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Cell Division in *Pseudomonas aeruginosa*: Participation of Alkaline Phosphatase

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Pseudomonas aeruginosa grows at an apparent reduced rate at 46 C as compared with the rate at 37 C, when growth is measured as an increase in absorbance. Cells at 46 C are long, plasmolyzed, nonmotile filaments. The filaments contain phase-dark material that may be chromosomal in nature. When the 46 C culture is shifted to 37 C, the filaments fragment at polar ends after flagella form, and the final number of cells is equal to the number of chromosomal "packets" observed within the filament. The outer envelope of the filament appears to be structurally complete as determined by biochemical, thin section, and freeze-etch examination. When filaments are treated with lysozyme, they form large spheroplasts, suggesting that the outer wall and the cytoplasmic membrane are continuous within the filament. Filaments produce little or no periplasm-located alkaline phosphatase (APase), but activity appears immediately after a shift to 37 C. Cells grown at 37 C and shifted to 46 C remain as single, nonmotile, rods or doublets, and the APase formed at 37 C remains stable at 46 C. The addition of APase or inorganic phosphate is partially or completely effective as an inducer of filament fragmentation at 46 C. The results suggest that periplasm-located APase is an important enzyme in the final stages of cell division when *P. aeruginosa* is cultured on inorganic phosphate-limiting media.

Filament formation is reported in a number of temperature-sensitive mutants of mesophilic gram-negative bacteria (2, 15, 19, 26) and gram-positive bacteria (7, 14, 23), as well as in the psychrophilic *Bacillus insolitus* (13). Filament formation takes place under nonpermissive conditions for cell division such as high temperature, the presence of certain antibiotics (17, 24, 27), and after ultraviolet irradiation (21). These treatments cause filament formation in the respective organisms as a direct result of inhibition of certain physiological activities of the organism such as inactivation of certain enzymes (17), changes in permeability of the cell wall, and inhibition of deoxyribonucleic acid synthesis (21).

The aim of the present communication is to demonstrate filament formation in wild-type *Pseudomonas aeruginosa* (ATCC 9027) at 46 C. A tentative model is presented which implies that alkaline phosphatase (APase) plays an important role in the cell division cycle of *P. aeruginosa*.

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MATERIALS AND METHODS

Organism and culture conditions. *P. aeruginosa* (ATCC 9027) was grown in medium of the following composition: 0.02 M NH_4Cl, 0.02 M KCl, 0.12 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, 0.5% glucose, 0.5% proteose peptone (Difco), and 0.0016 M $MgCl_2 \cdot 7H_2O$. The medium, without glucose and $MgCl_2$, was prepared, and the pH was adjusted to 6.8 with concentrated HCl. The medium was autoclaved and filtered, and the pH was then readjusted to 6.8, if necessary, and reautoclaved. Before inoculation, 2.0 ml of 25% glucose and 1.0 ml of 0.16 M $MgCl_2 \cdot 7H_2O$ solution per 100 ml of medium were added aseptically. This inorganic phosphate (P_i)-limiting medium is necessary to derepress the synthesis of alkaline phosphatase (3). The total P_i concentration of proteose peptone medium under these conditions is approximately 10^{-6} M. Growth studies were subsequently conducted at the temperatures specified in the particular experiment. In the case of "high phosphate" medium, the above-mentioned medium was supplemented, aseptically, with 1 mM KH_2PO_4. An inoculum of 1 ml from a 7- to 10-h culture was used per 100 ml of medium, and growth was carried out by incubation in a gyratory shaker (Psychrotherm shaker, New Brunswick Scientific Co., New Brunswick, N.J.) at 37 C or other specified temperatures. Growth rates

were determined after standard inoculation of 500 ml of medium with 5 ml of a culture grown to 1.2 to 1.4 optical density (OD) units (660 nm, Gilford model 300-N microsample spectrophotometer, 1-cm light path). *P. aeruginosa* forms filaments when cultivated at temperatures above 45 C, so that under this condition the OD at 660 nm was correlated with the total protein of the cell suspension (18).

Chemicals. *p*-Nitrophenylphosphate, Tris, lysozyme, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis, Mo. Noble agar and proteose peptone were purchased from Difco Laboratories, Detroit, Mich. [2-^{14}C]leucine and [2-^{14}C]uracil were obtained from New England Nuclear Corp., Boston, Mass.

