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DO SIGNIFICANT PHYSIOLOGICAL STRAINS OF *BACILLUS AMYLOVORUS* (Burr.) TREV. EXIST?

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From the plant pathologist's viewpoint, more knowledge of the existence of physiological strains of *Bacillus amylovorus* would be of value. Stewart (7) after a study of the organism in culture calls attention to slight variations in the fermentation reactions of the nine different isolated cultures with which he worked. Therefore, at the suggestion of Dr. H. E. Thomas of Cornell University, N. Y., this work was undertaken in an effort to contribute this information. The portion on acid tolerance has been checked in the laboratories of the State University of Iowa through the courtesy of Drs. G. Hansmann and W. F. Loehwing.

Cultures of the fire blight organism were gathered together from widely scattered sources as shown in table I, in order that any

Table I — Source and History of Cultures

CULTURE	DATE ISOLATED	LOCATION	SUSPECT
UR	8-21-'24	Utica, N. Y.	Apple twig
S	5-13-'25	Columbia, Mo.	Apple twig
Cal	5-15-'25	Davis, Calif.	Pear
SC	6-15-'25	Clemson College, S. C.	Pear twig
Mi	6-16-'25	Grand Rapids, Mich.	Apple twig
X	5- 5-'26	Ithaca, N. Y.	Kieffer pear twig
Ti	5-23-'26	Tifton, Ga.	Kieffer pear twig
TGa	5-23-'26	Tifton, Ga.	Kieffer pear twig
Ta	'26	Tifton, Ga.	Kieffer pear twig
Mont	8- '26	Montana	Apple twig
NB	8- '26	Clyde, N. Y.	Pear
N	10-18-'26	Clyde, N. Y.	Apple?
Q	10-18-'26	Clyde, N. Y.	Quince sucker
W	6-24-'27	Ithaca, N. Y.	Apple twig
C	7-23-'27	Ithaca, N. Y.	Crataegus twig
NZT	3- 3-'28	New Zealand	Pear or apple
Z	3-21-'28	New Zealand	Apple

variations, if existing, might be the more readily demonstrated. Before beginning culture work, the assembled cultures were twice inoculated into succulent apple shoots and reisolated to check their pathogenicity. Single cell colonies were chosen from suitable dilution plates of the second reisolation of each culture and transfers made to nutrient agar slants. Transfers were then made, as needed, from the stock nutrient slants to tubes of bouillon, which after

forty-eight hours incubation, were used as sources of inoculum. Methods outlined by the Committee on Pure Culture Study of the S. A. B. (1) were followed as closely as possible. About 25°C. was found to be near the optimum temperature for growth and was adopted as a standard incubation temperature. Determinations of pH. were made with the quinhydrone electrometric apparatus devised by Youden.

Previous workers, who have investigated the acid tolerance of *B. amylovorus,* necessarily expressed their results in Fuller's scale values as determination of hydrogen ion concentration was practically unknown at that time. Jones (3) obtained clouding in bouillon + 16 with hydrochloric acid only after four days incubation. Stewart (7) gave + 23 as the limit for growth in bouillon acidified with hydrochloric acid and + 25 as the limit when malic acid was used. To gain an idea of these values in terms of pH., the figures were converted by means of the formula of Quirk and Fawcett (4) $\text{pH.} = 8.2 - \frac{\text{Fuller value}}{10}$. On this basis the limits of tolerance found by Stewart would be pH. 5.9 for bouillon acidified with hydrochloric acid, pH. 5.7 for bouillon acidified with malic acid, and pH. 8.9 when potassium hydroxide was added to the bouillon. A first series was accordingly set up with the pH. range above pH. 5.0 and growth was observed in all tubes. Hence it was necessary to set up another series with a lower range of pH. values. The results are shown in table II; a plus sign signifying

Table II — Tolerance of H and OH Ions

Culture	Hydrochloric Acid				Malic Acid		KOH [1]		
	4.68	4.65	4.60	4.55	4.6	4.5	8.5	8.7	8.9
W	+	+	±	−	±	−	+	+	−
Cal	+	+	−	−	±	−	+	+	−
N	+	+	−	−	+	+	+	+	−
TGa	+	±	−	−	+	±	+	+	−
Mi	+	+	−	−	+	−	+	+	−
Mont	+	+	±	−	+	±	+	+	−
S	+	±	−	−	+	−	+	+	−
UR	±	−	−	−	−	−	+	+	−
X	+	+	±	−	+	±	+	+	−
Q	+	+	±	−	+	−	+	+	−
SC		±		−			+	±	−
Z		±		−			+	+	−

growth in all quadruplicate tubes, a minus sign no growth, and a plus-minus sign growh in some of the quadruplicate tubes.

