The advantage of the heated blood medium is that prolific growth is obtained with strains that will grow on NNN, and that the suspension of flagellates is free from erythrocytes. Work is in hand on identification of growth factors necessary for the growth of these flagellates.

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Acid Production by *Azotobacter vinelandii*

Azotobacter vinelandii has been characterized by its lack of acid production in culture[1]. In fact, most species of *Azotobacter* do not form acid, but appear to oxidize carbohydrates completely to carbon dioxide and water[2]. The notable exceptions to this are the acid-tolerant *A. indicum* (*Beijerinckia indica*)[3] and *A. macrocytogenes*[4] that are characterized by acid production. Other acid-tolerant species are not reported to be acid producers. There have been a few scattered reports of acid production by *A. vinelandii*, but these usually mention that it occurred during abnormal cultural conditions[5], or with little or no growth[6].

It was, therefore, of interest to find an acid reaction produced routinely when *A. vinelandii* was grown in broth. During the growth of *Azotobacter vinelandii* 3*A* in broth for purposes of examining extracellular polysaccharide, a distinct colour change was observed in the characteristic green fluorescent pigment. Johnstone and Fishbein[7] reported that the fluorescence of this pigment changed with *p*H. The colour change observed here was coincident with a reduction of *p*H. When this strain was cultured in shake flasks containing Burk's nitrogen-free broth[8], with 1·5 per cent sucrose, glucose or fructose, the reaction changed from an initial *p*H 7·0 to approximately *p*H 5·5 in about five days with abundant growth.

Another characteristic of most strains of *A. vinelandii* is the synthesis of copious amounts of highly viscous extracellular slime. Table 1 illustrates the direct relationship of slime to acidity produced by various strains of *A. vinelandii* after several days in Burk's broth. The medium contained 1·5 per cent sucrose and the initial reaction was *p*H 7·0. Cultures of the slime-forming strains became acid, and the non-slime-forming strains remained near neutrality. Thus, it appears evident that acid production is not unique and limited to one strain but has been observed with all slime-forming strains of *A. vinelandii* examined in this laboratory.

Table 1. Comparison of Slime and Acidity produced by Various Strains of *A. vinelandii*

Strain		Final *p*H	Slime
ATCC 9046		5·5	Present
ATCC 13705	Wilson strain *OP*	7·0	Absent
ATCC 12518	Wilson strain *O*	5·3	Present
ATCC 12837	Johnstone strain 3*A*	5·0	Present
121	Wilson strain *S*	6·8	Absent
GC-1	Vermont soil isolate	5·1	Present
GC-2	Vermont soil isolate	5·5	Present

In order to determine the effect of carbohydrate concentration on the ability to produce acid, shake cultures of strain 3*A* were grown for 10 days with each of three sugars, sucrose, glucose, and fructose, in concentrations of 1·0, 2·5, and 6·0 per cent. The results (Table 2) show a direct relationship between carbohydrate concentration and acidity attained. The greater the amount of carbohydrate available, the lower was the *p*H at the end of ten days, with an exception in the case of fructose. The cultures with the highest concentration of each sugar were also found to have the most extracellular polysaccharide.

Table 2. *p*H attained by *A. vinelandii* when grown in Burk's Medium for Ten Days

Percentage concentration	Sucrose *p*H	Glucose *p*H	Fructose *p*H
1	5·1	5·5	5·4
2·5	4·8	5·0	4·4
6·0	4·4	4·5	4·6

Concurrent electrophoretic and chemical analyses have revealed the polysaccharide slime of *Azotobacter vinelandii* 3*A* to be an acidic polymer, a major component of which is galacturonic acid[9]. Jensen[10] has suggested that the acid-forming *Azotobacter* possesses a metabolic block that prevents the further conversion of acid intermediates, resulting in accumulation of acid in the medium. Whereas this may be so, as supported by the experiments of Jensen[6], we offer another possible explanation. We suggest that the slime-forming *Azotobacter vinelandii* possesses a specific polymerase which combines the partially oxidized intermediates into an acid polymer. With sufficient carbohydrate, the acidic extracellular polysaccharide accumulates, thereby lowering the *p*H of the culture. In those strains that do not lower the *p*H, there may be no polymerase, thus permitting the intermediates to be completely oxidized with no acidity resulting.

Considering the voluminous literature on *A. vinelandii*, it is interesting to speculate as to why this phenomenon had not been reported. It might have been overlooked, because maximum cell proliferation is completed in a few days at most, long before marked acidity is developed. Another likely possibility is that several of the media used for their growth contain calcium carbonate, whereas Burk's medium does not. To see if a slime-forming strain would produce a culture with low *p*H and/or slime when grown with calcium carbonate, *A. vinelandii* 3*A* was grown for five days in Ashby's[8] (containing 0·5 per cent calcium carbonate) and Burk's medium to which 0·5 per cent calcium carbonate was added. Regular Burk's medium, serving as control for the added carbonate, produced abundant growth, copious slime, and *p*H 5·3. Whereas abundant growth also took place in both media containing carbonate, the cultures remained at neutrality with no accumulation of extracellular slime. It is not known at this time whether the calcium carbonate serves merely to neutralize the acid as formed, or whether it inhibits in some other manner the ability of the cell to synthesize the polysaccharide. Markovitz and Sylvan[11] have recently shown that certain salts markedly influence heteropolysaccharide synthesis by some soil bacteria.

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Colicine *A*

Antagonism between closely related bacteria has often been observed. In attempts to find a basis for antagonism between strains of *Escherichia coli*, Gratia[1] isolated a filtrable agent possessing potent antibacterial properties. The striking resemblance of this material to bacteriophage