THE RELATIONSHIP OF CARBON AND NITROGEN UTILIZATION TO THE TAXONOMY OF *STREPTOMYCES* SPECIES

by

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PREFACE

In April of 1950 while in a discussion with Dr. Campbell M. Gilmour, the writer asked Dr. Gilmour if he may have a suggestion for a thesis project. Because of Dr. Gilmour's interest in the Actinomycetales, he suggested work on a problem concerning the latter group. The final correction of this thesis has been accomplished by correspondence with Dr. Gilmour now on the faculty at Oregon State College. Without the cooperation and understanding of Dr. Gilmour, the author would never have been able to complete the manuscript.

The writer wishes to express his appreciation to Dr. S. A. Waksman who forwarded the two strains of Streptomyces griseus to Dr. Gilmour; to the members of the faculty of the Department of Bacteriology for their encouragement and advice during the preparation of this thesis; and to my wife for her constant encouragement and for the typing of the thesis.

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INTRODUCTION

The morphological, cultural, physiological, pathogenic and immunological characteristics of bacteria are remarkably constant. Yet the main characteristics of bacteria do vary to some extent and such variation must be taken into consideration in their characterization and classification. Any one of a pure culture's properties may be lost or, in some instances, a property may be gained. If the characteristic lost or gained should be a major one in the characterization of the organism, it often makes species differentiation quite difficult.

The present study was undertaken to investigate any major morphological or physiological differences in two strains of Streptomyces griseus. Both strains originated from the same parent culture and were separated on the basis of positive or negative streptomycin production.

REVIEW OF LITERATURE

A review of the literature on the nomenclature of the actinomyces group discloses that various terms have been used in the designation of genera and species types. At various time intervals the terms Streptothrix, Cladothrix, Oospora, Nocardia, Ray Fungi, Actinomyces, and Streptomyces have been used. For various reasons, these terms have either been discarded or their meaning narrowed. Under the present accepted classification (1, 12), the order Actinomycetales is made up of three families including Mycobacteriaceae, Actinomycetaceae and Streptomycetaceae. The family Actinomycetaceae is divided into two genera, the Nocardia without a true mycelium, aerobic and generally non-pathogenic and the Actinomyces with a true mycelium, anaerobic or microaerophilic, and pathogenic to man and animals. The family Streptomycetaceae is also divided into two gen-

era. The first genus, Streptomyces, shows a typical branched aerial mycelium and forms conidiospores in chains. On the other hand the second genus, Micromonospora, shows a fine non-septate mycelium and produces single spores at the tip of lateral branches.

As stated in Bergey's Manual of Determinative Bacteriology (1), rules for the differentiation of cultures or species are difficult to establish. A type culture was defined as one from which the original description was made. In a related sense, species were defined as follows: "A species of bacterium is the type culture or specimen together with all the other cultures or specimens regarded by an investigator as sufficiently like the type (or sufficiently closely related to it) to be grouped with it." In view of this definition, it then would appear that new species may be differentiated by an investigator, later to be reviewed and discussed by others before being accepted.

The term strain has no specific meaning in bacterial classification. However, the recommended meaning according to Bergey's Manual of Determinative Bacteriology (1) is that it refer to source but never to a biologic factor. It is commonly accepted that when two distinct characteristics appear in strains of a species, they may be called distinct species. Perhaps some of the more recent results will allow the differentiation into species of some of the presently regarded strains.

In this regard, the following characteristics are used for species differentiation in the order Actinomycetales: (1) pathogenicity, (2) appearance of colonies, their size, color, etc., (3) gelatin liquefaction, (4) presence of aerial mycelium, (5) acid-fastness, (6) presence of cystities (swollen cells) or not, (7) speed of growth, (8) color of pellicle, (9) solubility of pigment, (10) relative strength of proteo-

lytic action in milk, (11) hydrolysis of starch and (12) acid tolerance.

Within recent years, the production of acids from various carbohydrates has been given consideration for the differentiation of Streptomyces species (4, 8, 20). Gilmour (8) found that in the presence of dextrose, the Streptomyces species fall into two large groups according to their utilization of nitrogen sources. One group which had the ability to utilize both simple and complex nitrogen sources, demonstrated a definite alkaline trend. The second group, showed a marked preference for a simple or less complex nitrogen source, and exhibited a tendency toward various degrees of acidity. In this regard, Cochrane (4) reported that 50% of 100 named Streptomyces cultures did produce acid from glucose in four days, 20% did not produce acid, and 30% produced acid over extended periods.

Waksman (25) related that certain of the actinomyces in the presence of carbohydrates will produce organic acids. Sooner or later, these acids will decompose or the organisms will produce neutralizing substances, with the result that the reaction tends to be alkaline. The alkaline reaction was said to result from the presence of basic ions (Na, K) when nitrites are used in the medium as sources of nitrogen or to the formation of ammonium ions from proteins. However, Waksman (25) also stated that the presence of 1% glucose in buffered media may lower the pH due to the formation of organic acids. Further, in the presence of 2% glucose in unbuffered media, the pH levels may go down as low as 3.2 in two days.

There is some disagreement as to the action of carbohydrates as they affect nitrogen utilization. Gilmour (8) found that when dextrose was added to a medium the rate of breakdown of casein, alanine,

asparagine, and glycine was lowered. To accomplish this, the actinomyces apparently prefer the available carbon rather than the extensive decomposition of nitrogen for energy purposes. Gilmour (8) suggested that the rapid utilization of carbohydrate and the corresponding decreased hydrolysis of the nitrogen compound, indicates that, in the presence of dextrose, the various species exert a sparing action on the utilization of the nitrogen source. In this connection, Waksman (25) declares actinomyces prefer proteins to carbohydrates as a source of carbon. Apparently this effect was so pronounced that when a protein such as peptone is placed in a medium containing glucose, the actinomyces attacks the protein first, not only as a nitrogen source but also as a source of carbon, and liberates considerable waste nitrogen in the form of ammonia. Carbohydrates, therefore, were included in the medium mainly to buffer the ammonia produced during the breakdown of the protein. Waksman (25) also found that carbon utilization is greater in stationary cultures than in submerged cultures.

Schatz and Waksman (14) found sporulating colonies, regardless of color of mycelium, produced streptomycin. Non-sporulating strains apparently have a lower pH value namely 5.0 to 6.5, whereas filtrates of the sporulating types were alkaline, ranging from 7.5 to 8.5. The two could have been considered distinct species had they been isolated separately from a natural substrate.

Cochrane and Dimick (5) found that acids are not a significant product of Streptomyces griseus. No sharp drop in pH was observed. Waksman (25) found that some strains of Streptomyces griseus that have lost their capacity to form aerial mycelia produce more lactic acid than those that have not degenerated. Schatz and Waksman (14) also

stated that the combination of inactive and active strains does not inhibit streptomycin production. Streptomycin, however, apparently inhibits the growth of the inactive variants. These authors suggested the possibility that these variants might be regarded as a Nocardia.

Since the discovery of actinophages capable of lysing various species of the Streptomyces, ideas have been advanced regarding the possibility of their use in species differentiation.(3) Woodruff et al. (26) found the soil to be a poor source of actinophage. The data indicated that lysis was complete in submerged cultures with a spore suspension inoculum. In addition, Saudek and Collingsworth (13) found that actinophages completely inhibited streptomycin production. More recently, Gilmour and Buthala (9) isolated a number of actinophages from soils. In their study of phage specificity, the phages isolated proved to be lytic with 47 of 77 Streptomyces strains tested. In general, Buthala (3) found a high incidence of lysis among the members of the genus Streptomyces. Furthermore, the pattern of lysis shows no predelection for any one morphological or physiological group.

The phage studied by Waksman (11) was shown to lyse only streptomycin producing strains. However, phage 514-3, studied by Buthala (3), was found to be active against a rather large number of distinct Streptomyces species. This observation points to either a lack of phage specificity or to the physiological likeness of the tested species.

EXPERIMENTAL

A. Cultures

The following cultures were used:

1. Streptomyces griseus strain 3475. An active isolate obtained by Dr. C. M. Gilmour from Dr. S. A. Waksman. It is a strain isolated from Waksman's strain 3863-4 (isolate from original streptomycin-producing strain 18-16).
2. Streptomyces griseus strain 3478. A culture obtained from Dr. S. A. Waksman by Dr. C. M. Gilmour which produced an antibiotic of the non-streptomycin type.
3. Actinophage isolate number 514-3, isolated by D. A. Buthala, Oklahoma A & M College.
4. Forty Streptomyces species. Isolates from several Oklahoma soils by D. A. Buthala, Oklahoma A & M College.
5. Thirty named Streptomyces species from American Type Culture Collection.

All cultures of Streptomyces were carried in the asparagine-glucose media and transferred every five days. Actinophage was prepared from a lysed culture of Streptomyces griseus strain 3475.

B. Procedure

1. Utilization of Carbon and Nitrogen Sources

A basal salt medium was used throughout this study. This medium has the following composition:

K_2HPO_4 -- 0.5 gm.

$MgSO_4 \times 7\ H_2O$ -- 0.2 gm.

$FeCl_3 \times 6\ H_2O$ -- 0.01 gm.

