Penicillin Types Produced by *P. Chrysogenum* Q-176

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The organism *P. chrysogenum* Q-176 (*1*) has been widely used for the commercial production of penicillin. It has been recognized that this mold can produce several types of penicillin, although the literature (*4*) is not clear on the number of different penicillins actually produced.

It has now been found that Q-176, grown on the usual corn steep liquor-lactose medium apparently can produce at least 8 penicillins. These include the common types (*2*), namely, G-penicillin (benzylpenicillin), F-penicillin (Δ^2-pentenylpenicillin), what is probably dihydro-F-penicillin (n-amylpenicillin), K-penicillin (n-heptylpenicillin), and X-penicillin (p-hydroxybenzylpenicillin). In addition, three other penicillin types have been demonstrated to be produced in small amounts by this organism.

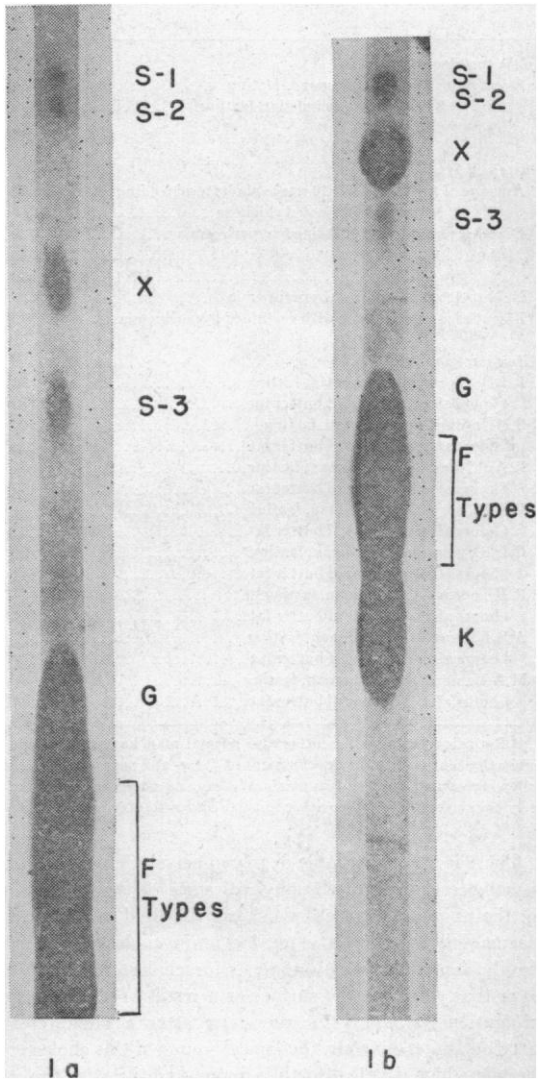
In proving the presence of the indicated penicillins, a paper partition chromatographic method similar to that described by Goodall and Levi (*3*) has been used. These workers obtained evidence for the existence of penicillins other than F (I), G (II), X (III), and K (IV), but did not state the origin of such penicillins.

In determining the types of penicillin present in Q-176 fermentation broth, the method of Goodall and Levi was modified. Thus, the chromatograms were developed at room temperature (about 24°C.), in a 2- to 4-hour period, using Whatman #4 paper strips buffered at pH 5.0 with 25 per cent phosphate buffer, and amyl acetate as the developing solvent. The fermentation broth was adjusted to pH 5.0 before taking an 0.008-ml. drop for analysis.

After developing the chromatograms, the strips were air dried and placed on FDA agar plates which had previously been seeded with *Staph. aureus* 209P. The plates were incubated at 37° C. for 18 hours.

In looking for penicillins which may be present in trace quantities in a fermentation broth, it was found necessary to remove the paper strips from the agar plates after termination of the period of bacterial growth. Upon removing the strips, one frequently notes the presence of zones of inhibition which were too small to extend beyond the covering paper strip; there is sufficient bacterial growth beneath the strip to delineate such zones clearly.

In Figs. 1a, 1b, and 2 are shown the results obtained using the paper chromatographic method of analysis as applied to 5-day-old *P. chrysogenum* Q-176 fermentation broth, the photographs being taken after removal of the paper strips from the agar plates. The organism Q-176 was grown in the usual corn steep liquor-lactose medium (4.5 per cent corn steep liquor by volume, 2 per cent lactose by weight) without addition of specific penicillin precursors.



FIGS. 1a and 1b: Chromatographic analysis of undiluted 5-day-old *P. chrysogenum* Q-176 fermentation broth.

In Fig. 1a two small, separate zones of inhibition, designated S-1 and S-2, can be noted. These zones are due to penicillin types not hitherto reported as being produced by Q-176. Proof that they are due to penicillin-type antibiotics will appear below.

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The third zone from the top has been shown to be due to penicillin X. This was accomplished by adding known penicillin X to a fermentation broth and then chromatographing. Only the third zone was enlarged by the added penicillin X (Fig. 1b). In this case the chromatogram was not developed sufficiently to show the complete separation of penicillins S-1 and S-2.

The fourth zone from the top, designated as S-3, represents a third new penicillin type.

