STUDIES ON THE PENTOSE METABOLISM BY MICROORGANISMS

1. A NEW TYPE-LACTIC ACID FERMENTATION OF PENTOSE BY LACTIC ACID BACTERIA

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The fermentation of various pentoses by lactic acid bacteria was first described by Fred, Peterson and Anderson⁽¹⁾, and the following formula was proposed for the reaction:

This formula has, heretofore, been admitted by many investigators. According to the formula equimolar quantities of lactic and acetic acids are formed from pentose, namely, the theoretical yield of lactic acid is 60% by weight of the consumed pentose. By an isotopic experiment using *Lactobacillus* sp.^{(2), (3), (4)} it has been established that the methyl and carboxyl groups of acetate arise from C-1 and C-2, while -COOH, -CHOH and -CH₃ groups of lactate are derived from C-3, C-4 and C-5 of pentose molecule respectively.

Fig. 1. Pathway of Pentose Fermentation by *Lactobacillus pentosus*.

Recently, the pathway of pentose fermentation by *L. pentosus* has been summarized by Horecker and his co-workers⁽⁵⁾ as shown in Fig. 1. In this

scheme each pentose phosphate ester is converted to D-xylulose-5-phosphate which is then cleaved by phosphoketolase into glyceraldehyde-3-phosphate and acetyl-phosphate. These resulting compounds are further converted to lactate and acetate respectively. By a comparative study on the fermentation of pentoses, however, we have observed the following two cases⁽⁶⁾: 1) some strains of lactic acid organisms metabolize various pentoses according to formula (I), and 2) the others produce lactic acid in a yield of more than 60% of consumed pentoses with a small amount of formate, but no detectable amount of acetic acid. For example, *Lactobacillus arabinosus* readily ferments L-arabinose and produces equimolar quantities of lactic and acetic acids as expected from formula (I), while on the other hand *Streptococcus faecalis* produces 1.45 moles of lactate and 0.23 mole of formate per mol. of L-arabinose without any detectable amount of acetate⁽⁶⁾. Furthermore, the cells of *S. faecalis* grown in a medium containing L-arabinose is able to produce lactic acid in a yield of 95% of the consumed L-arabinose (1.58 moles of lactate per mole of L-arabinose consumed), while volatile acids are not detectable. This finding gives a clear evidence that *S. faecalis* might involve a new pathway for pentose fermentation. We have much interests in those phenomena mentioned above, especially in the new pathway of pentose fermentation.

In this paper, it will be discussed that this new type of pentose fermentation may be formulated as follows:

METHODS

Strains and medium—Lactic acid bacteria used in the present experiments have been maintained as stab-culture on a nutrient bouillon-malt extract-CaCO₃ agar by transferring at 3 month-interval in our laboratory. The medium for seed culture and fermentation has the following composition: peptone 1 g; fish meat extract 1 g; NaCl 0.5 g; Difco's yeast extract 0.2 g; pentose 2-3 g; CaCO₃ 3 g; and tap water 100 ml. Sterilization was carried out as mildly as possible under a pressure of 10 lbs. for 1 min. in an autoclave in order to minimize the possible decomposition of pentoses into formate at a high temperature. Seed cultures were prepared by inoculating organisms from stock cultures and incubating them at 30° or 45° for 24 hours. The fermentations were generally carried out for 5 days at 30° after inoculating 5 ml of seed culture of each strain into 200 ml fermentation medium, except for *Lactobacillus thermophilus* which is cultured at 45°C.

Determination of Volatile Acids⁽⁷⁾—The cultures were centrifuged at 4,000 G for 10 minutes then the supernatant was acidified with sulfuric acid. Volatile acids were separated from the acidified broth by steam-distillation and identified paper-chromatographically. The quantitative determination was carried out by the fraction distillation method⁽⁸⁾.

Determination of Lactic Acid—After removal of volatile acids, the acidified broth was extracted with ether for 24 hours. Acids in the ether extract were estimated as lactic acid by two methods, i.e., alkali titration and colorimetry⁽⁹⁾. In fact, the nonvolatile acid contained in the ether extract was identified exclusively to be lactic acid by paperchromatography.

Determination of sugars—For the determination of pentoses the Bertrand method was applied, and for the identification and determination of ketoses the cystein-carbazol reaction⁽¹⁰⁾, FeCl_3 -orcinol reaction⁽¹¹⁾, and paperchromatography⁽¹²⁾ were employed.

