmalemma and the cell wall. There may be specific enzymes present which break the particular linkages between the polysaccharides and protein units of the glycoprotein. It therefore seems that the slime polysaccharides are synthesized attached to proteins. The protein may be membrane bound and act as an acceptor during the transfer of sugars. Slime polymer synthesis occurs in the membrane. At later stages of synthesis the protein carrier may be detached from the membrane before final secretion."

At this point it seems to this author that the description of the protein carrier in the later stages of synthesis may be describing the situation one finds as depicted by Costerton, et al, in the figure redrawn from their report. The drawing shows individual polysaccharide chains attached to the cell membrane by means of the enzymes (protein) and are terminated on inert surfaces,

animal cells by wall of lectins or cations, etc. Since the glycocalyx is, as previously described, a tangled mat of fibers, some polysaccharides will still be attached to the enzyme and possibly make contact with other surfaces. This is what is shown. But it also seems probable that free polysaccharides exist entwined in the tangled mat, having been detached from the "protein carrier", which will adhere too. Divalent cations might then enhance the development of the glycocalyx by "joining" the acidic terminals of the polysaccharides, adding to the complex network of the glycocalyx, in addition to the proposed linkage function between the polysaccharide fibers and other negatively charged surfaces.

Green and Northcote (10) continue then "it is important to distinguish the synthesis and secretion of slime polysaccharides from the formation of the polysaccharides deposited into the cell walls. The polysaccharides of the wall may not be carried by protein acceptors. Evidence comes from time course studies of glycoprotein and slime formation using radioactive labelling techniques with the sugar fucose. Fucose was not metabolized to other sugars so it could be used to indicate the relative amounts of various polymers containing it which were formed after a particular time. Total radioactivity incorporated reached a maximum after about 50 minutes. The amount of label in the glycoprotein rose sharply then fell to zero,

whereas in the slime polysaccharide it increased almost linearly with time.after a short lag period. It was then possible that the glycoproteins were synthesized and then converted into the slime which was continually secreted from the cells.

Many different substances have been reported to have an effect on the formation of the slime polysaccharide, some facilitating production and others decreasing or inhibiting it. The slime has been reported to be formed under specific cultural conditions, for example, when sulfur source was changed from sulfate to sulfite (15a), when the carbohydrate source was changed (either the CHO itself or concentrations of it)(3,17), and when the temperature of the culture was changed (11,17,17a). Cheng,et al, stated from their studies of S. bovis that the organism produces large amounts of extracellular dextran when supplied with high concentrations of sucrose (3), In contrast, Marshall, et al, (13) reported that extremely low levels of available carbon stimulated irreversible sorption (implies firmer adhesion of bacteria to a surface) while higher levels inhibited this process; this may be relevant to microbial ecology. In natural seawaters the available carbon levels are usually very low, and such conditions probably favor the firm adhesion of microorganisms to surfaces immersed in such environments. Sorption (the binding of one substance by another by any mechanism) of bacteria is

also affected by age of inoculum and by deletion of divalent cations (13). Marshall, et al, reports that omission of Ca^{++} and Mg^{++} prevented irreversible sorption. It was found by Humphreys and his associated (18) that when cells were soaked in Ca^{++}-Mg^{++}-free seawater, a component of the cell surface necessary for cell aggregation was presumably removed because cells would not aggregate. Then if the cells were mixed with the supernatant of the Ca^{++}-Mg^{++}-free seawater, the cells were able to reaggregate. Evidently these divalent cations are an integral part of the sorption, aggregation or adhesion processes. Possibly, if the synthesis model proposed by Green and Northcote is true and the slime polysaccharides which are excreted are most important in holding the cells together and to other surfaces,which the literature indicates, then maybe the divalent cations are integrated into the mass of fibers and their removal will, in effect, remove the slime polysaccharide layer. But, mere addition of Ca^{++} and Mg^{++} to a 2.5% NaCl-glucose medium did not stimulate the sorption of Pseudomonas R3 (13).

Research dealing with Streptococcus mutans lead Vicher, et al, to determine whether the effects to reduce the formation of polysaccharide by the organism could be separated fromother effects, particularly the inhibition of growth (21). They found a 28-fold decrease in mean yield of extracellular polysaccharide between the control cultures

and cultures to which 0.1% of Procion Blue had been added. The Procion Blue (a dye) has a crosslinking character which may or may not have an effect on the protein molecules of glucosyltransferase which, in turn, would not polymerize the glucose to dextran. Glucosyltransferase activity expressed as specific units showed some increase with the increased concentration of Procion Blue.in the medium. But when activity was expressed as specific units per viable count, viable counts being standard practice in microbiology, then the level of enzyme activity did not show a major change. So they belive it was accurate to state that no important change in the level of glucosyltransferase has occurred.

E. CHEMICAL CONTROL OF SLIMES

Asnoted earlier, industries such as power generating companies have a reduction in operation efficiency due to the accumulation of the slime layer on the surface of the conduits. They, like many other industries, have had to resort to chemical means in order to control or stop the formation of the slime. Some of the chemicals which have been used for this purpose are things such as chlorine (20) in 9-12 mg/l concentrations, hypochlorite, chlorine dioxide, chlorine-ammonia, and a calcium treatment.(2).

Characklis (2) states that a concentration of calcium hydroxide at 20mg per liter will have a "hardening" effect on the polysaccharide. Maybe this divalent ion, in excess,

ties up the negatively charged ends of the polysaccharide to an extent where the glycocalyx no longer adheres to other surfaces but to itself. Chlorine is frequently ineffective in destroying attached slime, and because of its reactivity, it is frequently dissipated into side reactions reducing its disinfectant power. It has been previously suggested that the gelatinous covering of the slime bacteria protects the cell from the lethal effects of the chlorine molecule. The chlorine dioxide has an oxidizing power 2.6 times that of chlorine. It oxidizes without chlorination but it destroys microorganisms by reacting with the cell structure and by accelerating metabolism to the detriment of the cell growth of by inhibiting protein synthesis. The chlorine dioxide is claimed by one author to clean away slime particles to which inorganic residues attach on surfaces of pipes and vats. In this way it removes the primary means of slime adhesion. Hypochlorite, which can cause denaturation of proteins, works by attacking glucose polysaccharides with extensive oxidation occuring at the C_2-C_3 position of D-glucose units with cleavage of the C_2-C_3 bond. Depolymerization may then occur. Hypochlorite presumably acts to bring about random splitting of the polysaccharide with production of a diversity of organic products of high molecular weight. Dydel (from 2) presents conclusive data that does indicate a greater decrease in suspended solids, with large amounts of cap-

sular material indicating that hypochlorite solubilizes portions of the microbial polysaccharide envelope. The effect of the OCl^- on attached microbial growths are attributed to the oxidation of polymers in the slime which are subsequently released from the surface.

