STIMULATION OF CELL DIVISION BY MEMBRANE-ACTIVE AGENTS

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The coordination of cell division is a complex regulatory process about which little is known. Evidence from several types of investigation implicates the plasma membrane as a site of regulation. For example, comparative studies of plasma membranes from dividing and nondividing cells have revealed differences in agglutination and in antigenic properties (3, 13). Other studies have shown changes in membrane proteins (10) and phospholipids (12, 17). Synthesis of the cell wall is also intimately associated with cell division. Localized thickening of the peptidoglycan is one of the earliest morphological indications of the initiation of cell division in bacteria (8), and as the peptidoglycan continues to grow inward, the cell is divided. Thus, the enzymes involved in the synthesis of the cell wall may regulate division.

Many of the enzymes involved in the synthesis of the bacterial cell wall are localized in the plasma membrane (1, 15), and some have obligate requirements for lipid cofactors (4, 15). In in vitro systems, compounds such as dimethyl sulfoxide and some phospholipids can serve as activators of these enzymes (14, 16). Compounds that are functionally analogous to such activators could act as positive effectors controlling the invagination of the cell wall in vivo.

In two previous papers we proposed that the initiation of the invagination of the cell wall and cell membrane in Agmenellum quadruplicatum strain BG1 is controlled by a diffusable substance acting on the cell membrane (6, 7)

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and that serpentine mutants are deficient in the production and accumulation of this effector. In this paper we report the stimulation of invagination in two multinucleoid, filamentous mutants by the addition of agents that decrease the stability of cellular membranes (e.g. dimethyl sulfoxide, short-chain alcohols, sodium oleate, and lysolecithin). The stimulation of cell division by these compounds can be partially antagonized by long-chain alcohols, such as 1-octanol, which increase the stability of cellular membranes. Thus, compounds that alter the physical properties of cellular membranes can be used to regulate cell division.

MATERIALS AND METHODS

Organisms and cultivation. A. quadruplicatum strain BG1 (a marine, unicellular, blue-green bacterium) was isolated into axenic culture by C. Van Baalen (18). Strains SN12 and SN29 are serpentine, filamentous mutants of strain BG1 isolated following mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (6, 7). The parent and mutants were grown autotrophically at 35 C as described previously (9). Under these conditions, cell mass doubles in approximately 4 hr.

Measurement of growth, cell number, and cell size. Cell mass was estimated by measurement of absorbance at 620 nm with a Spectronic 20 colorimeter. Cell number and size distribution were monitored with a Coulter Counter Model B (30-μm aperture) equipped with a Model J size plotter. Mean filament length was estimated by comparison of the cell number per turbidity unit of the mutant to that of a population of stationary-phase parent cells, as described previously (6). Light micrographs were prepared by both phase-contrast and bright-field microscopy.

RESULTS

Growth. The growth of A. quadruplicatum strain BG1 and of the serpentine mutant strain SN29 was inhibited by alcohols (Fig. 1). The degree of inhibition was directly related to the chain length. Figures 2A and 2B show growth curves for strain SN29 with various concentrations of ethanol and 1-butanol,

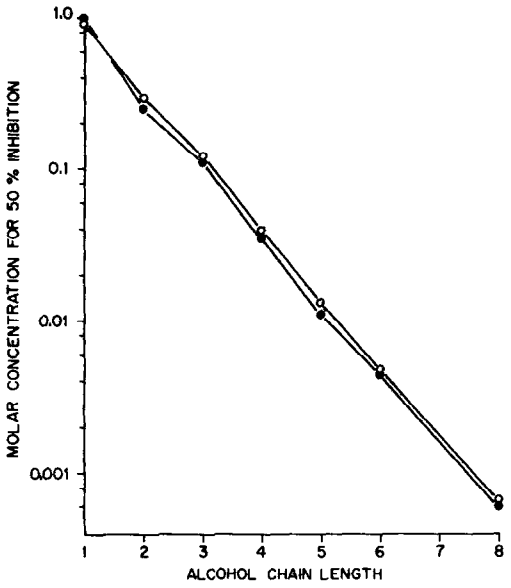

Fig. 1.