Enzyme assay and purification. APase was assayed by the method of Neu and Heppel (20). The enzyme sample or bacterial suspension was assayed at 22 C in a cuvette of 1-cm light path after the addition of 1 ml of 1 mM *p*-nitrophenylphosphate in Tris-Mg^{2+} buffer at pH 8.4 (Tris, 1 M; Mg^{2+}, 0.01 M, and pH adjusted to 8.4 with HCl). The change in OD at 420 nm, which indicates the release of *p*-nitrophenol, was measured in a Gilford model 300-N spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of *p*-nitrophenol per min under the prescribed conditions. APase was purified from *P. aeruginosa* as previously described (8), except that lysozyme was not used in the present procedure. The enzyme was purified to constant specific activity, 98 U per mg of protein, and shows a single band by polyacrylamide disc gel electrophoresis.

Estimation of protein. The method of Lowry et al. (18) was used to estimate protein concentrations with bovine serum albumin used as a standard.

Spheroplast formation. Cells of *P. aeruginosa*, grown 10 h on the standard medium, were used, and spheroplasts were obtained by three methods: (i) 10 or 20 ml of culture medium was adjusted to pH 8.4; (ii) cells from 10 or 20 ml of culture medium were collected by centrifugation (13,000 × *g* for 10 min) and suspended in an equal volume of 20% sucrose in 10 mM Tris-hydrochloride buffer, pH 8.4; and (iii) cells from a similar volume of culture medium were suspended in an equal volume of 200 mM $MgCl_2$, 10 mM Tris-hydrochloride buffer, pH 8.4. Cell suspensions were treated with 0.5 mg of lysozyme per ml and incubated at 25 C in a water bath, with gentle shaking, for 0.5 h. After incubation, the cells were harvested at 13,000 × *g* for 10 min, and the pellets were resuspended in equal volumes of 10 mM Tris-hydrochloride buffer, pH 8.4, which contained 10 mM magnesium. Spheroplast formation was monitored at regular intervals by phase-contrast microscopy of the cells mounted on slides coated with a thin layer of freshly prepared 1.5% agar containing 1.0% formaldehyde.

Electron microscopy. Preparation of cells for electron microscopy was carried out as described previously (10). Thin sections were prepared with an LKB Ultrotome I (LKB, Stockholm, Sweden). All sections were examined with an AEI EM-6B electron microscope at 60-kV acceleration voltage. Specimens were prepared for freeze-etching as previously described (9). Discs bearing droplets of thick cell pellets were immersed in Freon 22, and after freezing were placed on the specimen table of a Balzer 510M unit at −150 C and cleaved at −100 C.

Uptake of [2-^{14}C]leucine and [2-^{14}C]uracil. The uptake of [2-^{14}C]leucine was measured as the acid-precipitable incorporation of [2-^{14}C]leucine into protein. Twenty milliliters of a midlog culture (OD_{660} of 0.40 to 0.50) were centrifuged, and the cells were resuspended in 20 ml of fresh standard growth medium. The medium was divided equally into two 50-ml flasks, and one aliquot was incubated at 37 C, and the other was incubated at 46 C (in constant-temperature water baths). After a 10-min equilibration, 40 μl of [2-^{14}C]leucine (0.5 mCi/mmol) was added to each flask. Samples (0.1 ml) were removed and diluted into 5 ml of ice-cold trichloroacetic acid (10% [wt/vol]) at 10-min intervals, maintained at 4 C (for 16 h), and filtered through a membrane filter (0.45 μm; Millipore Corp.). The precipitates were washed twice with 10 ml of trichoroacetic acid (10% [wt/vol]). The filters were dried in scintillation vials under a heat lamp, and 10 ml of toluene liquid scintillation solution was added. Radioactivity was determined with a Nuclear-Chicago Isocap 300 scintillation spectrometer. The uptake of [2-^{14}C]uracil was followed under conditions similar to those described above, except that 0.2 ml of [2-^{14}C]uracil (specific activity, 56.6 mCi/mmol, 0.3 mM final concentration) was added per 10 ml of cell suspension.