Inhibition of growth of these microorganisms is not the desired effect of chemical control of slimes; rather, only control of slime production. From the preceding review, the only chemical which would seem to "fit the bill" is the hypochlorite, though probably when used in amounts which are satisfactory in decreasing of disrupting slime formation, the concentration would exceed the federal and/or state rejulations. Since the excessive concentration would be detrimental environmentally, this necessitates the discovery of a new kind of chemical control. For example, as given by figure 1, there is something that should be able to function at either the production level or adhesion level which will stop the slime formation without resulting in the death of the organism or the ecosystem it lives in. Possibly the answer is down at the genetic level; some factor must be responsible for "turning on" a gene to "make slime", because the bacteria are able to survive without it. One the question of "how bacteria stick" (5) is answered, the many problems presented by this slime formation can be solved.

IV. RESEARCH

A. MATERIALS AND METHODS

Laboratory research was conducted in the research lab of Dr. Donald A. Hendrickson, Cooper Life Building room 34.

1. Identification

Isolates were obtained from river water samples taken at various power generating companies in the Mid west. Initial isolation was done on TGEA. After isolation of pure cultures, the isolates were inoculated onto various differential, subjective and objective bacteriologic tests in order to attempt identification of the bacteria. The description and results follow in tabular form in the Appendix.

2. Minimal Salts Media

Since one of the objectives of this research was to attempt to determine what nutrients were needed by the bacteria to form the most prolific slime layer, and because in order to generate and maintain a glycocalyx energy must be expended (18), a minimal salts medium was used for the growth of bacteria. A minimal salts-carbohydrate medium was utilized since, in this system, the bacteria would be forced to synthesize all of its components required to live. Palumbo(17a) reported an increase in slime formation when the sulfur source was changed from sulfate to sulfite and strongly buffered at pH 8.2.

This research was done using a minimal salts medium with sulfate, but calculations for a proposed sulfite medium are included. The media and calculations are on the following pages:

1) Minimal salts medium-sulfate 23
2) Minimal salts-carbohydrate medium-sulfate 24
3) Calculations for molar concentrations of minerals and redicals in the sulfate medium 26
4) Minimal salts medium-sulfite 29
5) Minimal salts-carbohydrate medium-sulfite 30
6) Calculations of changed molar concentrations of minerals and radicals in the sulfite medium 31
7) Concentration comparisons between sulfate and sulfite media 34
8) Buffers used 35

3. Method for inoculation of minimal salts media.

Five different pure cultures which had been previously established were used to inoculated the test media. Isolates were transferred to sterile buffered water tubes, mixed thoroughly, and the number of cells per milliliter was found by using a Petroff-Hausser counting chamber (see figures 9 and 10). The average number of cells counted per grid was multiplied by 2 x 10^7 to yield the number of cells per ml. These tubes were then serially diluted to about 10^2-10^3 cells/ml, and the dilution was then used consistently.

Figure 3

MINIMAL SALTS MEDIUM - SULFATE

Solution C: Per liter of deionized water:

1. 1.0g. $(NH_4)_2SO_4$

2. 5.0 ml of 0.5M Na_2HPO_4

3. Concentrated base (stock solution) 20 ml.

Solution B: Per 100 ml:

Nitrilotriacetic acid	1.0g
$MgSO_4$	1.445g
$CaCl_2$ $2H_2O$	0.3335g
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	0.0009g
$FeSO_4$ $7H_2O$	0.0099g
Metals "44"	5.0 ml

In preparing solution 3, the nitrilotriacetic acid is dissolved and neutralized with KOH (about 0.73g), after which the rest of the ingredients are added. Metals"44" contains:

Solution A: Per 30 ml:

EDTA		0.075g
$ZnSO_4$ $7H_2O$	(75mg)*	0.3285g
$FeSO_4$ $7H_2O$	(30mg)	0.1500g
$MnSO_4$ H_2O	(15mg)	0.0462g
$CuSO_4$ $5H_2O$	(3mg)	0.0118g
$Co(NO_3)_2$ $6H_2O$	(1.5mg)	0.0074g
$Na_2B_4O_7$ $10H_2O$	(0.6mg)	0.0053g

A drop or two of H_2SO_4 is added to retard precipitation.

* indicates trace element

Figure 4

MINIMAL SALTS - CARBOHYDRATE MEDIUM - SULFATE

Contains per liter:

1)	One of the following:	
	a) 0.5% glucose	5.0g
	b) 1.0% glucose	10.0g
	c) 2.0% sucrose	20.0g
	d) 5.0% sucrose	50.0g
	e) 2.0% lactose	20.0g
	f) 5.0% lactose	50.0g
2)	1.5% agar	15.0g
3)	0.5M Na_2HPO_4	5.0ml
4)	Concentrated minimal salts	20.0ml
5)	$(NH_4)_2SO_4$	1.0g

The carbohydrate and agar were dissolved in about 75% of the total amount of deionized H_2O/d used, depending on how much media was needed. The remaining water was used to dissolve the $(NH_4)_2SO_4$ and dilute the base.

Sterilization was done by separating the phosphate from the sulfates. Two flasks were autoclaved; one containing the carbohydrate source, agar, and phosphate, and the other containing the base and ammonium sulfate.

Since all the carbohydrate media were made at the same time, the base-ammonium sulfate fractions were combined into one flask

and a graduated cylinder was sterilized and used to deliver the correct amount of minimal salts medium into the carbohydrate-agar flask.

When the media had cooled sufficiently to allow combination of the two fractions (about 55-60°C), they were poured together and then poured into labelled petri plates.

