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NOTES

Impaired Penicillin Production in Lysine Regulatory Mutants of *Penicillium chrysogenum*[1]

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Lysine-regulatory mutants of *Penicillium chrysogenum* which excrete lysine were found to be deficient in production of penicillin. A revertant recovered the ability to produce penicillin.

Depression of pencillin formation by addition of L-lysine to cultures of *Penicillium chrysogenum* (3) provides an interesting example of the control of secondary metabolism by a primary metabolite. Such a phenomenon can be expected to occur whenever a primary and a secondary metabolite are end products of a branched pathway (4). A second example has been recently reported, i.e., the depression of candicidin formation by aromatic amino acids (7).

Although the lysine effect is of considerable academic interest, we have wondered whether it is of any practical significance in the production of penicillin. High exogenous concentrations (10 to 20 mM) of the amino acid are required to lower penicillin production under fermentation conditions, and commercial media certainly do not contain such levels of free lysine. However, endogenous production of L-lysine could possibly limit productivity. If such were the case, the phenomenon could have practical importance since the modification of intracellular lysine pools by environmental or genetic means could lead to increased penicillin production.

To determine whether endogenous lysine production regulates penicillin formation, we obtained lysine-regulatory mutants (i.e., lysine excretors) and studied their penicillin-producing abilities. An inverse relationship between biosynthesis of lysine and penicillin was found.

Three lysine analogues, *S*-(2-aminoethyl)-L-cysteine (Cyclo Chemicals, Los Angeles, Calif.), δ-hydroxylysine (Sigma Chemical Co., St. Louis, Mo.), and 4,5-dehydrolysine were used to select for lysine regulatory mutants. Conidia of *P. chrysogenum* Wis. 54-1255 were mutagenized for 12 h with 0.8% ethylmethane sulfonate (9) and were spread on Czapek agar plates. Crystals of lysine antimetabolites were placed in the center of each plate followed by incubation of the plates at 30 C. *S*-(2-aminoethyl)-L-cysteine and δ-hydroxylysine were used at 300 mg per plate, whereas 50 mg of 4,5-dehydrolysine was added to each plate. A zone of inhibition developed around each antimetabolite in 3 to 4 days. On further incubation, 102 colonies which appeared in these clear zones were isolated onto slants as antimetabolite-resistant mutants. Of the three antimetabolites tested, 4,5-dehydrolysine was the most toxic and δ-hydroxylysine was the least toxic. The mutants were then screened on agar for overproduction of lysine by the agar-piece technique (6), using *Leuconostoc mesenteroides* ATCC 8042 as the lysine assay organism. Eighty-one of the mutants were found to excrete lysine to some extent and were considered defective in regulation of lysine biosynthesis. The nine mutants which produced the largest zones of growth with *L. mesenteroides* ATCC 8042 were studied in liquid medium to examine their ability to produce extracellular lysine and penicillin. In addition to parental strain Wis. 54-1255, we used as controls nine randomly selected colonies from the same mutagenized population used to isolate the regulatory mutants. The chemically defined fermentation medium and methods used for fermentation and for the determination of penicillin, lysine, and dry mycelial weight were those previously reported (1, 2, 5, 8). Whereas the nine random cultures failed to excrete more lysine than parent strain Wis. 54-1255, two of the nine regulatory mutants markedly overpro-

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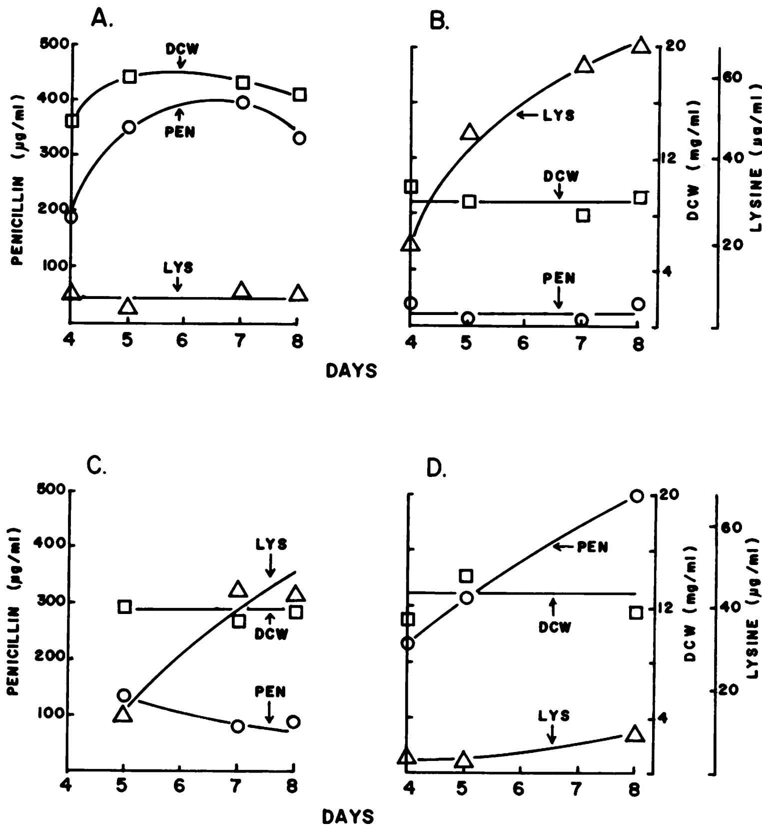

