# PENICILLIN AND POLYMYXIN EFFECTS ON THE CHROMOGENESIS OF *PSEUDOMONAS AERUGINOSA* STRAINS

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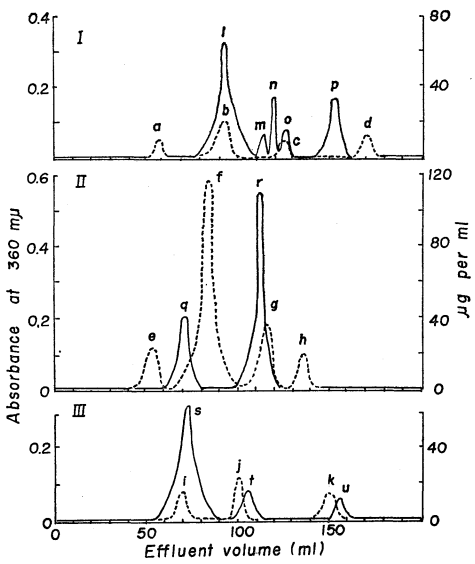
(Received for publication
December 8, 1970)

In the course of determining chromogenic variations of *Pseudomonas* species, some differences of pigmentation were observed[2]. Therefore, it seemed of interest to ascertain if these differences were caused by previous antibiotic treatment of the strains and if the effects depend on the antibacterial actions of some antibiotics. Some pigmentation differences are inherent to antibiotic sensitivity[5] and chromogenic variations have been observed in the presence of several antibiotics.[3,10]

In the course of our search, to pathogenic *Pseudomonas aeruginosa* strains (110 and 007 str.), isolated from bronchial infections and having different chromogenic patterns, were used. *P. aeruginosa* strains resistant to either penicillin or polymyxin were produced by either serial transfer on Penassay (Difco) medium with subinhibitory doses of drug

Fig. 1. Elution patterns, on Sephadex G-25F, of the different chromogene metabolites from the 007 (——) and 110 (·····) *Ps. aeruginosa* strains without antibiotics (I) and in presence of penicillin (II) and polymyxin (III). Letters on each peak represent fractions indicated in Table 1.




Figs. 2~7. Infrared spectra of purified chromogenes isolated from metabolic liquid of *Ps. aeruginosa* cultures either in the absence (Figs. 2 and 3) or in the presence of the indicated antibiotics.

Fig. 2.

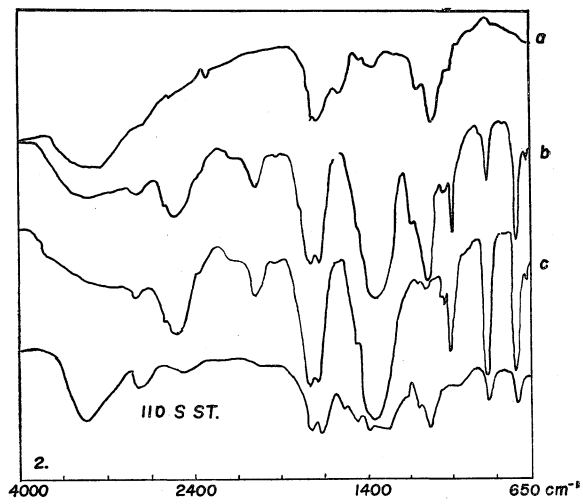
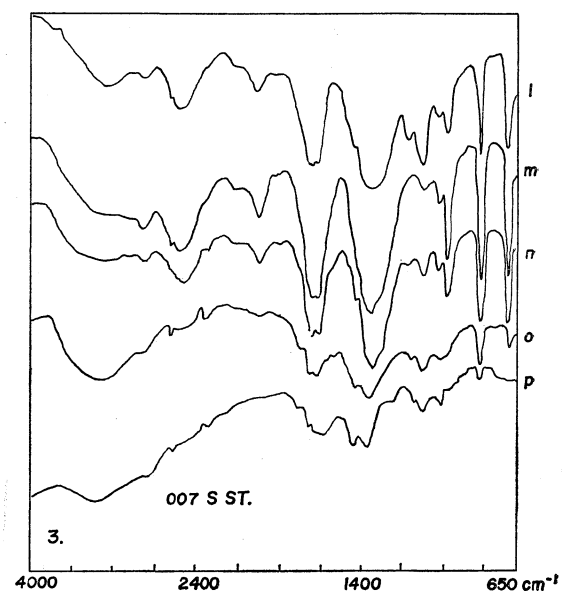



Fig. 3.



or by single exposure to higher antibiotic concentrations[8]; previously, minimal inhibitory concentration (MIC) were checked[9]. In all cases the mutants obtained were revertant types and their resistance levels went down in antibiotic-free media.

