Variation in the Acetic Acid-Lactic Acid Ratio Among the Lactic Acid Bacteria[1]

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The variability of lactic acid bacteria has been noted on numerous occasions. Pederson and Albury (1955) reported that approximately 10 per cent and sometimes as many as 50 per cent of the strains isolated from vegetable fermentations were not readily identified as strains of the lactic acid bacteria usually associated with such fermentations.

Considerable variation in the ratio of acetic to lactic acid has been observed in studies of commercial vegetable fermentations. The variation in ratio becomes apparent when the end products of fermentation from the several species of lactic acid bacteria are compared. In general, the higher the total acid produced, the lower will be the ratio of acetic to lactic acid. Since the end products of fermentation are responsible for quality characteristics of certain fermented products, a study of ratio variations seemed desirable. By using normally high acid producing strains of the different species and varying the sugar concentration in the various media, differences between high and low acid strains may be simulated.

It has been observed that the ratio of end products is influenced by the extent of utilization of sugars for growth and fermentation. In this study, attention is given to the effect of sugar concentration upon ratios of acetic to lactic acid.

Materials and Methods

A standard basic medium containing 0.5 per cent tryptone, 0.25 per cent yeast extract and 0.5 per cent each of A and B salts was used in these studies. Salt A has the following composition: K_2HPO_4, 100 g; KH_2PO_4, 100 g; distilled water, 1000 ml. Salt B has the following composition: $MgSO_4 \cdot 7H_2O$, 40 g; NaCl, 2 g; $FeSO_4 \cdot 7H_2O$, 2 g; $MnSO_4 \cdot 4H_2O$, 2 g; distilled water, 1000 ml. Glucose, fructose, and sucrose were used in the various media in concentrations ranging from 20 mg to 2000 mg per cent. Twenty-five ml of the broths were pipetted into one arm of the Eldredge tubes, which were plugged and sterilized. Since each arm of the Eldredge tube will hold about 50 ml of liquid, some oxygen was available to the organisms. The broths were inoculated with the various cultures; standardized $Ba(OH)_2$ solution was placed in the other arm of the tubes; and the tubes were then sealed and incubated at 32 C for a long enough period to insure complete fermentation. The amounts of sugars in the higher concentration sugar media were greater than the amounts that could be utilized by the organisms. Cultures used in the study include the two heterofermentative species *Leuconostoc mesenteroides* strain C33 and *Lactobacillus brevis* strains B155, L15, and L35, and the two homofermentative species *Pediococcus cerevisiae* strain E66 and *Lactobacillus plantarum* strain B 246.

After incubation, the arm of each Eldredge tube containing the $Ba(OH)_2$ was titrated rapidly with standard H_2SO_4, and, from these titrations, carbon dioxide production was calculated. The lactic and acetic acids were determined after separation by the column chromatographic technique of Marvel and Rands (1950) as adapted by Bulen *et al.* (1952). Uninoculated media were extracted and titrated in a similar manner as controls.

Results and Discussion

The homofermentative lactic acid bacteria produced small amounts of carbon dioxide and acetic acid in glucose, fructose, and sucrose regardless of the sugar concentration in the medium (table 1). The amount of lactic acid produced increased with increasing sugar concentrations. The ratio of acetic to lactic acid, therefore, decreased accordingly. In the higher concentrations of sugar, lactic acid accounted for from 90 to 95 per cent of the end products recovered. The amounts were slightly higher with the higher acid producing *Lactobacillus plantarum*. It may be noted that in the lower concentration sugar media the total carbon recovery as carbon dioxide and acids is slightly greater than the total carbon present in the sugar. This is most marked in the fructose media. These data suggest that part of the carbon recovered may have been derived from metabolism of the tryptone and yeast extract.

The heterofermentative lactic acid bacteria present a contrast, not only between two species, but also between these and the homofermentative species. *Lactobacillus brevis* strains B155, L15, and L35 produced increasing amounts of carbon dioxide and acetic acid, as well as lactic acid, with increasing sugar con-

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centrations (table 2). The lactic acid increased more rapidly than the carbon dioxide and acetic acid, except with the highest concentrations of fructose and sucrose, so that a general decrease in ratio still occurred. A

TABLE 1

Ratios of acetic to lactic acid obtained from increasing sugar concentration by Pediococcus cerevisiae and Lactobacillus plantarum