The penicillin types S-1, S-2, X, and S-3 are followed on the chromatogram by a multiple connected zone of inhibition, part of which may be seen in Fig. 1a. It has been found that this multiple zone consists of at least four parts, due to four penicillins: G, F, possibly dihydro-F, and K.

In order to show the component parts of the connected zone, it is advisable to use a diluted fermentation broth; otherwise, there may be a marked overlapping of zones which makes it difficult to see the true nature of their multiplicity

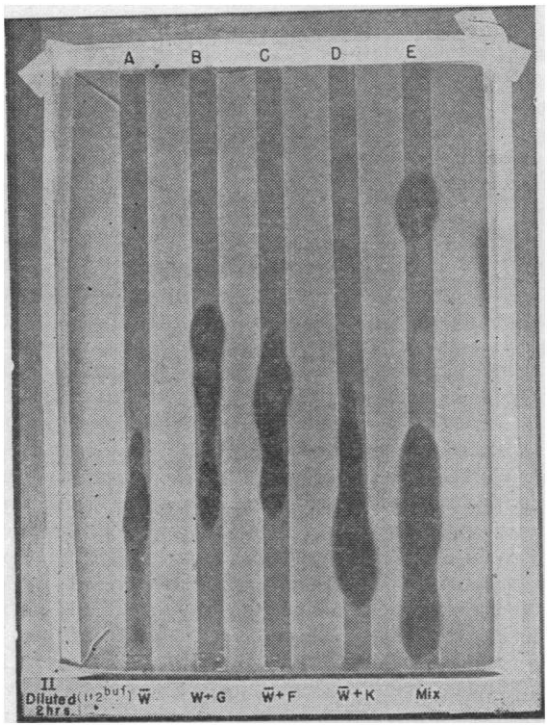

FIG. 2: Chromatographic analysis of 5-day-old *P. chrysogenum* Q-176 fermentation broth diluted to contain 75 units/ml.

Fig. 2 shows the results of an experiment designed to identify the component penicillins responsible for the connected zone of inhibition. In obtaining the results indicated in strip A, a sample of fermentation broth of Q-176 diluted to 75 units/ml. was chromatographed. Four zones, indicated by either separate ellipses or bulbous swelling formed by overlapping ellipses, show the presence of at least four different penicillin types.

These four zones were identified, by adding in turn to the same broth samples of pure, known penicillins, then chromatographing and observing which of the four zones was accentuated by the addition. The modified chromatograms so obtained revealed the identity of the penicillin types observed in the original chromatogram.

In this way, the first zone in chromatogram A of Fig. 2 was identified as being due to penicillin G (see modified strip B); the second zone was identified as being due to penicillin F (see modified strip C); the third zone was tentatively ascribed to dihydro-F, but without confirmation, because no unequivocal sample of dihydro-F was available for comparison; and the fourth zone was identified as being due to penicillin K (see modified strip D). It is to be noted that, due to some artifact, the K zone in strip A is smaller relative to the other zones than is usually the case. Thus, the K zone in strip B is more typical of the size and shape of the K zone relative to the other zones.

Strip E in Fig. 2, representing the results of chromatographic analysis of a mix of known penicillins X, G, F, and K, demonstrates the well-defined separation of the X zone and the overlapping tendency of the G, F, and K zones.

That all the zones observed in the paper chromatographic analysis of Q-176 broth are due to penicillin-type antibiotics is evidenced by the fact that these antibiotics are all destroyed by penicillinase.

It would appear, therefore, that *P. chrysogenum* Q-176 can produce no less than 8 penicillins when grown on the usual corn steep liquor-lactose medium. Four of these, S-1, S-2, X, and S-3, are produced in small quantities; in addition, four other well-known types, G, F, what is probably dihydro-F, and K, are also formed. The new penicillins S-1, S-2, and S-3 have not as yet been characterized chemically.

Addendum: Since submitting this paper, our attention has been called to a report (National Institute of Health release) presented by C. J. Salivar, V. V. Bogert, and E. V. Brown at the Conference on Antibiotic Research held in Washington, D. C., January 31 and February 1, 1947, under the auspices of the Antibiotics Study Section of the National Institute of Health. These workers have reported that Q-176 may produce Δ^2-pentenylpenicillin as well as possibly one or two additional penicillins which may be related to penicillin K. The method of analysis used by Salivar, *et al.* was a column chromatographic method supplemented by the Craig countercurrent distribution method, using ether as the solvent. We have found that the order in which the different penicillins appear in our paper chromatograms does not change whether ether or amyl acetate is used. Since the new penicillins we are reporting, namely, S-1, S-2, and S-3, have solubility characteristics more closely allied to penicillin X, they are probably not identical with those reported by Salivar, *et al.*, which are similar in solubility characteristics to F and K. Using the paper chromatographic method of analysis, only two F types and one K type of penicillin were observed. This may be due to the inability of the paper chromatogram to differentiate Δ^3-pentenylpenicillin and the new K-types suggested by Salivar, *et al.* It would seem, therefore, that according to our work and that of Salivar, *et al.*, Q-176 can produce as many as 11 penicillins.

References

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