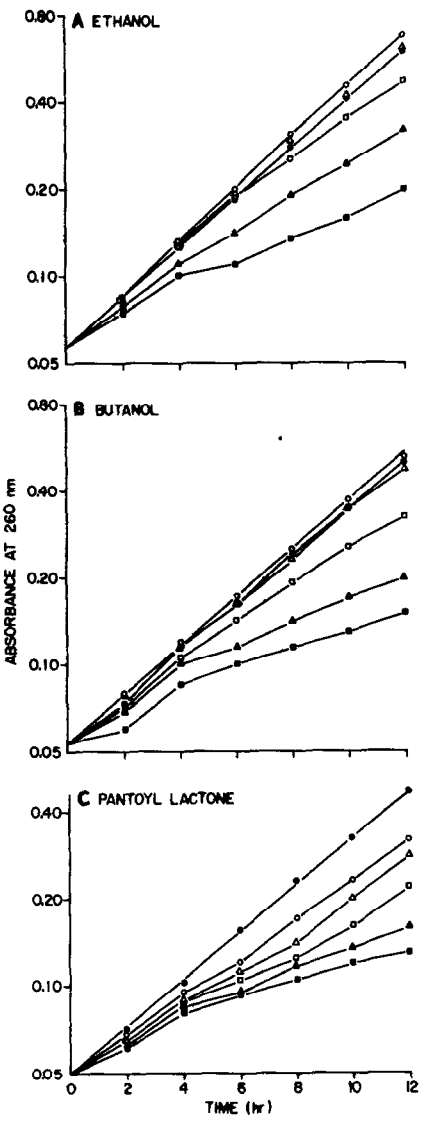

Fig. 2.

FIG. 1. Effect of chain length on molar toxicity of normal alcohols after incubation for 16 hr at 35 C. Parent, ; serpentine mutant strain SN29,

FIG. 2. Effects of various concentrations of agents that stimulate cell division on the growth of strain SN29. (A) Ethanol: no addition, ; 8.5×10^{-2} M, ; 1.7×10^{-1} M, ; 2.6×10^{-1} M, ; 3.4×10^{-1} M, ; 4.3×10^{-1} M, . (B) Butanol: no addition, ; 1.0×10^{-2} M, ; 2.1×10^{-2} M, ; 3.2×10^{-2} M, ; 4.3×10^{-2} M, ; 5.4×10^{-2} M, . (C) Pantoyl lactone: no addition, ; 1.5×10^{-2} M, ; 2.1×10^{-2} M, ; 3.0×10^{-2} M, ; 4.5×10^{-2} M, ; 6.0×10^{-2} M, .

and Fig. 2C shows the response to various concentrations of pantoyl lactone. Addition of dimethyl sulfoxide at concentrations from 0.80 to 0.25 M produced a similar range of toxic responses. At the maximum concentrations tested, sodium oleate (0.1 mg/ml, exceeds solubility) and lysolecithin (5 mM) did not impair growth. In all cases, the growth response of the parent was identical to that of the serpentine mutant.

Morphology and cell division. As described previously, the parent strain typically grows as short, rod-shaped unicells and divides by the simultaneous invagination of the plasma membrane and the multilayered cell wall (8). The serpentine mutant strain SN29 typically grows (at 35 C) as multinucleoid, coenocytic filaments, with sporadic division (7). At higher temperatures strain SN29 grows as shorter filaments, approaching normal size at 42 C.

Addition of ethanol to log-phase serpentine filaments of strain SN29 stimulates division into "unit cell equivalents" within 4-6 hr. Figure 3 shows phase-contrast micrographs of the parent strain (Fig. 3A), strain SN29 (Fig. 3B), and strain SN29 incubated with ethanol (Fig. 3C). This ethanol-stimulated cell division is readily detectable as an increase in cell number (Fig. 4) after incubation for 2 hr (half a generation time). After 6 hr, the size distribution of the ethanol-treated filaments is identical to that of the parent organism (Fig. 5). A comparable stimulation of cell division was observed for a variety of agents that decrease the stability of cell membranes (Figs. 3D and 6A). Table I summarizes these results. The data show two effective concentrations, one for the most rapid rate of recovery of normal morphology, regardless of the effects on growth, and a second for the most rapid rate in the absence of growth inhibition. Normal alcohols of chain length greater than five, tested over a wide range of concentrations, did not stimulate division.

