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Requirement of Potassium or Rubidium for Biosynthesis of Pigment by *Serratia marcescens*[1]

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All bacteria appear to require potassium ion (K^+) for growth, although it has been shown that rubidium ion (Rb^+) can usually spare or completely replace this requirement [E. E. Snell, p. 547, in J. H. Yoe and H. J. Koch (ed.) *Trace Analysis*, John Wiley & Sons, Inc., New York, 1957]. In this paper, we report the apparently complete replacement of the need for K^+ by Rb^+ for pigment biosynthesis by *Serratia marcescens*. The pathway of pigment formation appears complex (D. A. Morrison, J. Bacteriol. **91**:1599, 1966), and it was considered possible that the substitution might lead to variations in the relative proportions of pigment intermediates.

A pigmented strain of *S. marcescens* was used in this study. All glassware was repeatedly autoclaved in distilled water before use; the filter paper used was washed in three changes of distilled water. Cells were washed and resuspended in basal medium before inoculation. The basal medium (Table 1) was a modification of a synthetic medium previously used for metabolic studies of marine bacteria (R. A. MacLeod and E. Onofrey, J. Bacteriol. **71**:661, 1956). Sterilization was accomplished by membrane filtration. K^+ or Rb^+, as 0.1 M solutions of potassium or rubidium chloride in basal medium, were added as required. The K^+ content of the basal medium, as determined by flame photometry performed by B. von Spindler of the Department of Soil Science, University of British Columbia, did not exceed 1.5×10^{-5} M.

To determine the quantitative K^+ or Rb^+ requirements for pigment synthesis, cells were grown on 5.5-cm Whatman no. 1 filter-paper discs placed in the bottoms of 250-ml Erlenmeyer flasks. The filter paper was saturated with medium which had previously been adjusted to the required K^+ or Rb^+ concentration and then inoculated with cells. The flasks were tightly stoppered and incubated in the dark at 25 C for 24 hr. Pigment synthesis was not detectable until the concentration of either K^+ or Rb^+ was raised to approximately 4.8×10^{-4} M although growth was observed at 1.2×10^{-4} M.

TABLE 1. *Basal medium for determination of the K^+ or Rb^+ requirement for growth and pigment synthesis*

Ingredient[a]	Wt (g/liter of medium)
NaCl	5.7
$MgCl_2 \cdot 6H_2O$	2.7
$NaSO_4$	0.9
$CaCl_2$	0.3
Na_2HPO_4	0.2
$NaHCO_3$	0.05
NaBr	0.02
$SrCl_2$	0.008
H_3BO_3	0.006
$FeSO_4 \cdot 7H_2O$	0.005
Glucose	3.0
DL-α-Alanine	2.4
L-Glutamic acid	1.2
DL-Aspartic acid	0.96
1.0 N NaOH	to *p*H 7.0

[a] Reagent-grade materials obtained from Fisher Scientific Co., Pittsburgh, Pa., were used except as noted below. Reagent-grade glucose was obtained from Allied Chemical Canada, Ltd., Montreal, Que., Canada, and the amino acids were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Requirements for K^+ or Rb^+ were met by addition from 0.1 M stock solutions. RbCl of 99.9% purity was obtained from K & K Laboratories, Inc., Jamaica, N.Y.

For pigment analysis, cells were grown in 1-liter flasks containing 100 ml of basal medium brought to 10^{-3} M in either K^+ or Rb^+. The flasks were plugged with cotton and incubated in the dark at 25 C for 72 hr. Cells were then harvested and pigments were extracted from equal wet

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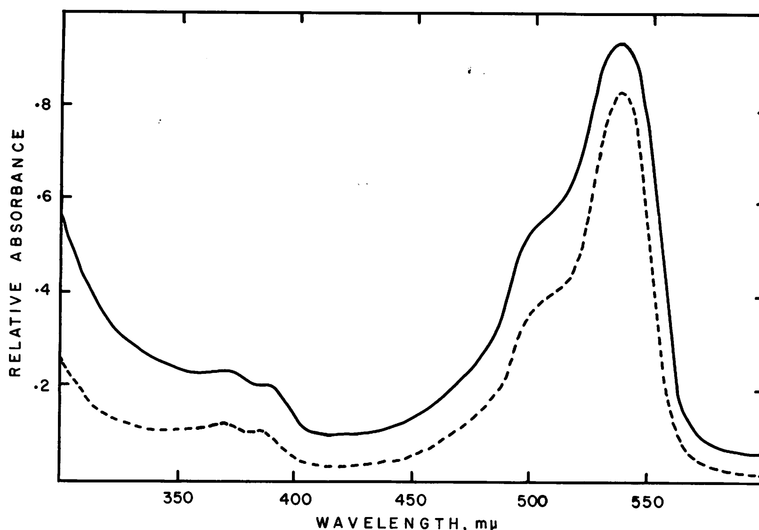

FIG. 1. *Spectrophotometric analysis of pigment extracted from cells grown in* 10^{-3} M K^+ *(solid line) or* 10^{-3} M Rb^+ *(dotted line).*

weights of cells grown under either condition. The extraction method used was the acetone to petroleum ether procedure (R. P. Williams et al., J. Bacteriol. **71**:115, 1956). These extracts were first dried and then each one was redissolved in a mixture of anhydrous ethyl alcohol and 1 N hydrochloric acid (9:1). Spectrophotometric analyses were performed on appropriate dilutions of these solutions, with the Bausch & Lomb Spectronic 505 spectrophotometer. The absorption maxima for either preparation occurred at 537, 387, and 368 mμ, and both preparations had a shoulder at 504 mμ (Fig. 1).

Equal portions of each extract were then further separated by descending paper chromatography using an ether-petroleum ether (1:2) solvent system. The ratio of red pigment (prodigiosin) at R_F 0.7 to orange pigment at R_F 0.9, obtained under either condition, was found to be approximately the same (Table 2). As expected, small quantities of two additional components, a blue pigment at R_F 0.2 and a second red pigment at R_F 0.5, were also noted in both preparations.

TABLE 2. *Optical densities (OD), in absolute ethyl alcohol-HCl, of the relative quantities of red and orange pigment fractions separated from extracts of Serratia marcescens*[a]

Ions added	Red pigment (OD at 537 mμ)	Orange pigment (OD at 504 mμ)
K^+ supplement	0.84	0.05
Rb^+ supplement	0.80	0.04

[a] Grown in the presence of 10^{-3} M K^+ or Rb^+.

All of the various reactions carried out by the cells during pigment biosynthesis, as indicated by the composition and ratios of the final products, remained essentially the same when Rb^+ was substituted for K^+ in the growth medium. Therefore, Rb^+ probably completely replaces the requirement for K^+ in the biosynthesis of prodigiosin by *S. marcescens*.

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