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Murein Components Rescue Developmental Sporulation of *Myxococcus xanthus*

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Murein (peptidoglycan) components are able to rescue sporulation in certain sporulation-defective mutants of *Myxococcus xanthus*. *N*-Acetylglucosamine, *N*-acetylmuramic acid, diaminopimelic acid, and D-alanine each increase the number of spores produced by SpoC mutants. When all four components are included they have a synergistic effect, raising the number of spores produced by SpoC mutants to the wild-type level. Murein-rescued spores are resistant to heat and sonic oscillation and germinate when plated on a nutrient-rich medium. They appear to be identical to fruiting body spores in their ultrastructure, in their protein composition, and in their resistance to boiling sodium dodecyl sulfate. Murein rescue of sporulation, like fruiting body sporulation, requires high cell density, a low nutrient level, and a solid surface.

Myxobacteria exhibit multicellular development that depends on communication among cells (12, 17). When *Myxococcus xanthus* is plated at a high cell density on a low-nutrient agar, then tens of thousands of cells move to a central location where they build a spore-filled fruiting body. There is evidence for at least five intercellular signals in the development of *M. xanthus*. Adenosine is excreted during development and may be one signal, because when its secretion is inhibited, fruiting bodies do not form unless adenosine is supplied exogenously (15). In addition there are four groups of nonsporulating mutants, each of which can sporulate when mixed with wild-type cells (8). The four groups behave as if defective in the production of different signals because members of each group sporulate when mixed with members of the other three groups, but not with members of their own group. These phenotypic complementations can occur through a membrane filter (LaRossa et al., submitted for publication), and adenosine will not rescue any of the groups.

Murein or its components, added to growing *M. xanthus* cells, induce a pattern of coordinated cell movement that normally occurs during fruiting body development (16). Here we show that the addition of the same murein components bypasses the developmental defect in all members of one group of nonsporulating mutants (group C). Development that is rescued by the addition of murein components appears normal in the sense that the myxospores formed are identical in structure to fruiting body spores. Moreover, the critical fruiting conditions, high cell density, a solid surface, and starvation, are also required for rescue. These properties suggest that murein components or biochemically related compounds serve as intercellular signals in *M. xanthus* development.

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MATERIALS AND METHODS

Cells and growth conditions. Table 1 lists the characteristics of the bacterial strains used in this study. The generalized transducing phage Mx8cp2 was used in the construction of these strains (14). Vegetative cultures were grown in CTT medium (9) or in the minimal medium A1 (2). Development was induced on CF agar (8).

Analysis of cell populations during fruiting body formation. Vegetative cells growing exponentially in CTT medium were suspended in distilled water and plated at a density of 8×10^6 cells per cm^2 on CF agar, or CF agar supplemented as described below, and incubated at 33°C. The numbers of rod-shaped cells and spores were determined by scraping cells off the surface of CF agar and suspending them in 2% (wt/vol) glutaraldehyde for 12 h or more. Glutaraldehyde-fixed cells were subjected to sonic oscillation for 30 s to disrupt cell clumps, and then rod-shaped cells and spores were counted in a Petroff-Hauser counting chamber (20). After 5 days of development, spores were purified on sucrose step gradients (11).

Glycerol induction. Glycerol was added to an exponential culture in CTT medium to a final concentration of 0.5 M, and the culture was incubated with shaking for 18 h at 33°C. Glycerol spores were purified in the same manner as fruiting body spores.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS)-gel electrophoresis was carried out with 17% acrylamide by the method of Anderson et al. (1). Spore samples were suspended in the solubilization solution and incubated in a boiling water bath for 30 s.