RESULTS

Effect of temperature on growth. *P. aeruginosa* (ATCC 9027) was cultivated at 37 and 46 C, and the growth response observed at these temperatures is illustrated in Fig. 1A. The generation times for cultures grown at 37 and 46 C were 40 and 60 min, respectively. The APase activity of the cell-free culture fluid (CFC), the cell-bound APase activity (3), and the pH of the cultures observed at various times of growth at the two temperatures are depicted in Fig. 1B. The highest level of enzyme activity in the CFC and in the cells was obtained at 37 C, whereas the lowest levels of the respective activities were observed at 46 C. The decreased enzyme activity observed in the CFC at later stages of growth at 37 C is due to the decreased pH of the culture medium. Previous studies (5) showed that APase dissociates into subunits under these conditions and that it is unstable.

P. aeruginosa formed small, motile, actively dividing, rod-shaped cells (1.5 μm in length) when grown at 37 C. When the culture was grown at 46 C, the OD increased slightly with time, and the cells elongated with less frequent division. The long, filamentous cells (Fig. 2A) were slightly plasmolyzed, undivided, and nonmotile. Such filaments varied in length from 20

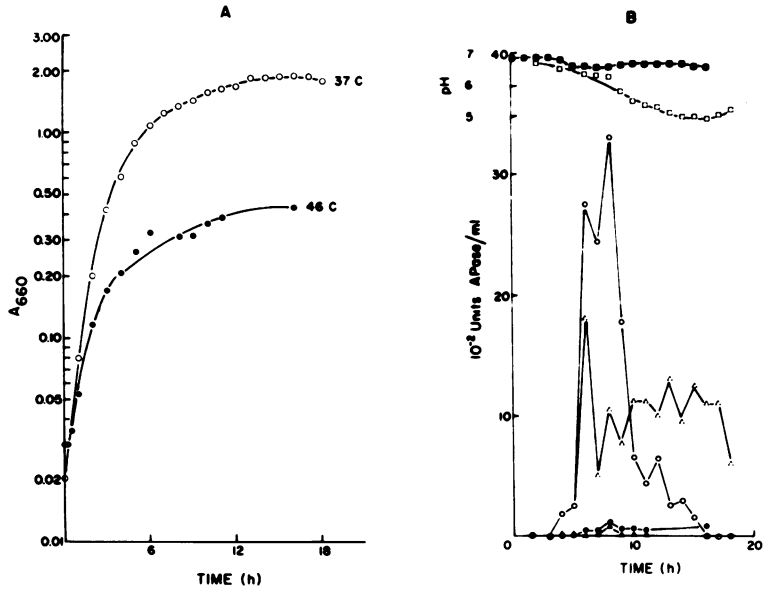

FIG. 1. *(A) Growth of P. aeruginosa at 37 C (○) and 46 C (●). (B) Effect of temperature on cell-free (○, ●) and cell-bound (△, ▲) APase and pH (□, ■) in P. aeruginosa grown at 37 C and 46 C. Samples were withdrawn at the indicated times for pH determinations. Centrifuged CFC were assayed for cell-free APase activity, and the cells were assayed for cell-bound APase activity as described in Materials and Methods. Hollow and solid symbols represent growth of cultures at 37 and 46 C, respectively.*

to 33 μm, i.e., 15 to 20 times longer than cells grown at 37 C.

The conversion of cells grown at 37 and 46 C to spheroplasts was studied in the culture medium, 20% sucrose and 200 mM Mg^{2+}. Very few cells formed spheroplasts when lysozyme was added directly to the culture medium, 20% sucrose, or 200 mM Mg^{2+}. After treatment with lysozyme, the cells were centrifuged and resuspended in 10 mM Mg^{2+}. After suspension of cells in this low concentration of Mg^{2+}, a few spheroplasts were produced from cells treated with lysozyme in the culture medium; however, after the 20% sucrose-lysozyme or 200 Mg^{2+}-lysozyme conditions, more than 99% conversion to spheroplasts was observed within a few minutes after resuspension. Spheroplasts of cells grown at 37 C were mainly phase dark (Fig. 2B) and had an average volume of 0.53×10^{-9} μl, whereas those from cultures grown at 46 C were extremely large (average volume, 20.6×10^{-9} μl), phase light, and showed scattered cytoplasmic material bound to the inner periphery (Fig. 2C). The volume of spheroplasts from cells grown at 46 C was approximately 40 times that of spheroplasts from cells grown at 37 C. Spheroplasts formed after the 200 mM Mg^{2+}-lysozyme treatment were stable for more than 1 h, whereas spheroplasts formed after 20% sucrose-lysozyme treatment lysed within 5 to 10 min.

