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Unique Developmental Characteristics of the Swarm and Short Cells of *Proteus vulgaris* and *Proteus mirabilis*

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Swarming cells of *Proteus mirabilis* and *Proteus vulgaris* could be distinguished from their short-cell counterparts by virtue of their synthesis (or lack of synthesis) of certain enzymes and outer membrane proteins. Urease synthesis was constitutive in swarm cells and uninducible in short cells. In contrast, phenylalanine deaminase was inducible in both short and swarm cells, demonstrating that transcriptional and translational processes were functional. During swarm cell development, the amount of one outer membrane protein (45 kilodaltons) fell and the amounts of two others (50 and 28.3 kilodaltons) rose significantly, the level of cytochrome *b* decreased, and the syntheses of cytochromes *a* and *d* were repressed. Respiratory activities of swarm cells were greatly diminished, suggesting that energy for swarming came from fermentation rather than from respiration. Widespread changes in the pattern of enzyme activities, in cytochrome composition, and in the composition and type of outer membrane proteins suggest that they are due to transcriptional regulation.

The swarming phenomenon associated with some members of the genus *Proteus* (*P. mirabilis* and *P. vulgaris*) has intrigued investigators since first reported by Hauser (9), yet the underlying mechanisms regulating this process remain elusive. Lominski and Lendrum (14) proposed chemotaxis as a mechanism for swarming, but recently Williams et al. (28) presented convincing evidence against this. The swarm cycle observed on solid media generally involves development of swarm cells from short, sparsely flagellated cells (short cells) followed by outward migration of the swarm cells over the agar surface. Masses of swarm cells (80 μm in length and profusely flagellated) move over the surface for a period of several hours, followed by consolidation (division into short cells), and this cycle is repeated until the surface of the medium is completely covered. Although several descriptive studies have appeared (for a review, see reference 29), studies into the genetics and biochemistry of the phenomenon have not followed. Moreover, there is presently no good working hypothesis that might explain swarming. In our view, swarming should be considered as an example of procaryotic differentiation. In this regard, stage 1 would involve those signals and receptors responsible for triggering swarm cell formation, stage 2 would involve the coordination of cellular processes to support the outward migration, and stage 3 would involve the signals and receptors responsible for termination of swarming and initiation of cell division.

In a previous study of swarm and short cells of *P. vulgaris* (10), we reported that the enzyme tryptophanase was not inducible in swarm cells. Because failure of swarm cells to induce this enzyme was not due to a block in transport of the amino acid or to lack of cyclic AMP, we sought to determine whether other enzymes also showed differing patterns of induction or expression in the two *Proteus* spp. cell types. The objectives of such a study were twofold. First, we sought to identify marker activities unique to one of the two cell types which could be used for studies of the induction of swarm cell formation, since microscopic observation is neither reproducible nor sensitive. In addition to identifying differences in enzyme activities, we have looked for changes in membrane proteins, respiratory capabilities, and cytochrome composition in swarm and short cells. Second, if there were widespread changes in enzymes, membrane proteins, or cytochromes between swarm and short cells, they might indicate general alterations in the pattern of transcription in short and swarm cells, as had been observed in differentiating cells of *Bacillus subtilis* (6), *Myxococcus xanthus* (20), and *Arthrobacter crystallopoietes* (18).

MATERIALS AND METHODS

Bacterial strains. *P. vulgaris* PV and *P. mirabilis* PM were obtained from the stock culture collection of Iowa State University, Ames, Iowa, and the F-*lac*$^+$ derivative of *P. mirabilis*, PM-1 (30), was obtained from Dennis Kopecko of the Walter Reed Army Institute of Research, Washington, D.C., and used for measurement of β-galactosidase activity.

Growth and isolation of short and swarm cells. Swarm and short cells were grown on either casein hydrolysate or phosphate-buffered Casitone (CPB) agar media. Casein hydrolysate medium contained (per liter): vitamin-free casein hydrolysate (Nutritional Biochemicals Corp.), 10 g; 10 mM potassium phosphate (pH 7.0); 1 mM $MgSO_4$; and nicotinic acid, 50 mg. Swarm cells do not migrate on casein hydrolysate agar unless 40 μg of Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) is added per ml to reduce surface tension in addition to 1.5% (wt/vol) agar. CPB medium contained (per liter): Casitone (Difco Laboratories, Detroit, Mich.), 10 g; 0.09 M NaCl; 10 mM potassium phosphate (pH 7.0); 1 mM $MgSO_4$; and nicotinic acid, 10 mg. Solid CPB agar medium contained 1.5% agar. After swarming had commenced, swarm cells were harvested from either casein hydrolysate or CPB agar medium by cutting out the central nonswarming, short cell-containing portion of the agar medium and washing the remaining surface with 50 mM potassium phosphate buffer (pH 7.0) to remove the swarm cells. The cells from a number of plates were pooled, centrifuged at 10,000 × *g* for 10 min, washed twice, and suspended in medium suitable for enzyme induction or membrane isolation. Short cells in the central portion of the agar plates were isolated by