Preparation of the cells for small-scale fermentation—The cells were prepared as follows: *S. faecalis* was grown in a medium containing L-arabinose and *L. thermophilus* T₁ in D-xylose medium. After 20 hours culture, the cells were harvested by centrifugation and washed three times with distilled water. About 2 g of wet cells of both organisms were obtained from each one liter of the culture broth.

Measurement of radioactivity—Radioactivities of all products were determined after conversion to salts or barium carbonate.

RESULTS

1) *Fermentation of pentoses by various strains of lactic acid bacteria*

From the results of fermentation shown in Table 1, types of pentose fermentation by lactic organisms were divided into following two types: Type 1) which produced equimolar lactic acid and acetic acid from pentose according to formula (I) and Type 2) which produced more than one mole of lactate and small amount of formate but no acetate from one mole of the substrate. The latter type of fermentation may be formulated as follows:

In these experiments it was observed that some strains were able to metabolize pentoses according to formula (I) and (III). For example, *Lactobacillus thermophilus* II was able to ferment L-arabinose as formula (I) and D-xylose as formula (III). These phenomena suggest the fact that the pentose metabolizing systems of lactic organisms are sometimes altered by natures of pentose used in the culture media. Further, as shown in Table 2, *L. xylosus* was able to ferment D-xylose and D-ribose through different metabolic systems. On the other hand, *L. thermophilus* T₁ metabolized various pentoses according only to formula (III) and *Leuconostoc mesenteroides* B07 followed exclusively to formula (I).

2) *Effect of carbonate on the pentose metabolism by S. faecalis and L. thermophilus T₁*

When a CaCO_3 -containing medium is employed, the broth may be maintained nearly saturated conditions with CO_2 and HCO_3^- throughout the fer-

Table 1. Fermentation of Pentoses by Lactic Acid Organisms.

a) Fermentation of L-arabinose

Strain	Initial sugar (g)	Consumed sugar (g)	Lactic acid (g)	Acetic acid (g)	Formic acid (g)	Yield of lactic acid (%)
<i>S. faecalis</i>	3.75	3.60	3.11	0	0.19	86.4
<i>S. glycerinaceus</i>	3.75	3.75	3.11	0	0.24	82.9
<i>L. arabinosus</i>	3.75	3.75	1.98	1.56	0	52.8
<i>L. thermophilus</i> T ₁	2.45	2.20	1.55	0	0.24	70.4
<i>L. thermophilus</i> II	2.80	1.93	1.18	0.52	0	61.2
<i>Leuc. mesent.</i> B07	3.75	3.20	1.76	1.33	0	55.0
<i>L. brevis</i>	3.75	2.35	1.13	1.04	0	48.0
<i>Pc. hennebergi</i> *	2.05	1.78	0.95	0.41	0	53.3
<i>Pc. lindneri</i> *	2.05	1.40	0.71	0.60	0	50.7

* 100 ml culture.

b) Fermentation of D-xylose

<i>S. lactis</i>	3.30	3.30	2.01	1.02	0	60.9
<i>L. xylosus</i>	4.12	3.06	1.63	1.10	0	53.2
<i>L. thermophilus</i> T ₁	3.24	2.85	2.34	0	0.29	82.1
<i>L. thermophilus</i> II	2.45	1.45	0.98	0	0.41	67.5
<i>Leuc. mesent.</i> B07*	14.2	8.5	4.52	2.88	0	53.1
<i>L. fermentum</i> H60	2.80	2.65	1.35	1.47	0	50.9
<i>Pc. hennebergi</i>	2.10	1.99	0.95	0.41	0	47.8
<i>Pc. lindneri</i>	2.10	1.72	0.84	0.80	0	48.9

* 700 ml culture.

c) Fermentation of D-ribose

<i>S. faecalis</i>	0.93	0.83	0.68	0	0.04	81.9
<i>S. lactis</i>	0.50	0.50	0.32	0	0.07	64.0
<i>L. xylosus</i>	0.80	0.67	0.50	0.03	0.07	74.6
<i>L. arabinosus</i>	0.50	0.50	0.22	0.13	0	44.0
<i>L. thermophilus</i> T ₁	0.93	0.93	0.66	0	0.04	70.9

50 ml culture.

mentation, because they are liberated from carbonate by organic acids formation from sugars. Therefore, two attempts were made in order to determine whether the carbonate fixation reaction was involved or not in this fermentation, namely 1) comparison of fermentations in the presence and absence of carbonate, and 2) investigation of the incorporation of C¹⁴-carbon into lactate by using NaHC¹⁴O₃.

Table 2. Pentose Fermentation Formula by Lactic Acid Organisms.