TABLE 1. Myxobacterial strains

Strain	Description[a]	Derivation	Reference or source
DK418	SpoA *spo-418*	UV on DK101	8
DK423	SpoB *spo-423*	UV on DK101	8
DK425	SpoD *spo-425*		8
DK426	SpoD *spo-426*		8
DK428	SpoB *spo-428*	UV on DK101	8
DK429	SpoD *spo-429*		8
DK433	SpoB *spo-433*		8
DK435	SpoB *spo-435*	UV on DK101	8
DK439	SpoD *spo-439*		8
DK440	SpoB *spo-440*	UV on DK101	8
DK442	SpoB *spo-442*	UV on DK101	8
DK445	Spo^- Gli^- *spo-445*	UV on DK101	8
DK448	Spo^- Gli^- *spo-448*	UV on DK101	8
DK452	Spo^- Gli^+ *spo-452*	UV on DK101	8
DK453	Spo^- Gli^+ *spo-453*	UV on DK101	8
DK460	SpoB *spo-460*	UV on DK101	8
DK468	SpoB *spo-468*		8
DK473	SpoA *spo-473*		8
DK478	SpoB *spo-478*		8
DK510	Spo^- Gli^- *spo-510*		13
DK631	SpoB *spo-631*	NTG on DK101	8
DK707	Spo^- Gli^+ *spo-707*	UV on DK101	8
DK739	SpoA *spo-739*		8
DK751	SpoB *spo-751*		8
DK753	SpoB *spo-753*		8
DK762	SpoA *spo-762*	UV on DK101	8
DK767	SpoA *spo-767*	UV on DK101	8
DK1525	SpoC *spo-741* Ω1519	Mx8 (DK1519) × DK741 → Km^r [Spo^-][b]	
DK1529	SpoC *spo-653* Ω1519		R. LaRossa
DK1531	Spo^+ Ω1519		
DK1536	SpoC *spo-731* Ω1519	Mx8 (DK1519) × DK731 → Km^r [Spo^-]	
DK1622	Spo^+		D. Morandi
DK2629	Spo^+ Ω1519	Mx8 (DK1525) × DK1622 → Km^r [Spo^+]	
DK2630	SpoC *spo-741* Ω1519	Mx8 (DK1525) × DK1622 → Km^r [Spo^-]	
DK2631	Spo^+ Ω1519	Mx8 (DK1529) × DK1622 → Km^r [Spo^+]	
DK2632	SpoC *spo-653* Ω1519	Mx8 (DK1519) × DK1622 → Km^r [Spo^-]	
DK2633	Spo^+ Ω1519	Mx8 (DK1536) × DK1622 → Km^r [Spo^+]	
DK2634	SpoC *spo-731* Ω1519	Mx8 (DK1536) × DK1622 → Km^r [Spo^-]	
DK2822	SpoA *spo-756* Ω1537	Mx8 (DK993) × DK1622 → Km^r [Spo^-]	

[a] Spo^- indicates inability to produce fruiting body myxospores. Gli^- indicates inability to produce glycerol-induced myxospores. Each independent insertion of the transposon Tn*5* is identified by an omega number that designates the insertion site.

[b] Strain DK1525 was prepared by transduction of DK741 with Mx8 grown on DK1519 and selection of a kanamycin-resistant Spo^- transductant.

Insoluble material was sedimented in an Ependorff centrifuge (10 s), and 20 μl of the soluble material was applied to the gel. Gels were stained with Coomassie blue.

Electron microscopy. Purified spores were fixed in 2.5% (wt/vol) glutaraldehyde and 1% (wt/vol) formaldehyde in 0.1 M cacodylate buffer (pH 7.25) for 2 h and then washed with three changes of cacodylate buffer. They were postfixed in buffered 2% (wt/vol) OsO_4 for 1 h, washed five times with glass-distilled water, and stained for 30 min in 2% uranyl acetate. Spores were dehydrated in a graded series of ethanol (once each with 30, 60, and 90% and three times with 100% [vol/vol]) and embedded in Spurr resin. Several days were required for the resin to permeate the fruiting body myxospores. Sections were examined in a Philips transmission electron microscope.

RESULTS

Rescue of SpoC mutants by murein components. SpoC strains belong to one of four groups of mutants that have lost the capacity to sporulate under fruiting conditions, but which can be rescued by mixing them with wild-type cells (8). Three independent SpoC mutants have been isolated. They fail to rescue each other, but when they are mixed with rescuable mutants of groups A, B, or D, then sporulation occurs. The

TABLE 2. Rescue of sporulation by Murein components[a]

Strain		Spores/cm^2	
Type	No.	CF	CF plus components
Wild type	DK1622	4.9×10^6	2.6×10^6
SpoC	DK2630	146	1.6×10^6
SpoC	DK2632	128	1.6×10^6
SpoC	DK2634	28	4.1×10^6

[a] Exponentially growing cells were plated on CF agar or CF agar supplemented with 2.5 mM each *N*-acetylglucosamine, diaminopimelate, and D-alanine and incubated for 5 days at 33°C. Plates were heated to 50°C for 2.5 h, and the cells were removed, dispersed by sonic oscillation for 60 s, and plated on CTT agar. Each colony appearing on CTT agar arose from a single viable spore.

three SpoC mutations map close to the Ω1519 insertion of Tn*5* in *M. xanthus* (LaRossa et al., submitted for publication). All three mutants produce myxobacterial hemagglutinin, a protein that is synthesized near the middle of the developmental process (5) (LaRossa et al., submitted for publication).

