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EFFECT OF PRODIGIOSIN ON RESPIRATION OF SERRATIA MARCESCENS

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Studies of the factors influencing the pigmentation of *Serratia marcescens* have been numerous. Recently Linnane and Still (1953) observed that unsaturated fatty acids stimulate pigmentation while Harned (1954) has developed techniques for producing prodigiosin in submerged culture. Weinberg (1951) found that glutamic acid suppressed pigmentation, but the suppression was neutralized by penicillin and bacitracin.

The non-homogeneity of pigmented material produced by these bacteria has been demonstrated by Williams *et al.*, (1956a) who resolved an acetone extract chromatographically into orange, red, and blue fractions. A subsequent study by the same group (Williams et al., 1956b) has encouraged the belief that the blue fraction is a dimer of red components binding iron. Earlier Kost (1942) postulated an iron-pigment-protein complex and suggested in addition the probability of a respiratory role for the complex.

Experiments described in this paper were undertaken to determine if pigmented extracts may exert a stimulatory effect on the respiration of suspensions of pigmented and pigmentless *S. marcescens* and on the rate of oxidation of several carbohydrates.

Materials and Methods

Bacteria. The pigmented strain of *S. marcescens* was used in a previous investigation (Payne *et al.*, 1953). The non-pigmented strain was that used by Smith and Johnson (1954) and kindly provided by the latter author.

Production and extraction of pigment. For the production of prodigiosin in quantity the pigmented strain was cultured on trypticase soy agar in large enamel pans. The pans were sterilized with paper covers and the tempered, sterile agar was added aseptically. Diluted broth cultures were spread evenly over the surface of the agar and the cultures incubated for 48 hr. at 25-30 C.

Deeply pigmented cells were scraped from the surface with bent glass rods and extracted with acid alcohol. The extract was filtered through paper and sintered glass and left to evaporate at room temp. The residue was taken up in ether and washed repeatedly with several changes of distilled water in a separatory funnel. Anhydrous Na_2SO_4 was added to dry the ether fraction after which it was decanted and again evaporated at room temp.

The solution used in the respiratory studies was prepared by adding to the residue from the evaporated ether only enough ethanol to give a saturated solution. An aliquot was diluted 1:100 with water. Residual HCl was neutralized with NaOH in the course of dilution. A lightly tinted, purplish-red solution resulted which was saturated by dint of the diluteness of the organic solvent.

The absorption spectrum of the pigmented extract was determined by diluting 1 ml of the saturated alcoholic solution 1:100 with ethanol. Readings were made over the interval from 200 to 600 microns using a Beckman Spectrophotometer, Model DU.

Respirometry. The pigmented and the colorless strains were cultivated in trypticase soy broth until the pigmented bacteria, growing as a pellicle, became barely pink at 18-20 hr. at room temp. Packed cells were twice washed with water and suspended in M/15 phosphate buffer at pH 6.8. Each manometer cup received 2 ml of cells (10 mg. dry wt.). The side arms were charged with 1 ml of the solution of pigment or 1 ml of water containing 1 ml of ethanol per 100 ml. The cells were equilibrated at 26C for 30 min. before readings were taken of the rate of endogenous respiration.

In a previous report (Payne and Kieber, 1954) it was noted that mannose, fructose, gluconic acid or ribose induced pigmentation of *S. marcescens* when provided as a sole source of carbon on washed agar, mineral salts media. The effect of prodigiosin on the rates of oxidation of these substrates was determined using vessels with two side arms-pigment or dilute ethanol being tipped in from the first and 1 ml of M/50 substrate from the other.

Results

The curves in Figure 1 indicate that the addition of prodigiosin to resting cells of a normally chromogenic strain of *S. marcescens* results in an increase in the rate of endogenous respiration. Addition of pigment to suspensions of cells of the non-pigmented strain did not result in stimulation. The endogenous QO_2 for the non-pigmented cells was 5.5 while the QO_2 of the endogenous plus pigment was 4.9. An extract of non-pigmented cells prepared in the same manner as the pigmented extract did not change the rate of endogenous respiration of cells of either strain. The curve in Figure 2 indicates that the acid alcohol extract showed peak absorption in the visual range at 550 microns and at 275 microns in the ultraviolet.