Therefore, throughout the experiments, NAVES' medium[7] was used with the sensitive bacteria while the resistant ones were cultivated on this medium with subinhibitory drug concentrations ($0.8 \times$MIC).

A standard inoculum of each strain was seeded into a series of individual 2-liter culture flask containing 400 ml of NAVES' me-

Fig. 4.

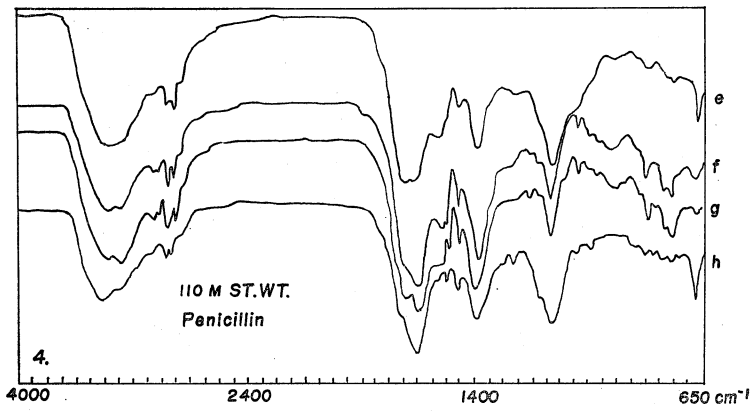



Fig. 5.

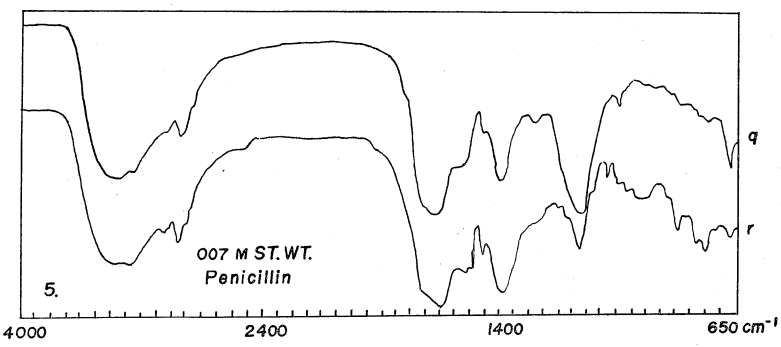



Table 1. Color and production of the different chromogene metabolites obtained from the strains used

| Culture conditions | | Chromatographic fractions eluted by Sephadex G-25F** | | | | | Total pigment*** |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | |
| *Ps. aeruginosa* 110 Str. | Without antibiotic | *a* Brown 0.27 | *b* Yellow 0.53 | *c* Brown reddish 0.35 | *d* Blue 0.35 | — | 0.016 |
| | With penicillin ($8\times10^3$ $\mu$g/ml) | *e* Yellow 1.6 | *f* Green yellow 17.6 | *g* Green 4.4 | *h* Pale blue 1.2 | — | 0.316 |
| | With polymyxin 37 $\mu$g/ml | *i* Yellow 0.5 | *j** Brown 1.0 | *k* Pale blue 0.35 | — | — | 0.048 |
| *Ps. aeruginosa* 007 Str. | Without antibiotic | *l* Green yellow 1.0 | *m* Pale pink 4.1 | *n* Pink yellow 1.2 | *o* Red pink 0.7 | *p* Blue 0.8 | 0.077 |
| | With penicillin ($8\times10^3$ $\mu$g/ml) | *q* Yellow 2.1 | *r* Brown yellow 12.3 | — | — | — | 0.219 |
| | With polymyxin 50 $\mu$g/ml | *s* Green yellow 2.2 | *t* Brown 0.9 | *u* Pale blue 0.3 | — | — | 0.040 |

* This pigment appears integrated by two fractions.
** In each case, letters indicate the relative position in the elution patterns and numbers correspond to mg/ml of the relative production rate on 2 ml of the metabolic liquid samples, previously concentrated.
*** Total pigment production in mg/ml of culture medium.

dium with and without antibiotics. After growth at 37°C for 48 hours, the culture fluid from the *Pseudomonas* was centrifuged and sterilized by passing through 0.22 $\mu$ Millipore filters and concentrated at 18~20°C in a Büchi vaccum rotary evaporator. Each concentrated specimen, with all the chromogene metabolites, was filtered through a Sephadex G-25F chromatographic column. Elution patterns at 360 m$\mu$ were determined in a Beckman spectrophotometer DB-G equipped with a liquid micro-aperture flow-cell (Model 97460) and the eluted fractions recovered using a Beckman automatic fraction colector. The different separated pigments were lyophilized and their weights determined before preparing the KBr pellets to study the IR spectra using a Perkin-Elmer model 257 spectrophotometer.