H_2O (Distilled) -- 1000 cc.

pH -- 6.8-7.0

In the first series of experiments d,l asparagine (0.1%) was used as the nitrogen source in conjunction with a number of carbohydrates. The latter included glucose, fructose, lactose, sucrose, maltose, galactose, glycerol, mannitol, soluble starch, dulcitol and raffinose. These sugars were prepared as 10% stock solutions and were sterilized by passing through a Sietz filter.

The asparagine medium was dispensed in 40 ml quantities in 250 ml flasks and 10 ml quantities in 125 ml flasks and then sterilized in an autoclave at 15 lbs. pressure for 20 minutes. The sugar solutions were then added by sterile pipettes so as to give a final concentration of 2.0%.

In a second series of experiments, 0.1% peptone was utilized in the place asparagine. However, in this case, only glucose, glycerol and soluble starch were tested as the carbohydrate source.

In the third series, 0.1% casein was substituted for asparagine along with glucose, glycerol and soluble starch. In each instance, the concentration of the carbohydrate was set at 2.0%. One ml of a 2-5 day old Streptomyces broth culture was inoculated into the 250 ml flasks and 0.25 ml into the 125 ml flasks. All inoculated and non-inoculated control flasks were incubated at room temperature (28-30° C.) for two to six days. Observations and determinations were made on the 2nd and 6th day intervals. All of the experiments were run in duplicate and an average taken of the results. These tests included:

1. Morphological observations: type of cellular growth.
2. Trend in pH: each flask was thoroughly shaken by hand and 10

ml removed by sterile pipette for this determination. The pH was determined by means of a Beckman pH meter, model H-2.

3. Titratable acidity: each flask was thoroughly shaken by hand and 5 ml removed by means of a sterile pipette. Titratable acidity of each sample was determined by titrating against 0.1 N NaOH using phenophthalein as the indicator.

4. Volume of growth: at the end of six days, all the growth in the 125 ml flasks was transferred into graduated and tapered centrifuge tubes. Then after five minutes of centrifugation at approximately 1500 revolutions per minute, the volume of settled growth was estimated in terms of cubic centimeters.

2. Utilization of Glucose-Asparagine Medium by Unknown Streptomyces Isolates

Glucose-asparagine medium was prepared as before and various unknown Streptomyces isolates were inoculated into the medium. Two and six day pH readings were taken for these organisms to determine the degree of variation.

3. Phage Specificity

In the experiments concerned with phage activity, the following medium was used:

Glucose -- 10.0 gm.
Beef Extract -- 5.0 gm.
Peptone -- 5.0 gm.
Water(Distilled) -- 1000.0 cc.

The above medium was poured in 60 ml aliquots into 250 ml flasks. After sterilization of the medium, 1 ml of a 2-5 day old broth culture of the test organism was added to the sterile medium. At the close of

a 24 hour incubation period, a 2 ml quantity of the phage filtrate (514-3) was added to the growing culture. Observations were made at 12 and again at 24 hours. Observable lysis, even though incomplete, was considered positive.

C. Experimental Results

1. Utilization of Various Carbohydrates

The ability of individual Streptomyces species to utilize carbohydrates can be determined by the drift in H-ion-concentration, titratable acidity and by the volume or weight of cellular growth. Any observed variation in one or more of the above mentioned criteria very often indicate a basic difference in the metabolism of the microorganism under study. It is also important to remember that the utilization of a particular carbohydrate is modified by the included nitrogen source. For this reason the following data on carbohydrates are discussed in respect to the nitrogen source used.

a. Asparagine medium

Reference to the pH values listed in Table 1 and Figure 1 discloses that little change in hydrogen ion concentration occurred with the various carbohydrates at the two day interval. However, strains 3475 and 3478 showed a slight alkaline trend with lactose, maltose, sucrose, dulcitol and raffinose. On the other hand, these strains produced a slight acid trend with glucose, fructose, glycerol and soluble starch. In general, the trends in pH are reflected in the titratable acidity values.

After six days incubation strains 3475 and 3478 exhibit more striking differences in the utilization of the carbohydrates under study. A

Table 1. Trend in H-ion Concentration in Asparagine Medium after 2 Days Incubation at 30°C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)		
Carbohydrate	Control	3475	3478	Control	3475	3478
None	7.00	7.28	7.35	1.0	--	--
Glucose	7.02	7.19	7.03	1.0	1.0	1.0
Fructose	7.30	7.15	7.17	1.0	1.5	2.0
Galactose	7.31	7.25	7.02	1.0	1.5	2.0
Lactose	7.26	7.42	7.42	1.5	1.5	1.5
Maltose	7.09	7.45	7.48	2.0	1.5	1.5
Sucrose	7.38	7.54	7.43	1.5	1.0	1.5
Glycerol	7.34	7.19	7.18	2.0	3.0	3.0
Mannitol	7.26	7.28	7.26	1.5	1.5	1.5
Sol. Starch	7.13	6.78	6.86	1.5	2.5	2.5
Dulcitol	7.25	7.42	7.43	1.0	1.5	1.5
Raffinose	7.32	7.42	7.38	1.0	1.0	1.0

Figure 1. Trend in H-ion Concentration in Asparagine Medium after 2 days Incubation at 30° C.

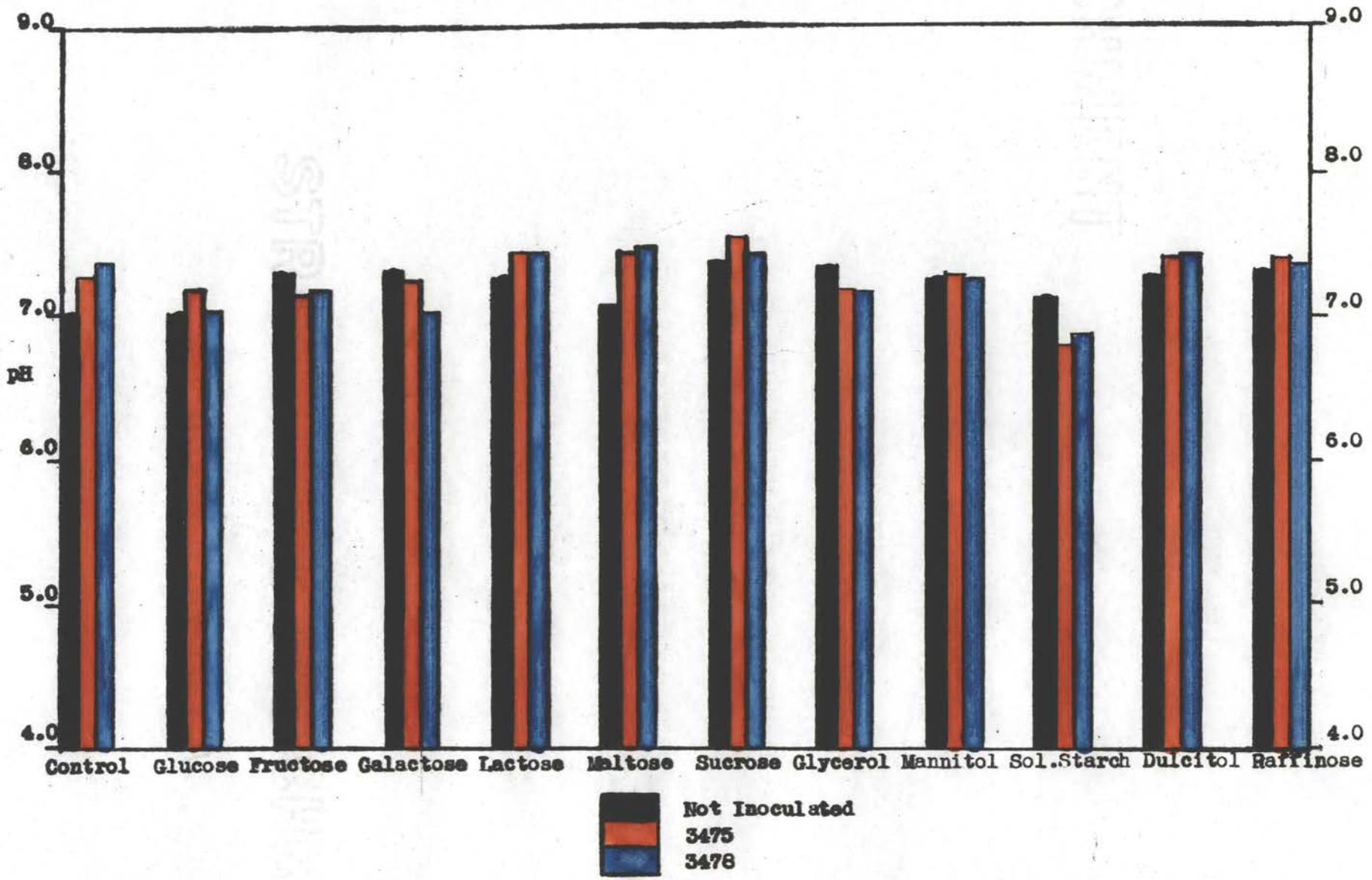
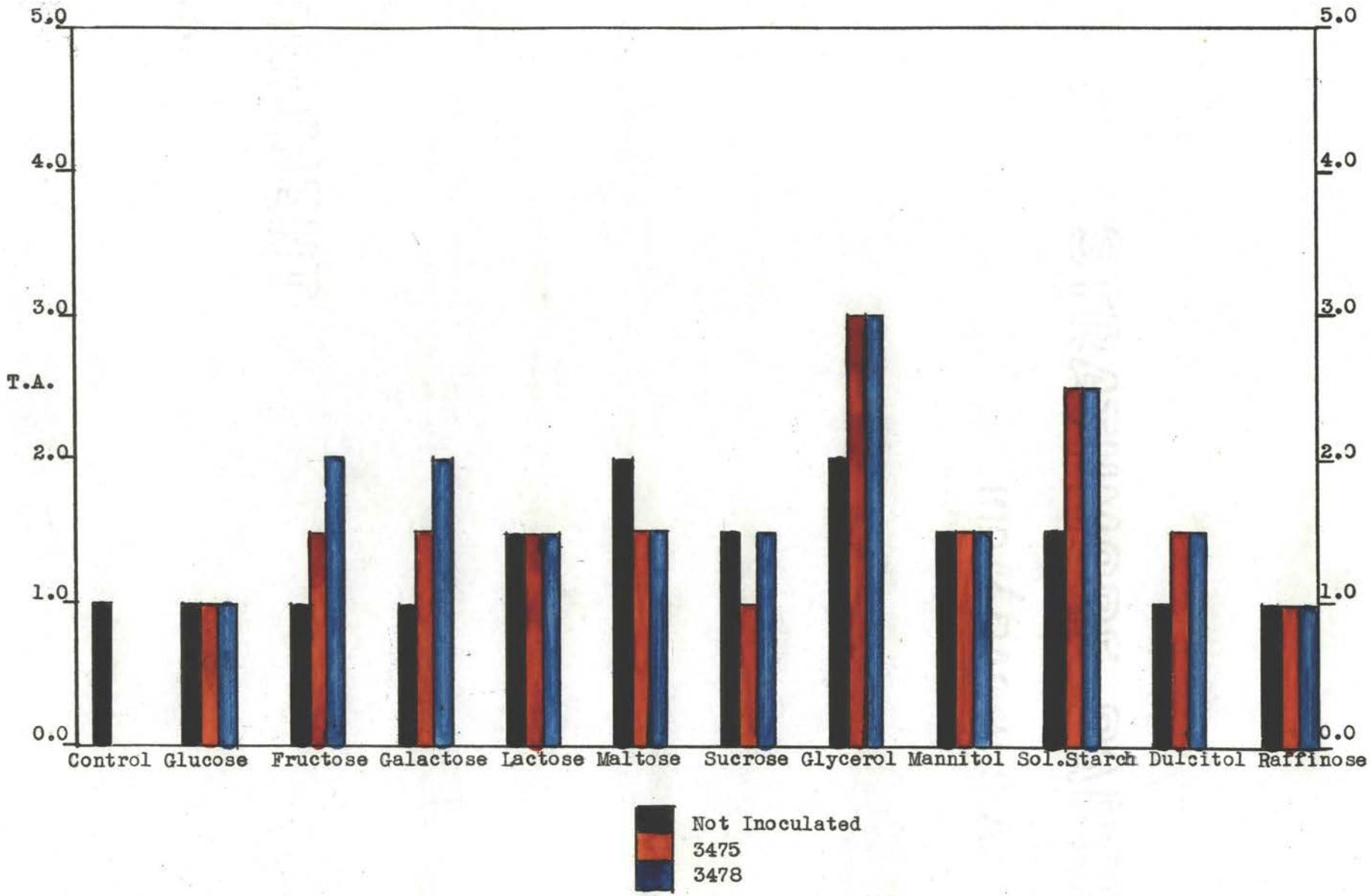