Sulfate

Mineral	Chemical	%age molecule	moles:	per:	x C.F. =	[M]	x %age =	[M]
S	$(NH_4)_2SO_4$	$\frac{32.06}{132.06} = 0.243$	.0076	1000ml	1	.0076	.243	$1.84x10^{-3}$
	$MgSO_4$	$\frac{32.06}{120.32} = 0.267$	.120	1000ml	1/50	.0024	.267	$1.11x10^{-4}$
	$FeSO_4 \cdot 7H_2O$	$\frac{32.06}{277.87} = 0.115$	.00035	1000ml	1/50	$7x10^{-6}$	.115	$8.08x10^{-7}$
	$ZnSO_4 \cdot 7H_2O$	$\frac{32.06}{287.40} = 0.112$	.0038	100ml	1/1000	$3.8x10^{-5}$	.112	$4.24x10^{-6}$
	$FeSO_4 \cdot 7H_2O$	$\frac{32.06}{277.87} = 0.115$	.0018	100ml	1/1000	$1.8x10^{-5}$	.115	$2.08x10^{-6}$
	$MnSO_4 \cdot H_2O$	$\frac{32.06}{168.96} = 0.190$	.0009	100ml	1/1000	$9.0x10^{-6}$	.190	$1.71x10^{-6}$
	$CuSO_4 \cdot 5H_2O$	$\frac{32.06}{249.57} = 0.128$	.00016	100ml	1/1000	$1.6x10^{-6}$	.128	$2.06x10^{-7}$
								$1.96x10^{-3}$
SO_4	$(NH_4)_2SO_4$	$\frac{96.02}{132.02} = 0.727$	.0076	1000ml	1	.0076	.727	$5.53x10^{-3}$
	$MgSO_4$	$\frac{96.02}{120.32} = 0.798$	.120	1000ml	1/50	.0024	.798	$1.92x10^{-3}$
	$FeSO_4 \cdot 7H_2O$	$\frac{96.02}{277.87} = 0.346$	.00035	1000ml	1/50	$7.0x10^{-6}$	.346	$2.42x10^{-6}$
	$ZnSO_4 \cdot 7H_2O$	$\frac{96.02}{287.40} = 0.334$	.0038	100ml	1/1000	$3.8x10^{-5}$	.334	$1.27x10^{-5}$
	$FeSO_4 \cdot 7H_2O$	$\frac{96.02}{277.87} = 0.346$	.0018	100ml	1/1000	$1.8x10^{-5}$	.346	$6.22x10^{-6}$
	$MnSO_4 \cdot H_2O$	$\frac{96.02}{168.96} = 0.568$	.0009	100ml	1/1000	$9.0x10^{-6}$	.568	$5.11x10^{-6}$

Table 1

Sulfate

Mineral	Chemical	%age / molecule	moles:	per:	x C.F. =	[M] x	%age =	[M]
SO_4	$CuSO_4 \cdot 5H_2O$	$\frac{96.02}{249.57} = 0.385$	.00016	100ml	1/1000	1.6×10^{-6}	.385	6.16×10^{-7}
								7.48×10^{-3}
P	Na_2HPO_4	$\frac{30.97}{141.91} = 0.218$	.0025	1000ml	1	.0025	.218	5.45×10^{-4}
PO_4	Na_2HPO_4	$\frac{94.93}{141.91} = 0.669$	.0025	1000ml	1	.0025	.669	1.67×10^{-3}
Cl	$CaCl_2$	$\frac{(35.45)2}{146.98} = 0.482$	.022	1000ml	1/50	4.4×10^{-4}	.482	2.12×10^{-4}
N	$(NH_4)_2SO_4$	$\frac{28.00}{132.02} = 0.212$	.0076	1000ml	1	7.6×10^{-3}	.212	1.6×10^{-3}
	$N(CH_2CO_2H)_3$	$\frac{14.00}{191.00} = 0.073$	.052	1000ml	1/50	1.04×10^{-3}	.073	7.59×10^{-5}
	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	$\frac{84.00}{1235.34} = 0.068$	.0000074	1000ml	1/50	1.48×10^{-7}	.068	1.0×10^{-8}
	NTA	$\frac{28.00}{371.98} = 0.075$	.00067	100ml	1/1000	6.7×10^{-8}	.075	5.02×10^{-7}
	$Co(NO_3)_2 \cdot 6H_2O$	$\frac{28.00}{290.87} = 0.096$	.000085	100ml	1/1000	8.5×10^{-7}	.096	8.16×10^{-8}
								1.68×10^{-3}
Fe	$FeSO_4 \cdot 7H_2O$	$\frac{55.85}{277.87} = 0.201$	.00035	1000ml	1/50	7.0×10^{-6}	.201	1.41×10^{-6}
	$FeSO_4 \cdot 7H_2O$	$\frac{55.85}{277.87} = 0.201$	.0018	100ml	1/1000	1.8×10^{-5}	.201	3.62×10^{-6}
								5.02×10^{-6}

Sulfate

Mineral	Chemical	%age molecule		moles:	per:	x C.F. =	[M] x	%age =	[M]
Na	Na_2HPO_4	$\frac{(22.99)2}{141.91}$	= 0.324	.0025	1000ml	1	.0025	.324	$8.1\text{x}10^{-4}$
	NTA	$\frac{(22.99)2}{371.98}$	= 0.264	.00067	100ml	1/1000	$6.7\text{x}10^{-6}$	.264	$1.77\text{x}10^{-6}$
	$Na_2B_4O_7 \cdot 10H_2O$	$\frac{(22.99)2}{381.15}$	= 0.120	.000046	100ml	1/1000	$4.6\text{x}10^{-7}$	.120	$5.52\text{x}10^{-8}$
									$8.12\text{x}10^{-4}$
Mg	$MgSO_4$	$\frac{24.30}{120.32}$	= 0.202	.120	1000ml	1/50	.0024	.202	$4.85\text{x}10^{-4}$
Ca	$CaCl_2 \cdot 2H_2O$	$\frac{40.08}{146.98}$	= 0.273	.022	1000ml	1/50	.00044	.273	$1.2\text{x}10^{-4}$
Mo	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	$\frac{(95.94)7}{1235.34}$	= 0.544	.0000074	1000ml	1/50	$1.5\text{x}10^{-7}$	.544	$8.05\text{x}10^{-8}$
Zn	$ZnSO_4 \cdot 7H_2O$	$\frac{65.38}{287.40}$	= 0.227	.0038	100ml	1/1000	$3.8\text{x}10^{-5}$	.227	$8.63\text{x}10^{-6}$
Mn	$MnSO_4 \cdot H_2O$	$\frac{54.94}{168.96}$	= 0.325	.0009	100ml	1/1000	$9.0\text{x}10^{-6}$	.325	$2.92\text{x}10^{-6}$
Cu	$CuSO_4 \cdot 5H_2O$	$\frac{63.55}{249.57}$	= 0.255	.00016	100ml	1/1000	$1.6\text{x}10^{-6}$	.255	$4.08\text{x}10^{-7}$
Co	$Co(NO_3)_2 \cdot 6H_2O$	$\frac{58.93}{290.87}$	= 0.202	$8.5\text{x}10^{-5}$	100ml	1/1000	$8.5\text{x}10^{-7}$	.202	$1.72\text{x}10^{-7}$
B	$Na_2B_4O_7 \cdot 10H_2O$	$\frac{(10.81)4}{381.15}$	= 0.113	$4.6\text{x}10^{-5}$	100ml	1/1000	$4.6\text{x}10^{-7}$	.113	$5.2\text{x}10^{-8}$
K	KOH	$\frac{39.09}{56.08}$	= 0.697	.130	1000ml	1/50	$2.6\text{x}10^{-3}$	.697	$1.81\text{x}10^{-3}$

Figure 5

MINIMAL SALTS MEDIUM - SULFITE

Solution C: Per liter of deionized water:

1. 1.02g of $(NH_4)_2SO_3\ H_2O$
2. 5.0ml of Sodium pyrophosphate-HCl buffer
3. Concentrated base (stock solution) 20ml.

