ORGANIC ACID PRODUCTION BY BASIDIOMYCETES

II. Acid Production from Xylose

By

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INTRODUCTION

In a previous paper[5], it was reported that 67 strains belonging to 47 species of Basidiomycetes were examined for their acid-producing abilities in glucose media in stationary and shake cultures and 20 strains of them were selected as oxalic-acid-producing strains. Among these Basidiomycetes, 9 strains, mostly brown-rot fungi, were able to produce oxalic acid, regardless of whether $CaCO_3$ was present in the medium ; eleven strains, mostly white-rot fungi, produced oxalic acid only when the medium contained $CaCO_3$. On the other hand, *Schizophyllum commune* and *Merulius tremellosus* were found to produce substantial amounts of L-malic acid as a main product in the $CaCO_3$-containing medium in shake culture.

Most of the above Basidiomycetes are known to be wood-decomposing fungi. Wood, especially hard wood, contains large amounts of xylan as a staple hemicellulose, so that xylose can be easily obtained on a commercial scale by hydrolysis of xylan in relation to the wood industry. However, there are relatively few reports relating to the formation of organic acids and other useful metabolic products from xylose by Basidiomycetes.

In this paper, therefore, the production of organic acids from xylose by Basidiomycetes selected as oxalic or malic acid-producing strains in the glucose medium is described.

MATERIALS AND METHODS

Organisms.

Corticium rolfsii 9404, *Fomitopsis officinalis* 9354, *Gloeophyllum trabeum* 9315, *Poria vaporaria* 9381, *Trametes gibbosa* 9389, *Trametes heteromorpha* 9395 and *Trametes sanguinea* 9392, known to be oxalic-acid-producing strains,

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and *Schizophyllum commune* 9384 and *Merulius tremellosus* 9371, found to be malic-acid-producing strains, were all obtained from the Laboratory of Applied Microbiology, Hokkaido University.

Cultural procedure.

All strains were grown at 27°C for 10 days on a mixture of 3 g of oak sawdust, 2 g of rice bran and 5 ml of tap water in 100-ml Erlenmeyer flasks. They were transferred to 40 ml of medium in 150-ml Erlenmeyer flasks, then stationary cultured at 27°C for 21 days. Each strain was also cultured in 50 ml of broth medium in 500-ml shake flasks on a reciprocal shaker at 27°C for 7 days. The broth medium contained 5% xylose, 0.2% peptone, 0.5% KH_2PO_4, 0.25% $MgSO_4 \cdot 7H_2O$, 0.2% yeast extract, and 1% malt sprouts extract. Two % $CaCO_3$ was added to some media.

Analytical methods.

Xylose was measured in accordance with the method of SOMOGYI[4]. Mycelial weight was determined after drying overnight in an oven at 100°C. The oxalic acid in the culture solution, which contained no added $CaCO_3$, was estimated by titration with 0.1 N NaOH. In the $CaCO_3$-containing medium, oxalic acid, which accumulated as insoluble calcium salt, was determined in the same manner as that described in a previous paper[5]. Soluble calcium in the broth was estimated by precipitation of calcium as oxalate and subsequent titration with $KMnO_4$ solution. Elution analysis of organic acid was made by the same method as that employed in the previous experiment[5].

RESULTS AND DISCUSSION

1. **Sugar consumption and growth.**

The per cent of sugar consumed by 9 strains of acid-producing Basidiomycetes and their mycelial weights in glucose and xylose media under four sets of cultural conditions are compared in Table 1. All strains grew in the xylose medium, but this sugar was found to be less effective than glucose in respect to sugar utilization and growth under every cultural condition studied, with only a few exceptions.

2. **Oxalic acid production.**

Comparison of the acid production from glucose and xylose by 7 oxalic-acid-producing strains is shown in Table 2. In the case of *C. rolfsii*, glucose was always more suitable for the acid production than xylose. However, with some strains, such as *G. trabeum*, *P. vaporaria* and *T. gibbosa*, xylose gave

TABLE 1 Utilization of glucose and xylose by acid-producing strains of Basidiomycetes.

Species	Sugar	Without $CaCO_3$				With $CaCO_3$			
		Stationary		Shake		Stationary		Shake	
		Sugar consumed (%)	Mycelial wt/100 ml (g)	Sugar consumed (%)	Mycelial wt/100 ml (g)	Sugar consumed (%)	Mycelial wt/100 ml (g)	Sugar consumed (%)	Mycelial wt/100 ml (g)
Corticium rolfsii	Glucose	98	2.2	85	3.0	86	1.8	95	3.0
	Xylose	72	1.6	47	0.5	66	0.9	53	0.6
Fomitopsis officinalis	Glucose	53	0.6	25	0.4	55	0.5	21	0.3
	Xylose	33	0.4	18	0.5	38	0.4	17	0.4
Gloeophyllum trabeum	Glucose	48	0.6	49	0.4	70	0.7	45	0.4
	Xylose	20	0.5	23	0.4	45	0.4	25	0.4
Poria vaporaria	Glucose	78	1.5	38	0.9	80	1.3	64	1.7
	Xylose	57	1.0	31	0.7	64	0.9	50	0.5
Trametes gibbosa	Glucose	81	1.7	88	3.2	62	1.4	82	1.5
	Xylose	67	1.2	48	1.7	41	0.5	40	0.8
Trametes heteromorpha	Glucose	97	1.8	82	3.1	63	1.5	70	1.6
	Xylose	77	1.3	46	1.6	39	0.4	44	0.4
Trametes sanguinea	Glucose	95	3.8	74	0.9	53	0.8	90	1.1
	Xylose	85	2.2	57	1.9	40	0.6	56	0.7
Schizophyllum commune	Glucose	100	1.8	91	2.2	98	2.3	98	1.5
	Xylose	97	1.7	87	1.4	72	0.9	86	0.8
Merulius tremellosus	Glucose	99	1.0	78	1.1	100	1.6	100	0.8
	Xylose	98	1.4	96	1.3	71	0.9	83	0.7