Similar results were obtained with a second serpentine mutant of strain BGI, strain SN12. As might be expected, serpentine mutants form colonies that are more normal on solid medium containing dimethyl sulfoxide or other agents that stimulate cell division (Fig. 6C and 6D).

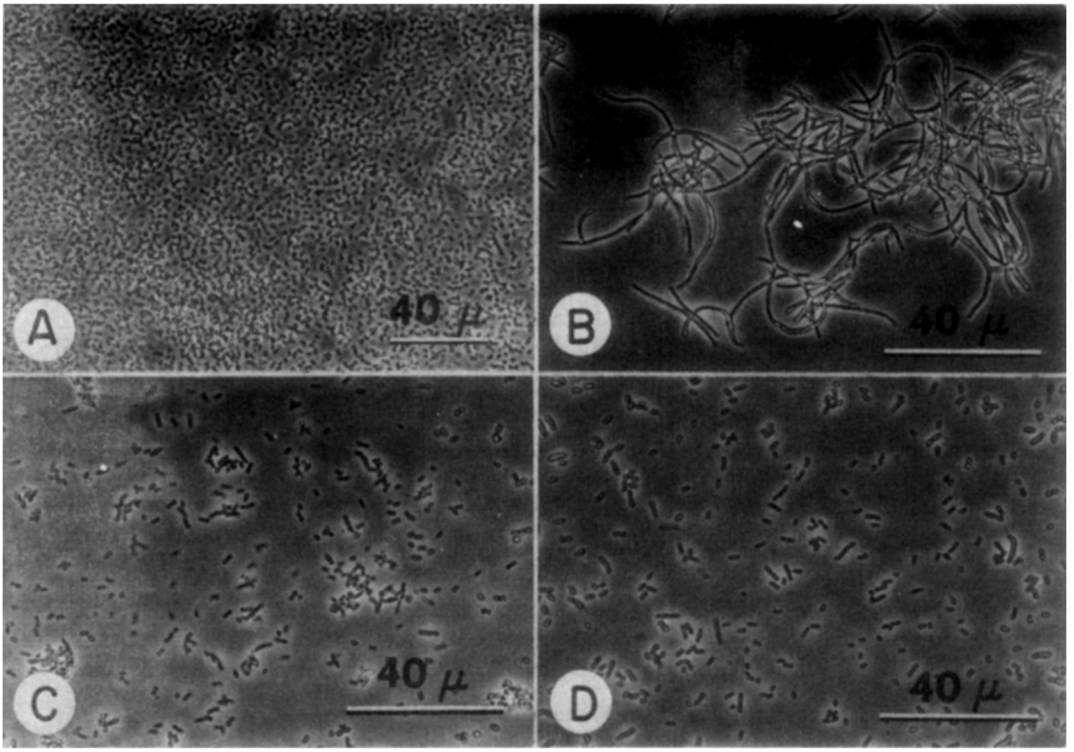

FIG. 3. Light micrographs of (A) parent strain BG1, (B) SN29 mutant, (C) strain SN29 after growth with 2.6 X 10^{-1} M ethanol for 6 hr, (D) strain SN29 after growth with 3.0 X 10^{-2} M pantoyl lactone for 16 hr.

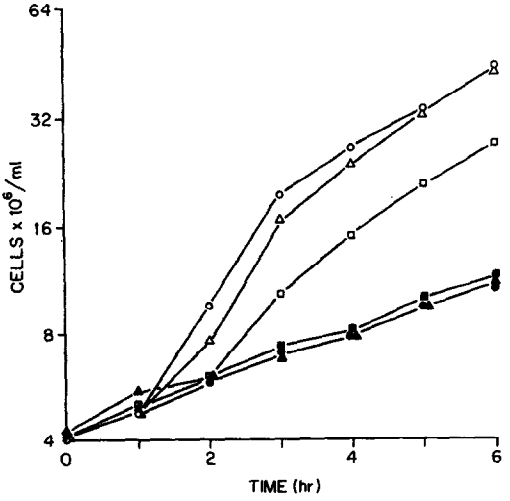

FIG. 4. Stimulation of cell division in serpentine filaments of strain SN29 by ethanol and its antagonism by 1-octanol. No addition, ; 3.4 X 10^{-1} M ethanol, ; 2.6 X 10^{-1} M ethanol, ; 1.7 X 10^{-1} M ethanol, ; ; 3.85 X 10^{-4} M 1-octanol, ; 1.7 X 10^{-1} M ethanol with 3.85 X 10^{-4} M 1-octanol, .