Sugar in Medium	Carbon Dioxide	Acetic Acid	Lactic Acid	Acetic/Lactic Acid
	Mg per cent carbon			
Pediococcus cerevisiae in Glucose				
20	8	14	9	1.55
40	8	9	25	0.36
80	14	17	51	0.33
160	14	17	127	0.13
320	11	17	249	0.07
400	12	15	288	0.05
Pediococcus cerevisiae in Fructose				
20	33	1	10	0.10
40	11	11	17	0.59
80	19	20	41	0.49
240	9	15	243	0.06
320	9	7	307	0.02
400	10	21	389	0.05
2000	11	29	351	0.08
Pediococcus cerevisiae in Sucrose				
21	6	8	12	0.67
42	8	8	48	0.16
168	11	23	143	0.16
252	6	13	228	0.06
421	9	16	292	0.06
2105	9	12	340	0.04
Lactobacillus plantarum in Glucose				
20	6	15	20	0.75
40	13	26	24	1.08
80	15	14	21	0.67
160	14	11	70	0.16
240	12	19	163	0.12
320	9	15	315	0.05
400	9	9	382	0.02
600	11	17	516	0.03
Lactobacillus plantarum in Fructose				
20	10	10	18	0.55
40	9	20	30	0.67
80	15	22	58	0.38
160	11	21	139	0.15
240	11	23	233	0.09
320	11	20	315	0.06
400	10	16	316	0.05
2000	13	23	620	0.04
Lactobacillus plantarum in Sucrose				
21	7	12	16	0.75
42	11	28	24	1.17
84	10	21	57	0.37
252	10	12	235	0.05
337	8	14	311	0.04
421	9	13	389	0.03
2105	9	16	588	0.03

TABLE 2

Ratios of acetic to lactic acid obtained in increasing sugar concentrations by Leuconostoc mesenteroides and Lactobacillus brevis

Sugar in Medium	Carbon Dioxide	Acetic Acid	Lactic Acid	Acetic/Lactic Acid
	Mg per cent carbon			
Leuconostoc mesenteroides in Glucose				
40	2	13	28	0.47
80	15	11	48	0.23
160	27	21	94	0.22
240	37	19	120	0.16
320	35	23	131	0.17
400	38	17	116	0.15
600	51	6	219	0.03
Leuconostoc mesenteroides in Fructose				
40	9	8	25	0.32
80	9	22	33	0.67
320	28	47	109	0.43
400	30	56	114	0.49
2000	40	85	124	0.68
Leuconostoc mesenteroides in Sucrose				
84	14	14	34	0.41
168	22	19	55	0.34
421	34	31	99	0.31
2105	40	80	128	0.62
Lactobacillus brevis B155 in Glucose				
20	9	20	9	2.22
40	17	31	9	3.44
40	19	31	8	3.87
80	32	54	11	4.90
160	42	60	54	1.11
240	—	73	91	0.80
320	—	65	136	0.48
400	—	49	186	0.26
600	—	36	296	0.12
600	71	39	294	0.12
Lactobacillus brevis B155 in Fructose				
20	41	4	6	0.67
40	45	14	8	1.75
80	28	42	9	4.67
160	38	53	44	1.20
240	41	68	85	0.80
320	46	70	114	0.61
400	50	69	112	0.61
2000	68	212	302	0.70
Lactobacillus brevis L15 in Fructose				
40	16	21	23	0.91
80	29	42	17	2.47
160	36	48	71	0.69
240	39	53	131	0.40
320	37	43	201	0.21
400	45	53	245	0.22
2000	68	210	333	0.63
Lactobacillus brevis L15 in Sucrose				
21	5	3	27	0.11
84	16	13	47	0.27
168	25	28	93	0.30
337	15	37	151	0.24
421	9	49	158	0.31
2105	41	192	460	0.41

TABLE 2—*Continued*

Sugar in Medium	Carbon Dioxide	Acetic Acid	Lactic Acid	Acetic/Lactic Acid
	Mg per cent carbon			
	Lactobacillus brevis L35 in Fructose			
20	35	2	27	0.07
40	20	27	6	4.50
80	29	33	13	2.53
160	36	42	59	0.71
240	43	35	106	0.33
320	46	51	178	0.28
400	45	58	222	0.26
2000	64	216	297	0.73
	Lactobacillus brevis L35 in Sucrose			
21	6	4	28	0.14
42	15	20	28	0.71
84	17	22	54	0.41
168	18	25	99	0.25
252	18	23	142	0.16
337	18	22	191	0.11
421	18	28	336	0.08
2105	51	105	468	0.22

trace of formic acid was always obtained in every fermentation regardless of the organism or sugar concentration.