TABLE 2 Comparison of oxalic acid production from glucose and xylose by some Basidiomycetes.

Species	Type of rot	Sugar	Oxalic acid yield based on sugar available			
			Without $CaCO_3$		With $CaCO_3$	
			Still (%)	Shake (%)	Still (%)	Shake (%)
Corticium rolfsii	?	Glucose	5.2	5.0	32.7	8.2
		Xylose	4.9	4.2	21.8	7.5
Fomitopsis officinalis	Brown	Glucose	11.5	4.4	14.2	5.7
		Xylose	7.8	5.6	15.3	7.3
Gloeophyllum trabeum	Brown	Glucose	2.6	4.7	30.2	5.8
		Xylose	2.7	3.9	34.2	6.2
Poria vaporaria	Brown	Glucose	5.9	17.1	13.0	30.8
		Xylose	5.5	20.8	27.5	33.5
Trametes gibbosa	White	Glucose	0	0	6.0	21.5
		Xylose	0	0	8.8	27.1
Trametes heteromorpha	Brown	Glucose	0	0	6.2	24.2
		Xylose	0	0	11.8	25.9
Trametes sanguinea	White	Glucose	0	0	14.6	21.3
		Xylose	0	0	14.0	40.1

somewhat higher yields of oxalic acid than did glucose, especially in $CaCO_3$-containing media. With regard to oxalic acid production by Basidiomycetes, SHIMAZONO[3] (1951) reported that *P. vaporaria* grew well in the medium containing xylose as well as in glucose, but the acid yield from xylose was much lower than that obtained from glucose. GOKSØYR[1] (1958) also indicated that oxalic acid production by *Merulius lacrymans* was less in xylose medium than in glucose medium, although the growth was better with xylose. On the contrary, NAGATA and HAYASHI[2] (1956) found that glucose was more suitable for the good growth of *Corticium centrifugum* than was xylose, but inferior to xylose for oxalic acid production. From these observations and our results, it is considered that the suitability of xylose and glucose for growth and oxalic acid production varies occasionally with different Basidiomycetes and under different cultural conditions.

C. rolfsii, *F. officinalis*, *G. trabeum* and *P. vaporaria*, which were previously included in one group, were able to produce oxalic acid from xylose regardless of whether $CaCO_3$ was present in the medium, although higher yields were generally obtained by the addition of $CaCO_3$. Three species of the genus *Trametes* belonging to the other group produced the acid only when $CaCO_3$ was added to the medium and it was observed that shake culturing was more favorable than stationary culturing for acid production with each strain. These reactions of two groups of Basidiomycetes in xylose media were quite similar to those found in glucose media.

Recently, TSAO[6] (1963) observed that the wood-rotting fungus *Pleurotus ostreatus* produced oxalic acid from xylose or arabinose in about half the yield that it did from glucose in $CaCO_3$-containing media in shake culture, then attempted to produce this acid directly from wood hemicellulose by using the same Basidiomycete. The results obtained in this experiment indicate the possibility that some Basidiomycetes examined here may be employed as promising strains for oxalic acid production from xylose or hemicellulose, as well as from glucose.