CF agar is a low-nutrient, starvation medium that induces wild-type *M. xanthus* to form fruiting bodies containing spores. When a SpoC mutant was plated on CF agar medium, the mutant commenced multicellular morphogenesis, but failed to develop to the point of spore formation. However when a SpoC mutant was plated on CF agar supplemented with components of murein, then many heat-resistant, sonication-resistant spores were formed (Table 2). The spores were genetically *spo*$^-$, and when they germinated their descendent cells retained the SpoC character. Evidently their sporulation is not the consequence of a genetic change, but instead results from a phenotypic bypass or rescue. The data of Table 2 show that all three SpoC mutants could be rescued by murein components. Of the individual components of murein diaminopimelic acid and D-alanine were the most effective, but complete rescue required a mixture of components (Fig. 1). A mixture of three components, diaminopimelate, D-alanine, and *N*-acetylglucosamine, at a concentration of 2.5 mM each was sufficient to bring the mutant strains close to the wild-type level of sporulation (Table 2).

All four compounds that are able to rescue sporulation of SpoC mutants are present in the peptidoglycan of *M. xanthus* (19). Further experiments showed that two other components of *M. xanthus* murein, L-alanine and D-glutamate, were not active over a concentration range of 1 to 16 mM. The action of the murein components was stereospecific, since D-alanine was active but L-alanine was not. Compounds unique to murein from other types of bacteria were also

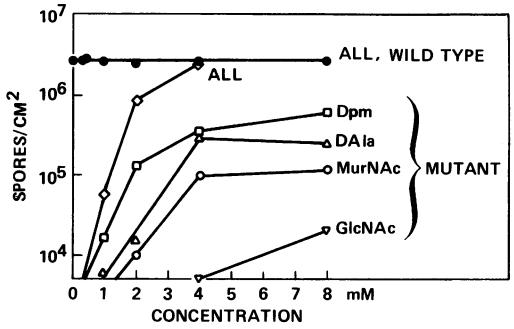

FIG. 1. Rescue of sporulation by the addition of peptidoglycan components to a SpoC mutant. The density of spores produced is plotted as a function of the concentration of component added. Mutant (DK2634) or wild-type (DK2633) cells were plated on CF agar supplemented as indicated and incubated at 32°C for 5 days. Spores were harvested and counted in the microscope as described in the text, each point representing the average of three experiments. The spore density of the SpoC mutant plated on unsupplemented CF was $<5 \times 10^3$/cm^2. Symbols: (□) mutant plus diaminopimelate; (△) mutant plus D-alanine; (○) mutant plus *N*-acetylmuramic acid; (▽) mutant plus *N*-acetylglucosamine; (◇) mutant or (●) wild type plus diaminopimelate, D-alanine, *N*-acetylmuramic acid, *N*-acetylglucosamine, L-alanine, and D-glutamate. The mutant plus L-alanine or D-glutamate (1 to 16 mM) produced $<5 \times 10^3$ spores per cm^2.

tested. None of the following compounds was able to rescue sporulation of the SpoC mutants over a concentration range of 1 to 20 mM: D-glutamine, L-lysine, L-ornithine, DL-homoserine, and L-glutamate. It appears that the only murein components that rescue sporulation of SpoC mutants are constituents of *M. xanthus* murein.

A possible explanation for the rescue of SpoC mutant sporulation by the addition of murein components would be a defect in the capacity of

TABLE 3. Growth in a minimal medium[a]

Type	Fully motile		Partially motile	
	No.	GT (h)	No.	GT (h)
Spo$^+$	DK2631	36	DK1531	31
SpoC 741	DK2630	72	DK1525	46
SpoC 653	DK2632	48	DK1529	31
SpoC 731	DK2634	34	DK1536	60

[a] Exponentially growing cells were removed from CTT medium, washed once in 10 mM Tris (pH 7.6)–8 mM $MgSO_4$, and suspended in A1 minimal medium. Cells were grown in A1 medium at 33°C with shaking for 24 h to allow them to adapt to A1 medium. Cells were diluted into fresh, prewarmed A1 medium, and the increase in cell mass was monitored with a Klett colorimeter for 6 days. Each *spoC* allele was tested in two different genetic backgrounds, a fully motile wild-type-like strain, and a partially motile strain containing sglA1. GT, is Generation time.