The data in Table 1 indicate that gluconic acid and ribose were oxidized more rapidly by pigment-producing cells in the presence of prodigiosin than the stimulation of the endogenous respiration would account for. Non-pigmented cells oxidized the substrates less rapidly in the presence of prodigiosin than in its

absence. No increase was observed in the QO_2 for mannose or fructose above that induced by the pigment.

Discussion

It appears that cells harvested at a time when synthesis of pigment was incipient were stimulated by additional prodigiosin, while cells adapted to living without pigmentation were not equipped to utilize the pigment. These findings are not in agreement with those of Tarantino (1939) who found that 24-36 hr. non-pigmented cells harvested from broth were stimulated by an ether-acetone extract of pigment. However, neither washed cells nor washed pigment were used for that study.

It would seem illogical to assume that stimulatory factors other than pigment may have been operative in our study. If this were true the non-chromogenic cells should have been stimulated, or the extract of non-pigmented cells should have increased the respiratory rate of both strains.

The absorption spectrum presented in Figure 2 is very like that for an acidified acetone extract described by Williams *et al.*, (1956a). The maxima are slightly removed toward the longer wave lengths reflecting perhaps the differences in extraction solvents or degree of acidification.

If prodigiosin or an iron-pigment complex prove to be involved in the oxidation of gluconic acid or its intermediates, as the data in Table 1 suggest, then presumably more than one pathway would exist. Two aerobic pathways have been demonstated for *Pseudomonas fluorescens* by Lenhoff *et al.*, (1956). Little is known of the intermediary metabolism of *Serratia*. Wasserman *et al.*, (1956) have shown that suspensions of glucose broth-grown *S. marcescens* oxidize glucose to gluconic acid to 2-keto-gluconic acid without phosphorylation. On the other hand, DeLey and Vandamme (1955) noted that extracts of *S. plymouthicum* phosphorylated 2-keto-gluconate at C_6.

TABLE 1

Effect of prodigiosin on rate of oxidation of substrates by *Serratia marcescens*

	QO_2 Chromogenic strain		QO_2 Non-pigmented strain	
Substrate	Without pigment*	With pigment**	Without pigment	With pigment
Gluconic acid	16.8	20.4	10.9	10.3
Ribose	3.3	5.5	6.6	5.6
Mannose	12.1	12.4	—	—
Fructose	9.8	10.2	—	—

* Values for endogenous respiration subtracted.