Figure 2. Trend in Titratable Acidity (X 10) in Asparagine Medium after 2 days Incubation at 30° C.



study of the data in Table 2 shows that both Streptomyces strains displayed strong alkaline trends with lactose, maltose, sucrose, dulcitol and raffinose. It is also interesting that in each of the latter instances very little volume of growth was recorded. This would indicate that the attack on lactose, maltose, sucrose, dulcitol and raffinose has been very limited. The rise in pH and negligible titratable acidity values can be attributed to the liberation of ammonia by the preferential use of asparagine as the source of carbon and nitrogen.

On the other hand, as evidenced by the data in Table 2 and Figures 3 and 5, both strains showed a decided acid trend and a far greater volume of growth in the presence of glucose, fructose, galactose, glycerol and soluble starch. This observation applies particularly to strain 3478 in which case a final pH of 4.87 was recorded with glucose as the carbohydrate. The generally stronger acid trends noted with strain 3475 may represent a significant physiological difference between strain 3475 and 3478. The greater cell mass illustrated in Figure 5 for strain 3475 with each carbohydrate studied, would also tend to infer that these isolates vary considerably in their ability to utilize the carbon and nitrogen sources. In this respect, strain 3475 showed an additional divergence from 3478 in the production of a soluble greenish pigment with glucose and mannitol. In all other instances the type of growth of the two strains appeared identical.

b. Peptone medium

In the following experiments the carbohydrates studied were limited to those which had produced a strong acid reaction with strain 3475. These included glucose, glycerol and soluble starch.

A study of the data in Table 3 substantuates the previous finding

Table 2. Trend in H-ion Concentration and Corresponding Growth in Asparagine Medium after 6 Days Incubation at 30° C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)			Volume of Growth	
Carbohydrate	Control	3475	3478	Control	3475	3478	3475	3478
None	7.00	7.72	7.70	1.0	--	--	.03	.01
Glucose	7.00	6.85	4.87	1.0	1.5	3.5	.45	.10
Fructose	7.20	7.23	6.12	1.5	1.0	2.0	.65	.22
Galactose	7.18	6.72	6.35	1.5	0.5	1.0	.85	.30
Lactose	--	8.10	7.85	--	1.5	1.5	.30	.10
Maltose	7.60	8.15	7.88	1.5	1.5	1.5	.10	.08
Sucrose	7.40	8.01	7.79	1.5	1.5	1.5	.08	.03
Glycerol	--	7.00	6.90	--	1.0	1.0	.70	.50
Mannitol	7.37	7.28	7.00	2.0	1.0	2.5	1.00	.30
Sol. Starch	7.20	6.60	6.63	3.0	1.0	1.0	.60	.35
Dulcitol	--	8.37	8.26	--	1.5	2.0	.30	.10
Raffinose	7.50	7.98	7.76	2.5	2.0	2.5	.03	.02

Figure 3. Trend in H-ion Concentration in Asparagine Medium after 6 days Incubation at 30° C.

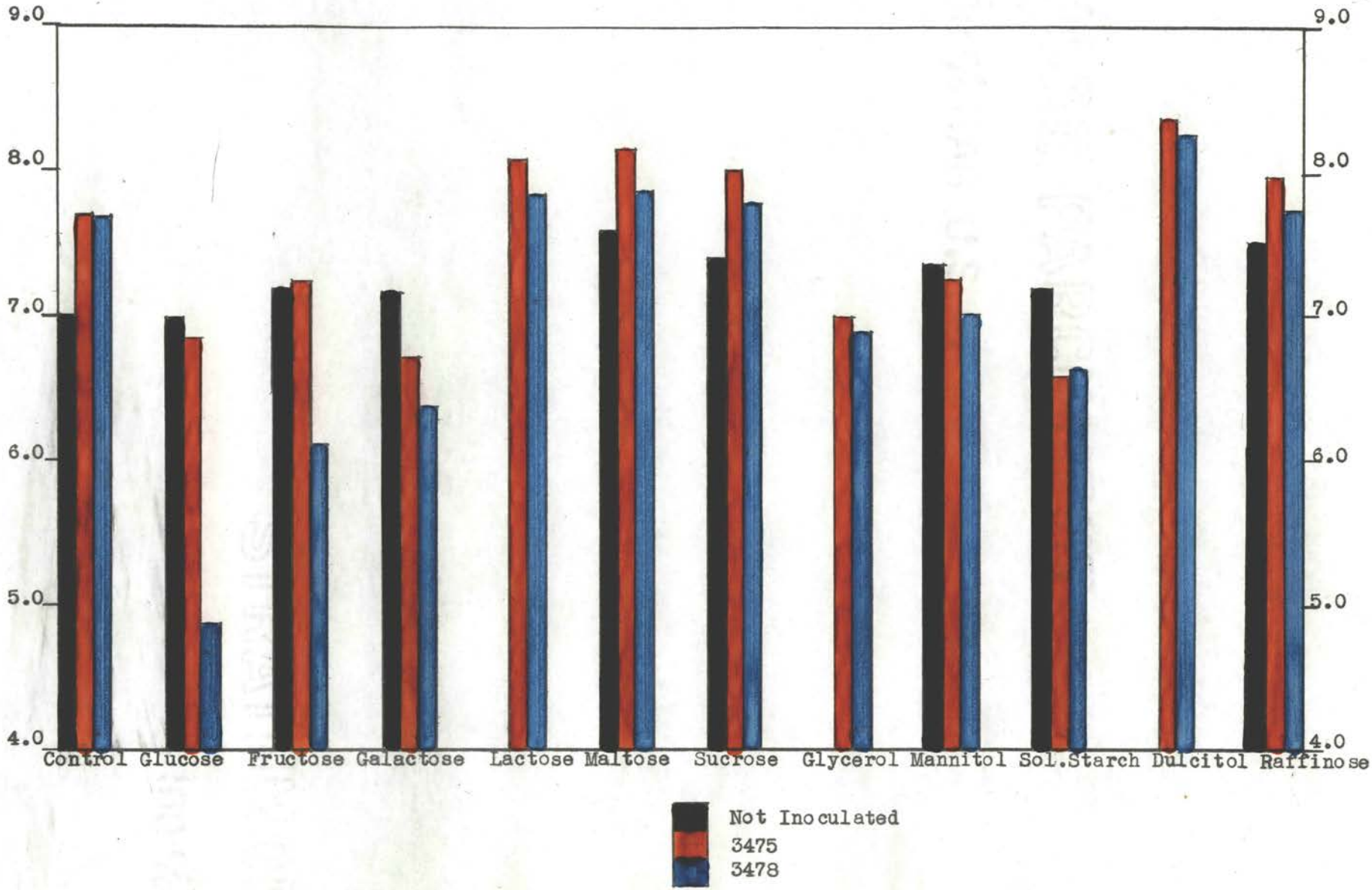


Figure 4. Trend in Titratable Acidity (X 10) in Asparagine Medium after 6 days Incubation at 30° C.