The results of Fig. 3 show that indeed long filaments of *P. aeruginosa* did exist in cultures grown at 46 C. Electron microscope examination of thin sections (Fig. 3A) suggests that approximately three or four cell equivalents are present in each filament. Due to problems arising from the plane of section, no more than this number of fragments was observed. Phase microscopy examination of this preparation, however, revealed that most filaments contained four or eight "chromosomal packets." In addition to the thin section examination, the filaments were studied by freeze-fracture, and the results of Fig. 3B indicate that the filament is continuous and possesses a uniform outer cell wall. Spheroplasts that arose by conversion of long filaments were also examined by thin section, but large spheroplasts were never observed. Small spheroplasts resulting from single or doublet cells were observed and were surrounded by large pieces of cell debris (Fig. 3C). It is believed that the large spheroplasts were unstable to the fixation procedure employed and that their disintegration gave rise to the debris. Such debris was observed only rarely in spheroplast preparations obtained from cells grown at 37 C.

Effect of inorganic phosphate. The data of Fig. 4 show that 1 mM P_i had no effect upon the growth of *P. aeruginosa* at 37 C, and the cells

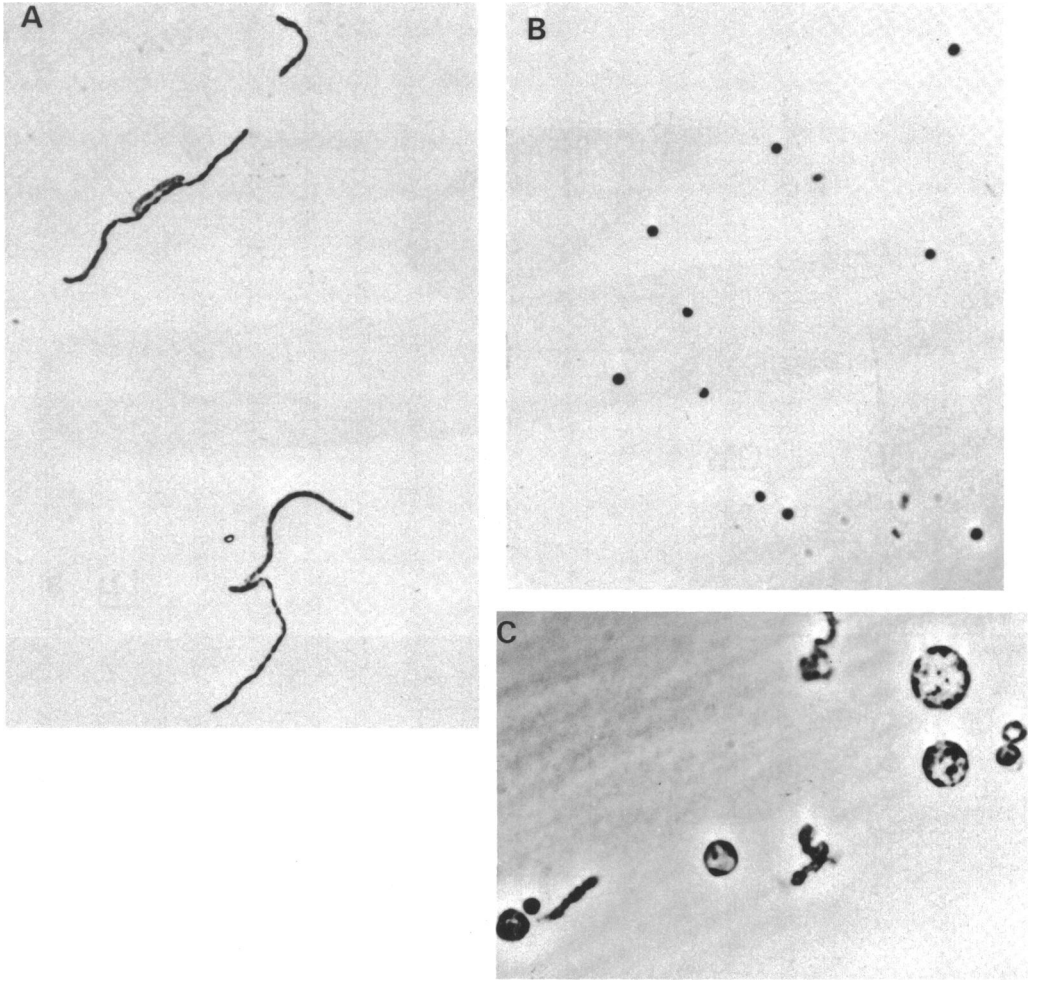

FIG. 2. *P. aeruginosa showing (A) long, beaded filaments in cultures grown in 46 C; (B) spheroplasts obtained from normal culture grown at 37 C; and (C) spheroplasts obtained from long, beaded filaments grown at 46 C.*

