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P S Hoffman and J O Falkinham 3rd


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Induction of Tryptophanase in Short Cells and Swarm Cells of *Proteus vulgaris*

PAUL S. HOFFMAN* AND JOSEPH O. FALKINHAM III

Department of Biology, Memphis State University, Memphis, Tennessee 38152, and Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

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Tryptophanase was noninducible in swarm cells of *Proteus vulgaris* despite transport of the inducer tryptophan. Further, cyclic AMP, which stimulated increased levels of tryptophanase in short cells, had no effect on swarm cells.

*Proteus vulgaris* undergoes a morphological change from short, sparsely flagellated cells to elongated, extensively flagellated swarm cells when grown on suitable solid media. Swarm cells are capable of migrating across the surface of most laboratory media, making *Proteus* spp. nuisances when other clinically important bacteria are being isolated. Despite numerous descriptive studies of the swarming phenomenon (for review, see reference 6), little has been reported concerning the physiology and genetics of this process, due largely to the difficulties encountered in obtaining sufficient quantities of swarm cells, which form only on solid media. The regulatory mechanisms controlling the various events in the swarm cycle are not understood, although Williams et al. (5) have concluded that a chemotaxis-related mechanism is unlikely. Williams and Schwarzhoff (6) have suggested that swarm cell development be considered a separate event from migration. In mature swarm cells, which are multinucleated and without septa (6), replication of DNA and synthesis of protein and cell wall material appear to have stopped (4). In addition, swarm cells appear to use endogenous energy reserves during migration (5). This conclusion is supported by the observation that swarm cells, when transferred to a nonnutrient (agar-water) medium containing a surfactant to decrease surface tension, continue to migrate over the medium. Thus, swarm cells might be considered vectors for carrying genetic material from an unfavorable to a more favorable environment. In this regard, we decided to test the possibility that the multiple genomes in swarm cells might be repressed for transcription. The inducible enzyme tryptophanase (EC 4.1.99.1) was monitored in swarm cells and in short cells of *P. vulgaris* in the presence of the inducer tryptophan. Further, the effect of cyclic AMP was also examined.

Swarm cells normally do not migrate on vitamin-free (tryptophan-deficient) casein hydrolysate (CH) medium (Nutritional Biochemicals, Inc.). However, the addition of Triton X-100 or Tween 80 to this medium permits swarm cells to migrate over the surface (5). CH medium contained, per liter, the following: vitamin-free CH, 10 g; 10 mM potassium phosphate (pH 7.0); 1 mM MgSO₄·7H₂O; and nicotinic acid, 50 mg. For experiments requiring tryptophan, 0.5 g/liter was used. Solid medium was prepared by the addition of 1.5% agar, and Triton X-100 was added at approximately 40 μg/ml. Short cells were grown on CH broth and harvested during the late logarithmic phase. Swarm cells were harvested by cutting away the nonswarming central portion of the CH agar and then washing the swarm cells off the surface with 50 mM potassium phosphate buffer (pH 6.8). The washings were pooled and centrifuged once (a similar procedure was used for short cells), suspended in induction medium (CH plus tryptophan), and incubated at 30°C. Samples (5 ml) were removed at various intervals, and the turbidity was determined with a Klett-Summerson colorimeter (blue filter, 420 nm). The cells were then centrifuged and suspended in 5 ml of 50 mM potassium phosphate buffer (pH 7.8), and 1.5 ml of this suspension was incubated with 0.5 ml of a 50 mM tryptophan solution for 5 min. The reaction was stopped by the addition of Ehrlich reagent (3). Tubes were allowed to stand at room temperature for 20 min to permit full color development, and the concentration of indole was determined spectrophotometrically at 565 nm by comparison with a standard curve. As seen in Fig. 1, tryptophanase induction occurred within 2 min in uninduced short cells, and maximal levels were observed within 20 min. Whereas short cells were readily inducible, swarm cells were not. In the presence of tryptophan, tryptophanase activity remained very low and nearly constant for 1 hour, with a gradual increase observed after 1 h. This increase corresponded with an increase in the number of short cells in
the suspension of swarm cells, as determined by microscopic examination. The swarm cell population used in this study was initially 90 to 95% swarm cells, but after 1 h, this amount had decreased to 80 to 85%. Swarm cells harvested from CH agar supplemented with tryptophan had levels of tryptophanase activity similar to those of swarm cells harvested from CH agar without tryptophan. Short cells harvested from the central portion of the CH agar supplemented with tryptophan had nearly maximal levels of tryptophanase activity. Crude cell-free extracts prepared by passage of swarm cells through a French pressure cell also had very low tryptophanase activity (0.23 μg of indole per min per mg of protein), as compared with induced short cells (17.2 μg of indole per min per mg of protein).

Several reports have suggested that membrane alterations might occur during swarm cell development (1, 4). Thus, it is possible that such membrane changes could affect tryptophan uptake and consequently tryptophanase activity. To test this possibility, we measured the transport of [3H]tryptophan with short and swarm cells grown on agar medium containing tryptophan. Short cells and swarm cells were collected as previously described with uptake medium. The uptake medium contained, per liter, the following: K2HPO4, 10.5 g; KH2PO4, 4.5 g; Na3 citrate·2H2O, 0.5 g; (NH4)2SO4, 1.0 g; MgSO4· 7H2O, 0.1 g; nicotinic acid, 7.5 mg; and chloramphenicol, 15 mg. Cells were washed twice in uptake medium, suspended to a final density of 0.3 mg (dry weight) per ml, incubated for 10 min at 30°C, and then stored on ice until used. To a 2-ml sample of cells warmed to 30°C, 0.02 ml of [3H]tryptophan (7 Ci/mmol) was added (final tryptophan concentration, 10⁻⁶ M). The suspension was mixed, and at 30-s intervals, 0.1 ml of the sample was withdrawn and filtered through 0.45-μm cellulose-acetate filters (Millipore Corp.) and washed twice with 2 ml of ice-cold uptake medium. The filters were dried, suspended in scintillation fluid, and counted in a liquid scintillation counter.

The results indicate that the transport of tryptophan was decreased in swarm cells (to 39% of that of short cells). Total tryptophan uptake in short cells was 0.077 nmol/mg (dry weight) per min, and that in swarm cells was 0.030 nmol/mg (dry weight) per min. This decrease was similar to that reported by Armitage et al. (2) for proline transport and suggests a general defect in transport in swarm cells. However, this decrease in the rate of tryptophan transport was not enough to result in the lack of tryptophanase induction when the high-induction concentration (0.02 M) was used.

Because it had been reported that swarm cells have lower concentrations of cyclic AMP, we investigated whether 1 mM cyclic AMP could restore tryptophanase induction to swarm cells. Although the addition of 1 mM cyclic AMP to suspensions of short cells increased the uninduced and induced levels of tryptophanase, cyclic AMP did not enable swarm cells to produce tryptophanase in the presence of an inducer.

The results of this study support the idea that swarm cells may serve as vehicles for carrying genetic information to a more favorable environment. It is reasonable to assume that considerable energy is required for migration and that it would be to the advantage of cells to repress...
nonessential energy-requiring activities. Although induction of tryptophanase could not be demonstrated in swarm cells, this does not rule out the possibility that selected transcriptional and translational events occur during swarm cell migration. We believe that tryptophanase could be a useful marker for studying the regulatory processes involved in the development of swarm cells as well as in the division of swarm cells to the short cell morphology again.

LITERATURE CITED