THE OXIDATION OF PENTOSES BY PSEUDOMONAS

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The production of acid when pentoses are supplied as carbon sources for members of the genus *Pseudomonas* has been reported by several investigators (Chernomordik, 1939; Sears and Gourley, 1928), but apparently no attempt has been made to identify the products. Foster (1944), studying manometrically the oxidation of *d*-ribose and *d*-arabinose by *P. riboflavina*, found that only about 40 per cent of the oxygen theoretically required for the complete oxidation to carbon dioxide was consumed. He concluded that the remainder of the sugars was assimilated, there being no indication of acid accumulation.

In almost all papers dealing with the fermentation of pentoses by bacteria, the configurations of the sugars studied are not indicated. It is generally assumed that the sugars studied were the common, naturally occurring enantiomorphs. Thus, except for arabinose, which most commonly occurs in the *l*-form, the pentoses studied were probably of the *d*-series.

The present investigations were made to determine whether there were differences in the ability of various species of *Pseudomonas* to utilize *d*- and *l*-arabinose, to identify the acids produced when various pentoses are oxidized by *Pseudomonas*, and to obtain yield data.

MATERIALS AND METHODS

In order to obtain data on the nature of the products of the metabolism of pentoses by *Pseudomonas* and to get yield data, 100-ml cultures were aerated with 100 ml of filter-sterilized air per minute in 250-ml test tubes equipped with aerator stones which dispersed the air in fine bubbles. All cultures were incubated at 30 C. The corn steep liquor, which was the commercial grade product widely used in industrial fermentation processes, contained about 50 per cent solids, and the mineral nutrients supplied were of cp quality. The basal nutrient solution used in the 100-ml cultures contained 2 g urea, 0.6 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, and 5 ml corn steep liquor per liter. Three drops of soybean oil were added to each culture to prevent excessive frothing. At the time of inoculation, sufficient sterile $CaCO_3$ was added to neutralize the pentonic acid which might be formed if quantitative conversion of the sugar to a pentonic acid took place. The $CaCO_3$ was sterilized dry. Since preliminary experiments with *d*-xylose had shown the formation of furfural in toxic concentration in culture media sterilized 20 minutes at 15 pounds' steam pressure, all culture media used in these experiments were sterilized by filtration through Seitz sterilizing pads.

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All cultures were inoculated with 6 ml of 24-hour broth cultures which contained 0.2 per cent pentose, 0.3 per cent tryptone, and 0.3 per cent yeast extract.

The *d*-arabinose used in these experiments, supplied by Dr. Ray Hann, National Institute of Health, Bethesda, Maryland, was prepared by degradation of gluconic acid. The *l*-arabinose and *d*-xylose were of a pure reagent grade. The *d*-ribose, for which polarimetric examination indicated a purity of 99.7 per cent, was obtained through the courtesy of Dr. J. A. Aeschlimann.

All cultures used were from the culture collection of the Fermentation Division, Northern Regional Research Laboratory, and the identifying numbers used here are those of this collection.

TABLE 1

The oxidation of d-arabinose by Pseudomonas

CULTURE	*d*-ARABINOSE* CONSUMED	*d*-ARABONIC ACID		
		Produced†	Yield based on *d*-arabinose	
			Consumed (theoret.)‡	Supplied (weight)§
	g/culture	*g/culture*	*per cent*	*per cent*
P. fragi 73	4.8	1.20	22.6	25.0
P. graveolens 14	2.7	0.73	24.6	15.2
P. synxantha 79	2.5	1.20	22.6	25.0
P. vendrelli 23	1.4	0.73	52.0	15.2

* 4.8 g *d*-arabinose supplied per culture, duration 7 days.

† Calculated from data on calcium in solution.

‡ Theoretical yield: 1.107 g *d*-arabonic acid per g *d*-arabinose.

§ $\frac{\text{Grams } d\text{-arabonic acid produced}}{\text{Grams } d\text{-arabinose supplied}}$.

EXPERIMENTAL

The oxidation of *d*-arabinose was first studied. In table 1 are data for *P. fragi* 73, *P. graveolens* 14, *P. synxantha* 79, and *P. vendrelli* 23, which utilized a substantial proportion of the 4.8 grams *d*-arabinose supplied per 100 ml and produced considerable quantities of a soluble calcium salt. This was identified as calcium *d*-arabonate by preparation of the phenyl-hydrazide of the free acid (mp 213) (Glattfeld, 1913). A sample, mixed with synthetic *d*-arabonic phenylhydrazide melted at the same temperature. Other cultures which, while making considerable growth, either failed to oxidize the *d*-arabinose supplied in this experiment or failed to produce appreciable acid were *P. ovalis* 8, *P. schuylkilliensis* 9, *P. mildenbergii* 21, *P. fluorescens* 6, *P. putida* 13, *P. pavonacea* 24, and *P. mephitica* 75. The last four probably used the protein constituents of the media for growth. It is of interest that *P. ovalis* 8 oxidizes glucose to gluconic acid, and *P. schuylkilliensis* 9, *P. putida* 13, and *P. mildenbergii* 21 produce 2-oxogluconic acid from glucose in good yields (Lockwood, Tabenkin, and Ward, 1941).