were small and motile. In the presence of 1 mM P_i at 46 C, the growth rate of *P. aeruginosa* was greater, as compared with the culture without added P_i. Filaments were obtained in the culture grown at 46 C in the absence of P_i, whereas the cells from the culture grown in the presence of P_i were slightly larger than normal cells and were dividing and nonmotile. Although the growth rate at 46 C was less than that observed at 37 C, the extent of growth at 46 C after 24 h approached that observed after 12 h at 37 C. The amount of APase produced after 12 h at 46 C in the absence of P_i was approximately 50-fold less than the amount produced at 37 C in the same time interval (Table 1). At the end of 24 h, when growth at 46 C approached that obtained at 37 C, the amount of APase produced at 46 C increased two- to fivefold, and the filaments were fragmented to large, single cells. It appears, therefore, that when the culture at 46 C produces APase, the filaments fragment.

Effect of APase and P_i on growth at 46 C. The results of Fig. 5 show the effect of purified APase and P_i on the growth of *P. aeruginosa* at 46 C. The addition of 1 mM P_i to the medium increases the growth rate of the organism as compared with the untreated control, and the addition of purified APase increases the rate to a value that is intermediate between the untreated control and the P_i supplement. Phase microscopy examination revealed that *P. aeruginosa* formed mostly (95%) long, nonmotile filaments in the absence of P_i at 46 C. In the