Solution B: Per 100ml:

Nitrilotriacetic acid	1.0000g
$MgSO_3\ 6H_2O$	2.5000g
$CaCl_2\ 2H_2O$	0.3335g
$(NH_4)_6Mo_7O_{24}\ 4H_2O$	0.0009g
$FeCl_2\ 4H_2O$	0.0707g
Metals "44""	5.0ml.

In preparing solution 3, the nitrilotriacetic acid is dissolved and neutralized with KOH (about 0.73g), after which the rest of the ingredients are added. Metals "44'" contains:

Solution A: Per 30ml:

EDTA		0.0750g
$ZnSO_3\ 2H_2O$	(75mg)*	0.2205g
$FeCl_2\ 4H_2O$	(30mg)	0.1071g
$MnSO_3$	(15mg)	0.0369g
$CuCl_2$	(3mg)	0.0064g
$Co(NO_3)_2\ 6H_2O$	(1.5mg)	0.0074g
$Na_2B_4O_7\ 10H_2O$	(0.6mg)	0.0053g

A drop or two of H_2SO_4 is added to retard precipitation.

* indicated trace element

Figure 6

MINIMAL SALTS - CARBOHYDRATE MEDIUM - SULFITE

Contains per liter:

1) One of the following:

   a) 0,5% glucose 5.0g

   b) 1.0% glucose 10.0g

   c) 2.0% sucrose 20.0g

   d) 5.0% sucrose 50.0g

2) 1.5% agar 15.0g

3) 1.0M Sodium pyrophosphate-HCl Buffer 5.0ml

4) Concentrated minimal salts base 20.0ml

5) $(NH_4)_2SO_3\ H_2O$ 1.02g

The medium is made as in the sulfate medium.

Figure 7

CALCULATIONS FOR SULFITE MEDIUM

$MgSO_3 \cdot 6H_2O$ = 212.33g/mole

$$\frac{Mg}{MgSO_3 \cdot 6H_2O} = \frac{24.30}{212.33} = .114 \cdot X = 2.92mgMg$$

X = 25g $MgSO_3 \cdot 6H_2O$/1000ml conc base

$ZnSO_3 \cdot 2H_2O$ = 181.86g/mole

$$\frac{Zn}{ZnSO_3 \cdot 2H_2O} = \frac{65.38}{181.86} = .34 \cdot X = 250mgZn$$

X = 735.0 mg $ZnSO_3 \cdot 2H_2O$/100ml metals "44"

$MnSO_3$ = 135g/mole

$$\frac{Mn}{MnSO_3} = \frac{54.94}{135.0} = .407 \cdot X = 50mg\ Mn$$

X = 122.85mg $MnSO_3$/100ml metals "44"

$(NH_4)_2SO_3 \cdot H_2O$ = 134.03g/mole

$$\frac{NH_4}{(NH_4)_2SO_3} = \frac{36.0}{134.03} = .268 \cdot X = .273mgNH_4$$

X = 1.02g $(NH_4)_2SO_3 \cdot H_2O$/1000ml total

$CuCl_2$ = 134.45g/mole

$$\frac{Cu}{CuCl_2} = \frac{63.55}{134.45} = .47 \cdot X = 3mg\ Cu$$

X = 6.38mg $CuCl_2$

$FeCl_2 \cdot 4H_2O$ = 198.81g/mole

$$\frac{Fe}{FeCl_2 \cdot 4H_2O} = \frac{55.85}{198.81} = .28 \cdot X = 30mg\ Fe$$

X = 107.1mg $FeCl_2 \cdot 4H_2O$

.28·X = 19,8mg Fe

X = 70.7mg $FeCl_2 \cdot 4H_2O$

New calculations for Sulfite Medium:

Mineral	Chemical	%age / molecule	moles:	per: x	C.F. =	[M] x	%age =	[M]
S	$(NH_4)_2SO_3 \cdot H_2O$	$\frac{32.06}{134.03} = 0.239$	.0076	1000ml	1	.0076	.239	.0018
	$MgSO_3 \cdot 6H_2O$	$\frac{32.06}{212.33} = 0.151$	.1177	1000ml	1/50	.0024	.151	$3.6x10^{-4}$
	$ZnSO_3 \cdot 2H_2O$	$\frac{32.06}{181.86} = 0.176$	.004	100ml	1/1000	$4.0x10^{-5}$	.176	$7.04x10^{-6}$
	$MnSO_3$	$\frac{32.06}{135.00} = 0.237$	.0009	100ml	1/1000	$9,0x10^{-6}$	.237	$2.13x10^{-6}$
								$2.17x10^{-3}$
SO_3	$(NH_4)_2SO_3 \cdot H_2O$	$\frac{80.03}{134.03} = 0.597$	.0076	1000ml	1	.0076	.597	$4.5x10^{-3}$
	$MgSO_3 \cdot 6H_2O$	$\frac{80.03}{212.33} = 0.377$	.1177	1000ml	1/50	.0024	.377	$9.0x10^{-4}$
	$ZnSO_3 \cdot 2H_2O$	$\frac{80.03}{181.86} = 0.4400$	.004	100ml	1/1000	$4.0x10^{-5}$	.440	$1.76x10^{-5}$
	$MnSO_3$	$\frac{80.03}{135.00} = 0.593$	.0009	100ml	1/1000	$9.0x10^{-6}$	.593	$5.34x10^{-6}$
								$5.42x10^{-3}$
P_2O_7	$Na_4P_2O_7 \cdot 10H_2O$	$\frac{173.87}{445.83} = 0.389$	.005	1000ml	1	.005	.389	$1.94x10^{-3}$
P	$Na_4P_2O_7 \cdot 10H_2O$	$\frac{(30.97)2}{445.83} = 0.139$	.005	1000ml	1	.005	.139	$6.95x10^{-4}$
Cl	$CaCl_2 \cdot 2H_2O$	$\frac{(35.45)2}{146.98} = 0.482$	.022	1000ml	1/50	$4.4x10^{-4}$	.482	$2.12x10^{-4}$
	$FeCl_2 \cdot 4H_2O$	$\frac{(35.45)2}{198.81} = 0.356$	.0035	1000ml	1/50	$7.0x10^{-5}$	.356	$2.49x10^{-5}$