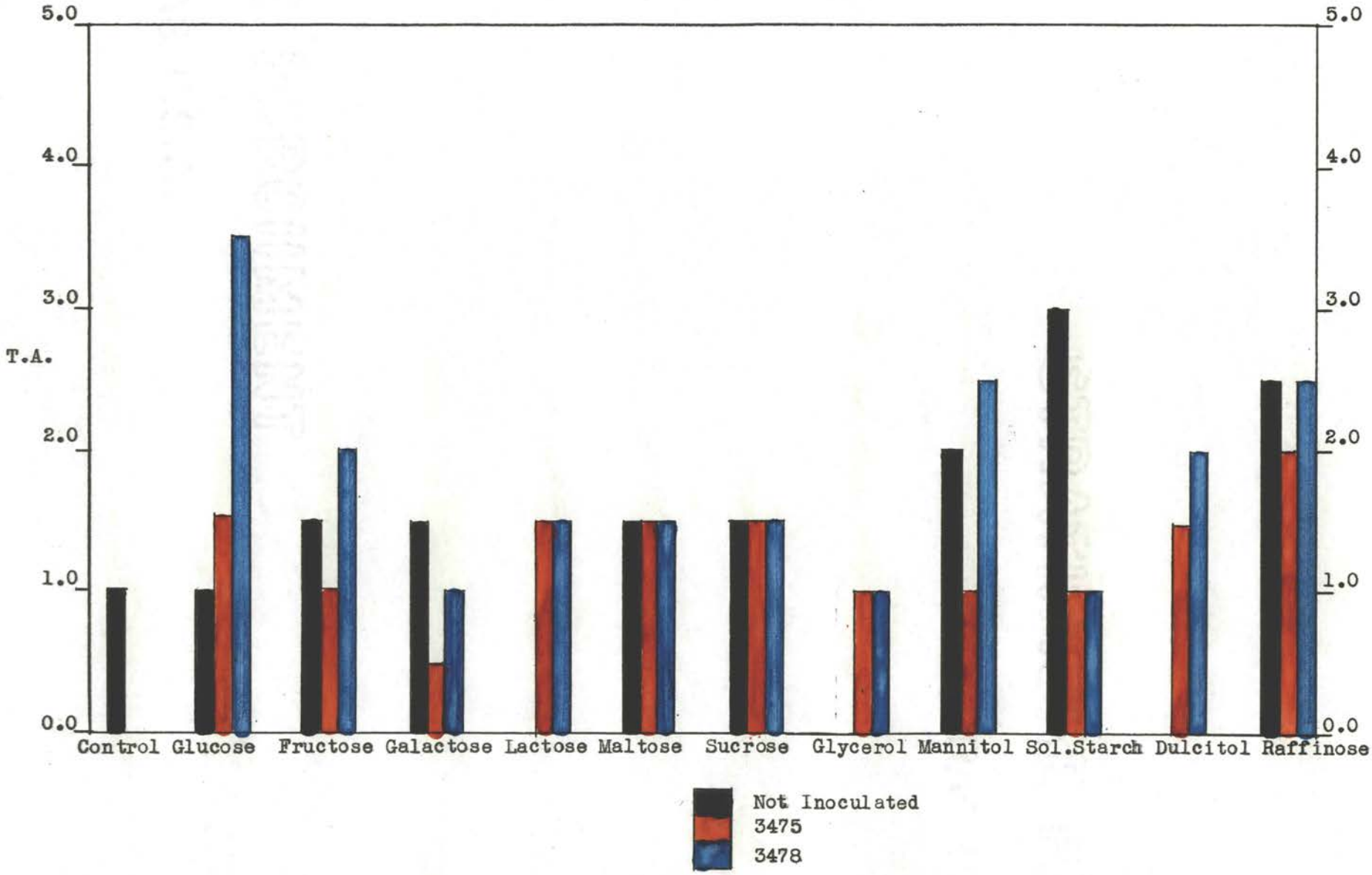


Figure 5. Volume of Growth in Asparagine Medium after 6 days Incubation at 30° C.

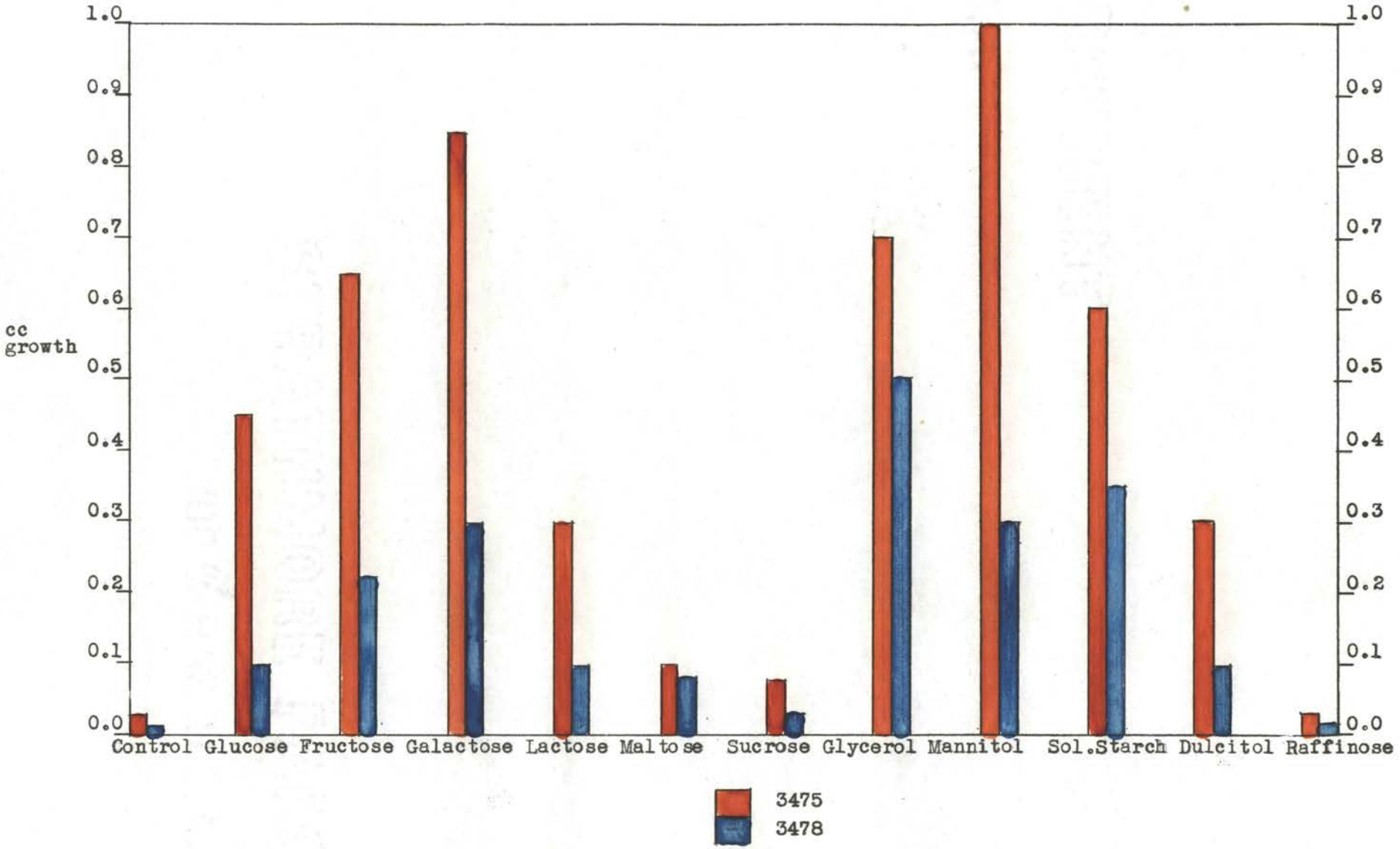


Table 3. Trend in H-ion Concentration in Peptone Medium after 2 Days Incubation at 30° C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)		
Carbohydrate	Control	3475	3478	Control	3475	3478
None	7.00	7.65	7.86	1.5	2.0	2.0
Glucose	7.30	7.31	7.02	1.0	1.5	1.5
Glycerol	7.34	7.14	6.89	1.0	1.5	1.5
Sol.Starch	7.21	6.91	6.70	1.5	2.5	2.5

that a slight acid trend begins at the 2 day period. Apparently the inclusion of peptone in place of asparagine as the source of nitrogen has not changed this observation. This observation finds complete corroboration in the definite acid reactions noted in Table 4 and Figures 6 and 7 for both Streptomyces strains. Here again, strain 3478 displays an unusually low pH of 4.78 in the presence of glucose. Whereas strain 3475 showed only a slight acid reaction of pH 6.90. In general, the respective volume of cell mass with each carbohydrate is approximately equal for both strains at the six day period. (Table 4 and Figure 8).