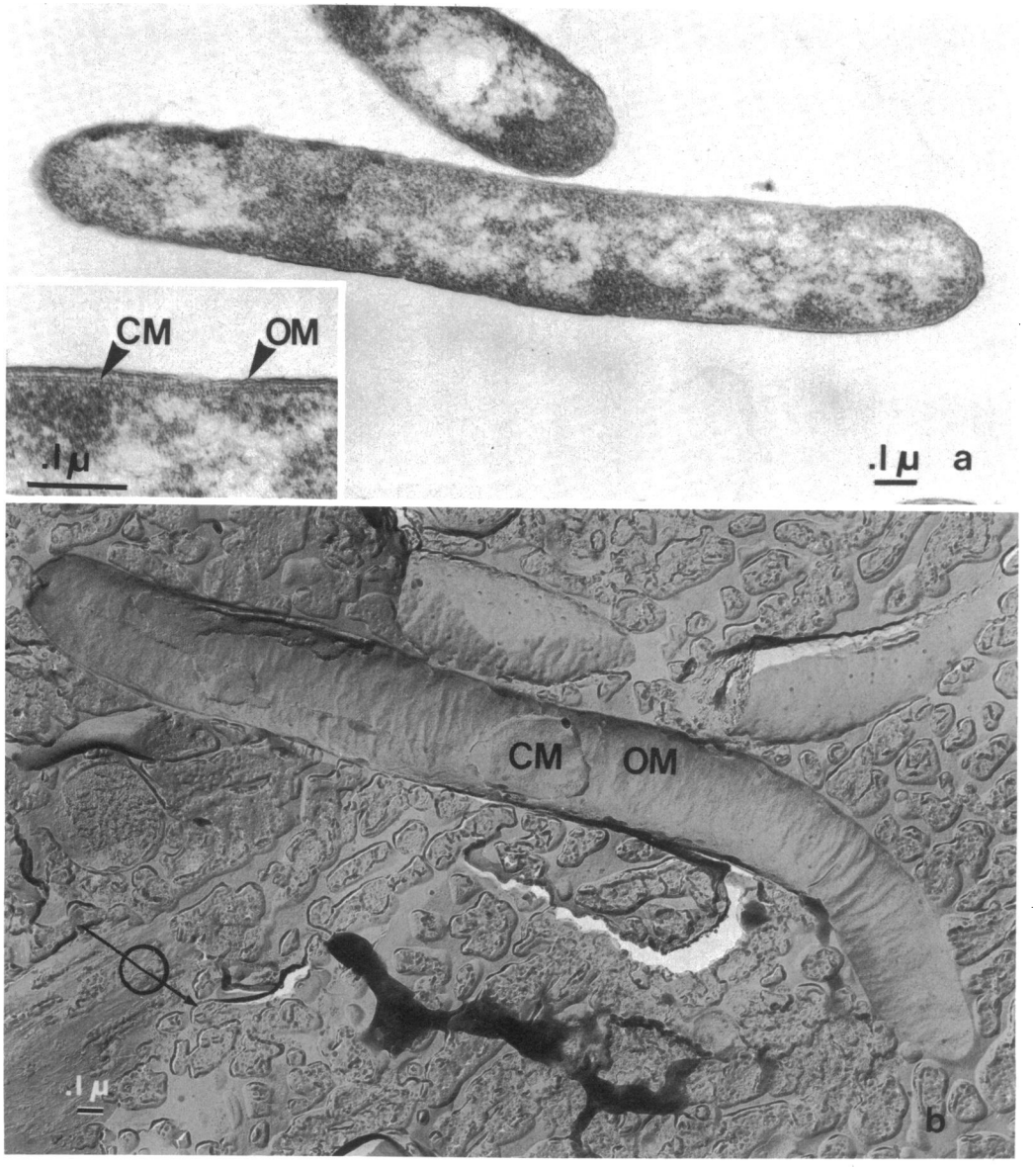

FIG. 3. *Electron microscopy examination of (a) thin section of filament obtained at 46 C; (b) freeze-etch of filament at 46 C; and (c) thin section of spheroplast and debris obtained from filament at 46 C. The bar in all cases equals 0.1 μm. Abbreviations: OM, Outer membrane; CM, cytoplasmic membrane.*