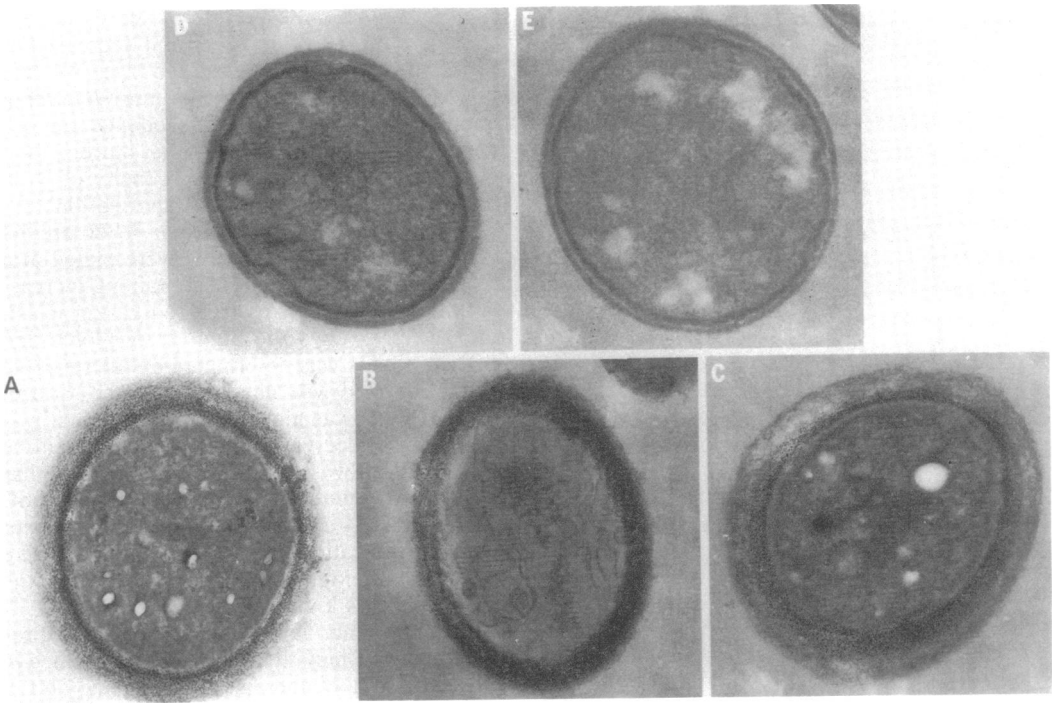

FIG. 2. Morphology of myxospores produced by the rescue of a SpoC mutant with peptidoglycan components compared with wild-type fruiting body myxospores and spores induced by glycerol. Myxospores were fixed, embedded, and thin sectioned as described in the text. Panels: (A) wild-type (DK2631) fruiting body myxospore formed on CF agar; (B) wild-type fruiting body myxospore formed on CF agar in the presence of 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate; (C) mutant (DK2632) fruiting body myxospore rescued on CF agar by 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate; (D) wild-type glycerol-induced myxospore; (E) mutant glycerol-induced myxospore. Bar in panel A, 0.3 μm.

SpoC mutants to synthesize murein components. However, the data presented in Table 3 show that all three SpoC mutants grew vegetatively in a minimal medium (A1) whose only organic constituents are pyruvate, asparagine, leucine, isoleucine, valine, and methionine. Although Table 3 shows some strain-to-strain variation in growth rate, there was no substantial difference correlated with the *spoC* allele. Since vegetative growth includes cell wall synthesis, SpoC mutants did not behave like vegetative murein auxotrophs.

Characterization of rescued myxospores. Like myxospores from fruiting bodies, the spores formed by SpoC mutants in the presence of murein components are optically refractile and heat and sonication resistant. *M. xanthus* forms a second type of spore when glycerol is added to exponentially growing vegetative cells (7). Both fruiting body and glycerol-induced spores are optically refractile and sonication and heat resistant, but they differ in biochemical composition and ultrastructure (11). To determine whether rescue by murein components is related to fruiting body development or to glycerol-induced sporulation, the structure of rescued spores was compared with that of glycerol-induced spores on the one hand and that of fruiting body spores on the other. Spores prepared from the wild type and an isogenic SpoC mutant were sectioned, stained, and observed by transmission electron microscopy. Murein-rescued spores from the SpoC mutant, DK2632, had thick spore coats (Fig. 2C), like the coats of wild-type (DK2631) fruiting body myxospores (Fig. 2A) and unlike the thin coats of glycerol-induced spores of the wild type (Fig. 2D). SpoC mutants retain glycerol inducibility (8), and glycerol-induced spores of DK2632 have thin coats (Fig. 2E), like the wild type. The addition of murein components did not change the structure of wild-type fruiting body spores (Fig. 2B). Thus, murein-rescued SpoC mutant spores morphologically resemble fruiting body myxospores rather than glycerol-induced myxospores.