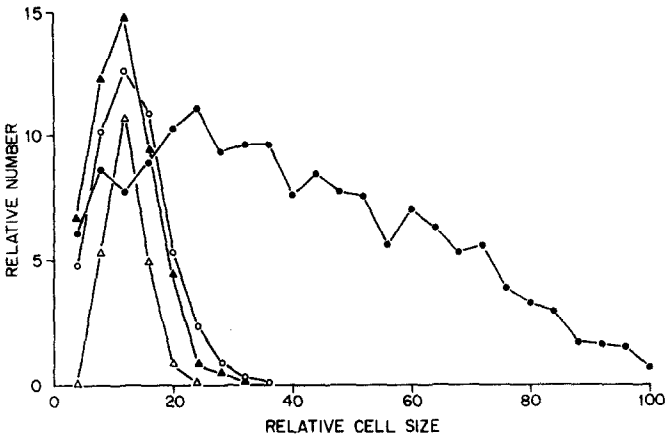

FIG. 5. Comparison of cell sizes. Log-phase parent, ; strain SN29 untreated control, ; strain SN29 after growth with 2.6 X 10^{-1} M ethanol for 6 hr, ; strain SN29 after growth with 3.4 X 10^{-1} M ethanol for 6 hr,

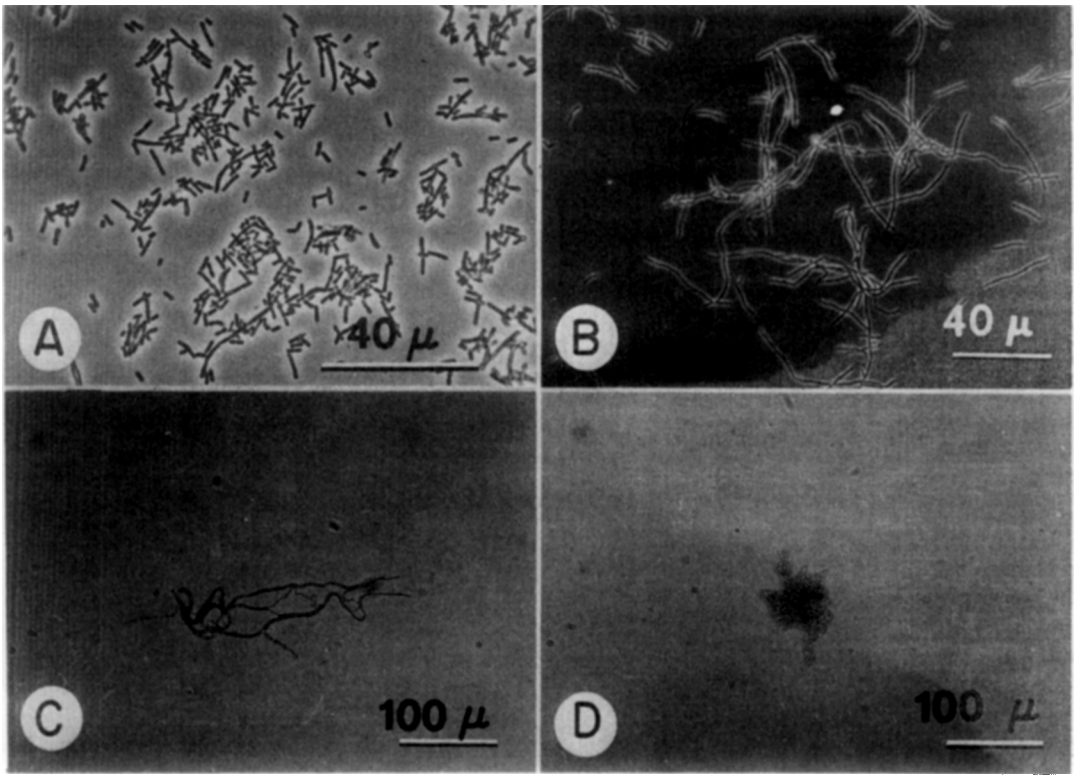

FIG. 6. Light micrographs of (A) strain SN29 after growth with 5.0 X 10^{-4} M lysolecithin for 16 hr, (B) strain SN29 after growth with 3.8 X 10^{-4} M 1-octanol for 16 hr, (C) microcolony of strain SN29 following incubation for 48 hr on solid medium, (D) microcolony of strain SN29 following incubation for 48 hr on solid medium containing 2.8 X 10^{-1} M dimethyl sulfoxide.

