grew in sea-water medium but not in freshwater medium, revealed that after two to five years' maintenance in sea-water media virtually all of them would grow in freshwater medium.

At first this seems contrary to the observations of Kluyver and Baars[4] that bacteria become less adaptable to adverse environmental conditions during prolonged laboratory cultivation. Moreover, it is contrary to the accepted beliefs concerning the adaptability of organisms in general. However, there are several factors which must be taken into account in the interpretation of this apparent paradox. According to Rahn[5] it is established that physiologically young bacteria are more sensitive to adverse conditions than old ones. The frequent transfer of the cultures during the attempts to acclimatize them to hypotonic solutions tends to keep them physiologically young, while the stock cultures becomes senescent.

Data on the salt tolerance of bacteria from different strata of mud cores collected from the deep sea bottom lend credence to this "tolerance with senescence" theory. The majority of the bacteria found in the topmost few millimeters of sedimentary material where conditions favor their multiplication are typical marine species which grow in sea-water but not in freshwater media upon initial isolation.[6] However, in the deeper strata where the aerobic bacteria have probably been buried in a state of suspended animation for a long time lacking both oxygen and food for their multiplication, the majority of them grow equally well on sea-water and freshwater media. Similarly, the senescent bacteria from these deeper mud strata will grow over a wider temperature range than the physiologically young psychrophilic bacteria found in the topmost layers of mud.

It has been suggested that these bacteria require for growth certain allelocatalysts or accessory growth substances, which are present in sea water but not in fresh water. In an old culture, these substances may accumulate to such an extent that an inoculum therefrom may carry into a new culture an amount sufficient to promote growth, even though the new medium fails to contain the necessary substances. This, perhaps, is the reason for the greater tolerance of these senescent cultures to fresh water. This possibility, as well as others, is being investigated in order to account for the phenomena reported above.

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[4] A. J. Kluyver and J. K. Baars, *Proc. Koninklijke van Wetenschappen te Amsterdam*, 35: 1, 1932.

[5] O. Rahn, "Physiology of Bacteria," P. Blakiston's Son, Philadelphia, 1932.

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THE PROTEIN CONTENT OF MOSAIC TOBACCO[1]

THE concentration of virus protein in tobacco infected with mosaic and the proportion of virus protein to normal proteins are data necessary to an adequate understanding of the synthesis of virus protein. According to Bawden and Pirie,[2] mosaic plants may contain five to ten times as much protein as normal tobacco, and according to Stanley,[3, 4] as much as 80 per cent. of the total protein of diseased tissue may be virus protein. Either an enormous accumulation of virus protein or an almost complete change of normal into virus protein has to be assumed. Such estimates are based, however, on the yield of protein obtained by extraction, and, especially with healthy plants, the extractable protein accounts for only a small fraction of the total nitrogen. On the other hand, Stanley is less concerned with the comparison of healthy and diseased tissues than with the examination of the diseased tissues as such. However, in spite of an increase in extractable protein in virus-bearing plants, a comparison of diseased and control plants made from the recent data of Stanley[3] appear to show no significant increase in total nitrogen due to the disease. With some strains of mosaic a decrease has occurred. This follows from the published data when the residual nitrogen in the press cakes is taken into account. For instance, in plants infected with common mosaic, the total nitrogen was 7.25 mg/g, while control plants contained 6.90 mg/g. Plants infected with aucuba mosaic contained 2.92 mg/g, as compared with the same control, thus indicating a substantial decrease.

The extraction of the protein, on the other hand, may have been incomplete. The quantities obtained from healthy plants are barely a tenth of the protein content of tobacco reported by Vickery and Pucher[5] from an extensive series of analyses, also on healthy plants. The highest yield recorded by Stanley shows 22 per cent. of the total nitrogen as protein in diseased plants, while the lowest yield was only 5 per cent. in control plants.

By applying the observation[2, 6] that virus protein is not attacked by crude trypsin, although normal tobacco proteins are hydrolyzed, it has been possible to develop a method of distinguishing between trypsin-resistant virus protein and normal proteins without resorting to extraction of the tissue.

[1] Food Research Division Contribution No. 362.

[2] F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc.*, B 123: 274, 1937.

[3] W. M. Stanley, *Phytopath.*, 27: 1152, 1937. (Particularly page 1152 together with page 1155.)

[4] W. M. Stanley, *Jour. Biol. Chem.*, 121: 205, 1937.