Cultures of *P. fluorescens* 6, *P. fragi* 25, *P. mildenbergii* 21, *P. putida* 13, *P.*

synxantha 79, and *P. vendrelli* 23 were grown on 4.9 per cent *l*-arabinose nutrient solution. The first four species were harvested after 2 days' aeration, but the last two species oxidized the *l*-arabinose much less rapidly and were not harvested until the sixth day. At harvest, all cultures contained substantial amounts of a soluble calcium salt, which was in each case identified as calcium *l*-arabonate by preparation of the brucine salt (mp 152 C); when this was mixed with synthetic brucine *l*-arabonate, the melting point showed no depression. Nef (1907) reported the melting point of the brucine *l*-arabonate to be 155 C. As a further check on the identity of the material, the *l*-arabobenzimidazole derivative was prepared. This melted at 235 to 236 C, and when it was mixed with purely synthetic material, the resulting mixture melted at the same point (Moore and Link, 1940). Examination of X-ray diffraction patterns confirmed the identity

TABLE 2

The oxidation of l-arabinose by Pseudomonas

CULTURE	AGE AT HARVEST	*l*-ARABINOSE CONSUMED*	*l*-ARABONIC ACID		
			Produced†	Yield based on *l*-arabinose	
				Consumed‡ (theoret.)	Supplied§ (weight)
	days	*g/culture*	*g/culture*	*per cent*	*per cent*
P. fluorescens 6	2	4.9	1.38	25.4	28.2
P. fragi 25	2	4.9	1.75	32.2	35.7
P. mildenbergii 21	2	4.9	1.41	26.0	28.8
P. putida 13	2	4.9	2.34	43.1	47.8
P. synxantha 79	6	4.3	0.86	18.0	17.5
P. vendrelli 23	6	4.9	2.22	40.9	45.4

* Four and nine-tenths g *l*-arabinose supplied per culture.

† Calculated from data on calcium in solution.

‡ Theoretical yield: 1.107 g *l*-arabonic acid per g *l*-arabinose.

§ $\frac{\text{Grams } l\text{-arabonic acid produced}}{\text{Grams } l\text{-arabinose supplied}}$.

of the benzimidazole derivatives of *l*-arabonic acids of synthetic and bacterial origin. Yields of *l*-arabonic acid are given in table 2.

Cultures of *P. fluorescens* 6, *P. ovalis* 8, *P. putida* 13, *P. graveolens* 14, *P. mildenbergii* 21, and *P. fragi* 25 grown on 6.7 per cent *d*-xylose nutrient solution for 6 days contained much soluble calcium salt, which was found to be calcium *d*-xylonate. The identity of the compound was established by preparation of the brucine salt (mp 176 C) (Nef, 1914). Yield data are presented in table 3.

In table 4 are presented the data for experiments in which fermentations were conducted on 5.9 per cent *d*-ribose. Cultures of *P. fluorescens* 6, *P. fragi* 25, *P. graveolens* 14, *P. mephitica* 75, *P. mildenbergii* 21, *P. ovalis* 8, *P. pavonacea* 24, *P. putrifaciens* 76, *P. synxantha* 79, and *P. vendrelli* 23 were employed. When the reducing action toward Shaffer-Hartmann copper reagent (Shaffer and Hartmann, 1921) was nil, or after 9 days, cultures were harvested. *P. pavonacea* 24 consumed little *d*-ribose, and little $CaCO_3$ was dissolved in either this culture or

in the culture of *P. mephitica* 75. *d*-Ribonic acid was identified as the product of the oxidation of *d*-ribose by preparation of the benzimidazole derivative which

TABLE 3

The oxidation of d-xylose by Pseudomonas

CULTURE	*d*-XYLOSE* CONSUMED	*d*-XYLONIC ACID		
		Produced†	Yield based on *d*-xylose	
			Consumed‡ (theoret.)	Supplied§ (weight)
	g/culture	*g/culture*	*per cent*	*per cent*
P. fluorescens 6	5.9	4.59	70.4	68.0
P. ovalis 8	5.9	2.66	41.2	39.5
P. putida 13	5.4	4.40	73.4	65.3
P. graveolens 14	6.2	3.78	55.5	56.2
P. mildenbergii 21	6.3	4.87	70.1	72.4
P. fragi 25	5.5	1.89	31.0	28.1

* Six and seven-tenths g *d*-xylose supplied per culture, duration 6 days.
† Calculated from data on calcium in solution.
‡ Theoretical yield: 1.107 g *d*-xylonic acid per g *d*-xylose.
§ $\frac{\text{Grams } d\text{-xylonic acid produced}}{\text{Grams } d\text{-xylose supplied}}$.