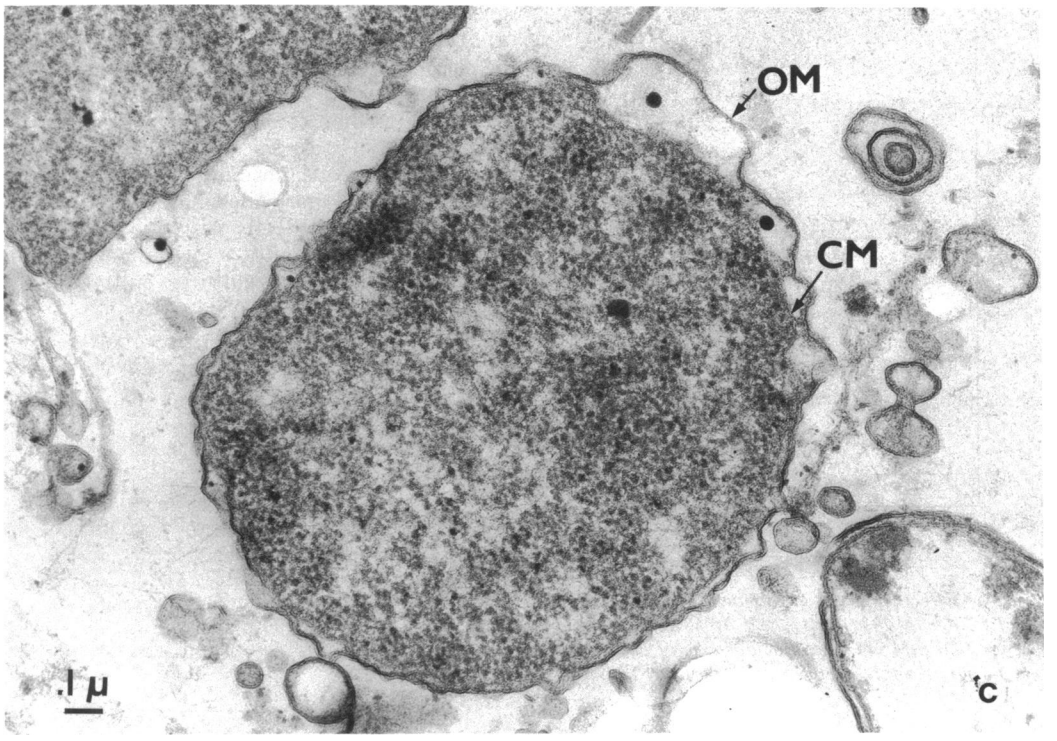

FIG. 3C

presence of P_i, only a few long filaments were observed, and most cells were large, single or doublet, nonmotile rods. In the presence of purified APase, about 80% of the cells were filaments, and the remainder were doublets. A few small, motile rods were also observed in this preparation. The fact that APase is not as effective as P_i in promoting filament fragmentation is not a surprising observation. The enzyme is added to the incubation mixture and there is no reason to assume a priori that it repenetrates the outer membrane to assume its original location within the periplasm. However, this experiment, in conjunction with others reported in this study, suggests that APase plays a role in the terminal stages of cell division.

Effect of temperature shift on growth and APase activity of *P. aeruginosa*. The results presented in Fig. 6A show the effects of a temperature shift on the growth of *P. aeruginosa*. The shift of temperature is indicated by a vertical arrow. The culture initially grown at 37 C before a shift to 46 C showed a normal growth response and cell morphology, i.e., cultures at 37 C formed small, motile rods. Cells grown initially at 46 C were in the form of long, beaded, plasmolyzed filaments. When these cultures were transferred from 46 to 37 C, there was an initial lag of 30 min, and the growth response and cell morphology were similar to the cultures initially grown at 37 C. When cultures were transferred from 37 to 46 C, there was a reduction in the growth rate, and the cells at this stage were small, nonmotile, plasmolyzed, dumbell-shaped doublets. A few chains were also observed. In some cases, phase microscopy showed that cytoplasmic material was clearly localized in one region of the doublet. Phase microscopy examination of filaments obtained from cultures grown initially at 46 C and shifted to 37 C showed that the filaments began to fragment from the polar ends. It was observed that one polar region would become motile and the terminal cell, obviously attached to the filament, moved in all planes with respect to the stationary filament. After 5 min, the terminal cell was released and moved away. Within a few minutes, the alternate polar cell developed the same motion and subsequently migrated.

The results in Fig. 6B show the APase activities before and after a temperature shift. Cells grown initially at 37 C had high enzyme activity (a, solid bar), and a temperature shift to 46 C caused little change in the enzyme activity (b). However, the enzyme activity of the CFC

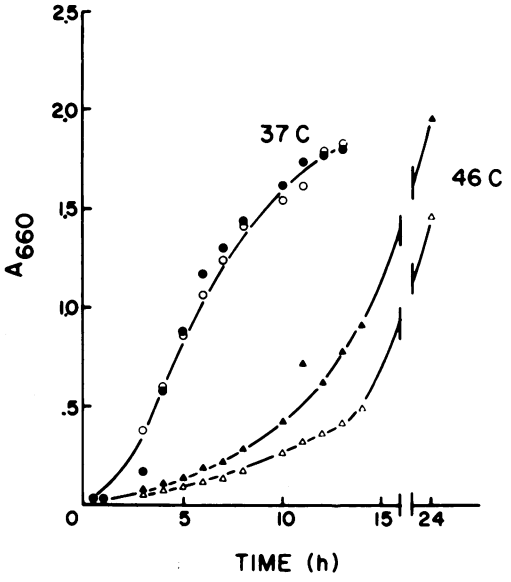

FIG. 4. *Effect of a P_i supplement on the growth rate of P. aeruginosa at 37 and 46 C grown in the presence, 1.0 mM (●, ▲), and absence of P_i (○, △). Circles and triangles represent growth at 37 C and 46 C, respectively.*

TABLE 1. *Effect of temperature on APase production by P. aeruginosa*

Time of growth (h)	Temp of growth (C)	Sp act (units/mg of protein)
12	37	41.2×10^{-3}
12	46	0.82×10^{-3}

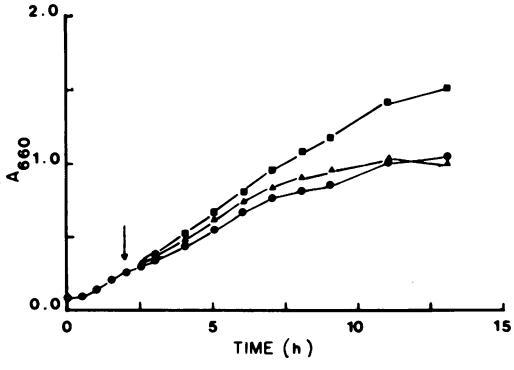

FIG. 5. *Effect of P_i and APase on the growth of P. aeruginosa at 46 C. The culture was grown at 46 C for 2 h and aseptically divided into three 20-ml portions in 50-ml flasks. To one was added 0.1 ml of purified APase to give 0.074 units of enzyme activity per ml (▲). To the second flask was added 0.1 ml of P_i to give a final concentration of 1 mM (■); no addition was made to the third flask (●).*

