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Nitrogen Metabolism of Penicillium-Chrysogenum - Q-176
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Nitrogen Metabolism of *Penicillium Chrysogenum* – Q 176

In the course of our studies on the mechanism of the biosynthesis of penicillin by *Penicillium chrysogenum*–Q 176, it became desirable to ascertain the day-to-day changes in the nature of the free amino acids present in the medium and the mycelium during the growth of the organism. The methods previously described were not considered very convenient in view of the very large number of estimations involved but an opportunity was provided by the recent development of the simple circular paper chromatographic technique in this department by GIRI and his co-workers[1] for rapid detection and estimation of amino acids.

[1] J. W. FOSTER, H. B. WOODRUFF, D. PERLMAN, L. F. MACDANIEL, B. J. WILKER, and D. HENDLIN, J. Bact. *51*, 465, 695 (1946). – P. E. HALPERN, D. SIMINOVITCH, and W. D. MACFARLANE, Science *102*, 230 (1945). – A. G. C. WHITE, L. O. KRAMPITZ, and C. H. WERKMAN, Arch. Biochem. *8*, 303 (1945). – R. P. COOK and M. B. BROWN, Nature *159*, 376 (1946). – S. G. KNIGHT and W. C. FRAZIER, Science *102*, 617 (1945).

Although many nutritional aspects of the growth of *P. notatum* and *P. chrysogenum*, particularly in relation to the production of penicillin have been described by numerous investigators[1], our knowledge of their nitrogen metabolism is still inadequate to formulate any clear-cut mechanism of biosynthesis of penicillin. That penicillin is not necessarily closely correlated with the growth of the mould has been shown by the observations that on certain media these fungi grow well yet produce negligible amounts of penicillin, but there can be no doubt that it is intimately connected with nitrogen metabolism in view of the various observations, e.g., the increased production of antibiotic by supplementation with some amino acids, viz., histidine, arginine, glutamic acid. In this connection a clear understanding of the

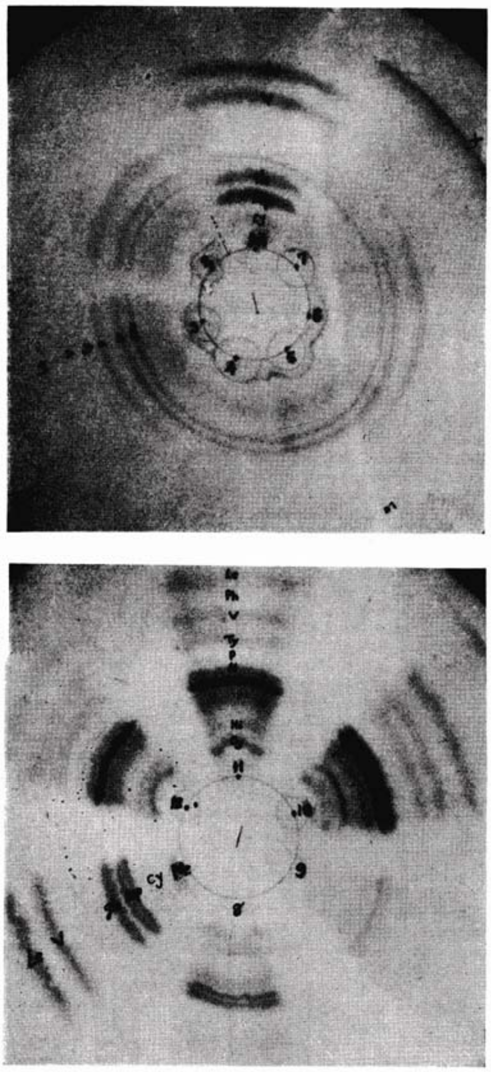

Fig. 1.

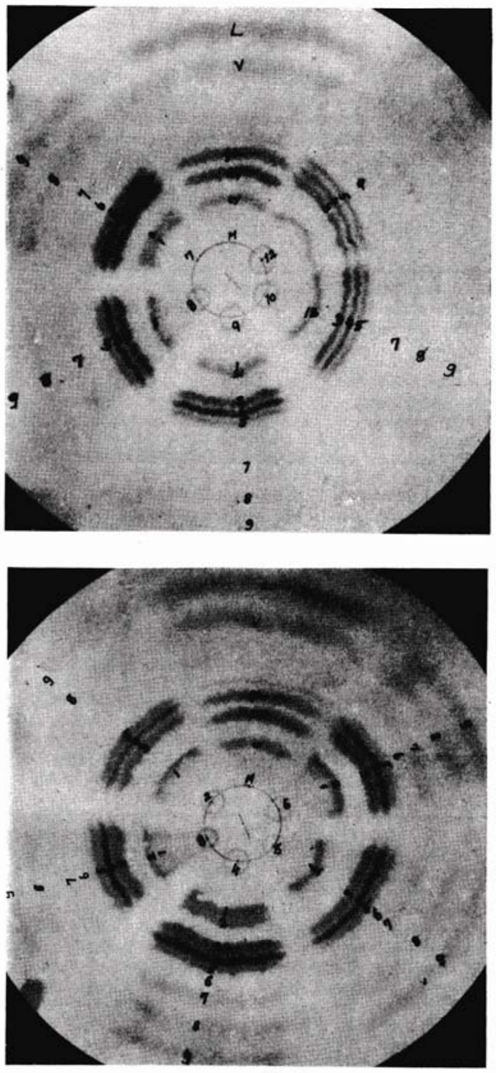

Fig. 2.