TABLE 4

The oxidation of d-ribose by Pseudomonas

CULTURE	AGE AT HARVEST	*d*-RIBOSE CONSUMED*	*d*-RIBONIC ACID		
			Produced†	Yield based on *d*-ribose	
				Consumed‡ (theoret.)	Supplied§ (weight)
	days	*g/culture*	*g/culture*	*per cent*	*per cent*
P. fluorescens 6	5	5.7	4.11‖	65.8	70.0
P. ovalis 8	5	5.7	1.15	18.3	19.6
P. graveolens 14	3	5.8	2.37‖	37.0	40.4
P. mildenbergii 21	5	5.6	1.01	18.2	18.7
P. vendrelli 23	7	5.6	4.56‖	75.5	77.8
P. pavonacea 24	9	0.4			
P. fragi 25	5	5.8	3.12‖	49.2	54.4
P. mephitica 75	9	1.8			
P. putrifaciens 76	9	3.5	0.78	20.2	13.3
P. synxantha 79	5	5.8	2.57‖	40.5	43.9

* Five and nine-tenths g *d*-ribose supplied per culture.
† Calculated from data on calcium in solution.
‡ One g *d*-ribose can yield 1.107 g *d*-ribonic acid.
§ $\frac{\text{Grams } d\text{-ribonic acid produced}}{\text{Grams } d\text{-ribose supplied}}$.
‖ Benzimidazole derivatives prepared and identity established by X-ray.

melted at 191 C (Dimler and Link, 1943); when this was mixed with a sample of synthetic material, there was no depression of the melting point. The identity

of the compounds was further established by comparison of X-ray diffraction patterns.

DISCUSSION

Many strains among the various species of *Pseudomonas* are capable of oxidizing pentoses to the corresponding pentonic acids. Similar oxidations have been known for many years anong the acetic acid bacteria. Bertrand (1898*a*, 1898*b*) demonstrated the production of xylonic acid from xylose and arabonic acid from arabinose by the sorbose bacterium (*Acetobacter xylinum*). Further similarities between bacteria of the genera *Acetobacter* and *Pseudomonas* lie in the facts that glucose is oxidized to gluconic acid by many species of both genera, and that gluconic acid is further oxidized by bacteria of each genus to 2-oxogluconic acid although, among the acetic acid bacteria, 2-oxogluconic acid is accompanied by the 5-oxogluconic acid, which is generally the major metabolic product (Lockwood, Tabenkin, and Ward, 1941; Prescott and Dunn, 1940). Further study of the oxidative capacity of bacteria of the genus *Pseudomonas* will doubtless reveal as interesting and valuable a series of biochemical conversions as are now known among bacteria of the genus *Acetobacter*.

It is noteworthy that cultures of *P. fragi*, *P. synxantha*, and *P. vendrelli* oxidized both the *d*- and *l*-enantiomorphs of arabinose.

No effort has been made to find the conditions under which the maximal yields of pentonic acids may be obtained. It is probable, however, that bacterial oxidation with *Pseudomonas* may prove a convenient method for the preparation of these acids from the corresponding sugars.

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SUMMARY

Pseudomonas fragi, *P. graveolens*, *P. synxantha*, and *P. vendrelli* oxidized *d*-arabinose to *d*-arabonic acid when grown in aerated corn steep liquor solutions in the presence of $CaCO_3$.

Pseudomonas fluorescens, *P. fragi*, *P. mildenbergii*, *P. putida*, *P. synxantha*, and *P. vendrelli* oxidized *l*-arabinose to *l*-arabonic acid when grown in aerated corn steep liquor solutions in the presence of $CaCO_3$.

Pseudomonas fluorescens, *P. fragi*, *P. graveolens*, *P. mildenbergii*, *P. ovalis*, and *P. putida* oxidized *d*-xylose to *d*-xylonic acid when grown in aerated corn steep liquor solutions in the presence of $CaCO_3$.

Pseudomonas fluorescens, *P. fragi*, *P. graveolens*, *P. mildenbergii*, *P. ovalis*, *P. putrifaciens*, *P. synxantha*, and *P. vendrelli* oxidized *d*-ribose to *d*-ribonic acid when grown in aerated corn steep liquor solutions in the presence of $CaCO_3$.

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