TABLE 1. Effects of various agents on cell division in the serpentine mutant strain SN29[a]

Agent	Molar conc.	Reversion[b] period (hr)	Percent growth[c]	Molar conc.	Reversion[b] period (hr)	Percent growth[c]
Methanol	4.9×10^{-1}	6	95	3.7×10^{-1}	10	100
Ethanol	3.4×10^{-1}	4	86	2.6×10^{-1}	6	100
1-Propanol	9.0×10^{-2}	4	92	4.5×10^{-2}	6	100
1-Butanol	3.2×10^{-2}	4	85	2.1×10^{-2}	10	100
1-Pentanol	1.8×10^{-2}	4	39	Growth-inhibitory at all effective concentrations		
Sodium oleate	0.1 mg/ml[d]	14	100	0.1 mg/ml[d]	14	100
Lysolecithin	5×10^{-4}	10	100	5×10^{-4}	10	100
Dimethyl sulfoxide	4.3×10^{-1}	8	88	2.8×10^{-1}	12	100
Pantoyl lactone	3.0×10^{-2}	6	43	Growth-inhibitory at all effective concentrations		

[a] After incubation for 16 hr at 35 C.

[b] Time required for filaments to divide into cell equivalents.

[c] Relative to untreated control culture.

[d] Exceeds solubility.

Long-chain alcohols have a stabilizing effect on cellular membranes (5). In the presence of 1-octanol, strain SN29 formed even longer filaments (Fig. 6B). Further, the addition of 1-octanol to filaments of strain SN29 antagonized the ethanol-stimulated cell division (Fig. 3). Similar antagonism was observed for dimethyl sulfoxide, lysolecithin, and pantoyl lactone (Table 2).

TABLE 2. 1-Octanol antagonism of induced cell division[a]

Treatment	No antagonist		1-Octanol[b]	
	Percent growth[c]	Filament length[d]	Percent growth[c]	Filament length[d]
None	100	11.8	95	12.7
Ethanol (1.3×10^{-1} M)	100	2.9	83	11.3
Dimethyl sulfoxide (2.1×10^{-1} M)	100	3.6	81	8.3
Lysolecithin (5.0×10^{-4} M)	100	3.8	77	11.2
Pantoyl lactone (3.0×10^{-2} M)	62	4.2	40	7.8

[a] After incubation for 16 hr at 35 C.

[b] 3.85×10^{-4} M.

[c] Relative to untreated control culture.

[d] Expressed as stationary-phase parent cell equivalents (log-phase parent is 2.4 cell equivalents).

DISCUSSION

In two previous papers (6,7) we described a model for the positive regulation of one step in the process of cell division. We suggested that the invagination of the cell wall and cell membrane results from a transient alteration in the membrane at a potential division site and is controlled by a diffusable positive effector. A transient alteration in permeability was suggested to explain the accumulation of this effector in the medium surround-

ing dividing cells. The plasma membrane was proposed as the site of regulation, so treatments that alter the physical properties of the plasma membrane should have a predictable effect on cell division. King and Grula (11) have proposed that the resumption of division in serpentine filaments of Micrococcus lysodeicticus treated with pantoyl lactone or spermine may result from physical interaction of these compounds with the plasma membrane. At high concentrations, pantoyl lactone stimulates cell division in strain SN29.

The ability of short-chain alcohols, lysolecithin, sodium oleate, dimethyl sulfoxide, and elevated temperature to decrease the stability and increase the permeability of membranes is well established (2, 5). Similarly, long-chain alcohols and low temperatures have been shown to increase membrane stability and decrease permeability. Exposure to short-chain alcohols, lysolecithin, sodium oleate, or dimethyl sulfoxide stimulates the serpentine filaments of strain SN29 to divide into normal cellular equivalents. The kinetics of such stimulation of cell division by ethanol closely resemble those previously observed after addition of the positive effector recovered from spent medium (7). Similar stimulation was obtained when serpentine filaments grown at 35 C were shifted to 42 C. The stimulation of cell division by the various agents that decrease membrane stability can be antagonized by membrane-stabilizing agents, such as 1-octanol. These results are interpreted as evidence for the involvement of the physical properties of the plasma membrane in the regulation of the invagination of the cell wall and cell membrane.