Circular paper chromatograms showing the changes in the amino acids present in the broth filtrate (Fig. 1) and the mycelium (Fig. 2) during the growth of *P. chrysogenum*–Q 176. The numericals in the centre circle indicate the day after inoculation. The mixture of known amino acids is spotted at *M*. *Cy* = cystine; *Asp* = asparagine; *Glu* = glutamic acid; *L* = leucine.

formation and utilisation of the amino acids by the mould appears desirable in order to arrive at a correlation of the synthesis of penicillin with nitrogen-metabolism. Earlier workers[1] have shown that many amino acids are synthesised by the mould but their study was based on the hydrolysis of the proteinous material present in the mycelium and no data is available on the continuous formation and utilization of amino acids *in situ* by the organism.

In a preliminary communication MACKENZIE and COOK[2] have reported the formation of glutamic acid followed by valine by *P. notatum*–1249 B 21 grown in surface cultures for 20 days.

Experimental: *P. chrysogenum*–Q 176 was grown in shake cultures (R.P.M. 90 ± 5) on chemically defined medium of JOHNSON and JARVIS[3] for 12 days. The mycelium and the culture fluid were separated every day and the distribution of free amino acids in both the fractions was determined chromatographically. The day-to-day changes in the amino acid pattern are shown in the chromatograms (Fig. 1 and 2). Table I shows the changes in the mycelial weight, pH and penicillin titre. Table II gives the amino acids identified.

There is a prominent band marked *X* in Figure 1 which appears to be due to penicillin in the solution. Besides, those mentioned in the table above there is evidence for the presence of two more unidentified amino acids.

Results: There appears to be mainly free amino acids present in the mycelium and broth filtrates in general agreement with the observations of MACKENZIE and COOK[1] on the nitrogen metabolism of *P. notatum*. Both the fractions contained considerable amounts of free amino acids even after 24 h. The early stages of growth (1–4 days) is marked by rapid accumulation of glutamic acid, alanine, proline, followed by leucine, valine, tyrosine and other amino acids. One of the striking observation made during this study is the rapid disappearance of virtually all amino acids at the peak period of formation of penicillin (5–8th day) in the broth filtrate while there does not appear to be correspondingly any marked variation in the mycelium. The changes in the proline content of the culture medium appears to be specially noteworthy. These facts lend support to the view that the formation of penicillin is intimately connected with the utilization of amino acids which have accumulated during the early stages of growth, thus confirming the findings of WOLF[2], on the rapid oxidation of many amino acids by 6½ days' old mycelial suspensions. The third stage, viz., 9th–12th day, is marked with the re-elaboration of the amino acids into the broth filtrate probably due to the breakdown of cellular material. The quantitative aspects are still under investigation. A full discussion of the significance of these findings will be published shortly.

Our sincere thanks are due to Prof. K. V. GIRI for his keen interest in the progress of these investigations.

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Zusammenfassung

Es wird über die täglichen Veränderungen in den freien Aminosäuren des Kulturfiltrats und des Myzels,

[1] Y. YOKOYAMA, J. Antibiotics (Japan) *4*, 95 (1951); C. A. *45*, 9130 (1951).

[2] R. M. MACKENZIE and R. P. COOK, J. Biochem. *50* [2], III (1951).

[3] M. J. JOHNSON and E. G. JARVIS, J. Amer. chem. Soc. *69*, 3010 (1946).

[1] L. c.

[2] F. T. WOLF, Arch. Biochem. *16*, 143 (1948).

Table I

Day after inoculation (counting day of inoculation as 1)	2	3	4	5	6	7	8	9	10	11	12
Mycelial weight (Wet) in g	0·13	0·26	0·54	0·63	0·65	0·80	0·85	0·85	0·82	0·85	0·83
pH	6·0	6·2	6·4	6·5	6·7	6·9	7·4	7·4	7·3	7·2	—
Penicillin titre in Oxford units/ml	—	—	32	40	64	160	220	180	160	—	—

Table II

Amino acid detected	Days after inoculation (counting day of inoculation as 1)																					
	2		3		4		5		6		7		8		9		10		11		12	
	C	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*	*C*	*My*
Cystine	±	+	+	+	+	++	+	+	+	+	−	+	+	+	?	+	+	+	++	..	+	+
Histidune . . .	−	−	−	+	−	++	−	+	−	+	−	+	+	−	+	+	+	+	+	..	+	−
Asparagine . .	+	−	±	−	+	−	+	−	−	−	−	−	+	−	−	−	+	−	+	..	+	−
Glutamic acid .	+	+	+	+	+	++	+	++	+	++	+	++	++	++	+	++	++	+	++	..	++	+
Proline	+	−	+	+	−	+	−	+	±	+	−	+	−	−	−	−	+	−	+	..	+	−
Alanine	+	+	+	++	+	++	+	++	+	++	+	++	++	++	+	++	++	+	++	..	+	+
Leucine	−	+	+	+	−	+	+	+	−	+	−	+	+	+	−	+	++	+	+	..	+	−
Valine	−	+	+	+	−	+	+	+	−	+	−	+	+	+	−	+	+	+	+	..	+	−
Tyrosine	−	−	+	−	−	+	−	+	−	+	−	+	+	+	−	+	+	+	+	..	+	−
Phenylalanine .	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	..	+	−

C = Culture Medium; *My* = Mycelium; + = present; ++ = Prominent; — = Absent; ± = Faint; ? = Doubtful; .. = not determined.