below	{	15	2.0	5.4
20		30	9.0	24.2
		40	12.0	32.2

(3) Yields from various concentration of glucose.

Merck's glucose is used (its purity: 98.98%) and the quantity of glucose which is consumed is determined by the method of Bertrand. 400 ccm. of medium are taken in one culture flask, so 10% solution of glucose in one culture flask corresponds to 39.50 g. of glucose, 20 % to 79.18 g. 30% to 118.77 g.

Glucose (%)	Peptone (%)	Day	Temp. (C°)	Ca-Gluconate (g) (with 1H ₂ O)	Yield (theoretical%)	
					from glucose used as medium.	from glucose consumed.
10	0.1	30	30	35.5	72.0	82.9
20	0.1	"	"	47.5	48.2	82.0
20	0.2	"	"	34.0	34.5	57.8
30	0.1	"	"	10.0	6.8	
30	0.3	"	"	10.0	6.8	

3. Conclusion.

(1) By *Asp. glaucus*, gluconic acid is produced from glucose used as carbon source and gluconic acid is identified as its lactone and phenylhydrazide.

(2) 0.1% of peptone as the nitrogen source, 10% of glucose as the carbon source and cultivation during 30 days at 30°C are the optimum conditions to produce gluconic acid.

(3) Under this optimum condition, the yield of gluconic acid from glucose used as the medium is 72.0 % of the theory which corresponds to 82.9 % of the theory from glucose consumed by the fungi.

STUDIES ON THE FERMENTATION PRODUCTS BY MOULD FUNGI. VII. PENICILLIUM OLIVACEUM.

By

YUSUKE SUMIKI.

(*Agricultural Chemical Laboratory, Tokyo Imperial University*).

(Received Nov. 22nd, 1929).

1. Condition of cultivation.

Penicillium olivaceum is cultured in the following media at 30°C during

29~126 days.

Glucose 10%, Pepton 0.2%, K_2HPO_4 & KH_2PO_4 each 0.015%, $MgSO_4 \cdot 7H_2O$ & $CaCl_2$ each 0.01%, NaCl & $FeCl_3$ each trace, dist. Water 90%, $CaCO_3$.

No.	Pepton (%)	Total (l.)	Day	
1	0.7	8	120	} for qualitative determination.
2	0.2	3	39	
3	1.0	6	29	
4	0.05	4.5	90	
5	0	4	126	
6	2.0	6	100	
7	0.1	9	90	
8	0	1.6	15~90	} for quantitative determination. 400 ccm. of medium are taken with 15 g. of $CaCO_3$ in one culture flask and one of these flasks is put to use at each test.
9	0.05	"	"	
10	0.1	"	"	
11	0.5	"	"	
12	1.0	"	"	
13	2.0	"	"	

2. Isolation and identification of fermentation products.

(1) By steem distillation, the volatile substances in the fermented medium are isolated from the non-volatile substances. This distillate gives no reactions of acid and of alcohol, so it is ascertained alcohol and volatile acid are not produced. The residue by steem distillation, is evaporated, acidified with sulphuric acid and extracted with ether. From the ethereal solution, organic acids are obtained and their Ca-salts are prepared to isolate each acid. From the ether insoluble part, gluconic acid is obtained. (see author's report, Bull. Agri. Chem. Soc., Japan, **4**, 13, 1928).

(2) The fermented medium is filtered and the filtrate, if it gives acid reaction, is neutralized with Ca-hydroxide accurately. The ppt. is the Ca-oxalate (ppt. A). When the filtrate from the Ca-salt of oxalic acid is evaporated to small bulk, the Ca-salt of citric acid crystallizes out (ppt. B). After filtration while it is hot, the filtrate is added with the excess of alcohol to precipitate aliphatic acids as their Ca-salts. These salts are acidified with sulphuric acid, filtered and extracted with ether. The ether insoluble part is neutralized with the excess of $CaCO_3$ by boiling. After cooling and filtering, the filtrate is evaporated, added with alcohol and gluconic acid is precipitated as its Ca-salt (ppt. C).

The mycelium and the ppts. isolated by the first filtration of fermented medium are macerated with dil. HCl in the boiling water bath. The macerated solution is neutralized roughly with $CaCO_3$, precisely with $Ca(OH)_2$. Then only oxalic acid is ppt. ed as its Ca-salt (ppt. D). The filtrate from this Ca-oxalate, is evaporated, put together with ppt. B and dissolved in water. From this solution, citric acid is ppt.ed as its Pb-salt by adding Pb-acetate and the free citric acid is obtained by treating with H_2S .

Ppts A and D are put together, added with sulphuric acid, filtered and extracted with ether. From this ethereal solution, free oxalic acid is obtained.

(a) Citric acid.

The crystal recrystallized from boiling water and dried in the desiccator over sulphuric acid, melts at $153\sim 6^{\circ}$.