A second quality that distinguishes glycerol-induced spores from fruiting body myxospores is their stability in boiling SDS: glycerol myxospores dissolve readily, releasing many different protein species, whereas fruiting body myxospores release fewer proteins (11). Figure 3 shows an SDS-polyacrylamide gel of protein

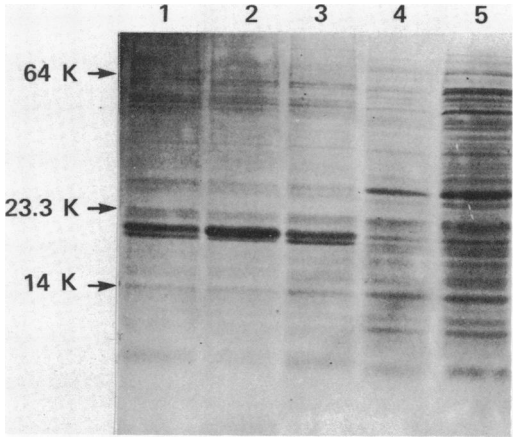

FIG. 3. Gel electrophoresis of proteins extracted from spores with boiling SDS; comparison of mutant and wild type. Lanes: 1, wild type (DK2631), 10^9 fruiting body spores formed in the presence of 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate; 2, wild type, 10^9 fruiting body spores; 3, mutant (DK2632), 10^9 fruiting body spores rescued with 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate; 4, mutant, 10^8 glycerol-induced spores; 5, wild type, 10^8 glycerol-induced spores. Arrows denote position of molecular weight markers: BSA (64K), trypsin (23.3K), and lysozyme (14K).

released by boiling spores in SDS. The spectra of proteins released from murein-rescued spores of the mutant (lane 1) and the wild type (lane 3) resembled each other as well as those of proteins released from fruiting body myxospores formed in the absence of murein components (lane 2). In all three cases two proteins having a molecular weight around 20,000 were the major protein species released. By contrast, glycerol-induced myxospores from the mutant (lane 4) and the wild type (lane 5) released more total protein per spore, and the proteins released had a greater variety of molecular weights than fruiting body myxospores. By this standard as well, the myxospores of SpoC mutants rescued by murein components resembled fruiting body myxospores and differed from glycerol-induced myxospores.

Relation of rescue by murein components to normal development. *M. xanthus* has three general requirements for fruiting body sporulation: a high cell density, low nutrient levels, and a solid surface (15). If rescue of sporulation in SpoC cells by murein components followed the normal developmental pathway, then rescue should have these same three requirements. To test the effect of cell density, cells were plated at different densities on nonnutrient agar, and after 5 days the number of spores and rod-shaped cells was determined by phase-contrast microscopic examination. The ratio of spores to rods was high at high cell densities and decreased as the initial cell density decreased (Fig. 4). The SpoC mutant in the presence of murein components exhibited a similar dependence on cell density. The data also suggest that murein components may lower the critical density of wild-type cells that is necessary for sporulation.

To test the effect of nutrient level on the rescue of SpoC mutants, cells were plated at a uniform density on CF agar containing increasing concentrations of hydrolyzed casein (Casitone). After 5 days the number of spores and vegetative cells was determined by microscopic count. For wild-type development, the ratio of spores to rods was high when little nutrient was available, but decreased rapidly as the Casitone concentration was raised (Fig. 5). A similar pattern was seen for the rescue of the SpoC mutant DK2634 in the presence of murein components, indicating that mutant rescue, like wild-type development, occurs only when the nutrient level is low.

To test whether a solid surface is required for rescue of SpoC mutants, cells were suspended in liquid CF medium and agitated or plated on CF agar. After 5 days the number of spores and vegetative cells was determined by microscopic count. The rescued sporulation of the mutant did not occur in liquid, but did occur on agar, thereby resembling wild-type development (Table 4).

