Microbiological Assay with *Lactobacillus arabinosus* after Storage for Three Years Lyophilized and in Conservation Medium.* (17594)

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Nymon *et al.*(1) have reported that the response of *Lactobacillus casei* and *Lactobacillus arabinosus* to riboflavin and niacin is essentially unchanged by the lyophile process and by storage for 3 months in the dehydrated condition. Other workers (Riesen *et al.*)(2) have shown that lyophilized cultures of lactic acid bacteria remain viable during a year of storage. Although it has been reported in the *Difco Manual*(3) that lactobacilli may be kept for 6 months in a cooked meat medium, it does not appear that maintenance of lactic acid bacteria in this or other conservation media has been employed with stock cultures for microbiological assays. Since it may be advantageous in some laboratories to maintain assay organisms by one of these methods rather than by frequent subculture, it seemed desirable to determine whether such maintenance might influence the performance of bacteria in microbiological assays. In the present study cultures of *Lactobacillus arabinosus* stored approximately 3 years lyophilized and in conservation medium have been employed to determine leucine in casein, gelatin, silk fibroin, and an amino acid mixture.

Experimental. The stock culture of *Lactobacillus arabinosus* was carried as described by Dunn *et al.*(4) One transplant from the stock culture was lyophilized essentially according to the procedure of Nymon *et al.*(1) and stored at room temperature (averages, 23°C) for 32 months. A second transplant was incubated 24 hours at 35° in Bacto-cooked meat medium. The culture tube was tightly closed with a screw cap and allowed to stand at room temperature for 38 months. After the stipulated storage periods the two cultures were transferred by stab to Bacto-tomato juice agar (Difco product),† and were stored in the refrigerator (4°) a few days before use. In preliminary experiments inocula were prepared by transferring directly from the stab cultures to the inoculum medium (Dunn *et al.*)(4) In the other experiments all 3 cultures were carried through 10 consecutive daily subcultures in the inoculum medium before use. For convenience the stock culture, lyophilized culture and cooked meat medium culture have been designated as cultures S, L, and CM, respectively. The assay methods for leucine and valine were those of Dunn *et al.*(4) The results are given in Fig. 1 and 2 and in Tables I and II.

Discussion. Leucine and valine standard curves obtained with the 3 cultures in a preliminary experiment are shown in Fig. 1 and 2. It may be seen that the curves were nearly identical with cultures S and L, but were considerably lower with culture CM, indicating that storage in the cooked meat medium may have reduced the vitality of this culture. Since the work of Nymon and Gortner(5) suggested that such a condition might be alle-

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1. Nymon, M. C., Gunsalus, I. C., and Gortner, W. A., *Science*, 1945, v102, 125.

2. Riesen, W. H., Spengler, H. H., Robblee, A. R., Hankes, L. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, v171, 731.

3. *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures*, Difco Laboratories, Inc. Detroit, eighth edition, 1948, pp. 64-65.

4. Dunn, M. S., Camien, M. N., Malin, R. B., Murphy, E. A., and Reiner, P. J., *Univ. Calif. Publ. Physiol.*, 1949, v8, 293.

† The lyophilized culture was rehydrated with the addition of 2 ml of inoculum medium (Dunn *et al.*(4) and incubated 24 hours at 35° before transferring to the agar.

5. Nymon, M. C., and Gortner, W. A., *J. Biol. Chem.*, 1946, v163, 277.

TABLE I.
Response of Three Cultures of *L. arabinosus* to Leucine in Casein Hydrolysate.*

Hydrolysed casein,† γ/tube	Ml 0.09 *N* NaOH to titrate contents of triplicate tubes‡			% leucine found†		
	Culture CM	Culture S	Culture L	Culture CM	Culture S	Culture L
74.6	4.00	5.29	5.39	9.54	9.22	9.33
149.2	6.33	8.45	8.57	9.15	9.17	9.06
223.8	8.41	11.02	11.19	9.31	9.06	9.35
298.4	10.19	13.27	13.27	9.31	9.31	9.52
373.0	11.80	14.62	14.57	9.25	9.62	9.57
			Avg	9.31	9.28	9.37

* Culture CM was stored 38 months at room temperature in cooked meat medium, culture S was the stock culture control, and culture L was stored 32 months at room temperature in the lyophilized condition.

† Values calculated for the moisture and ash-free product.

‡ Titration procedure described by Dunn *et al.*(4)

TABLE II.
Leucine Found in Proteins and Amino Acid Mixture by Microbiological Assay with Three Cultures of *L. arabinosus.**

Sample	Leucine found or recovered†					
	Culture CM		Culture S		Culture L	
	%	MDM‡	%	MDM‡	%	MDM‡
Casein	9.3	.09	9.3	.15	9.4	.14
Silk fibroin	.79	.007	.81	.013	.83	.019
Gelatin	3.1	.02	3.2	.02	3.2	.06
Amino acid mixture§	97	.7	101	1.5	104	1.6

* Culture CM was stored 38 months at room temperature in cooked meat medium, culture S was the stock culture control, and culture L was stored 32 months at room temperature in the lyophilized condition.

† Values calculated for protein on moisture and ash-free basis.

‡ Mean deviation from the mean of values obtained at 5 dosage levels.