Table 2

Sulfite

Mineral	Chemical	%age / molecule	moles:	per:	x C.F. =	[M]	x %age =	[M]
Cl	$FeCl_2 \cdot 4H_2O$	0.356	.0018	100ml	1/1000	$1.8x10^{-5}$	.356	$6.41x10^{-6}$
	$CuCl_2$	$\frac{(35.45)2}{134.45}$=0.527	.00016	100ml	1/1000	$1.6x10^{-6}$	.527	$8.43x10^{-7}$
	HCl	$\frac{35.45}{36.45}$ ==0.972	$5.2x10^{-3}$	1000ml	1	$5.2x10^{-3}$	.927	$5.09x10^{-3}$
								$5.33x10^{-3}$
Fe	$FeCl_2 \cdot 4H_2O$	$\frac{55.85}{198.81}$ = 0.281	.0035	1000ml	1/50	$7.0x10^{-6}$	.281	$1.97x10^{-6}$
	$FeCl_2 \cdot 4H_2O$	0.281	.0018	100ml	1/1000	$1.8x10^{-5}$	.281	$5.06x10^{-6}$
								$7.03x10^{-6}$
Na	$Na_4P_2O_7 \cdot 10H_2O$	$\frac{(22.99)4}{445.83}$=0.206	.005	1000ml	1	.005	.206	$1.03x10^{-3}$
	$Na_2B_4O_7 \cdot 10H_2O$	$\frac{(22.99)2}{381.15}$=0.120	$4.6x10^{-4}$	100ml	1/1000	$4.6x10^{-7}$	.120	$5.52x10^{-8}$
								$1.03x10^{-3}$
Mg	$MgSO_3.6H_2O$	$\frac{24.30}{212.33}$ = 0.114	.1177	1000ml	1/50	$2.4x10^{-3}$	.114	$2.69x10^{-4}$
Zn	$ZnSO_3 \cdot 2H_2O$	$\frac{65.38}{181.86}$ = 0.359	.004	100ml	1/1000	$4.0x10^{-5}$	.359	$1.44x10^{-5}$
Mn	$MnSO_3$	$\frac{54.94}{135.00}$ = 0.407	.0009	100ml	1/1000	$9.0x10^{-6}$	.407	$3.66x10^{-6}$
Cu	$CuCL_2$	$\frac{63.55}{134.45}$ = 0.473	$1.6x10^{-4}$	100ml	1/1000	$1.6x10^{-7}$	.473	$7.57x10^{-8}$

Table 3

Concentrations of each element or molecule in moles/l (M).

SULFATE		SULFITE	
SO_4	7.47×10^{-3}M	SO_3	5.42×10^{-3}M
S	1.95×10^{-3}M	CO_3	5.33×10^{-3}M
K	1.81×10^{-3}M	S	2.17×10^{-3}M
N	1.68×10^{-3}M	P_2O_7	1.94×10^{-3}M
PO_4	1.67×10^{-3}M	K	1.81×10^{-3}M
Na	8.12×10^{-4}M	N	1.68×10^{-3}M
P	5.45×10^{-4}M	Na	1.03×10^{-3}M
Mg	4.85×10^{-4}M	P	6.95×10^{-4}M
Cl	$2,12 \times 10^{-4}$M	Mg	2.69×10^{-4}M
Ca	1.20×10^{-4}M	Ca	1.20×10^{-4}M
Fe	5.02×10^{-6}M	Zn	1.44×10^{-5}M
Zn	8.63×10^{-6}M	Fe	7.02×10^{-6}M
Mn	2.92×10^{-6}M	Mn	3.66×10^{-6}M
Cu	4.08×10^{-7}M	Cu	7.57×10^{-8}M
Co	1.72×10^{-7}M	Co	1.72×10^{-7}M
B	5.20×10^{-8}M	B	5.20×10^{-8}M
Mo	8.05×10^{-8}M	Mo	8.05×10^{-8}M

To get quantities of compounds when changing from sulfate to sulfite the minimal trace element (ie. Zn) quantity was held constant (ie. Zn = 250mg/100ml of metals "44").

Figure 8

Phosphate Buffer (for dilutions)

A: 0.2M solution of monobasic sodium phosphate (2.78g in 100ml dd H20)

B: 0.2M solution of dibasic sodium phosphate (5.37g in 100ml)

X ml of A + Y ml of B, diluted to a total of 200ml.

For a pH of 7.0, X = 39.0ml, and Y = 61.0ml.

Pyrophosphate-HCl Buffer pH 8.2

Tetrasodium pyrophosphate - pK' = 8.22. To get a pH of 8.2 necessitates the use of the Henderson-Hasslebach Equation:

$$pH = pK' = \log \frac{[\text{proton acceptor}]}{[\text{proton donor}]}$$

$$8.2 = 8.22 - \log \frac{[P.A.]}{[P.D.]} \qquad -0.02 = \log \frac{[P.A.]}{[P.D.]} = 0.955$$

$$\frac{50ml\ [PA]}{x} = \frac{0.955\ [PD]}{1} \qquad x = 52.35ml$$

So:

A: 2M Pyrophosphate solution (44.58g in 100ml)

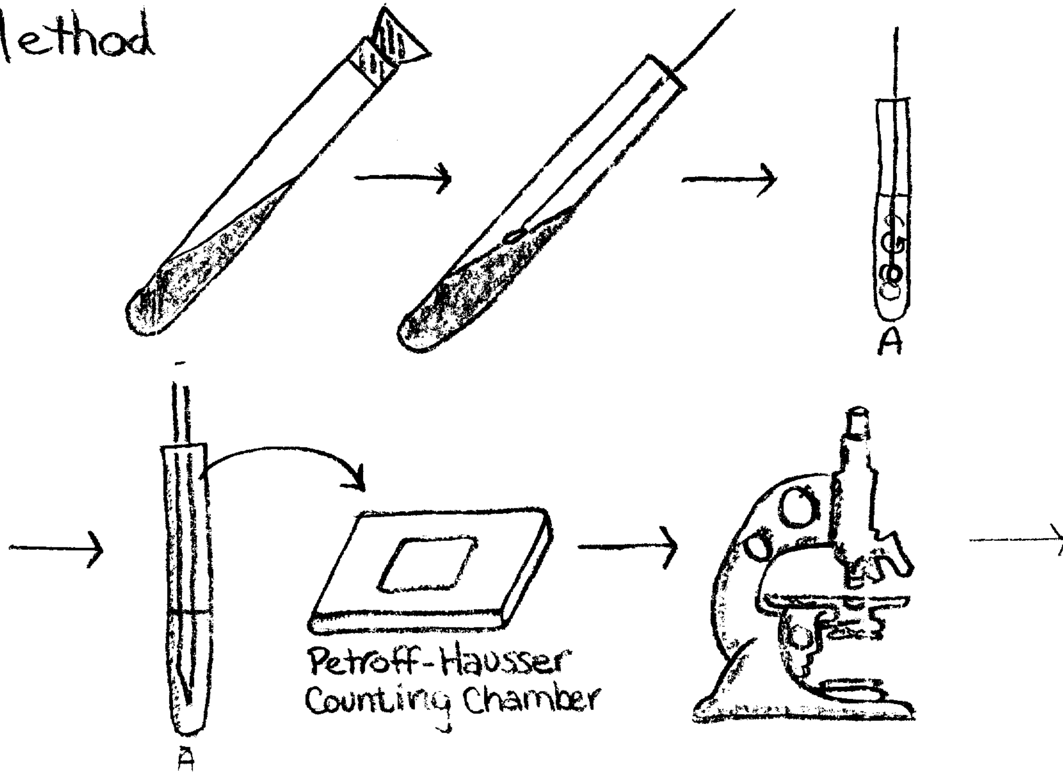
B: 2M HCL (Concentrated HCl is 12.1M)