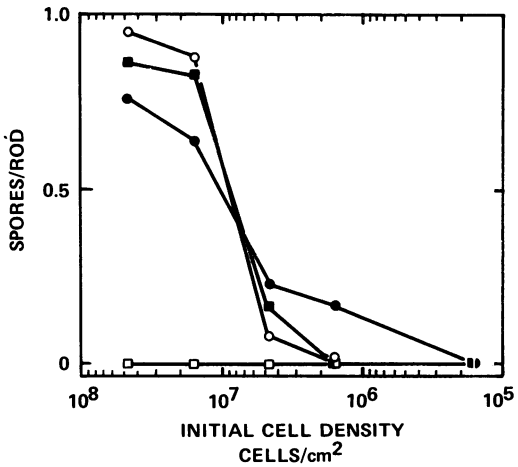

FIG. 4. Effect of cell density on myxosporulation. Cells were plated at the cell densities indicated on 1.5% agar containing 10 mM Tris (pH 7.6)–8 mM $MgSO_4$ with or without 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate. Symbols: (□) mutant (DK2634), (○) mutant plus supplements, (■) wild type (DK2633), (●) wild type plus supplements. Spores and rods were counted after 5 days, and the ratio of spores to rods is plotted.

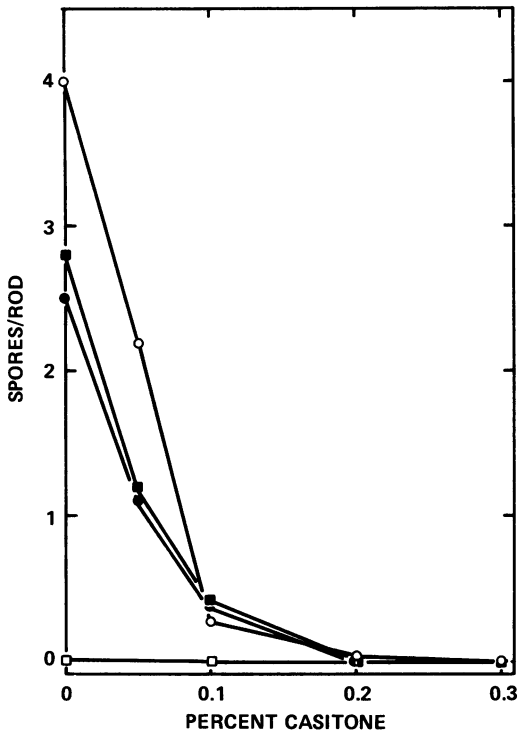

FIG. 5. Effect of the concentration of nutrient on myxosporulation. Cells were plated on CF agar containing the indicated concentrations of Casitone with and without 2.5 mM each *N*-acetylglucosamine, D-alanine, and diaminopimelate. Symbols: (□) mutant (DK2634), (○) mutant plus supplements, (■) wild type (DK2633), (●) wild type plus supplements. Spores and rods were counted after 5 days, and the ratio of spores to rods is plotted.

During development many cells die and lyse (20, 21). The data of Fig. 6 illustrate this process with a Spo$^+$ strain, DK2633; spores represent approximately half the starting number of cells or 12% of the peak number. The addition of murein components to DK2633 had little effect on growth, lysis, or myxospore formation. On the other hand, the SpoC mutant grew, but failed to lyse and failed to sporulate. G. Janssen and M. Dworkin have also observed the failure of SpoC to lyse (unpublished results). The data in Fig. 6 show that murein components rescue lysis of SpoC mutants.

Six different classes of sporulation-defective mutants were tested for their ability to be rescued by murein components (Table 5). Only strains that were rescued by mixing with viable wild-type cells could be rescued by murein components. Of the four groups of mutants whose sporulation was rescued by wild type cells, all members of group C and about a third of the members of group B were rescued by murein components.

DISCUSSION

The major new finding reported here is that a mixture of peptidoglycan (murein) components, *N*-acetylglucosamine, *N*-acetylmuramic acid, diaminopimelic acid, and D-alanine, added to the medium overcomes the sporulation defect in all SpoC mutants and many SpoB mutants (Fig. 7).

Several lines of evidence support the proposition that petidoglycan components restore normal fruiting body sporulation to SpoC mutants. The first line concerns the structure of the spores that are formed. When *M. xanthus* is plated on a low-nutrient agar at high cell density, cells aggregate and differentiate myxospores inside the fruiting body. Sporulation can also be induced by the addition of 0.5 M glycerol, or certain other substances, to an exponentially growing culture (7). Is the rescue of sporulation by murein components related to fruiting body development or do murein components act like glycerol? SpoC mutants do retain glycerol inducibility (8). Glycerol spores are readily distinguished from fruiting body spores by their microscopic ultrastructure and sensitivity to boiling SDS (11). Fruiting body spores have a thick coat, and few proteins are released when these spores are boiled in SDS. In contrast, glycerol spores have a thin coat and dissolve readily in boiling SDS. Comparison of isogenic pairs of *spo*$^+$ and *spo*$^-$ strains showed that