c. Casein medium

As was the case with asparagine and peptone, a slight acid drift can be observed with casein and the included carbohydrates. The results in Table 5 show little difference in the two strains except a slightly greater acid trend of 3478 with glucose at the end of two days. This trend is increased considerably at six days shown on Table 6 and Figure 9 as was the case with asparagine and peptone with glucose. The increase in titratable acidity of 3478 over 3475 from 1.5 to 2.5 is also of significance on Table 6. The volume of cell mass shown on Table 6 and Figure 11 indicates the carbohydrate has very little effect on strain 3478 but the amount of growth is significantly less with glycerol and soluble starch insofar as strain 3475 is concerned. The absence of increased growth due to the carbohydrates does not parallel the acid trend produced by these carbohydrates.

d. Growth curve experiment

This experiment was conducted in glucose-asparagine medium with the two strains of Streptomyces, 3475 and 3478, to determine the

Table 4. Trend in H-ion Concentration and Corresponding Growth in Peptone Medium after 6 Days Incubation at 30° C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)			Volume of Growth	
Carbohydrate	Control	3475	3478	Control	3475	3478	3475	3478
None	7.30	8.35	8.50	1.5	2.0	2.0	.26	.21
Glucose	7.42	6.90	4.78	1.0	1.0	2.5	.32	.20
Glycerol	--	6.92	6.59	--	1.0	1.0	.32	.35
Sol.Starch	7.28	6.60	6.57	1.5	1.0	2.0	.40	.45

Figure 6. Trend in H-ion Concentration in Peptone Medium after 2 and 6 days Incubation at 30° C.

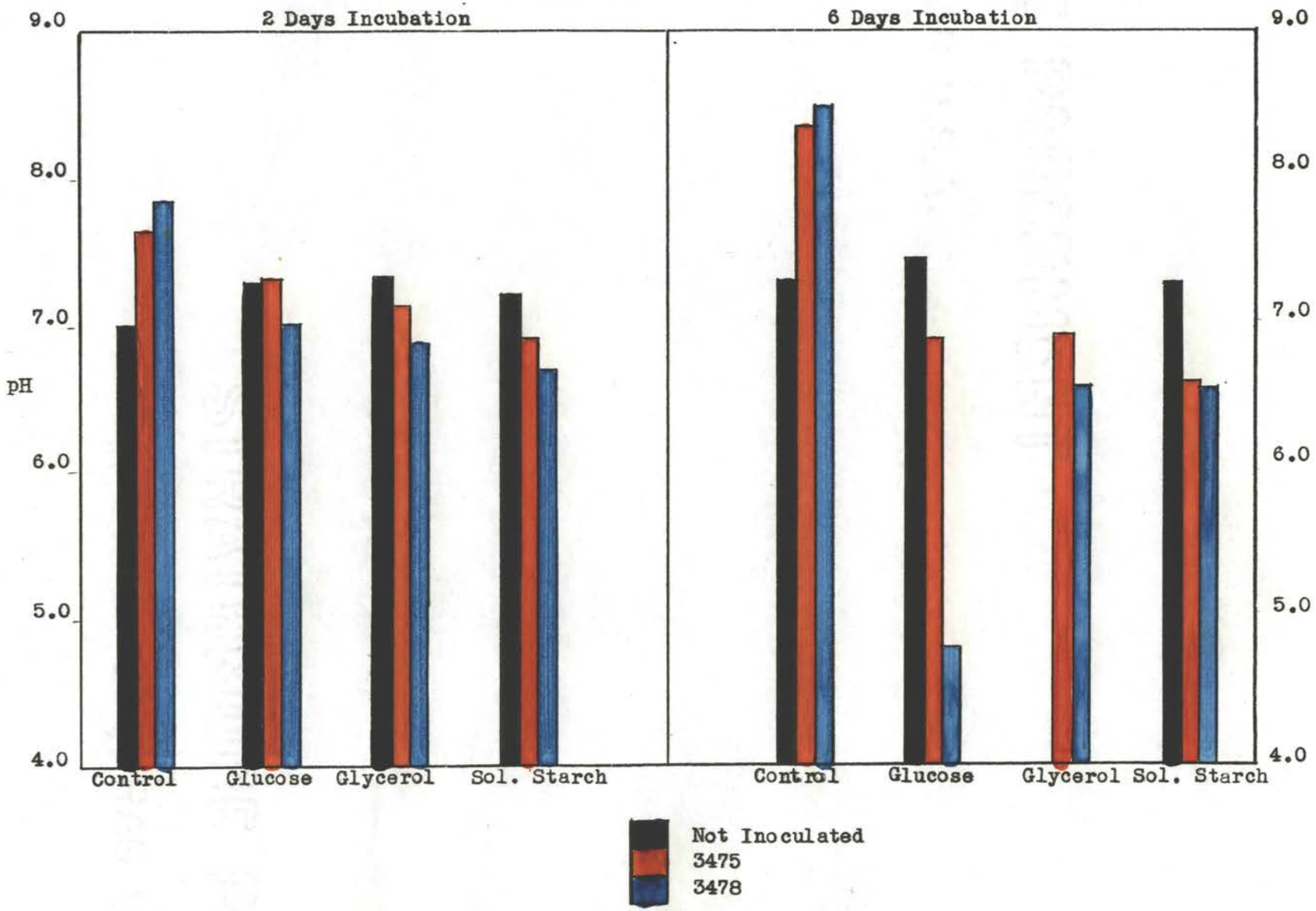


Figure 7. Trend in Titratable Acidity (X 10) in Peptone Medium after 2 and 6 days Incubation at 30° C.

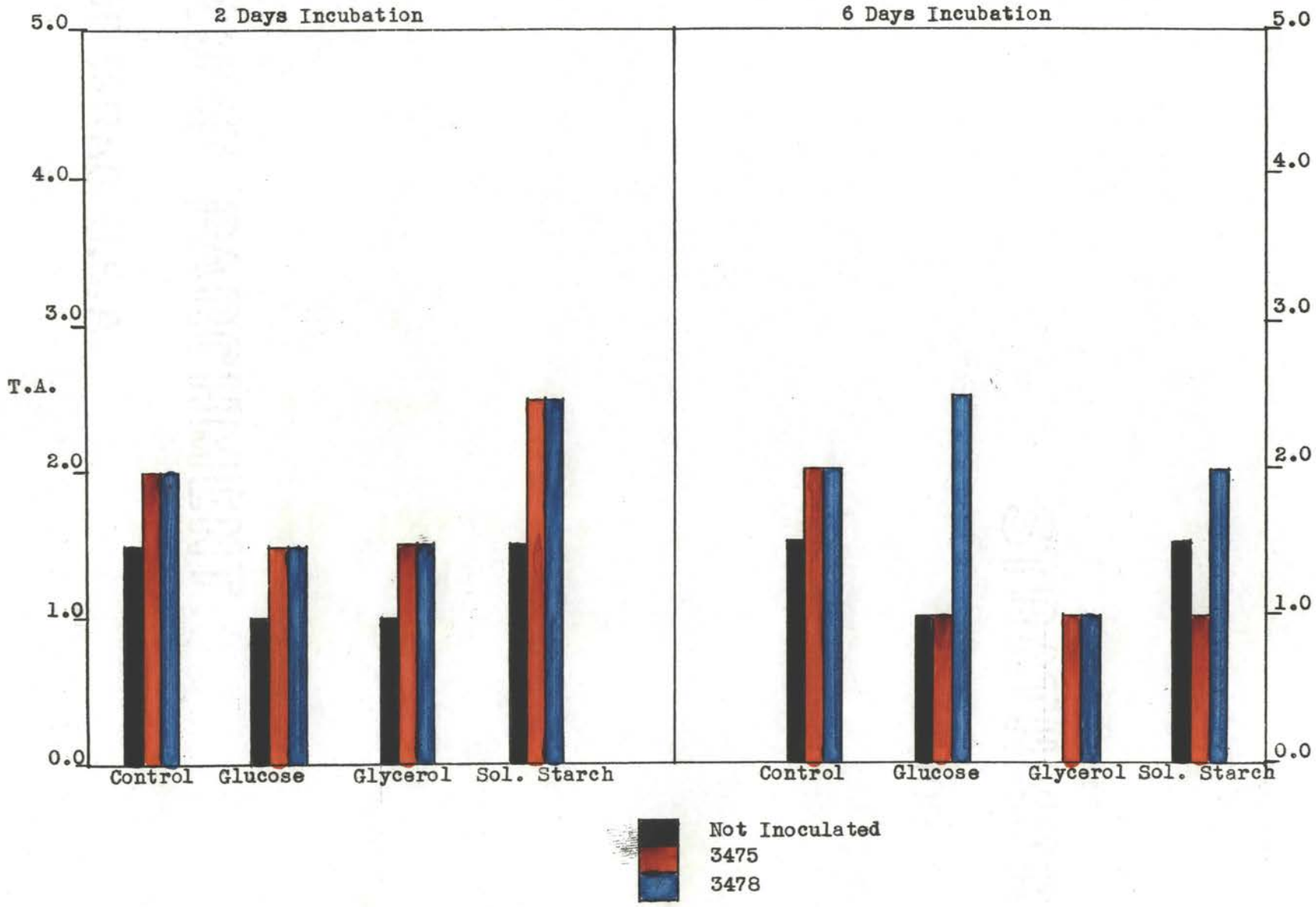


Figure 8. Volume of Growth in Peptone Medium after 6 Days Incubation at 30° C.