Strain	Substrate	L-Arabinose	D-Xylose	D-Ribose	D-Glucose
<i>S. faecalis</i>		III	non-ferment.	III	homo
<i>S. glycerinaceus</i>		III	non-ferment.	—	homo
<i>S. lactis</i>		non-ferment.	I	III'	homo
<i>L. xylosus</i>		non-ferment.	I	III'	homo
<i>L. arabinosus</i>		I	non-ferment.	I	homo
<i>L. thermophilus</i> T ₁		III	III	III	homo'
<i>L. thermophilus</i> II		I	III	non-ferment.	homo'
<i>L. brevis</i>		I	non-ferment.	—	hetero
<i>L. fermentum</i> H60		non-ferment.	I	—	hetero
<i>Leuc. mesent.</i> B07		I	I	non-ferment.	hetero
<i>Pc. hennebergi</i>		I	I	—	homo
<i>Pc. lintneri</i>		I	I	—	homo
<i>L. pentosus</i> *		I	I	—	—

* by Horecker et al.

non-ferment., non fermentable.; —, not examined.; III', almost III.

1) In the absence of carbonate, lactic acid fermentation was proceeded by neutralizing the broth with freshly prepared N-NaOH at a 12-hours interval. As is shown in Table 3, *S. faecalis* produced 1.35 moles of lactate from one mole of L-arabinose and *L. thermophilus* T₁ produced 1.22 moles of lactate from one mole of D-xylose, and at the same time small amounts of formate were formed by both strains. These results are similar to those of fermentations obtained by using Ca-carbonate containing medium (see Table 1), and suggests that carbonate has no effect on the pathways of pentose metabolism by *S. faecalis* and *L. thermophilus* T₁.

2) In the isotopic experiments for the carbonate fixation, NaHC¹⁴O₃ was used. One half of the NaHC¹⁴O₃ used in this case was added at the initial

Table 3. Fermentation of Pentoses in the Absence of Carbonate.

Strain	<i>S. faecalis</i>	<i>L. thermophilus</i> T ₁
Substrate	L-Arabinose	D-Xylose
Initial sugar (g)	1.80	3.05
Consumed sugar (g)	1.68	1.05
Lactic acid (g)	1.37	0.77
Formic acid (g)	0.11	0.18
Carbon dioxide (g)	trace	trace
Yield of lactic acid (%)	81.6	73.3
Do. mol./mol.	1.35	1.22

mol./mol.: moles of produced lactate per moles of consumed sugar.

stage of fermentation process and another half at the middle-stage. As shown in Table 4, the distribution pattern of C^{14} among the fermentation products gave evidence that lactic acid fermentation from pentose by lactic organisms did not involve carbonate fixation reaction, but carbonate was apparently fixed into other ether-soluble non-volatile acid which was not yet identified. From these findings, we may conclude that carbonate has no effect on lactic acid production from pentoses by lactic organisms except that it merely acts as a neutralizer.

Table 4. Incorporation of Carbon Atom from Carbonate into the Fermentation Products.

Strain	<i>S. faecalis</i>	<i>L. thermophilus</i> T ₁
Substrate	L-Arabinose	D-Xylose
NaHC ¹⁴ O ₃	613,440 (c.p.m.)	8,020,000 (c.p.m.)
Initial sugar	1.27 (g)	1.90 (g)
Consumed sugar	0.75	1.78
Lactic acid*	0.56 1,400	1.34 145,600
Zn-lactate**	0	0
Formic acid	0.04 0	0.12 0
CO ₂ ***	512,310	0.34 5,400,000
Residue	29,600	(not measured)
Recovered C ¹⁴	541,910	

* Ether-soluble non-volatile acid was calculated as lactic acid.

** Zn-lactate was prepared in a crystalline form.

*** CO₂ was liberated from carbonate by acid-formation.

3) Lactic acid fermentation by the intact cells grown in media containing pentoses.

As was shown in Table 1, lactic acid was produced in a yield of about 87% by weight of pentose utilized, a small amount of formate being formed at the same time as a by-product by *S. faecalis*. The proportions of formate to lactate were, however, varied by the conditions of fermentation. As the systems employed in the above culture experiments seem to be inappropriate to discuss the real fate of pentose metabolism, we must employ a more simplified experimental technique to meet this purpose. Thus, we carried out lactic acid fermentation anaerobically using the intact cells which were grown in the media containing pentoses.