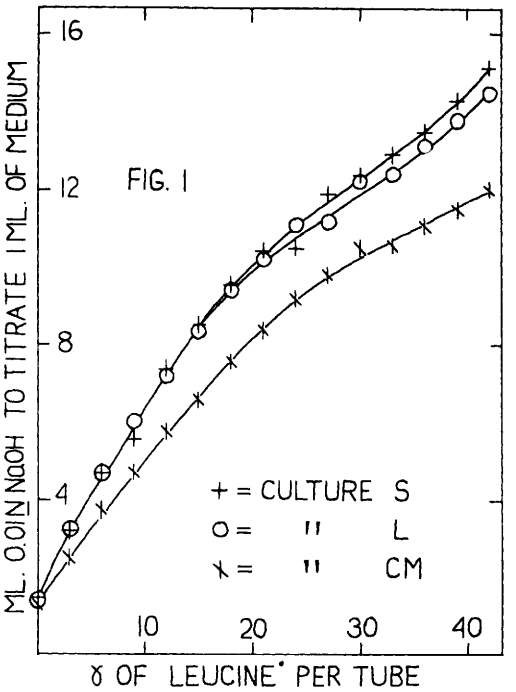
§ 4.85% L-leucine. DL-Alanine, L-aspartic acid, L-arginine • HCl, L-cystine, L-glutamic acid, glycine, L-histidine • HCl • H_2O, DL-isoleucine, L-lysine • HCl, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tyrosine, and DL-valine were present in proportions similar to those found in animal proteins (Dunn *et al.*) (4)

viated by a series of frequent subcultures in a nutrient-rich medium, all three cultures were carried through 10 consecutive daily transfers in the inoculum medium of Dunn *et al.*(4) No change was noted in any of the cultures, however, and the final turbidity attained in the liquid medium by culture CM was only about two-thirds that produced by cultures S and L throughout the series of transfers.

Nymon *et al.*(1,5) have implied that the quality of microbiological assays may be predicted from the appearance of the standard assay curves. According to this view it might be anticipated that microbiological assays with culture CM would be of relatively poor quality. The assay values given in Tables I and II failed to support this hypothesis, however. The agreement of results with the 3 cultures was well within anticipated experimental error, although acid production was considerably lower with culture CM (Tables I and II). The percentages found or recovered in assays with cultures CM, S, and L, respectively, were as follows: casein, 9.3, 9.3, 9.4; silk fibroin, 0.79, 0.81, 0.83; gelatin, 3.1, 3.2, 3.2; and amino acid mixture,‡ 97, 101, 104. It may be noted that these values are in close agreement with each other and with the values obtained previously for the same protein samples with *Lactobacillus arabinosus* and a different assay medium (Camien and

Dunn).(6)

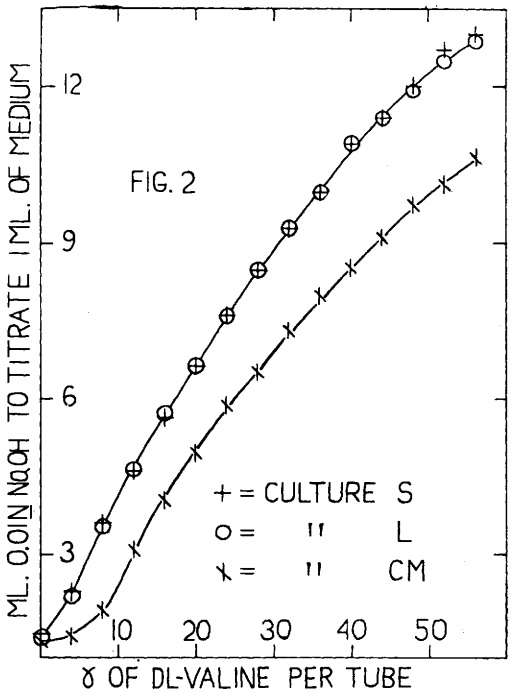
It appears, therefore, that the slope of the assay curve and the quantity of acid produced by the test microorganism may be relatively unimportant provided that nearly optimal assay conditions and medium are employed. This is contrary to the view of Nymon and Gortner(5). It may be concluded that *Lactobacillus arabinosus* and perhaps other lactic acid bacteria can be stored successfully for relatively long periods lyophilized or in cooked meat medium. The lyophile process



Response of 3 *Lactobacillus arabinosus* cultures to L-leucine. Culture CM was stored 38 months at room temperature in cooked meat medium, culture S was the stock culture control, and culture L was stored 32 months at room temperature in the lyophilized condition. The assay method was that of Dunn *et al.*(4)

‡ 4.85% L-leucine. DL-alanine, L-aspartic acid, L-arginine • HCl, L-cystine, L-glutamic acid, glycine, L-histidine • HCl • H_2O, DL-isoleucine, L-lysine • HCl, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tyrosine, and DL-valine were present in proportions similar to those employed previously (Dunn *et al.*)(4)

6. Camien, M. N., and Dunn, M. S., *J. Biol. Chem.*, 1948, v173, 137.



Response of 3 *Lactobacillus arabinosus* cultures to DL-valine. Culture CM was stored 38 months at room temperature in cooked meat medium, culture S was the stock culture control, and culture L was stored 32 months at room temperature in the lyophilized condition. The assay method was that of Dunn *et al.*(4)

may be preferable to the conservation medium method since the former procedure apparently does not affect the acid producing ability of the organism.

Summary. Cultures of *Lactobacillus arabinous* have been stored at room temperature for 32 months lyophilized and for 38 months in Bacto-cooked meat medium (Difco product). The assay performance of the cultures was not impaired by this treatment, although acid production in the cooked meat medium culture was reduced. It was concluded that these methods of storage may be substituted successfully for frequent subculture with *Lactobacillus arabinosus* and perhaps other lactic acid bacteria.

7. Dunn, M. S., and McClure, L. E., *J. Biol. Chem.*, 1950, in press.

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