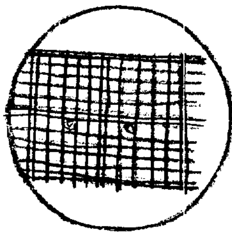
X ml of A + Y ml of B, diluted to a total of 100ml.

For a pH of 8.2, X = 50.0ml, and Y = 52.35ml.

This buffer is used for the Sulfite minimal salts medium.

Method





counts repeated 10 times and averaged. The average was multiplied by 2×10^7 to yield $\frac{\text{cells}}{\text{ml.}}$

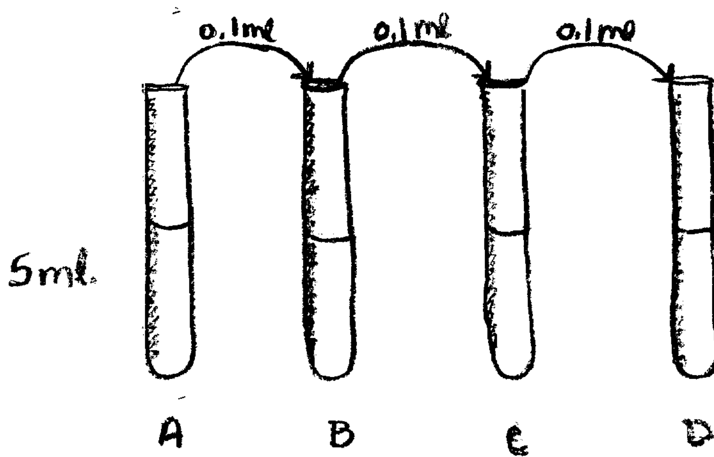


Figure 9

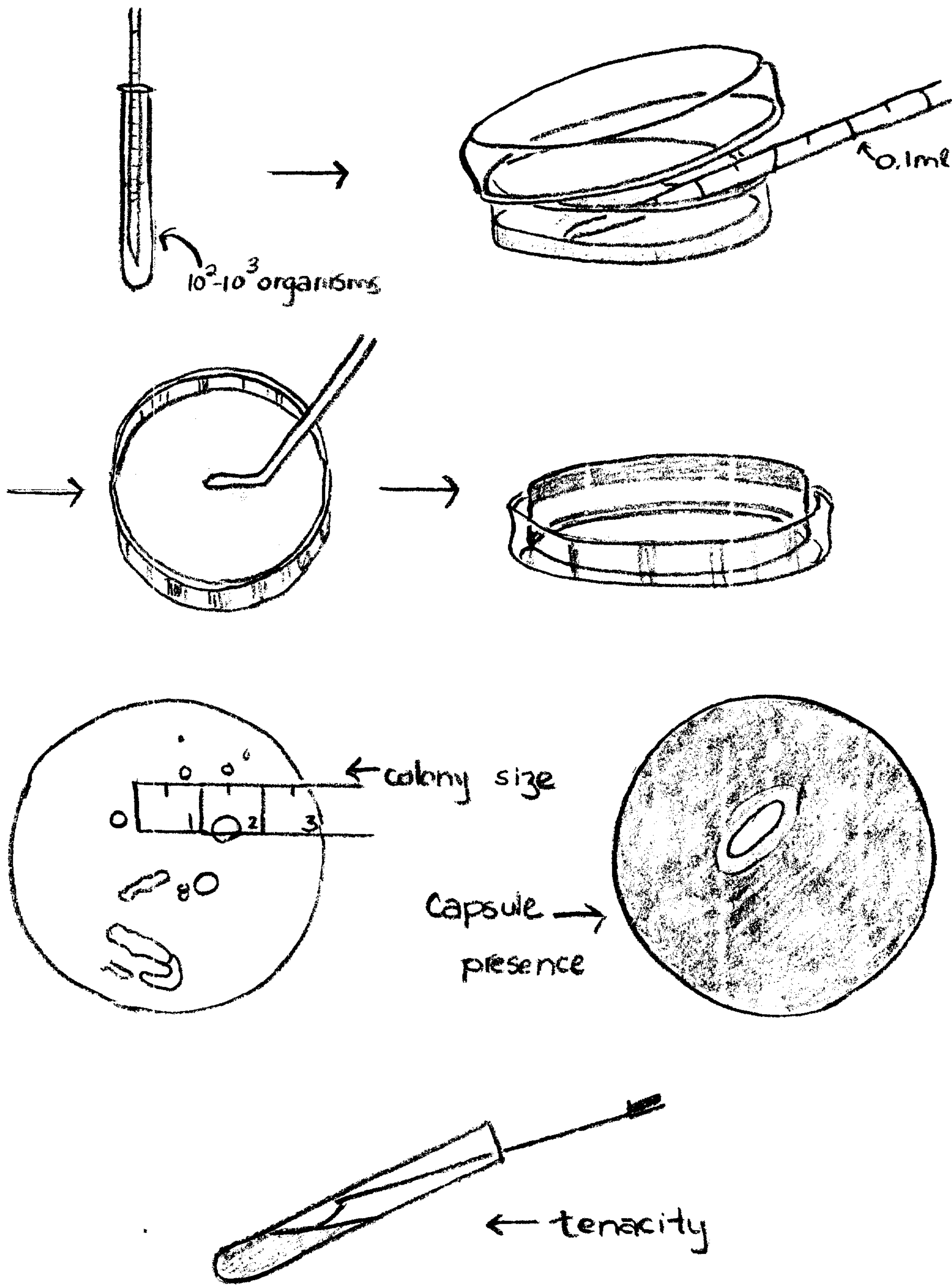


Figure 10

When the desired inoculum dilution was obtained, the plates were inoculated using a standard inoculum, which in this case was 0.1ml.,delivered by sterile pipettes. Then a "hockey stick" (curved glass rod) was sterilized and used to spread the drop across the agar surface. The plates were inverted and incubated at the given temperatures for five days, triplicates being done on all test media.