As shown in Tables 5 and 6, the cells of *S. faecalis* grown in L-arabinose medium produced lactic acid in a yield of 95% by weight of the consumed L-arabinose under anaerobic condition and the cells of *L. thermophilus* T₁ grown in D-xylose medium converted 93% of consumed D-xylose to lactic acid under the same condition, but no detectable formation of formate was

observed in each case. CO₂ evolution and glycerol formation were both undetectable, however, small amounts of L-xylulose, L-ribulose and D-xylose were formed by *S. faecalis*, and D-xylulose by *L. thermophilus* T₁. O₂ consumption and CO₂ evolution from L-arabinose were observed when intact cells of *S. faecalis* were used aerobically. Studies on ketose formation and pentose metabolizing system under aerobic condition will be published in the next paper.

Table 5. Fermentation of Pentoses by the Intact Cells of *S. faecalis* and *L. thermophilus* T₁.

Strain	<i>S. faecalis</i>		<i>L. thermophilus</i> T ₁			
Substrate	L-Arabinose		D-Xylose		L-Arabinose	
	50.0 mg	333.3 μM	50.0 mg	333.3 μM	50.0 mg	333.3 μM
Consumed sugar	50.0	333.3	42.0	280.0	36.0	240.0
Lactic acid	47.5	530.0	39.1	434.4	32.0	355.6
Formic acid		<20		<20		—
Yield of lactic acid mol./mol.	1.59		1.55		1.48	
mol./mol.:	formed lactate μM per μM of consumed substrate.					
	For example, 1.667 is theoretical yield by formula (II).					
Reaction mixture:	cell suspension (ca. 60 mg dried cells) 5 ml; buffer solution (M/10 phosphate, pH 7.0) 5 ml; substrate (crystal) 50 mg; total volume 10 ml					
Reaction period:	18 hours					

Table 6. Gas Balance in the Fermentation of L-Arabinose by the Intact Cells of *S. faecalis*.

Gas phase	N ₂	Air
CO ₂ evolution μM	0	5.58
O ₂ consumption μM	0	5.02
Formed lactate μM	6.66	2.88
Reaction mixture:	cell suspension (ca. 15 mg dried cells)	
	buffer solution (M/15 phosphate, pH 7.0)	
	L-arabinose 5 μM	
	dist. water	
	total volume	
Reaction period:	1 hour	
Reaction temperature:	35°C	
Gas balance were measured manometrically.		

From these findings, we wish to propose the following fermentation formula (II) for the anaerobic pentose metabolism by *S. faecalis* and *L. thermophilus* T₁.

The above formula could be accounted for only by a recycling of carbon, possibly through pentose phosphate pathway, for regeneration of C_3 -compound as a lactate precursor.

4) *Comparative studies on fermentation of glucose, pentoses and sedoheptulose by S. faecalis and L. thermophilus T₁*

As *S. faecalis* and *L. thermophilus* T₁ are so called homo lactic acid fermenters, so the theoretical yields of lactic acid from glucose by these organisms must be amounted to 100% of consumed sugar. Actually, however, lactate is not produced theoretically because lactate formation is accompanied with cell-growth and other by-paths in fermentation. For example, as shown in Table 7, the yield of lactic acid from glucose by *S. faecalis* and *L. thermophilus* T₁ was 87.5% and 90.0% respectively, and in both cases formate formation was observed but not acetate production. Comparison of the above data with the results obtained from pentose fermentation may suggest that there is no noticeable difference between the fermentations of pentoses and those of glucose in *S. faecalis* and *L. thermophilus* T₁.

Table 7. Comparison of Fermentation upon Glucose, Pentoses and Sedoheptulose by *S. faecalis* and *L. thermophilus* T₁

Strain	<i>S. faecalis</i>			<i>L. thermophilus</i> T ₁		
	Glucose	L-Arabinose	Heptulose	Glucose	D-Xylose	Heptulose
Initial sugar (g)	3.45	2.32	0.22	2.80	2.80	0.22
Consumed sugar (g)	3.45	2.32	0.22	2.80	2.80	trace
Lactic acid (g)	3.02	1.91	0.20	2.52	2.18	
Formic acid (g)	0.13	0.10	0.04	0.13	0.21	
Acetic acid (g)	0	0	0	0	0	
Yield of lactic acid (%)	87.5	82.3	90.9	90.0	77.9	
Recovery of carbon (%)	92.1	87.9	109	96.0	87.1	

Sedoheptulose medium was 25 ml.

Supposing the new pentose metabolizing system of lactic organisms involves the so-called pentose phosphate pathway⁽¹³⁾, sedoheptulose-7-phosphate and hexose-6-phosphate should be formed as intermediates. These compounds were not detected by paperchromatography throughout our fermentation processes. It may be reasonably assumed that these compounds are so reactable that they are unable to accumulate in detectable amounts.