Leuconostoc mesenteroides did not show a marked change in ratio. In glucose, the ratio varied from only 0.03 to 0.47. Because of the variable behavior of this organism, it has become the subject of many separate studies. DeMoss *et al.* (1951) found that aldolase and isomerase did not seem to be present in this organism. Gunsalus and Gibbs (1952) stated that the Leuconostoc species fermentation departs from the classical Meyerhof-Embden glycolytic pathway, and suggested a monophosphate shunt pathway, based on experience with labeled C^{14} glucose. Studies by Johnson and McCleskey (1957) on the aerobic carbohydrate metabolism of *Leuconostoc mesenteroides* establish marked variability between strains of this organism.

The highest ratio of acetic to lactic acid, 0.68 in the highest concentration of fructose, seems related to mannitol production. In each case in which fructose or sucrose is fermented, a higher ratio was obtained in the 2000 mg per cent carbon (5 per cent sugar) than in the 400 mg per cent carbon (1 per cent sugar) concentration. This seemed illogical at first. But, mannitol is produced from fructose and sucrose, and is not fermented further as long as sufficient sugar is available. Therefore, since mannitol is a reduced product, one would expect a proportionate swing from the reduced product, ethyl alcohol, to the oxidized product, acetic acid.

Lactic acid recovered in homofermentations practically equalled the sugar added. Carbon recovery in the heterofermentations exceeded slightly the total sugar available only in the lower sugar concentration. Ethyl alcohol, a major end product, was not determined. If it had been determined, carbon recovery may have been greater. But since these fermentations were conducted in the presence of small amounts of air, as in many commercial or pure culture test tube fermentations, little ethyl alcohol would be expected in at least the lower sugar concentration. These fermentations were repeated in 240 mg per cent glucose media with alkaline pyrogallol in an arm of the Eldredge tube to reduce the oxygen content. Acetic acid was not produced under these anaerobic conditions either in the heterofermentations in which lactic acid produced amounted to 136, 168, and 168 mg carbon or in homofermentations in which lactic acid amounts of 252, 324, and 332 mg carbon were obtained.

SUMMARY

The amount of acetic acid and carbon dioxide produced by the homofermentative lactic acid bacteria is more or less constant regardless of sugar concentration in the medium. The amounts produced by the heterofermentative species increases as the sugar concentration increases. Therefore, the ratio of acetic acid to lactic acid varies with the amount of sugar fermented. Higher ratios are obtained with the heterofermenters than with the homofermenters. Carbon recovery exceeded the total carbon in the sugar added in the lower sugar concentration media. This indicates that some of these products arise from amino acid metabolism. The high ratios of acetic to lactic acid were obtained in the highest concentrations of fructose and sucrose media. Mannitol produced in high concentration fructose media by *Leuconostoc mesenteroides* is not readily metabolized. Thus, in order to retain a normal oxidation reduction balance, acetic acid rather than alcohol would be produced in larger amounts.

These differences in ratios of acetic to lactic acid account for differences between strains and quality differences often encountered in certain industrial fermentations.

REFERENCES

BULEN, W. A., VARNER, F. E., AND BURRELL, R. C. 1952 Separation of organic acids from plant tissues. Anal. Chem., **24,** 187–190.

DEMOSS, R. D., BARD, R. C., AND GUNSALUS, I. C. 1951 The mechanism of heterolactic fermentation: A new route of ethanal formation. J. Bacteriol., **62,** 499–511.

GUNSALUS, I. C. AND GIBBS, M. 1952 The heterolactic fermentation. II. Position of C^{14} in the products of glucose dissimilation by *Leuconostoc mesenteroides*. J. Biol. Chem., **194,** 871–875.

JOHNSON, M. K. AND MCCLESKEY, C. S. 1957 Studies on the aerobic carbohydrate metabolism of *Leuconostoc mesenteroides*. J. Bacteriol., **74,** 22–25.

MARVEL, C. S. AND RANDS, R. D. 1950 Separation of organic acids. J. Am. Chem. Soc., **72,** 2642–2646.

PEDERSON, C. S. AND ALBURY, M. N. 1955 Variation among the heterofermentative lactic acid bacteria. J. Bacteriol., **70,** 702–708.