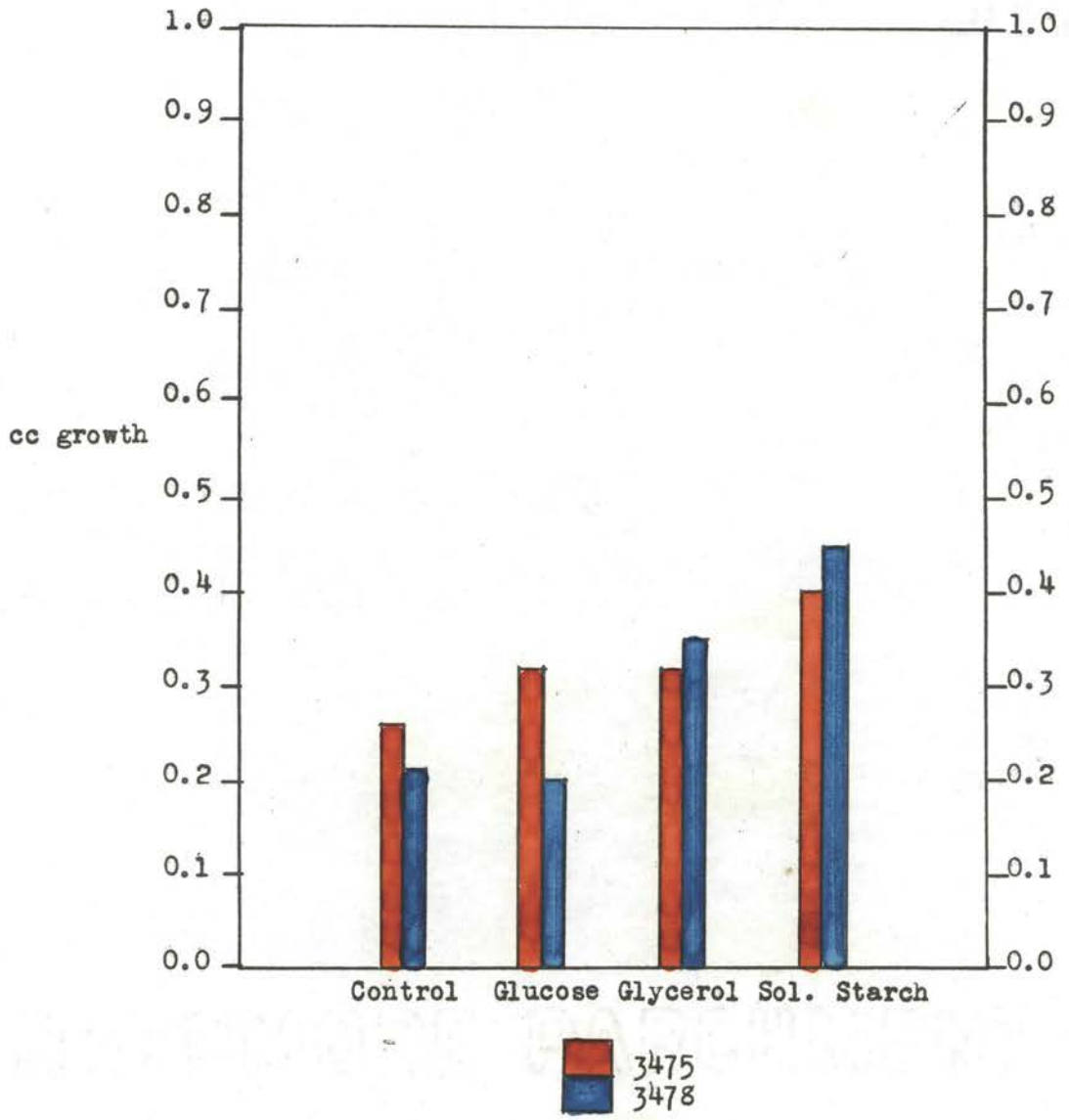


Table 5. Trend in H-ion Concentration in Casein Medium after 2 Days Incubation at 30° C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)		
Carbohydrate	Control	3475	3478	Control	3475	3478
None	7.40	7.45	7.47	1.0	2.5	1.5
Glucose	7.41	7.21	7.01	1.0	1.5	1.5
Glycerol	7.42	7.04	7.03	1.0	1.5	1.5
Sol.Starch	7.18	6.81	6.81	1.5	2.0	2.0

Table 6. Trend in H-ion Concentration and Corresponding Growth in Casein Medium after 6 Days Incubation at 30° C.

	pH Value			Titratable Acidity X 10 (N/10 NaOH)			Volume of Growth	
Carbohydrate	Control	3475	3478	Control	3475	3478	3475	3478
None	7.50	8.17	8.09	1.0	1.5	1.5	.36	.20
Glucose	7.56	7.10	4.82	1.0	1.5	2.5	.34	.20
Glycerol	7.50	7.01	6.95	1.0	1.5	1.0	.13	.22
Sol. Starch	7.38	6.89	6.67	1.5	2.0	1.5	.15	.20

Figure 9. Trend in H-ion Concentration in Casein Medium after 2 and 6 days Incubation at 30° C.

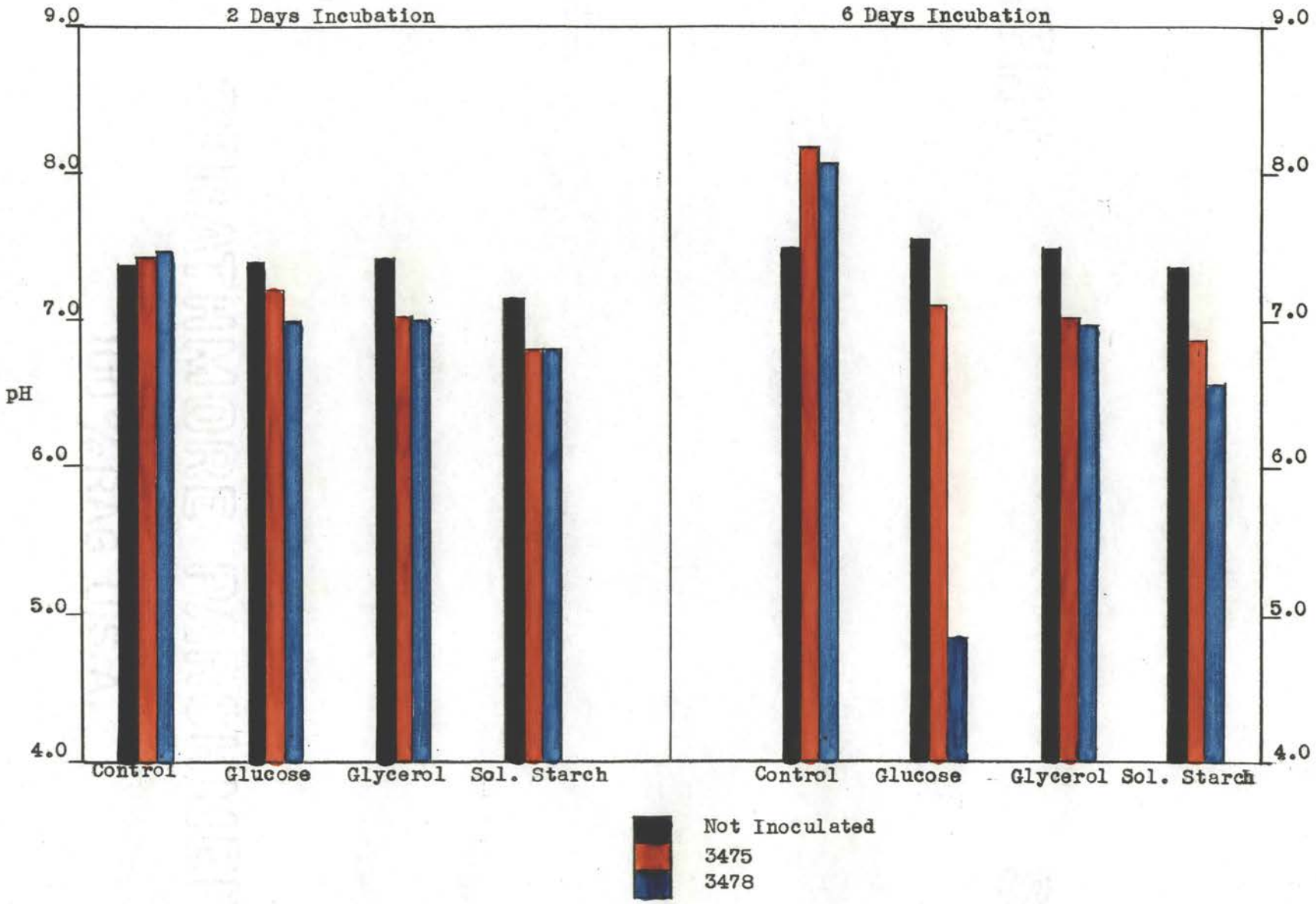


Figure 10. Trend in Titratable Acidity (X 10) in Casein Medium after 2 and 6 days Incubation at 30° C.