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swirling agar plugs in buffer, followed by centrifugation and washing as described for swarm cells. Short cells grown in CPB broth were harvested in mid- to late-log phase and treated as described for agar-grown cells. Isolated cell suspensions (swarm or short cells) were judged by microscopic examination to be morphologically over 90% pure. Both were motile.

Enzyme assays. β-Galactosidase activity of whole cells of strain PM-1 was assayed by the method of Miller (19). Phenylalanine deaminase activity was also measured in whole cells by following the formation of phenylpyruvate as described by Ben-Hamida and Le Minor (5). Urease activity of whole cells was measured by determining the amount of ammonia formed enzymatically from urea by the phenol-hypochlorite method of Russell (22). Values for enzyme activities were normalized by measurement of either cell dry weight or protein. Protein was measured by the method of Lowry et al. (15).

Isolation and characterization of outer membrane proteins. *P. vulgaris* PV was grown on CPB agar medium. Short and swarm cells, isolated as described above, were washed and suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer (Calbiochem-Behring, La Jolla, Calif.) (pH 7.4). Outer membrane fraction was recovered by Triton X-100 extraction as described by Schnaitman (23). Polyacrylamide gel electrophoresis of the outer membrane fraction was performed as described by Lugtenberg et al. (16). Coomassie blue-stained gels were scanned with a Helena Labs Quick Scan Jr. densitometer.

Cytochromes and respiration rates. Membrane vesicles were prepared from short and swarm cells by two passages through a French pressure cell (20,000 lb/in^2), followed by a low-speed centrifugation to remove cell debris (10,000 × *g*) and then ultracentrifugation at 100,000 × *g* for 90 min to sediment the membrane vesicles. Cytochrome content of membrane vesicles was measured by difference spectroscopy, using an Aminco DW-2 spectrophotometer (American Instrument Co., Inc., Silver Spring, Md.). In this method, the contents of the sample cuvette were reduced with a few crystals of sodium dithionite, and the contents of the reference cuvette were oxidized with either oxygen or potassium ferricyanide. The types of cytochromes and their concentrations were determined as described elsewhere (11, 12).

Respiration rates were determined by monitoring oxygen consumption with an oxygen electrode system. To a 1.35-ml cuvette housed in a waterjacket (30°C) was added 50 mM potassium phosphate buffer (pH 7.0), membrane vesicles, and substrates such as NADH (1 mM), malate (2 mM), or succinate (5 mM). Respiration rates were determined with a YSI oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio), interfaced to a microcomputer. Reduction of triphenyl tetrazolium chloride (TTC) by short cells and swarm cells was determined with whole cells grown on CPB medium supplemented with 0.01% (wt/vol) TTC. Washed cells were also examined for their ability to reduce TTC. Protein concentration was estimated by the biuret method (7).

RESULTS

Enzyme activities of short and swarm cells. β-Galactosidase activity was constitutive in the F-*lac*$^+$ derivative of *P. mirabilis* PM-1 (Table 1), as observed previously by Wohlhieter et al. (30). However, as previously documented for tryptophanase in *P. vulgaris* (10) and shown in Table 1 for comparison, the relative activity of β-galactosidase in short cells of *P. mirabilis* PM-1 was approximately fivefold higher than that observed in swarm cells. Phenylalanine deaminase activity was inducible in both short and swarm cells of *P. vulgaris* and *P. mirabilis*. Interestingly, higher activities were achieved with swarm cells, despite reports that swarm cells may be biosynthetically dormant (2, 4, 10). By contrast, urease activity was negligible in short cells of both *Proteus* species, regardless of whether the cells were obtained from broth or from agar medium. Urease activity was constitutive in swarm cells. The slightly higher values for urease activity after 30 min of induction (urea) were not considered significant. Urea was not present in the CPB medium used for collection of swarm cells. Neither swarm nor short cells of either species of *Proteus* had arginase activity measurable by the techniques of Hagan and Dallam (8) or Ward and Srere (27).

