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Genetic Mapping of a Locus Which Regulates the Production of Pigment Associated with Spores of *Bacillus subtilis*

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A map of gene locations in *Bacillus subtilis* was constructed by D. Dubnau et al. (J. Mol. Biol. **11**:476, 1967) on the basis of density transfer procedures and transduction with bacteriophage PBS1. However the map is confined to genetic markers for purine, pyrimidine, amino acid, and ribonucleic acid biosyntheses and to markers for cellular resistance to antibiotics and other inhibitors. More recently, M. Rogolsky and J. Spizizen (*unpublished data*) have added to the map numerous loci which control spore formation. This note reports the mapping of a genetic site which regulates the production of a dark-brown pigment associated with the spores of sporulating colonies of *B. subtilis* on specific media. The color of the pigment depends upon the Mn^{++} content of the sporulation agar, and it becomes more intense with increased Mn^{++} (E. Iichinska, Mikrobiologiya **29**:145, 1960). Colonies unable to sporulate are also unable to produce pigment so that the pigment appears to be dependent on spore formation. However, colonies (*pig*⁻) which consist of viable, heat-resistant spores can lack pigment and have been called albinos by P. Schaeffer and H. Ionesco (Compt. Rend. **251**:3125, 1960).

The chromosomal localization of the *pig* marker was accomplished by transduction with PBS1 phage. Donor cells for transduction were shaken for 3 hr in Penassay Broth (Difco) at 37 C and then mixed with PBS1 phage so that 10 ml of Penassay Broth contained 5×10^7 cells per ml and 5×10^6 plaque-forming units per ml. The resulting mixture was shaken 1 hr at 37 C, chloramphenicol was added to a final concentration of 5 μg/ml, and shaking was continued for 2 more hr. After sitting overnight at 37 C, the donor lysate was treated with deoxyribonuclease (50 μg/ml) and passed through a Millipore filter. Recipients for transduction were streaked into a few drops of 0.3% soft agar on Tryptose Blood Agar Base (Difco) to allow motile cells to swarm. PBS1 adsorbs only to motile cells (T. M. Joys, J. Bacteriol. **90**:1575, 1965). After 24 hr of incubation at 37 C, a loopful of the cells, which swarmed on the soft agar, was inoculated into 3 ml of Penassay Broth and shaken for 5 hr at 37 C. A 0.5-ml amount of the motile recipient cells was combined with 0.5 ml of the donor lysate and shaken at 37 C for 30 min. The mixture was then centrifuged at $4,000 \times g$, the cells were resuspended in 1 ml of Spizizen minimal medium, and 0.1 ml of the latter was spread onto Spizizen minimal agar fortified with 10^{-4} M $MgCl_2$, selecting for one of the recipient markers. Recombinant colonies with the *pig*⁻ marker appeared white among dark-brown *pig*⁺ types after 3 to 5 days of incubation at 37 C. The nomenclature and origin of the established map markers used below are identical to those used by D. Dubnau et al. (J. Mol. Biol. **11**:476, 1967). Albino mutants Y1 and 18TB were gifts from P. Schaeffer and CH from J.

TABLE 1. *Joint transduction of the pig and pur B6 markers*[a]

Unselected donor marker[b]	Selected marker	Total no. of *pur*⁺ recombinants	Total no. of white *pig*⁻ recombinants	Cotransfer (%)
pig Y1 (Schaeffer)	*pur* B6	965	729	76
pig 18TB (Schaeffer)	*pur* B6	572	418	73
pig UT683....	*pur* B6	580	436	75
pig UT682....	*pur* B6	631	473	75
pig UT686....	*pur* B6	790	608	77
Wild type.....	*pur* B6	530	0	—

[a] Donor strains were defective only in their ability to produce pigment; the recipient strain was defective only in purine biosynthesis and required either adenine or guanine to grow in minimal medium.

[b] No *pig* marker cotransduced with either *ery* or *thr*.

Szulmajster. All other albino strains were isolated as spontaneous mutants in this laboratory.

All the markers composing the present map of the *B. subtilis* genome, except *pur* B6, were ordered into four separate linkage groups, defined by cotransduction with PBS1; *pur* B6 unable to cotransduce with any other marker was excluded from a linkage group but was placed between groups I and II as a result of its behavior during density transfer (D. Dubnau et al., J. Mol. Biol. **11**:476, 1967). Table 1 illustrates that *pig* markers from five different albino strains cotransduce with *pur* B6. An approximate 75% cotransfer of *pur* B6 and *pig* separates the two markers by 25 map units. Since *pig* cotransduces with neither *ery* nor *thr*, it was not possible to bridge the gap between *pur* B6 and either linkage group I or linkage group II. A sixth *pig* marker, CH (Szulmajster), did not cotransduce with any other map marker tested; however, this mutant was leaky and produced pigment after prolonged incubation. In a transduction between a wild-type donor and a *pur* B6$^-$, *pig* Y1$^-$ recipient, 72% of the *pur*$^+$ recombinants were *pig*$^+$.

The *pig* marker is important because it represents a genetic locus which is linked to *pur* B6 and which is repressed until many hours after both cell division and deoxyribonucleic acid synthesis cease. Pigment production does not begin until the final stages of sporogenesis. Refractile sporangia were observed in cells of *B. subtilis* colonies which produced no pigment until 6 to 12 hr of further incubation. Since the genes for sporogenesis are scattered over the entire length of the *B. subtilis* chromosome (M. Rogolsky and J. Spizizen, unpublished *data*), an attempt is being made to determine whether *pig* markers may also occupy additional chromosomal sites.

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