TABLE 4. Sporulation requires a solid surface[a]

Strain		Murein components	CF liquid		CF agar	
Type	No.		Rods/ml	Spores/ml	Rods/cm^2	Spores/cm^2
Wild type	DK2633	−	7.0×10^7	$<10^5$	2.8×10^6	7.9×10^6
	DK2633	+	5.1×10^7	$<10^5$	3.5×10^6	8.6×10^6
SpoC	DK2634	−	9.0×10^7	$<10^5$	6.7×10^7	$<10^4$
	DK2634	+	6.4×10^7	$<10^5$	2.0×10^6	7.9×10^6

[a] Exponentially growing cells were washed once in 10 mM Tris (pH 7.6)–8 mM $MgSO_4$ and suspended in CF liquid at 5×10^7 cells per ml or plated on CF agar at 8×10^6 cells per cm^2. Supplements were 2.5 mM *N*-acetylglucosamine, D-alanine, and diaminopimelate. After 5 days at 33°C, cells were counted in a Petroff-Hauser counting chamber.

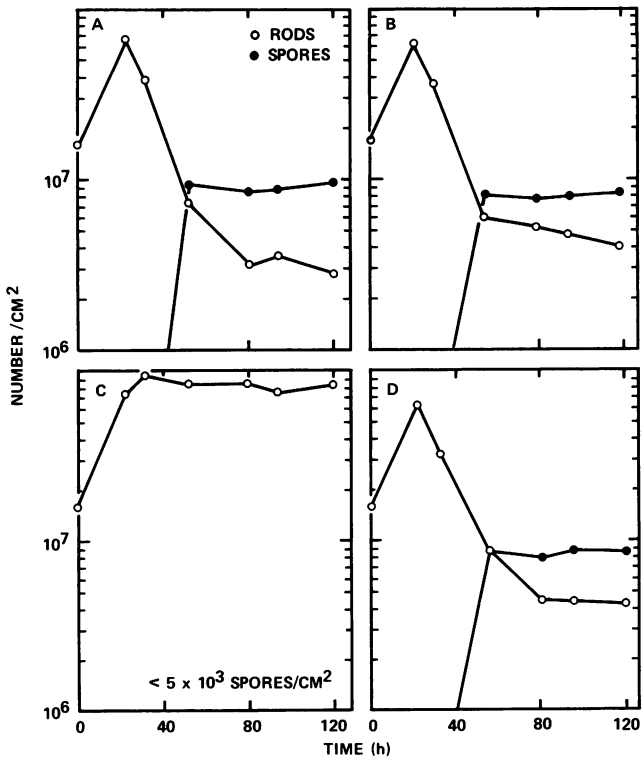

FIG. 6. Rescue of developmental lysis in a SpoC mutant by peptidoglycan components. Changes in number of rod-shaped cells (○) and spores (●) during development. Cells were plated on CF agar with and without 2.5 mM each *N* acetylglucosamine, diaminopimelate, and D-alanine and harvested at intervals, and the harvests were examined microscopically. Panels: (A) wild type (DK2633), (B) wild type plus supplements, (C) mutant (DK2634), (D) mutant plus supplements.

spores rescued by murein components resemble fruiting body spores and are unlike glycerol-induced spores both microscopically and in the proteins released by boiling SDS.

A second line of evidence that peptidoglycan components restore normal development to SpoC mutants is that a high cell density, a solid surface, and a low nutrient level are required for the rescue. These are the three general requirements of fruiting body development in wild-type cells. A high density of cells may facilitate the exchange of molecules that coordinate development. Cells require a solid surface for gliding motility which is essential for constructing the fruiting body (10). A solid surface should also stabilize contacts between cells (3). Low nutrient levels appear to initiate fruiting body development (13). None of these conditions is required for glycerol-induced sporulation, suggesting that they are specific prerequisites for fruiting body sporulation.

A third argument is that murein components overcome the failure of SpoC mutants to lyse. Normal fruiting body development is accompanied by death and lysis of nearly 90% of the cells (20, 21). Lysis does not occur during glycerol-induced sporulation, however (7).

The final argument relating rescue by peptidoglycan components to normal development is that rescue is specific to certain groups of developmental mutants. All SpoC mutants can be rescued. *spoC* mutations map at one locus and are likely to represent a single type of defect (LaRossa et al., submitted for publication). In addition about one-third of the SpoB mutants can be rescued, but members of groups A and D cannot. Since A and D mutants are blocked early in development (LaRossa et al., submitted for publication), completion of certain early developmental steps that are completed by B and C mutants, but not by A or D mutants, may be necessary for rescue by murein components.