Subst. 0.1188 g.	CO ₂ 0.1600 g.	H ₂ O 0.0478 g.
C ₆ H ₈ O ₇	Cal. C=37.50%,	H=4.17%.
	Fou. C=36.73%,	H=4.47%.
0.2122 g. of subst. requires 33.4 ccm. of NaOH.		
33.4 ccm. of NaOH=32.8823 ccm of N/10 H ₂ SO ₄ .		
M.W.=65×3=195 (cal. 192),		

(b) Oxalic acid.

The crystal recrystallized from hot water, melts at 100° alone or in admixture with authentic oxalic acid.

Subst 0.5384 g.	AgCl 0.5026 g.
Ag ₂ C ₂ O ₄	Cal. Ag=71.05%.
	Fou. Ag=70.26%.

(c) Gluconic acid.

The crude Ca-salt of gluconic acid (ppt. C), is recrystallized from boiling water twice, added with the theoretical quantity of oxalic acid and filtered. By treating with the HCl-salt of phenylhydrazine and Na-acetate, the filtrate produces phenylhydrazide of gluconic acid. After recrystallization from boiling water, hydrazide melts at 198° .

Subst. 0.0682 g.	N 5.7 ccm (18.0°, 762.0 mm).
C ₁₂ H ₁₈ O ₆ N ₂	Cal. N=9.79%.
	Fou. N=9.82%.

3. Quantitative determination of fermented products.

The condition of cultivation is already described on section 1. The method of quantitative determination is as follows. By filtration, the fermented medium is separated in 2 parts, the mycelium & the ppt. and the filtrate. The filtrate is acidified with sulphuric acid, evaporated, filtered and extracted with ether. From ethereal solution, one part of citric acid (A) is obtained. The ether insoluble solution is neutralized primarily with Ba-hydroxide using congo red paper as indicator only sulphuric acid, secondarily with Ca-carbonate boiling them, filtered, evaporated and again filtered. The filtrate is added with 2 volumes of alcohol (80%). The produced ppt. of Ca-gluconate, is filtered, dried at 100° and weighed as the crystal with one molecule of crystal water.

The mycelium and the ppt. are macerated completely in the boiling water bath with dil. HCl and the macerated solution is neutralized roughly with Ca-carbonate, then accurately with Ca-hydroxide and filtered. The ppt. is dried at 100° and weighed as the Ca-oxalate. The filtrate is evaporated, acidified with sulphuric acid and extracted with ether. From ethereal solution

the remaining part of citric acid (B) is weighed.

In the following table, the yield from 400 ccm. of medium is shown.

No.	Day	Mycellium (g.)	Citric acid (g.)			Oxalic acid (g.)		Gluconic acid (g.)	
			A	B	Total	Salt	Free	Salt	Free
8	15	0.15	0.25	0.05	0.30	0.15	0.10	1.3	1.13
9	"	0.75	0.1	0.05	0.15	"	"	1.95	1.71
10	"	1.7	0.3	"	0.35	"	"	2.0	1.75
11	"	3.6	0.8	0.3	1.1	0.2	0.14	3.4	2.98
12	"	8.6	"	0.4	1.2	0.25	0.17	3.6	3.15
13	"	10.4	"	0.3	1.1	"	"	5.4	4.73
8	30	0.15	0.3	0.05	0.35	0.15	0.10	1.35	1.13
9	"	0.65	0.4	"	0.45	"	"	1.9	1.68
10	"	1.4	0.3	1.05	1.35	"	"	2.1	1.83
11	"	5.2	0.7	0.5	1.2	0.25	0.17	3.4	2.98
12	"	8.9	0.4	"	0.9	0.5	0.35	3.05	2.67
13	"	12.7	0.55	0.15	0.7	"	"	4.7	4.11
8	50	0.15	0.3	0.05	0.305	0.15	0.10	2.0	1.75
9	"	1.0	"	0.5	0.8	"	"	2.9	2.54
10	"	1.25	0.35	0.45	"	0.25	0.17	"	"
11	"	7.3	0.7	1.15	1.85	0.35	0.24	3.0	2.63
12	"	8.1	0.3	0.6	0.9	0.6	0.41	1.9	1.68
13	"	9.3	0.45	0.2	0.65	1.0	0.69	4.1	3.59
8	90	0.15	0.45	0.05	0.50	0.15	0.10	2.0	1.75
9	"	1.2	0.3	0.65	0.95	"	"	2.5	2.19
10	"	1.9	0.3	"	"	0.3	0.21	2.3	2.01
11	"	7.1	0.35	0.75	1.10	0.45	0.31	3.0	2.63
12	"	9.1	0.3	1.0	1.3	0.4	0.28	4.2	3.68
13	"	12.55	0.35	1.1	1.45	0.5	0.35	5.2	4.56

4. Conclusion.

(1) The fermentation products from glucose by *Penicillium olivaceum* is studied.

(2) As the fermentation products, citric, gluconic and oxalic acids are isolated and identified.

(3) The yield of each acid from the medium containing 0~2% of peptone is also determined quantitatively.

(4) The volatile acid and alcohol are not produced.