FIG. 1. *Growth, lysine production, and penicillin formation by strains of Penicillium chrysogenum. (A) 'arental strain Wis. 54-1255; (B) regulatory mutant R-23; (C) regulatory mutant R-1; (D) R-1-REV, a revertant f R-1. Abbreviations: LYS, lysine; PEN, penicillin; DCW, dry cell weight. Cultures were grown in chemically efined production medium and the flasks were removed periodically for the measurement of penicillin, lysine, nd the dry cell weight. The entire contents of a flask were sacrificed for each time point.*

luced the amino acid. These two mutants were he only isolates which were defective in penicilin formation. Figure 1 shows the fermentation lynamics of these two mutants (R-1 and R-23) is well as those of their parent. Also shown in he figure is the performance of a revertant of R-1 that no longer excretes lysine. Reversion back to lysine regulation was accompanied by a 'eturn to normal penicillin production.

Figure 2 summarizes the inverse relationship between overproduction of lysine and formation of penicillin. A clue to this relationship can be 'ound in our earlier results concerning the effect of lysine on the incorporation of [^{14}C]valine into penicillin by washed suspensions of *P. chrysogenum* Wis. 54-1255 (8). In those experiments, we added lysine either to the growth medium or to the [^{14}C]valine incorporation system. When added only to the growth medium, subsequent penicillin formation was stimulated. When lysine was added to the incorporation medium, penicillin production was markedly inhibited. Our working hypothesis (A. L. Demain, Lloydia, in press), is shown in Fig. 3. We assume that lysine causes feedback inhibition of the first step of the pathway (i.e., homocitrate synthase) and represses an enzyme after the

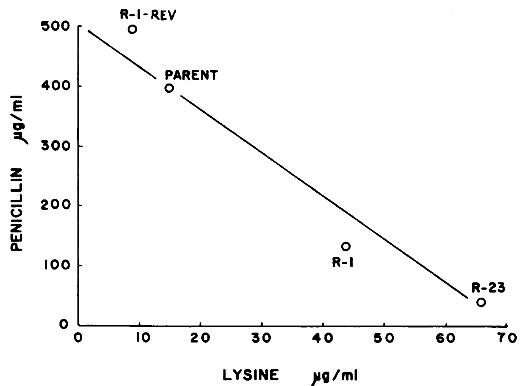

FIG. 2. *Inverse relation between lysine and penicillin production by strains of Penicillium chrysogenum. Figures represent the maximum production for each culture.*

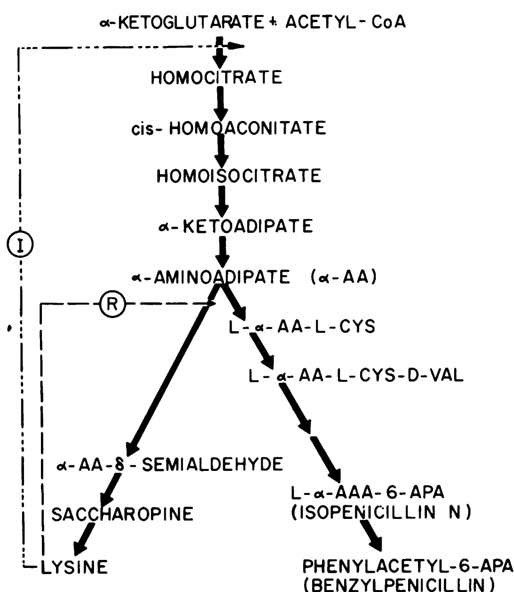

FIG. 3. *Postulated branched pathway to lysine and benzylpenicillin in Penicillium chrysogenum. R, Repression; I, inhibition.*

branchpoint. Feedback inhibition of homocitrate synthase would thus inhibit penicillin synthesis. Conversely, repression after the branchpoint could increase the pool of α-aminoadipic acid and stimulate formation when lysine is present solely during growth. We have provided evidence of in vivo inhibition of homocitrate synthase by lysine (A. L. Demain and P. S. Masurekar, J. Gen. Microbiol., in press) but the late repression control is still a matter of conjecture. If the working hypothesis is correct, it appears that the regulatory mutants obtained in this study have been derepressed after the branchpoint. Such an event would be expected to lead to lysine overproduction and penicillin underproduction. Whatever the mechanism involved, the present results point to an intimate intracellular relationship between biosynthesis of lysine and penicillin, a relationship which has practical significance. This relationship encourages our continued examination of lysine regulatory mutants in the hope of finding those mutants desensitized to lysine inhibition of homocitrate synthase and which may be superior producers of penicillin.

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