Changes in outer membrane proteins. Appreciable differences were observed in the amounts of major outer membrane proteins in short and swarm cells of *P. vulgaris* PV (Table 2). Short cells typically exhibited three major outer membrane proteins corresponding to molecular weights of 45,000, 40,000, and 36,400, with a minor outer membrane protein at 34,000. In outer membrane preparations from swarm cells, the 36.4- and 34-kilodalton (kd) proteins (probably porin proteins) were unchanged, whereas the 45-kd protein seen in preparations from short cells decreased to trace levels in swarm cells (16.5-fold decrease). Interesting-

TABLE 1. Enzyme activities of short and swarm cells of *Proteus* spp.

Proteus sp. and type of cells	Activity of the following enzyme:					
	β-Galactosidase[a] (constitutive)	Tryptophanase[b] (inducible)	Phenylalanine deaminase[c] (inducible)		Urease[d] (constitutive)	
			0 min	30 min	0 min	30 min
P. vulgaris						
Short	NT[e]	60 ± 1	<0.01 ± 0.01	0.11 ± 0.01	<0.1 ± 0.1	<0.1 ± 0.1
Swarm	NT	1.0 ± 0.5	<0.01 ± 0.01	0.21 ± 0.02	16.1 ± 1.7	17.8 ± 3.4
P. mirabilis						
Short	5,850 ± 237	NT	<0.01 ± 0.01	0.12 ± 0.01	0.4 ± 0.4	<0.1 ± 0.1
Swarm	1,360 ± 93	NT	0.03 ± 0.01	0.25 ± 0.02	18.5 ± 1.0	21.1 ± 0.9

[a] Expressed as units per milligram of protein ± range of duplicate determinations.
[b] Expressed as micrograms of indole produced per minute per milligram (dry weight) (10).
[c] Expressed as millimoles of phenylpyruvate produced per milligram of protein ± range of duplicate measurements.
[d] Expresed as micrograms of ammonia liberated per milligram of protein ± range of duplicate measurements.
[e] NT, Not tested; activity absent in both cell types.

ly, two outer membrane proteins present in trace amounts in short cells increased dramatically in swarm cells: the 50-kd (8-fold) and 28.3-kd (12-fold) proteins.

Cytochromes and respiratory activities. An examination of the cytochrome content of membrane vesicles from short and swarm cells of *P. vulgaris* also revealed differences in type and level (Table 3). Short cells contained high levels of cytochromes of the *b*, *a*, and *d* types. Membrane vesicles from swarm cells contained diminished levels of *b*-type cytochromes and were deficient in cytochromes of the *a* and *d* types. No differences were seen with either ferricyanide or oxygen as oxidant in this study. Consistent with decreases in levels and types of cytochromes in swarm cells was a lower respiration rate. Respiration rates with NADH, malate, or succinate were lower in membrane vesicles from swarm cells than in those from short cells (Table 4). The activity of NADH dehydrogenase decreased by a factor of one-third; the other respiratory activities of swarm cells were decreased to a greater extent. Swarm cells also failed to reduce TTC, suggesting possible decreases in flavoproteins or flavin adenine dinucleotide. This result is consistent with low succinate dehydrogenase activity observed with membrane vesicles from swarm cells. Short cells readily reduced TTC regardless of whether cells were harvested from agar or from broth. Short cells on agar medium seeded with TTC readily reduced TTC, whereas the swarm cells migrating over the agar surface failed to reduce the dye. No attempt was made to quantify the reduction of TTC for either cell type.

DISCUSSION

Because it is possible to separate short and swarm cells of *P. mirabilis* and *P. vulgaris* with over 90% efficiency (due to the use of strains which swarm across the entire surface of suitable media without reverting to short cells), it has been possible to identify enzymatic activities unique to each cell type. In an earlier study (10), we identified tryptophanase as a useful marker for short cells because it was uninducible in swarm cells. By contrast, urease was constitutive and present in high levels in swarm cells and was uninducible and of marginal activity in short cells. The possibility that the short cells, collected from the center of the growth medium, were devoid of urease activity because they were in stationary phase was ruled unlikely by the finding that broth-grown short cells also had low or undetectable levels of urease activity (data not shown). Further, the fact that short cells are motile, produce β-galactosidase, induce tryptophanase (10), and transport phenylalanine (2) argues against the possibility that their lack of urease activity is due to their nongrowing state.

Urease may not be under strong repression in short cells since Senior reported urease activity in short cells of *P. mirabilis* cultured in nutrient broth supplemented with urea (24). It should be pointed out that measurable levels of urease activity were not seen until 1 h or later in induction experiments, and the levels reported were relatively low in comparison with the specific activities reported for swarm cells. Although not described in this study, urease repression in short cells can be overcome by culturing *P. vulgaris* on a glucose-minimal salts medium with urea as the sole source of nitrogen. On that same medium, urease activity was constitutive in swarm cells. Thus, it appears that urease repression is abolished in swarm cells.