3. **Malic acid production.**

S. commune and *M. tremellosus* selected as malic-acid-producing strains

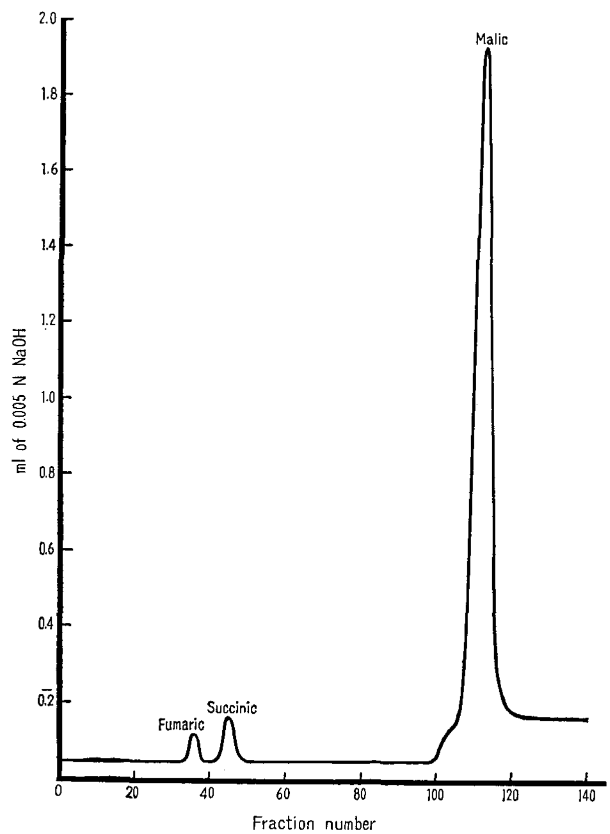

Fig. 1. Chromatogram of acids produced by *S. commune.*

did not produce any significant amount of acid in xylose media without the addition of $CaCO_3$, but these strains appeared to produce relatively large quantities of organic acid other than oxalic acid only in the $CaCO_3$-containing media in shake culture as observed in glucose media, because of substantial amounts of soluble calcium in the broth. Each culture filtrate containing soluble calcium salts was then subjected to column chromatography after treatment with cation-exchange resin. The chromatograms having the three peaks shown in Figs. 1 and 2 were very similar to those previously obtained with glucose medium using *S. commune*. The location of each acid peak was in good agreement with those of fumaric, succinic and L-malic acids and each acid has already been confirmed to be produced in glucose medium by both strains in the previous experiment[5]. From the results of chromatography, it is obvious that *S. commune* and *M. tremellosus* produce relatively large amounts of L-malic acid as a main product together with smaller quantities of fumaric and succinic acids from xylose.

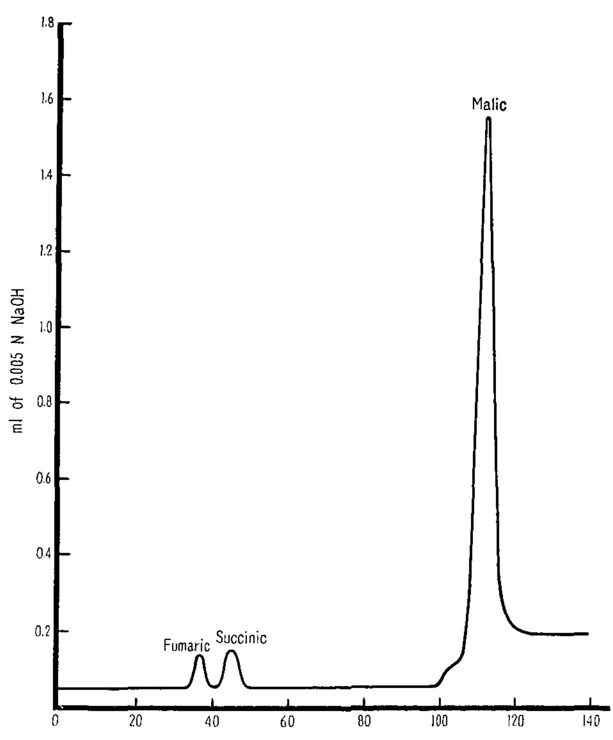

Fig. 2. Chromatogram of acids produced by *M. tremellosus*.

TABLE 3 Organic acid production by *S. commune* and *M. tremellosus* in $CaCO_3$-containing medium in shake culture for 7 days.

Species	Yield (%) based on available xylose			
	Fumaric	Succinic	Malic	Oxalic
Schizophyllum commune 9384	0.3	0.5	12.4	7.6
Merulius tremellosus 9371	0.3	0.4	10.2	3.0

Table 3 indicates the yields of four organic acids, based on the available xylose, obtained by these two species. Fumaric, succinic and malic acids were estimated from titratable acidity for each peak effuluent, and oxalic acid was determined from insoluble calcium salts. Higher yields of malic acid should be attained by altering cultural condition ; thus, these species of Basidiomycetes may be used for malic acid production not only from glucose but also from xylose or wood hydrolyzate.

SUMMARY

Nine species of Basidiomycetes, which produced relatively large amounts of oxalic or L-malic acid from glucose, were examined for their abilities to produce organic acids from xylose in both the presence and absence of $CaCO_3$, in stationary and shake cultures.

With most of the strains, sugar consumption and growth in xylose media were somewhat inferior to those in glucose media under each different cultural condition.

Among 7 strains of oxalic-acid-producing Basidiomycetes, *Corticium rolfsii*, *Fomitopsis officinalis*, *Gloeophyllum trabeum* and *Poria vaporaria* produced oxalic acid from xylose regardless of whether or not $CaCO_3$ was present in the medium, whereas *Trametes gibbosa*, *T. heteromorpha* and *T. sanguinea* accumulated the acid only when $CaCO_3$ was a constituent of the medium. These characteristics of oxalic acid production by these strains were quite similar to those found in glucose media.

Schizophyllum commune and *Merulius tremellosus* produced L-malic acid as a main product from xylose as well as from glucose in $CaCO_3$-containing medium in shake culture.

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