After five days three measurements were taken:

1) Colonial size using a metric ruler
2) Capsule formation using a negative stain
3) Tenacity by touching colonies with a probe

B. DATA AND RESULTS

The data and results are tabulated on the next page.

C. CONCLUSIONS

From the results of the experimentation, one can see that the medium used to enhance more tenacity, or slime, was inhibitory for isolates 3 and 5B, and somewhat for isolates 7A and 7B, considering these isolates did produce slime upon initial isolation. The medium had no effect on the slime formining abilities of isolate 4A (no slime-forming ability to begin with). The reason for the inhibition may be because of the lack of a nutrient like an amino acid, as is suggested by Roseman (16). Further work with this media will involve finding the missing nutrient before any of the proposed objectives of this research can be carried out.

			4A
0.5% GLUCOSE (.5G)	20	1. .5-1.mm white,round,convex,entire 2. no tenacity 3. no capsule 4. rods 3-4 x width; length varies	1. .5-1.mm white,translucent,round,convex, entire,smooth,shiny like 3-20-.5G 2. no tenacity 3. no capsule 4. length 3-4 x width
	35	No growth	No growth
1.0% GLUCOSE (1G)	20	1. .5-1.mm look smaller,transparent, entire,round,slightly convex 2. no tenacity 3. no capsule 4. rods3-4 x width; length varies	1. .5-1mm transparent,entire,round,slightly convex, exactly like 3-20 2. no tenacity 3. no capsule 4. noticibly thinner-some like .5G
	35	No growth	No growth
2.0% SUCROSE (2S)	20	1. pinpoint,transparent 2. no tenacity 3. no capsule 4. seems smaller, lengths vary;chains	1. pinpoint,transparent 2. no tenacity 3. no capsule 4. much smaller and thinner
	35	No growth	1. .5-1.mm small,round,shiny,smooth,convex, entire,transparent,milky 2. no tenacity 3. no capsule 4. length about 2 x width
5.0% SUCROSE (5S)	20	1. pinpoint,transparent with milky hint, convex, shiny, round 2. no tenacity 3. no capsule 4. small rods, lengths vary	1. pinpoint,shiny,round,transparent,milky, 2. no tenacity 3. no capsule 4. much smaller and thinner than .5G
	35	No growth #3	1. .8-1.mm whiter than 2S,small,round, shiny,smooth,convex,entire,milky 2. no tenacity 3. no capsule 4. slighty shorter #4A

5B		7B
1. pinpoint,transparent. almost looks like no growth 2. no tenacity 3. no capsule 4. small rods 2-4 x width	1. .2-.8mm white,transparent,round,convex,shiny,smaller than 35 2. no tenacity 3. no capsule 4. varying lengths and widths	1. .8-2.mm white,convex,shiny,entire, smooth 2. tenacity may be due to surface tension 3. no capsule 4.varying lengths and widths
No growth	1. .5-1.8mm transparent,milky,shiny, smooth,convex 2. no tenacity 3. no capsule 4. length 2-3 x width	1. .2-2.mm white,shiny,smooth,round, whiter than 20 2. somewhat tenacious - debatable 3. no capsule 4.length 2-3 x width; some much smaller
1. .2-.6mm. growth more evident than .5G round,regular,shiny 2. no tenacity 3. no capsule 4. small rods 2-4 x width	1. .5-1.8mm larger than .5G,still transparent,similar to 35,wetter looking 2. some tenacity 3. no capsule 4. variable lengths and widths	1. .5-1.5mm smaller,more transparent, white,convex,shiny,entire,smooth 2. some tenacity, similar to .5G 3. no capsule 4. varying lengths and widths
No growth	1. 2-3mm flat,transparent,white,somewhat shiny,slight haloing 2. no tenacity 3. no capsule 4. length varies; thinner than .5G	1. .2-2.2mm identical to .5G. more cream tint;whiter than at 20 2. no tenacity 3. no capsule 4. length 2-3 x width
1. pinpoint,transparent,almost imperceptible. like ,5G 2. no tenacity 3. no capsule 4. thicker; some curved	1. .2-1.mm smaller than 35,shape and color similar 2. poss. tenacity; almost imperceptible 3. no capsule 4. widths vary	1. .5-2.5mm more opaque than .5G,white, convex,shiny,entire,smooth 2. no tenacity 3. no capsule 4. varying lengths and widths;more large
1. pinpoint,transparent,milky,shiny,round, raised 2. no tenacity 3. no capsule 4. length 3-4 x width	1. .5-1.8mm transparent,milky,shiny, smooth, convex 2. no tenacity 3. no capsule 4. much wider; look blocklike	1. .5-3.mm white,flat or depressed,round, dry,slighty whiter than 20 2. no tenacity 3. no capsule 4. varying lengths and widths
1. .2=.4mm more convex and shinier; as others 2. no tenacity 3. no capsule 4. short and thick,not curved like 2S	1. .2-1.mm white with opaque centers, "haloing" around body 2. tenacious better than 1G 3. no capsule 4. size varies	1. 1.5-2.5mm cream,shiny,smooth,entire, convex,round 2. tenacity like 1G;string seems thicker 3. no capsule 4. bacteria much shorter than previously
1. .2-.6mm white,translucent(more perceptible) slighty larger, shiny 2. no tenacity 3.no capsule 4. length 3-4 x width #5B	1. 1.2-3mm very white,opaque,"haloing" edges irregular 2. no tenacity 3. no capsule 4. thinner than 2S #7A	1. .5-3.mm white,flat or depressed,round, somewhat dry-looking.some not depressed 2. no tenacity 3. no capsule 4. lengths vary greatly #7B

LITERATURE CITED

1. Bennett, H. Stanley. "Morphological Aspects of Extracellular polysaccharides." Journal of Histoe chemistry and Cytochemistry, 11,(1):14-23

2. Characklis, William G. "Attached microbial growths-II. frictional resistance due to microbial slimes." Water Research, 7:1249-1258.

3. Cheng,K-J., R. Hironaka, GA Jones, T. Nicas, and J.W. Costerton. "Frothy feedlot bloat in cattle: production of extracellular polysaccharides and development of viscosity in cultures of Streptococcus bovis." Canadian Journal of Microbiology, 22:450-459 (1976).