The induction of phenylalanine deaminase by swarm cells is even more striking in light of the decreased transport of this amino acid by swarm cells (2). This enzyme was inducible in both short and swarm cells and appeared to be regulated differently from tryptophanase and urease. Moreover, the finding of an inducible enzyme in swarm cells reinforces the view that swarm cells are not metabolically dormant. The induction of phenylalanine deaminase and the constitutive formation of urease in swarm cells verifies that the machinery of transcription and translation is functional. Our findings that tryptophanase is uninducible, that urease is constitutive, and that phenylalanine deaminase is inducible suggest that selective transcription occurs in swarm cells. However, transcription and translation are not necessary during the migration of swarm cells on agar since migration proceeds normally on medium supplemented with rifampin and chloramphenicol (3; unpublished data), though division of swarm cells leading to the short-cell morphology is inhibited in the presence of these antibiotics, as described by Armitage et al. (3).

The changes in enzyme activities also suggest that they are due to a response to a common regulatory signal mediated through changes in transcription. Alterations in the pattern of RNA transcripts and in RNA polymerase have been demonstrated in differentiating cells of *B. subtilis* (6) and *M. xanthus* (20). The contention that changes in transcription in

TABLE 2. Outer membrane protein differences of short and swarm cells of *P. vulgaris*

Apparent protein mol wt[a] (10^3)	Amt of protein (densitometer units)[b]		Short/swarm ratio
	Short	Swarm	
64.5	3.6	9.8	0.36
50	5.0	38.0	0.13
45	16.5	<1.0	>16.5
40	23.5	41.0	0.57
36.4	77.0	77.0	1.0
34	7.5	7.2	1.04
28.3	3.5	41.0	0.08

[a] Molecular weights were determined by comparison with mobilities of standards.

[b] Amount of protein is expressed in arbitrary densitometer units of area under peaks, with identical amounts of outer membrane fractions applied to gel.

TABLE 3. Cytochrome content of membranes isolated from short and swarm cells of *P. vulgaris*

Cytochrome	Cytochrome concn[a]		Short/swarm ratio
	Short cells	Swarm cells	
b (560 nm)	0.518	0.15	3.45
o (558 nm)	0.116	.057	2.04
a_1 (600 nm)	0.043	<0.001	>43.
d (630 nm)	0.42	<0.001	>420.

[a] Expressed as nanomoles per milligram of protein.

TABLE 4. Respiration rates for short and swarm cells of *P. vulgaris*

Substrate	Oxygen consumption[a]		Short/swarm ratio
	Short cells	Swarm cells	
NADH	340	230	1.48
Malate	11	2	5.5
Succinate	95	21	4.52

[a] Expressed as nanomoles of O_2 consumed per milligram of protein per minute.

swarm cells are responsible for the changes in the pattern of enzyme activities and outer membrane protein composition is supported by the evidence that a fraction of rifampin-resistant mutants of either *P. vulgaris* or *P. mirabilis* are incapable of swarming (data not shown). Similar evidence has been found and correlated with changes in DNA-dependent RNA polymerase in *M. xanthus* (21) and *B. subtilis* (26). Though the data suggest that swarm cell formation involves changes in transcription, we still have no clue to the identity of the inducer of swarm cell formation.

The diminished respiratory activity of swarm cells, decreased cytochrome content of membrane vesicles prepared from swarm cells, and absence of TTC reduction by swarm cells compared with short cells raise questions concerning the source of energy during swarm cell motility across the agar surface. At 30°C, swarm cells take 12 to 18 h to migrate to the edge of the agar medium. The energy for swarming is probably derived from fermentation, since swarming also occurs under strict anaerobic conditions and aerobically in the presence of 2 mM sodium cyanide to the same extent as observed aerobically (data not shown). Williams (personal communication) has suggested from preliminary work that an intracellular storage compound composed of glucuronic acid serves as an energy source. In *Pseudomonas aeruginosa*, anaerobic motility can be driven by arginine catabolism (1), and it is possible that the induction of urease and amino acid decarboxylases and deaminases by swarm cells of *P. vulgaris* and *P. mirabilis* also provides the necessary ATP to generate a *trans* membrane proton potential and drive motility (13, 17, 25). Clearly, the energy source is intracellular because exogenous energy sources are not required for swarm cell migration over agar surfaces (29).

Swarming species of *Proteus* are regarded as common causes of urinary tract infections, and urease is considered a virulence factor (24). Our finding that urease is maximally produced in swarm cells may implicate this cell type in urinary pathogenesis. In light of the significance of urease in urinary pathogenesis, the role of swarm cells in urinary tract infections should be reexamined.

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