The data indicates that particular cultures may vary slightly in their toleration of H and OH ions. If the organism was able to

[1] pH values also checked with a LaMotte Roulette comparator and found to be 0.1 to 0.2 of a pH. unit lower than when determined by the quinhydrone method.

grow at all, the reaction of the medium became more favorable for growth with time. Growth decreased quantitatively within a given time interval as the acidity or alkalinity approached the limits of tolerance. Potter (4) wrote "that strong malic acid promotes the growth of *B. amylovorus* while it is altogether inhibitory to most other bacteria." This statement is questioned in view of the results of this investigation. However, the actual acidity of his "strong malic acid" is not given. Smith (6) says that the organism would not grow in beef broth + 80 Fuller's scale but that it would grow in potato broth acidified to + 45 with malic acid. If the Quirk and Fawcett formula is applied, the equivalent pH. value obtained is much lower than that which I have found the organism able to tolerate. The organism does seem slightly more tolerant of malic acid than of hydrochloric acid. The acid tolerance of *B. amylovorus* expressed in pH. units is now more exactly fixed than before.

RELATIVE FERMENTATION OF SUGAR BROTHS

Harding and Morse (2) in studying morphologically similar strains of *B. carotovorus* differentiated six races on their ability to ferment sugars as indicated by the amount of gas formed. Since *B. amylovorus* produces acid but no gas, it seemed likely that significant differences in acid formation might furnish a valid basis for the differentiation of strains. The rate of fermentation was followed by determining the change in hydrogen ion concentration of cultures under standardized conditions. The sugars, dextrose, fructose, sucrose, lactose, and maltose were added to nutrient broth. Four test tubes of each sugar broth were inoculated with each of the cultures. The tubes were then placed in the 25°C. incubator and observations made at intervals. Table III presents in summary form a typical portion of the data obtained. The time elapsing until the first change in acidity, ephemeral differences in growth habit, and the pH. of the medium after thirty days incubation are omitted.

In determining the pH. of the medium after ten days incubation, as shown in table III, two test tubes of each culture were removed from the racks, shaken to obtain a uniform sample, and two determinations made from each tube. This gave four values for each culture, the mean of which is given in the table.

The variation in the pH. of quadruplicate tubes inoculated with a given culture was generally slight — many times less than 0.05 of a pH. unit and infrequently varying more than 0.1 of a pH. Although the comparative fermenting power of two particular

Table III — Change in pH. of Medium After Ten Days Incubation

Culture	Medium					
	Dextrose	Fructose	Sucrose	Lactose	Maltose	Asparagin
Mi	5.23	5.08	5.32	6.83	6.91	7.05
X	5.07	5.20	5.34	7.05	7.05	6.88
W	5.01	5.28	5.30	7.07	7.24	6.90
Ta	5.23	5.25	5.54	7.05	7.15	7.03
UR	4.91	5.32	5.49	7.08	7.24	6.98
N	5.20	5.22	5.09	7.12	7.07	6.98
Q	5.03	5.28	5.09	7.15	7.27	6.98
S	5.18	5.15	5.23	7.17	7.24	7.05
NB	5.08	5.19	5.28	7.17	7.07	6.95
Cal	5.11	5.35	5.17	7.22	7.15	6.73
TGa	4.77	5.06	5.37	7.27	7.25	7.05
Mont	5.19	5.24	5.40	---	7.08	6.93
Range in pH	.46	.29	.45	.44	.36	.33
Initial pH.	7.24	5.91	7.28	7.25	7.07	6.50