* The pentose metabolism by formula (II) may be designated homo lactic acid fermentation upon pentose just like that upon hexose.

The abilities of fermenting sedoheptulose by various strains were also examined. As the results shown in Tables 7 and 8, *S. faecalis* and *S. glycerinaceus* readily fermented sedoheptulose just as glucose, giving 90% yield of lactic acid and small amount of formate from consumed sugar. On the other hand, *L. thermophilus* T₁ could not produce any significant amount of acid in spite of its utilizing ability upon sedoheptulose (see Table 8).

Table 8. Utilization of Sedoheptulose by Lactic Acid Bacteria.

Strain	Acidity	Cell-growth
<i>S. faecalis</i>	1.00	++
<i>S. glycerinaceus</i>	0.10	+
<i>S. liquefaciens</i>	0.95	++
<i>S. faecalis</i> B06	0.35	+
<i>L. arabinosus</i>	0	(+)
<i>L. thermophilus</i> T ₁	0.10	+
<i>L. brevis</i>	0	(+)
<i>Leuc. mesenteroides</i> B07	0	(+)

Acidity: number of N/10 NaOH ml per 2 ml of broth containing 1% sedoheptulose after 4 days' culture.

Cell-growth: ++ good growth (normal growth).
+ moderate growth.
(+) slight growth.

These results may suggest that *S. faecalis* can ferment pentoses and sedoheptulose through the pentose phosphate cycle while *L. thermophilus* T₁ metabolizes pentose according to another pathway.

DISCUSSION

Clear evidence of the presence of a new pathway differing from the Fred-Peterson-Anderson scheme in the fermentation of pentoses by adapted cells* of *S. faecalis* and *L. thermophilus* T₁ was given from the studies of substantial balance in fermentation and tracer experiment. *S. faecalis* was also capable of fermenting sedoheptulose with the production of lactic acid in a yield of 90% by weight of consumed substrate. Therefore, the pentose metabolizing system of *S. faecalis* may be presented as follows:

or,

* Recently, we have concluded that the formation of new pentose metabolizing systems in lactic acid bacteria are adaptively induced.

In this scheme, a carbon recycling system, possibly the pentose phosphate pathway, and Emoden-Meyerhof scheme may be involved. Consequently, pentoses may probably be metabolized via hexose-6-P. In the case of *L. thermophilus* T₁, although sedoheptulose was assimilated it was not fermented. So it is assumed that lactate might be produced via hexose-6-P through the other cyclic pathway of pentose, but not via sedoheptulose-7-P. Recently, E. Racker and E. Schroeder⁽¹⁴⁾ reported that ribose-5-P was converted to fructose-6-P through octulose-8-P by transaldolase. There are some possibilities for the existence of a new cyclic pathway of pentose in *L. thermophilus* T₁. But in order to establish such a pathway, further investigation should be carried out.

SUMMARY

1) Pentose fermentation by various lactic acid bacteria can be classified into the following two types: a) Formula (I) which proceeds according to the Fred-Peterson-Anderson system, and b) formula (III) which supposedly involves a new pathway of pentose metabolism. Almost all of the examined organisms metabolized various pentoses according to either formula (I) or (III) exclusively. But, some exceptional strains fermented different pentoses by different schemes. For example, *Leuconostoc mesenteroides* B07 fermented various pentoses only according to formula (I) and *L. thermophilus* T₁ according to formula (III) exclusively. Whereas, *L. thermophilus* II was able to ferment L-arabinose by formula (I) and D-xylose by formula (III).

2) From comparative study of pentose fermentation in the presence and absence of carbonate and an isotopic experiment for incorporation of carbonate into lactate, it was concluded that carbonate fixation reaction is not involved in lactate formation from pentoses.

3) Anaerobically, the cells of *S. faecalis* grown in a L-arabinose medium produced lactic acid from L-arabinose in a yield of 95% by weight of consumed sugar, and likewise those of *L. thermophilus* T₁ grown in D-xylose medium converted 93% of consumed D-xylose to lactic acid. In both cases no formate and CO₂ were formed. As a consequence of these experiments, we proposed a new formula (II) as an alternative scheme of (III).

4) From the analysis of yields of lactate from glucose, pentose and sedoheptulose, no noticeable difference in yield among these fermentations was observed. Consequently, we have concluded that so-called pentose phosphate pathway may have no inconsistency for accounting for the pentose metabolizing system of *S. faecalis*. This hypothesis, however, can not be applied for such a strain like *L. thermophilus* T₁ which unable to ferment sedoheptulose.

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