In our model of cell division, membrane-labilizing agents can be interpreted as decreasing the threshold concentration of the positive effector required to initiate invagination. Similarly, membrane-stabilizing agents could antagonize these changes. Alternatively, labilizing agents may be functionally similar to the positive effector and thus reduce the amount of effector that must accumulate at the division site. That is, a localized alteration in the membrane at a division site, similar to the change promoted by agents that reduce membrane stability, may be the molecular event normally responsible for

the initiation of the invagination of the cell wall and cell membrane.

The enzyme systems involved in the synthesis and assembly of both the lipopolysaccharide and peptidoglycan components of the cell envelope are apparently localized on the outer surface of the plasma membrane (1, 15). Many of these enzymes, as well as other membrane-bound enzymes, require lipid components for activity (4). Both dimethyl sulfoxide and relatively hydrophilic lecithins (with two unsaturated fatty acids or with two cyclopropane fatty acids) can serve as activators, and this activation has been attributed to the formation of a complex of membrane lipid, protein, and activator lipid (15, 16). Formation of this type of complex in the periplasmic region could accelerate peptidoglycan synthesis at a division site and produce the localized thickening of the peptidoglycan that is the first visible sign of division. As division continues, the activated enzyme complex might continually lose the relatively hydrophilic lipid component by dissociation of the complex and subsequent diffusion of the activator into the surrounding medium.

SUMMARY

Agents that decrease membrane stability (e.g. dimethyl sulfoxide, lysolecithin, sodium oleate, and short-chain alcohols) stimulate multinucleoid, serpentine filaments of Agmenellum quadruplicatum strains SN12 and SN29 to divide into cellular equivalents within approximately one generation time. Agents that increase membrane stability (e.g. long-chain alcohols) antagonize this timulation. Thus, the physical properties of the cell membrane appear to be involved in the regulation of cell division. These observations suggest that the invagination of the cell wall may be regulated by agents that interact with the plasma membrane and with enzymes involved in peptidoglycan synthesis.

ACKNOWLEDGMENTS

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REFERENCES

1. Anderson, R. G., H. Hussey, and J. Baddiley, Biochem. J., 127, 11-26 (1972).
2. Brooks, S. C., and M. M. Brooks, The Permeability of Living Cells, Edwards Brothers Inc., Ann Arbor, Mich., 1944.

3. Burger, M. M., In B. L. Horecker and E. R. Stadtman (eds.), Current Topics in Cellular Regulation, Vol. III, Academic Press, New York, N. Y., 1971.
4. Cronan, J. E., Jr., and P. R. Vagelos, Biochim. Biophys. Acta 265:25-60 (1972).
5. Davson, H., and J. F. Danielli, The Permeability of Natural Membranes, pp. 246-262, Cambridge University Press, London, 1943.
6. Ingram, L. O., and W. D. Fisher, J. Bacteriol., In Press a.
7. Ingram, L. O., and W. D. Fisher, J. Bacteriol., In Press b.
8. Ingram, L. O., and E. L. Thurston, Protoplasma 71:55-75 (1970).
9. Ingram, L. O., and C. Van Baalen, J. Bacteriol. 102:784-789 (1970).
10. Inouyé, M., J. Mol. Biol. 63:597-600 (1972).
11. King, R. D., and E. A. Grula, Can. J. Microbiol. 18:519-529 (1972).
12. Nunn, W. D., and B. E. Tropp, J. Bacteriol. 109:162-168 (1972).
13. Pardee, A. B., In Vitro 7:95-104 (1971).
14. Rothfield, L., and M. Pearlman, J. Biol. Chem. 241:1386-1392 (1966).
15. Rothfield, L., and D. Romeo, Bacteriol. Rev. 35:14-38 (1971).
16. Sandermann, H., Jr., F.E.B.S. Letters 21:254-258 (1972).
17. Starka, J., and J. Moravova, J. Gen. Microbiol. 60:251-257 (1970).
18. Van Baalen, C., Bot. Mar. 4:129-139 (1962).