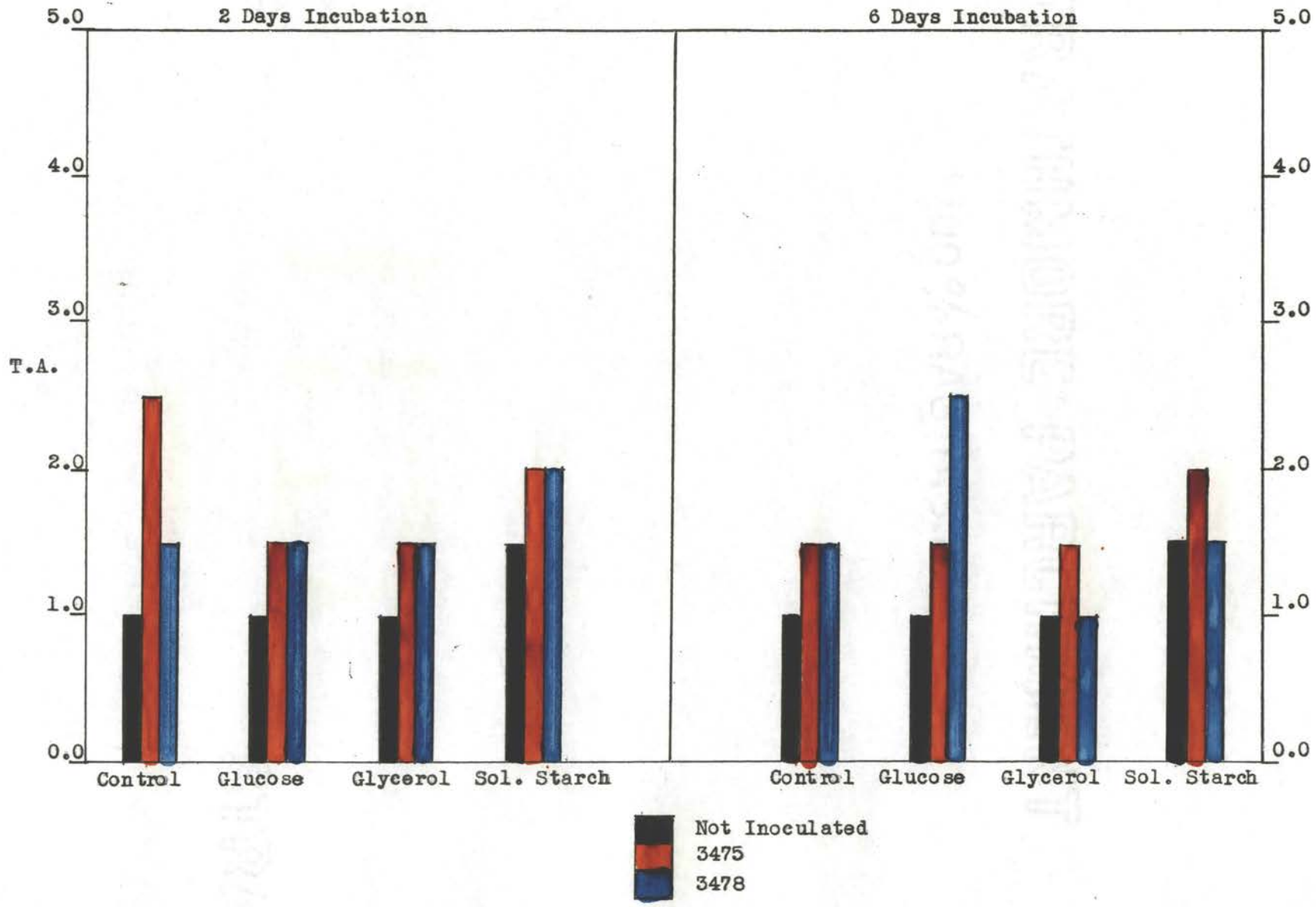
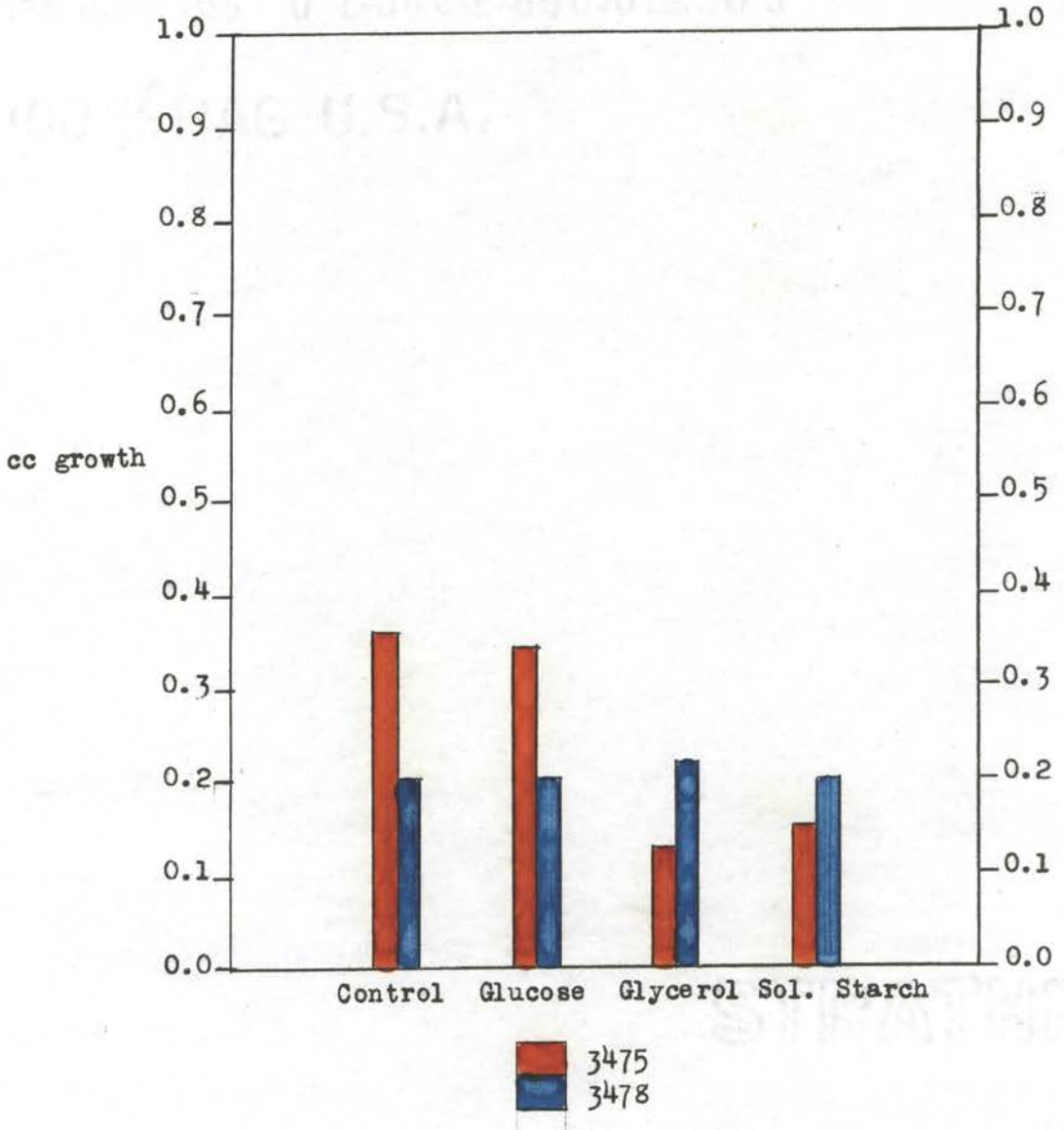


Figure 11. Volume of Growth in Casein Medium after 6 Days Incubation at 30° C.



rapidity in the drop of pH over the six day period. The results as shown on Table 7 and Figure 12 indicate the drop in pH is gradual with the greatest variation shown by 3478 from 4½ to 5 days. The growth in this experiment again exhibited the greenish discoloration of the medium with strain 3475 on the fifth day of incubation. The spores appearing on the cultures of 3478 appeared to be in smaller clumps than those of 3475. In each case, there was a heavy submerged growth.

2. Utilization of Glucose-Asparagine Medium by Unknown Streptomyces Isolates

The data shown on Table 8 indicate that within a group of unknown Streptomyces isolates, there are three rather well defined groups based on pH drift over a six day incubation period. There are those which show a drift below a pH of 6.8 producing a definite acid trend; and those that the pH is approximately neutral between 6.80 and 7.20; and those that indicated a definite basic trend of greater than 7.20. All species producing a basic trend were in the neutral group at the end of the two day incubation period while most of those showing a final acid trend, were found to have an acid pH at two days incubation. Of the 35 unknown Streptomyces isolates, 15 showed the acid trend, 9 in the neutral group, and 11 were in the basic trend group.

3. Phage Specificity

Reference to the data given in Tables 9 and 10 makes obvious the high incidence of lysis among both the unknown and known Streptomyces species. It is especially significant that more than one known Streptomyces species (Table 10) has undergone lysis by a phage regarded as being specific for Streptomyces griseus. This might infer that many of the listed Streptomyces species are more closely related than has been

previously realized. In this instance strain rather than species differences might be inferred.