[5] H. B. Vickery and G. W. Pucher, *Conn. Agr. Exp. Sta. Bull.* No. 324, 1931.

[6] L. F. Martin, H. H. McKinney and L. W. Boyle, SCIENCE, 86: 2234, 380–381, October 22, 1937.

In general, the method consists of determining total nitrogen and nitrogen soluble in 10 per cent. trichloracetic acid, the latter before and after digestion with commercial trypsin.

When corrected for the soluble nitrogen of the trypsin employed, the results are independent of the amount of enzyme used, although this should be fairly large in any case. The method permits the quantitative determination of crystalline common mosaic virus added to normal tobacco tissue.

Only a small amount of nitrogen is removed by water from healthy tobacco tissue; but about two thirds of the total nitrogen of the plant becomes soluble in trichloracetic acid solution after tryptic digestion. It seems probable that the material attacked by trypsin represents protein or protein-bound nitrogen. The amount of nitrogen rendered soluble in trichloracetic acid by trypsin acting on healthy tobacco tissue also corresponds well to the protein content of such tissue as found by Vickery and Pucher.[5] About 20 per cent. of the nitrogen of the normal plant is not rendered soluble by trypsin, however. We are not yet certain as to the character of the residual nitrogen, but in the diseased plant this amount is increased by the quantity of virus protein present. It is apparent that substances other than virus protein may be included in the trypsin-resistant fraction, but all the virus protein is included. The results obtained therefore represent an upper limit for the quantity of virus protein present.

Results were obtained by this method on three varieties of tobacco and three mosaics, as shown in Table I. These combinations were selected because of differences in the severity with which the mosaics attack the varieties in question. Wisconsin-Havana tobacco (No. 1, 6) with common mosaic developed a severe light green mottling, with yellow mosaic (No. 5) a severe yellow mottling, and with mild mosaic a mild light green mottling (No. 3). Common mosaic on the other hand did not induce symptoms in Ambalema tobacco (No. 2), although the juice was very infectious for more susceptible varieties. Tobacco 448A (No. 4) when inoculated with common mosaic virus developed no symptoms. The virus increased in the young plants, but at a later stage there was no detectable virus in the leaves used for protein determinations.

The following conclusions are drawn from the data in Table I:

TABLE I

No.	Tobacco variety	Mosaic	–A– Total N mg./g.	–B– Soluble N mg./g.	–C– Protein N and undig. N mg./g.	–D– Digestible protein N mg./g.	–E– Undigestible N mg./g.	–F– Virus-Protein N mg./g.	–G– Proportion of virus to total protein per cent.
1	Wis.-Hav.	Common	5.58	0.37	5.21	2.74	2.47	1.10	29
1c	Wis.-Hav.*	.....	5.53	0.62	4.91	3.54	1.37	...	..
2	Ambalema	Common	5.65	0.86	4.79	3.64	1.15	0.32	8
2c	Ambalema*	.....	5.65	0.99	4.66	3.83	0.83	...	..
3	Wis.-Hav.	Mild	6.42	0.86	5.56	3.78	1.78	0.18	5
3c	Wis.-Hav.*	.....	6.72	0.71	6.01	4.41	1.60	...	..
4	448A	Common	5.84	0.81	5.03	3.86	1.17	– 0.08	0
4c	448A*	.....	6.64	0.77	5.87	4.62	1.25	...	..
5	Wis.-Hav.	Yellow	5.83	0.88	4.95	3.52	1.43	– 0.06	0
6	Wis.-Hav.	Common	5.01	0.48	4.53	2.66	1.87	0.38	12
5–6c	Wis.-Hav.*	.....	5.06	0.55	4.51	3.02	1.49	...	..

* Healthy controls.

Seed and plant material of Ambalema and 448A supplied by E. E. Clayton.

(1) The total nitrogen of the plants (Column A) was found to be very little changed from the normal, irrespective of the severity of the disease.

(2) The total protein (Column C) seems also to have undergone little if any change but the accuracy of the results is probably not high enough to demonstrate small variations, since they are calculated by difference. This suggests that the virus protein is produced at the expense of the normal protein, though not necessarily directly from it.

(3) In the case of the common mosaic the trypsin-resistant protein (Column F), regarded by us as virus protein, exists in smaller proportion than has been supposed previously. The amount of resistant protein was found to be greater in a susceptible variety of tobacco than in those generally considered less vulnerable. We have no proof at present, however, that the yellow mosaic virus is resistant to trypsin.

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