STUDIES ON PIGMENTATION OF *SERRATIA MARCESCENS*

IV. ANALYSIS OF SYNTROPHIC PIGMENT

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Rizki (1954a) and Williams and Green (1956) have demonstrated that certain mutants produced by ultraviolet light irradiation of *Serratia marcescens* are capable of inducing red pigmentation in receptive colorless mutants of the same organism. This phenomenon has been termed syntrophic pigmentation (Williams and Green, 1956). Spectral analyses carried out on syntrophic pigment extracted from mixed cultures suggested that the induced pigment was similar to the red pigment, prodigiosin, of the wild-type parent organism (Rizki, 1954b). Williams et al. (1956) established that prodigiosin could be separated into four fractions by paper chromatography. The spectrum of the combined pigment fractions was identical to that of unfraccionated prodigiosin as published by Hubbard and Rimington (1950). In order to confirm the identity of syntrophic and natural pigment, it was thought necessary to investigate the chromatographic as well as the spectral properties of the induced pigment.

EXPERIMENTAL METHODS

The parent, wild-type strain employed in this investigation was *S. marcescens* strain Nima. An orange and a white variant were produced from the parent strain by ultraviolet light irradiation. The orange variant (Williams and Green, 1956) is capable of inducing red pigmentation in the white variant.

For the production of syntrophic pigment, the orange and white variants were grown together for 7 days at 27°C on the medium described by Williams et al. (1956). The organisms were harvested, washed by centrifugation, and then pigment was extracted from the mixed syntrophic red and orange organisms. This pigment will be referred to as fed-white pigment. Nima, orange, and white organisms were grown individually under identical conditions, and subjected to the same extraction procedure in order to obtain material for pigment comparisons. The methods employed for pigment extraction, paper chromatography, and spectrophotometry were identical to those previously described (Williams et al., 1956). Equal parts of ethyl ether and petroleum ether were used as the solvent for the paper chromatogram presented in this report.

RESULTS

Figure 1 is a circular paper chromatogram prepared with material extracted from the four different cultures. The paper was divided into four sectors, and the extracted material from each culture was placed at the origin within its own sector. The entire chromatogram was developed in the ethyl ether-petroleum ether solvent. The R_f values refer to those given in figure 1 of a previous report (Williams et al., 1956).

From an examination of figure 1 it is apparent that the white organism contains no pigment when grown by itself, and that the orange variant contains pigment which moves as a single band in the ethyl ether-petroleum ether solvent. However, when the orange and white organisms are grown together, the fed-white pigment extracted from the mixture exhibits four distinct bands when chromatographed. Three of these bands, the orange, and the two reds, have R_f values identical to the same colored pigment fractions present in the wild-type red pigment. Additional evidence establishing the identity of the orange and red bands present in the fed-white and wild-type pigments was provided by spectrophotometric analyses. The individual orange band and the combined red bands of the pigment fractions were cut from the paper chromatogram, and eluted with absolute ethanol. Spectral analyses carried out under acid, alkaline, and neutral conditions demonstrated that the spectra of the

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fractions present in the fed-white pigment were identical to those of the wild-type pigment (Williams et al., 1956).

The fourth band present on the chromatogram of the fed-white pigment was a slow-moving fraction which possessed an Rf value intermediate between the blue fraction of wild-type pigment and the pigment of the orange variant. This band was eluted from the chromatogram with chloroform and unsuccessful attempts were made to subject the eluant to further paper chromatography in order to separate the pigment into possible blue and orange fractions. No solvent system was discovered which could effect a clean separation. Spectrophotometric analysis resolved the difficulty of pigment separation and demonstrated that the slow-moving band consisted of both blue and orange pigment. Spectrophotometric identification of mixed blue and orange variant pigments was possible because the two substances exhibit different peaks in the visible spectrum when the pigments are examined in acid solution. The blue fraction of wild-type pigment has a peak in acid solution at 540 μm (Williams et al., 1956), whereas the orange variant pigment has a sharp peak under the same conditions at 500 μm (Williams and Green, 1956). Figure 2 illustrates that under acid conditions the spectral curve of the slow-moving band of the fed-white pigment contains peaks at both 500 μm and 540 μm. These facts demonstrate that the band contains both the blue fraction of wild-type pigment and the orange variant pigment.

**DISCUSSION**

Efforts to extract from orange variant cultures a substance that will induce red pigmentation in the white variant have been unsuccessful. The
The presence of orange variant pigment in material extracted from cultures of mixed orange and white organisms indicates that the orange pigment itself is not changed into natural pigment. It seems likely that the orange organism produces a diffusible substance which induces red pigmentation in the white organism. The relationship between the diffusible substance and the production of syntrophic pigment has been discussed in a previous report (Williams and Green, 1956). This report also contains data indicating that the orange variant pigment is a different substance from the orange fraction of natural and syntrophic pigment.

Santer and Vogel (1956) have isolated from a prodigiosin deficient mutant of \textit{S. marcescens} a substance capable of inducing red pigmentation in another colorless mutant. They state that the induced pigment has the same \( R_f \) value and visible absorption spectrum as authentic prodigiosin. Their report contains no information relative to what fractions might be present in the induced red pigment other than the statement that the major pigment produced was prodigiosin. The results of the present investigation demonstrate that the pigment induced in the white variant by the orange organism is identical to natural prodigiosin and contains the same four fractions.

**SUMMARY**

Syntrophic prodigiosin produced by growing together inducing orange variant organisms with susceptible white variant organisms has been demonstrated to be identical with the pigment of the wild-type organism and to consist of the same four fractions. The orange and the two red fractions of syntrophic pigment were chromatographically and spectrophotometrically identical to the same fractions of wild-type prodigiosin. The blue fraction of syntrophic pigment was admixed with pigment from the orange variant, but could be identified by spectrophotometric methods. When grown by itself, the white variant organism contained no extractable pigment.

**REFERENCES**


Rizki, M. T. M. 1954b. The nature of the pig-

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Figure 2. Spectral properties of slow-moving band eluted from paper chromatogram of fed-white pigment. Curve obtained in absolute ethanol + HCl. Arrows denote spectral peaks at 500 \( \mu \) and 540 \( \mu \).