Fig. 6.

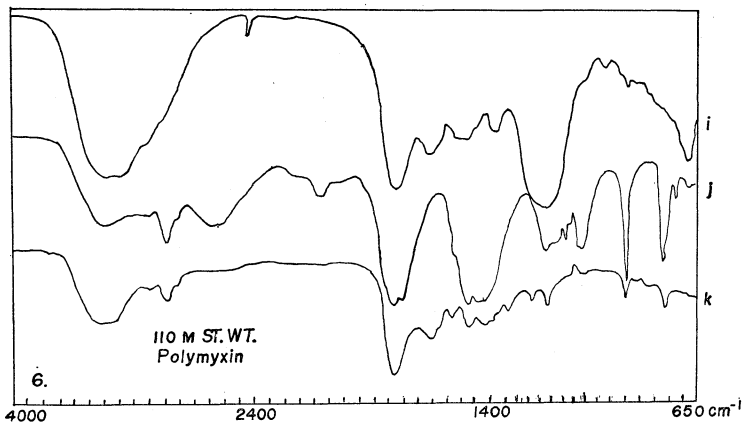
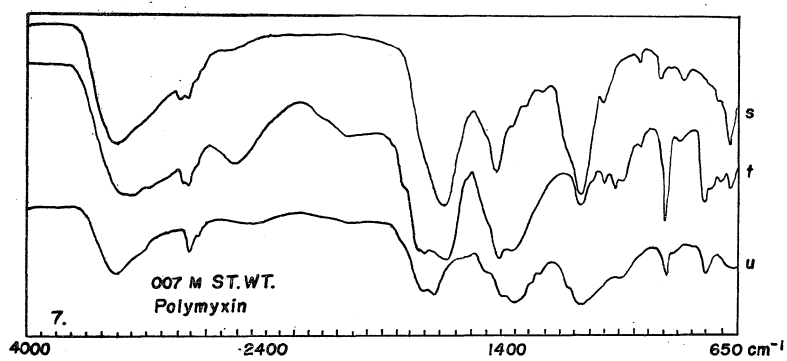



Fig. 7.




Comparative pigment production by the different *P. aeruginosa* normal and antibiotic resistant strains are shown in Table 1.

The results indicate that chromogenesis in the sensitive strains was inhibited by the presence of penicillin and polymyxin B in the media because only a faint trace of yellow pigmentation was observed ; this was not the case with the bacteria which had become antibiotic resistant "*in vitro*". In any case it may be observed that the pigment production was markedly modified when the *P. aeruginosa* was grown in media containing subinhibitory amounts of both antibiotics.

Fig. 1 shows the differences in elution patterns seem in experiments with normal and resistant *P. aeruginosa* strains. Furthermore, other molecular modifications are produced by penicillin and polymyxin B on the synthesized chromogenes as shown in Figs. 2~7 comparing their infrared specra in each case.

Four and five chromogenes, respectively, were distinguished from 110S and 007S *P. aeruginosa* strains. Their IR spectra revealed distinct molecular characteristics (Figs. 2 and 3). Identity of each substance was obtained by its characteristic frequencies in the molecule[1] and data are in agreement with previous reports.[4,6] The presence of different pyocyanine molecules (*d* and *p* fractions), acetamido-oxiphenoxazones (*b*, *c*), other oxi-phenoxazone compounds (*l*, *m*, *n*) and intermediate oxi-phenoxazonic substances not well identified (*a*, *o*) were detected.

In the case of *P. aeruginosa* penicillin resistant strains grown in the presence of this antibiotic, there is an increase in the production rate of total pigments accompanied by a change in their relative propor-

tions; there is a marked prevalence in unknown intermediate phenoxazonic compounds (*f*, *q*, *r*) and an increase of the oxiphenoxazone fractions (*e*, *g*) while phenazine molecules are totally inhibited or strongly modified (*h*).

Polymyxin B, in similar circumstances can increase or decrease the total pigments depending on the bacterial strains but in any case several strong molecular modifications of the phenazine (*k*, *u*) and phenoxazone (*i*, *j*, *s*, *t*) fractions are observed.

Acknowledgements

The authors wish to thank Pfizer, S. A. for the grant which has supported in part of this work.

We would like to thank Dr. VAZQUEZ for his valuable advices during the preparation of this manuscript. The technical assistance of Miss MA. T. ALDA and MA. L. DEL POZO is gratefully acknowledged.

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