4. Cook, G.M.W., and R.W. Stoddart. Surface carbohydrates of the Eucaryotic Cell. Academic Press, 1973 pp. 257-293.

5. Costerton, J.W., G.G. Geesey, and K.-J. Cheng. "How Bacteria Stick." Scientific American, 238 (1):86-95.

6. Cox, Susan M., P.S. Baur, and Brenda Haenelt. "Retention of the glycocalyx after cell detachment by EGTA." The Journal of Histochemistry and Cytochemistry, 25(12):1368-1372.

7. Doetsch, R.N., and T.M. Cook. Introduction to Bacteria and their Ecobiology. University Park Press 1973, p. 24.

8. Fletcher, Madelyn, and G.D. Floodgate. "An electron-microscopic Demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces." Journal of General Microbiology, 74:325-334.

9. Jones, H.C., I.L. Roth, W.M. Sanders, III. "Electron Microscopic Study of a Slime Layer." Journal of Bacteriology, 99(1):316-325.

10. Green, John R., and D.H. Northcote. "The structure and function of Glycoproteins synthesized during slime-polysaccharide production by membranes of the root-cap cells of maize." Biochemical Journal, 170:599-608.

11. Loginova, L.G. et al., "Thermophilic aerobic microflora forming slime in paper manufacture." Applied Biochemistry and Microbiology (1973) pp.701-709.

12. Manly, Richard S., ed., Adhesion in Biological Systems, (1970), Academic Press

13. Marshall, K.C., Ruby Stout, and R. Mitchell. "Mechanism of the Initial Events in the Sorption of Marine Bacteria to Surfaces." Journal of General Microbiology, 68:337-348.

14. McCowan, R.P., K.-J. Cheng, C.B.M. Bailey, J.W. Costerton. "Adhesion of Bacteria to Epithial Cell Surfaces Within the Reticulo-Rumen of Cattle." Applied and Environmental Microbiology, 35(1):149-155.

15. Patterson, H., R. Irvin, J.W. Costerton, K.-J. Cheng. "Ultrastructure and Adhesion Properties of Ruminococcus albus." Journal of Bacteriology, 122:278-287.

15a. Palumbo, S.A. "Role of iron and sulfur in pigment and slime formation by Pseudomonas aeruginosa." Journal of Bacteriology, Aug. 1972, p. 430-436.

16. Roseman, Saul. "The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion." Chemistry and Physics of Lipids, 5(1970):270-297.

17. Sanders, J.D. "Studies on slime producing bacteria from a sugar refinery." Texas Journal of Science, 17:113-121 (1965).

17a. Sanders, W.M. III. "Oxygen utilization by slime organisms in continuous culture." Air and Water Pollution, 10:253-276, (1966).

18. Slavin, Harold C., ed., The Comparative Molecular Biology of Extracellular Matrices, Academic Press, 1972, pp. 77-138.

19. Sosa, A. H. Giron, S. Alva, and L. Calzada. "Presence and Nature of a glycocalyx-like coat on the external vesicular membrane of Cysticercus cellulosae. A High resolution histochemical study." Life Sciences, 21:1021-1032.

20. Taylor, C.B. "Slime forming organisms inan industrial cooling system." Journal of Bacteriology, (1946). p. 43

21. Vicher, E.E., M. Iqbal, J.P. Waterhouse. "The effect of Procion Blue on certain Metabolic Activities of *Streptococcus mutans*." Journal of Dental Research, 56(8):977,982.

APPENDIX

Group	TEST	3	4A	5B	7A	7B
	indole	—	—	—	+	+
	MR	—	—	—	+	+
	VP	—	—	—	—	—
	citrate	+	+	+	+	+
	H_2S	—	—	—	—	—
motility	SIM	—	—	—	—	—
motility	hanging drop	+	+	+	+	?
KIA	slant	Alk	Alk	Alk	A	A
KIA	butt	NC	NC	NC	AG	AG
	nitrate	—	+	—	+	—
	Zn	—		—		—
	oxidase	+	+ around edge	—	+	—
	catalase	+	— or wk +	—	+	—
	urease	—	—	—	—	—
	malonate	+	+	+	+	+
Phenol Red	inositol	—	—	—	+ 19hr	+ 19hr
Phenol Red	rhamnose	—	—	—	+ 19hr	+ 7.5h
Phenol Red	raffinose	—	—	—	+	+
Phenol Red	mannitol	—	—	—	+ 7.5h	+ 8.5h
	fluoresc.	+	+	+	+	—
	tenacity	+	—	+	+	+
	Gr. rxn	—	—	—	—	—
	pigment	diffusable green	diffusable green	—	— *	—

*after a few days, the agar under the colonial mass was acquiring a brown tint.

Colonial morphology on TGEA

3- colonies about 0.4mmin diameter, convex, round,entire, shiny, cream in color, smooth. diffusable pigment(green) is quite apparent. fluorescence. Two days after inoculation the colonies were 2.0mm. Five days- motile rods no tenacity initially.

4A-colonies about 0.5-0.8 mm. convex, round, entire, shiny, smooth, creamy. Diffusable pigment(green). More outstanding than #3. fluorescence. Two days after inoculation colonies were about 2.0 mm. Motile rods, no tenacity.

5B-colonies about 1.0 mm, round, very convex, entire, shiny, smooth, creamy. no diffusable pigment. After two days slight fluorescence, lines of demarcation between coloniew. About 4.0 mm in diameter. motile rods. tenacious.

7A-colonies about 2.0 mm, round, convex, entire, shiny, creamy. no diffusable pigment. After a few days the medium was seen turning brown under the colonial mass. About 3.0 mm, motile short! rods or coccobacilli. tenacious, fluorescence

7B-colonies about 2.0 mm round, convex, entire, shiny, creamy, no diffusable pigment, no fluorescence, After a few days colony 4.5 mm, tenacious

Colonial morphology on EMB

3- Didn't grow well. somewhat tenacious; streak very flat, dried looking; colonies very small. Either white or pinkish from the media. Nonlactose fermenter.

4A-Pink, small colonies. Growth mainly at the beginning of the streak. No tenacity

5B-Pink where growth is thick. shiny thin to transparent. somewhat tenacious.

7A-pink,dark pink, and purple, viscous, watery. tenacious

7B-pink, dark pink, and purple, viscous, watery. More tenacious than 7A or 5B

From the results of these characteristics, it has been decided that the organisms were identified as: 3 and 4A-two different species of Pseudomonas; 5B-possibly Alcaligenes sp.; 7A-Aeromonas sp.;7B-identification could not be determined from available test results, but they do indicate that the organism is different from the others.