How do peptidoglycan components rescue SpoC mutants? Since all of these SpoC mutants grow vegetatively on unsupplemented minimal medium at rates similar to the wild type, and since vegetative growth requires murein synthesis (19), SpoC mutants are not vegetative auxo-

TABLE 5. Rescue of nonsporulating mutants[a]

Mutant group	Rescued by viable wild-type cells	Rescued by components[b]		Not rescued	
		Total no.	Strain nos.	Total no.	Strain nos.
A	+	0	473	8	418, 455, 739, 753, 762, 767, 2822, 473[c]
B	+	4	423, 468, 631, 751	7	428, 433, 435, 440, 442, 460, 478
C	+	3	2630, 2632, 2634	0	
D	+	0		4	425, 429, 439, 426[c]
Gli^{-}[d]	−	0		3	445, 448, 510
Gli^{+}	−	0		3	452, 453, 707[c]

[a] All strains listed failed to sporulate on CF agar.

[b] Washed exponentially growing cells were spotted on CF agar and CF agar containing 2.5 mM *N*-acetylglucosamine, D-alanine, and diaminopimelate at a density of 8×10^6 cells per cm^2. After 5 days at 33°C plates were heated to 50°C for 2.5 h and overlaid with 4% CTT medium containing 0.7% agar to germinate the myxospores. Strains producing 50 to 100% the number of spores as the wild type on supplemented CF agar, but not on CF agar, were considered to be rescued.

[c] Strains marked with superscript *c* produced more spores in the presence of murein components than in their absence, but less than 10% of wild-type levels.

[d] Gli^{-} indicates inability to produce glycerol-induced myxospores.

trophs for murein components. Rather, we suggest that peptidoglycan components serve as developmental regulators or special substrates for development. Peptidoglycan and peptidoglycan components activate several different events associated with development. Campos and Zusman (4) reported that the addition of diaminopimelate to starved wild-type cells increased the number of fruiting bodies formed. In an accompanying paper (16) we report that ripples, parallel ridge-like associations of cells which normally accompany fruiting body development, are induced by the addition of peptidoglycan components to quiescent wild-type cells. The same four components that induce rippling rescue sporulation. SpoA and SpoD mutants can be induced to ripple, but, as shown in the accompanying paper (16), SpoC mutants do not ripple spontaneously, nor can they be induced to ripple by the addition of peptidoglycan components (16). Even the rescue of C mutant development by peptidoglycan components does not induce them to ripple, showing that the addition of peptidoglycan components does not correct the SpoC defect, but serves instead to bypass the defect in development. In fact the bypass is very efficient: as many spores are formed by a SpoC mutant in the presence of murein components as are formed by a Spo^{+} strain in the absence (or presence) of the components. The lysis during development of SpoC mutants in the presence of murein components again suggests an activating or regulatory role for these compounds.

Peptidoglycan components may also be necessary substrates for spore murein synthesis. Murein precursors from outside the sporulating cell may be needed for the murein remodeling and synthesis that are required to transform a rod-shaped cell into a spherical, thick-walled myxospore. Dawson and Jones found extensive turnover of diaminopimelate in the peptidoglycan of sporulating *M. xanthus* cells (6). Peptidoglycan

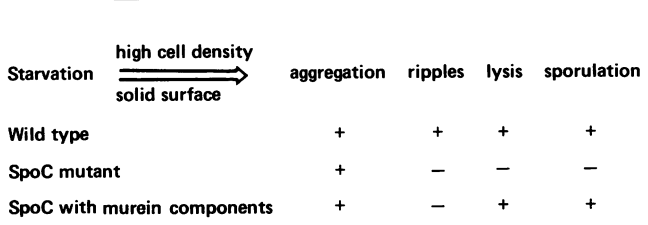

FIG. 7. Comparison of the developmental behavior of the wild type and group C sporulation-defective mutants.

would normally be released during fruiting body formation as a large proportion of the cells lyse (20, 21) (Fig. 6). At least three extracellular murein hydrolases have been identified in *M. xanthus*: an endo-*N*-acetylglucosaminidase, an *N*-acetylmuramyl-L-alanine amidase, and a peptidase active on D-alanyl-diaminopimelate (18). These enzymes could reduce murein at least to the level of peptides and disaccharide units, which might then or after further hydrolysis be incorporated into spores. The observation of Wireman and Dworkin that lysis precedes sporulation is consistent with this view (20, 21).

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