Table 7. Trend in pH of Strains 3475 and 3478 on Glucose-Asparagine Medium over a Six Day Period

Days	3475	3478
1	7.20	7.30
1½	7.00	7.05
2	7.02	6.90
2½	7.01	6.85
3	6.85	6.55
3½	6.90	6.43
4	6.98	6.50
4½	6.85	6.30
5	7.00	5.80
5½	7.11	5.50
6	6.89	5.40

Figure 12. The pH Drift of Strains 3475 and 3478 on Glucose-Asparagine Medium

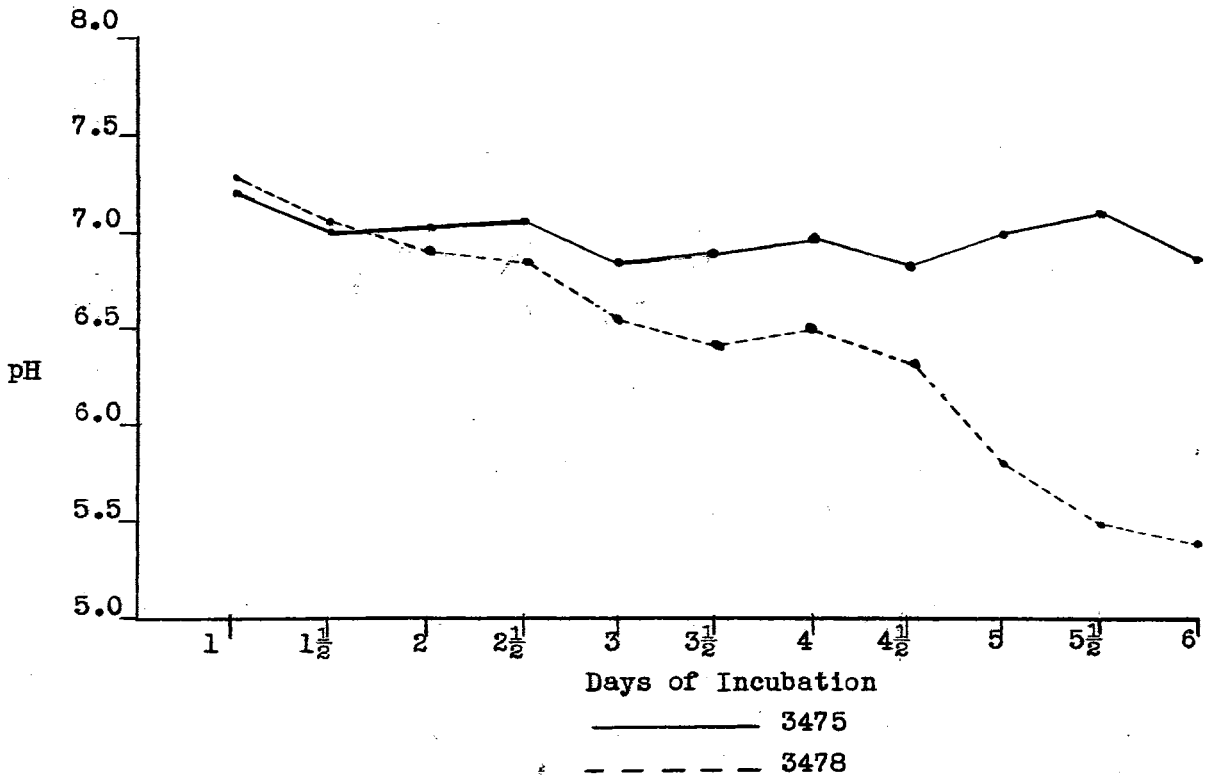


Table 8. Utilization of Glucose-Asparagine Medium by Unknown *Streptomyces* Isolates

	Incubation Period	
Organism	2 Days	6 Days
Control	7.25	7.30
401	6.90	5.65
402	6.72	6.75
406	6.75	5.15
505	7.19	4.18
515	6.40	5.50
519	6.41	5.65
520	6.70	4.90
521	6.58	5.50
522	7.60	6.80
524	7.25	6.80
526	7.10	7.60
530	6.22	5.05
532	6.05	4.50
533	6.91	7.52
534	7.00	7.28
535	7.05	7.60
539	6.88	7.10

	Incubation Period	
Organism	2 Days	6 Days
600	6.60	4.65
602	6.70	4.65
603	7.20	7.25
605	6.75	4.90
609	6.92	6.90
702	7.13	7.50
703	7.31	7.10
705	6.80	7.00
802	7.05	7.33
804	7.08	7.85
805	7.10	7.10
807	5.96	5.85
808	7.10	7.65
901	6.10	5.15
903	7.13	6.95
904	7.08	7.40
907	6.55	5.15
908	7.09	7.45

Table 9. Number of Lysed and Non-Lysed Cultures

Filtrate	Number		
	Lysed	Not Lysed	Total
512-3	32	45	77
513-5	25	52	77
514-3	36	41	77
514-5	33	44	77

Table 10. Lysis of Various Streptomyces Species with Phage 514-3

Organism	Time of Lysis (hrs.)	
	24	48
S. phaeochromogenus 3388	***	***
S. californicus 3312	-	-
S. albus 3004	***	***
S. antibioticus 10382	-	-
S. olivochromogenus 3336	***	***
S. cacaoi 3082	-	-
S. lavendulaie 8664	-	-
S. diastaticus 3315	-	-
S. purpeochromogenus 3343	***	***
S. microflavus 3332	-	-
S. olivaceus 3335	-	***
S. cellulosae 3313	***	***
S. coelicolor 10147	***	***
S. roseochromogenus 3347	-	-
S. flavovirens 3320	***	***
S. gibsonii 6852	***	***
S. babiliae 3310	-	-
S. willmorii 6867	-	-
S. flaveolus 3319	-	-
S. albosporus 3003	***	***
S. lipmanii 3331	-	-
S. griseolus 3325	***	***
S. rutgersensis 3350	-	-
S. viridochromogenus 3356	-	-
S. griseus 10137	***	***
S. griseus 3475	***	***
S. griseus 3478	**	***
S. flavus 3369	-	-

*** Lysis complete.
- No lysis.
All controls were negative.

GENERAL DISCUSSION

The experimental data resulting from this study leads one to believe that some physiological properties of the Streptomyces may be used for species identification. Three of the properties used in this work demonstrated the possibility by showing significant differences in the two strains of Streptomyces griseus, 3475 and 3478.

The first property, that of hydrogen ion concentration, showed 3478 to produce a decided acid pH of 4.87 in the glucose-asparagine medium while 3475 produced nearly a neutral pH of 6.85 after six days incubation. The nature of the organic acids produced were not known. It is well to point out that in Waksman's work (25), the lower pH levels were found with unbuffered media, while in the present investigation, the glucose-asparagine medium contained buffering salts. In Stephenson's text (15), Bacterial Metabolism, the following statement is found: "The presence of a fermentable carbohydrate in a cultural medium inhibits the production of proteases, but whether this action is due to the production of acid or to the inhibition of the formation of proteolytic enzymes is uncertain." It would seem that in the case of the Streptomyces the production of acid might be more correct. It is important to note that regardless of the protein utilized in the medium, i.e. asparagine, peptone, or casein, the results were very similar with glucose as the carbohydrate. No other carbohydrate produced the same pH drift as did glucose, even though the trend was similar. In some instances the pH of the two organisms was approximately equal at the end of six days incubation, but usually strain 3478 showed

an acid trend.

The second property, titratable acidity, was found to be significant only with glucose in asparagine, peptone or casein medium after six days incubation. With the other carbohydrates, neither strain consistently produced a greater titratable acidity than the other.

The third property, volume of growth, showed that strain 3475 grew more extensively than did strain 3478. Just why strain 3478 produces a lower pH and greater titratable acidity with a lesser cell growth is not known. It may be due to the lack of or inhibition of proteolytic enzymes. Because the low pH values resulted with the three varied nitrogen sources in buffered media, it would seem that the pH value is a direct result of the glucose added to the medium. This conclusion is drawn because both strains showed an alkaline trend in the media without glucose.

The addition of glucose to the asparagine medium brought about a striking response in cell growth. The gain in strain 3475 (Table 2) was from .03 centimeters to .45 centimeters with the addition of glucose in six days incubation. The addition of glucose to the peptone and casein media did not produce this effect. The complexity of the protein thereby showing an effect on the amount of growth. Apparently, glucose was not utilized for increased volume growth with peptone and casein as the sources of protein.

A fourth property is illustrated by the action of actinophages on Streptomyces. By using several phage isolates, various known and unknown Streptomyces were found to be lysed by each of the isolates. Consequently, the phage isolates used were not as specific as early

workers found with their phages. Variation in phages may have caused some conflicting results, but the fact that phages were used with such a wide range of lysis, would indicate either close similarity of Streptomyces species or a wider range of action of the phages.

SUMMARY

The four physiological properties of Streptomyces utilized in this experiment were found to be helpful in distinguishing Streptomyces species that may appear morphologically similar. They are a step forward to finding a means of classifying the Streptomyces on a physiological basis.

These characters -- H-ion concentration, titratable acidity, volume of cell growth, and phage sensitivity -- represent a few of the physiological characters that may be utilized for differentiation of strains and species.

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