** Values for respiration of endogenous plus pigment subtracted.

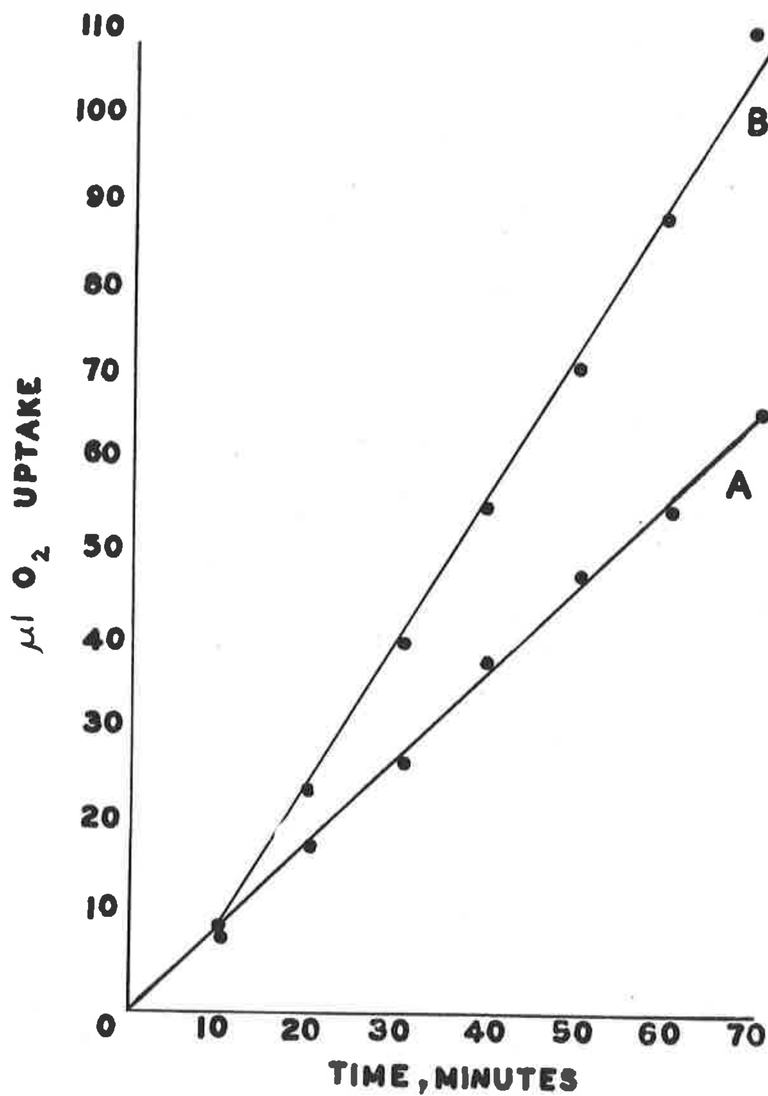

Figure 1. Effect of cellular extracts on the endogenous respiration of pigment producing *Serratia marcescens*. A. Endogenous or added extract of pigmentless cells. B. Prodigiosin added.

Disregarding the effects of the pigment, differences were noted in the oxidative metabolism of pigment producing and non-pigment producing strains. For example, the non-pigmented cells were able to begin an immediate, linear oxidation of ribose while the pigment producing cells were unable to oxidize the pentose for 30 minutes without the addition of pigment. Gluconic acid was oxidized less rapidly by the leuco-

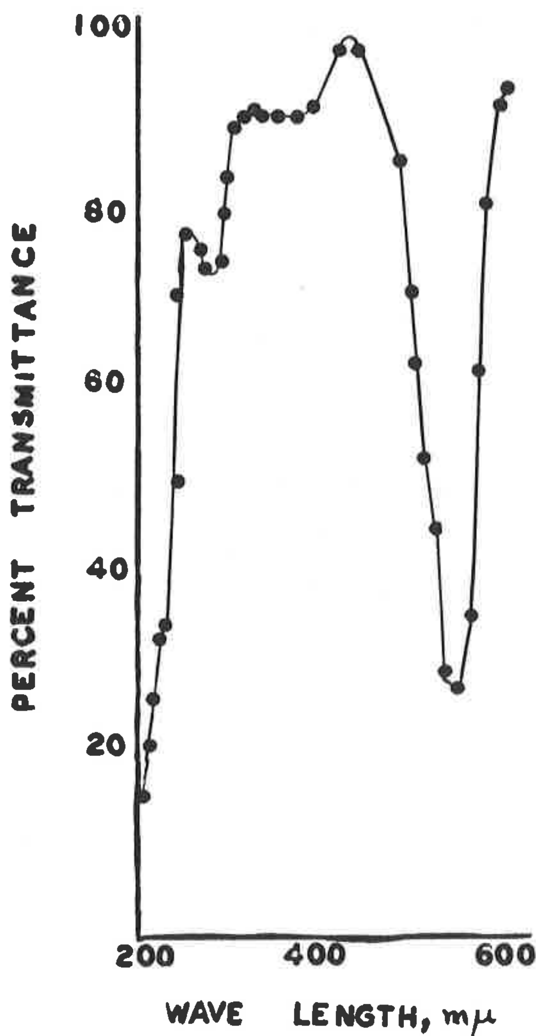

Figure 2. Absorption spectrum for prodigiosin in ethanol following extraction with acid alcohol.

strain. Classification of members of the Serrateae depends to a great extent on chromogenesis. Differences in the metabolic profiles of red and white varieties might be of some value taxonomically.

Summary

An acid alcohol extract of prodigiosin was found to increase the rate of endogenous respiration of 18 hr, pigment producing *Serratia marcescens* but not that of a pigmentless strain. An extract of colorless cells was inactive. The absorption spectrum of the pigment in ethanol closely resembled several previously reported.

The rates of oxidation of gluconic acid and ribose by chromogenic, but not non-pigmented, cells were increased by the pigmented extract more than the increase in endogenous respiration would account for. The rate of oxidation of mannose and fructose by color producing cells was increased by the pigmented extract no more than was the endogenous respiration.

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