cultures on a sugar broth may seem to show a significant difference, however, other cultures will be found to give an intergrading series, and thus it is felt that these differences hardly afford a valid basis for separating the cultures into strains. Some cultures gave a higher mean pH. of the medium after thirty days incubation than after ten days incubation indicating a reversion of reaction. Other cultures gave the opposite condition. Still no positive correlation could be shown between the bringing about of a reversion of reaction and the fermenting power or growth. For example, the first change in acidity of Andrade dextrose bouillon was apparent within forty-eight hours after inoculation with culture TGa; after ten days incubation the mean pH. of the dextrose bouillon was 4.77, and after thirty days incubation the mean pH. was 5.01. Culture Ta also showed a visible change in the acidity of the Andrade dextrose bouillon within forty-eight hours, but after ten days incubation the mean pH. of the medium was 5.23, and after thirty days incubation it was pH. 4.75. Culture Cal required seventy-two hours to show a visible change in the acidity of Andrade dextrose bouillon, yet the pH. after ten days was 5.11 and it had reverted to pH. 5.34 after thirty days incubation.

Waite (8) says that growth of *B. amylovorus* "is most on maltose cultures becoming strongly acid, and is slightly less so on cane sugar, dextrose, and levulose." The pH. values found indicate that maltose cultures do not become acid.

RELATIVE PATHOGENICITY OF THE CULTURES

In this study an attempt was made to determine whether differences could be shown in the relative pathogenicity of the various

cultures under experiment. Inoculations were made by the needle prick method using a twenty-four or forty-eight hour bouillon culture as the inoculum. Only the tips of succulent, rapidly growing shoots were used as infection courts. Table IV shows the

Table IV — Pathogenicity of Cultures of Bacillus amylovorous

Culture	King Apple		McIntosh Apple		Kieffer Pear		No. Spy Apple		Number of Inoculations	
	+	—	+	—	+	—	+	—	+	—
NB	3	0	3	0	2	1	9	0	17	1
N	3	0	3	0	2	1	3	0	11	1
W	5	0							5	0
X	8	0			2	1	8	1	18	2
S	3	0	3	0	2	1	3	0	11	1
Q	3	0	3	0	0	4	3	0	9	4
Cal	3	0	3	0	0	3	3	0	9	3
UR	3	0			1	2	4	1	8	3
TGa	1	2	2	1	1	2	2	0	6	5
Ta	3	0	1	2	1	2	2	1	7	5
Mont	3	0	3	0	0	3	2	1	8	4
Mi	7	2	1	2	3	0	3	0	14	4
Total infection	45	4	22	5	14	20	42	4	123	33

pathogenicity of the various strains expressed as positive infection (+) and negative infection (—) on the various suscepts. The history of the cultures may be found in table I. The data presented, while obviously not extensive enough to warrant definite conclusions, are at least suggestive. The total of infection brings out a difference in the relative resistance of certain varieties to infection. About ten percent of the inoculations in King and Northern Spy apples failed to cause infection, about twenty-five percent failed in the case of McIntosh apples and sixty-five per cent of the Kieffer pear terminals did not blight when inoculated. The culture Mi blighted Kieffer terminals very readily. In order that individual difference in resistance of a tree might not play a part, only one terminal on a tree was inoculated with a particular culture although several cultures were tried on the same tree.

Another point worth calling attention to is the difference in the time elapsing between the time of inoculation and the development of visible symptoms or signs. This incubation period was three to four days for King terminals, three days for Baldwin terminals, five to nine days for McIntosh terminals, and six to ten days for Northern Spy. Yet, from the data on the number of positive infections on each variety, there seems to be no correlation between the length of this period and the susceptibility of a given variety.

In conclusion some slight differences have been shown to occur in the physiological reactions of the various cultures. Attempts to correlate these reactions, that strains might be shown to exist, have proved fruitless. Therefore, on a physiological basis sufficient differences have probably not been demonstrated, as yet, to warrant the assumption that strains of *Bacillus amylovorus* occur. Apparently individuals have been dealt with. This species might be considered exceptionally constant when the widespread sources of the cultures are considered.

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