(open bar) was lower at 46 C (b) than at 37 C (a). This decreased activity is probably due to the decreased pH of the growth medium, a condition that leads to an irreversible loss of APase activity (5). When cells were transferred from 46 C (c) to 37 C (d), the enzyme activity was comparable to cells normally grown at 37 C, and the CFC had more phosphatase activity (d, open bar) than the cells (d, solid bar). The decline in pH of the culture medium as growth proceeded was less in cultures cultivated at

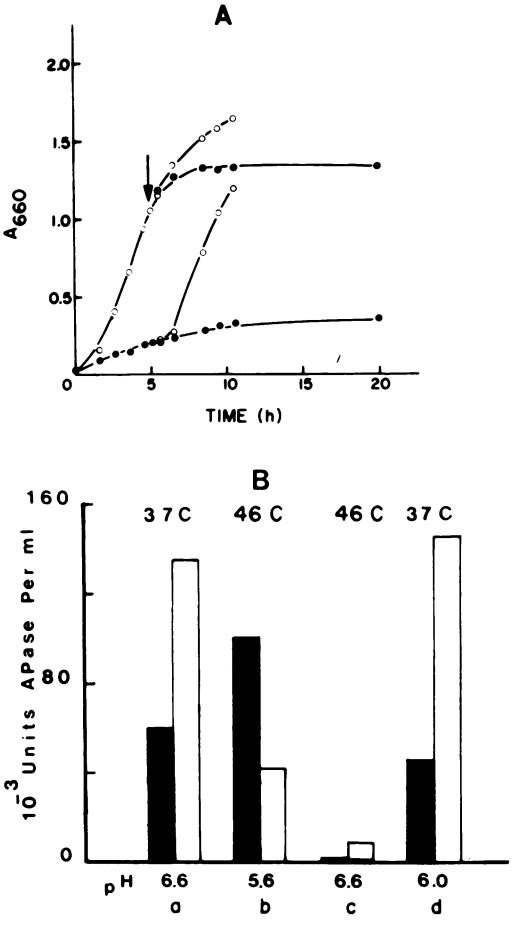

FIG. 6. *Effect of temperature shift from 37 to 46 C and 46 to 37 C on growth and APase production in P. aeruginosa. (A) cultures were grown at 37 and 46 C. After 5 h of growth they were divided into two equal portions, and one flask at 37 C was shifted to 46 C, and one flask at 46 C was shifted to 37 C. Growth was monitored at the indicated intervals. Hollow and solid circles represent cultures at 37 and 46 C, respectively. (B) Histogram showing cell-free (□) and cell-bound (■) APase activity observed at 5 h of growth at 37 C (a) and 46 C (c); (b) and (d) represent enzyme activity after 10 h of growth at 37 and 46 C.*

46 C when compared with those cultivated at 37 C.

Effect of NaCl, deoxycholate, and sucrose on filaments of *P. aeruginosa*. Results obtained with some temperature-sensitive divisionless mutants of *Escherichia coli* show that certain agents induce cell division within the filaments even at the nonpermissive temperature (22, 24). None of these agents, NaCl, deoxycholate, or sucrose, has any effect upon the filaments at 46 C as compared with the untreated control (data not shown). These results were substantiated by phase microscopy, which showed that mostly filaments were present in each case after treatment.

Macromolecular synthesis. Figure 7A shows the incorporation of L-[2-^{14}C]leucine into the protein of *P. aeruginosa* cultivated and incubated at either 37 or 46 C. The rate of incorporation for 20 min was approximately the same at both temperatures, with an increase in the rate of incorporation at 37 C and a decreased rate in the cultures at 46 C. The rate of incorporation of [2-^{14}C]uracil at 46 C was substantially decreased when compared with the rate of incorporation of [2-^{14}C]uracil into cells grown and incubated at 37 C (Fig. 7B).

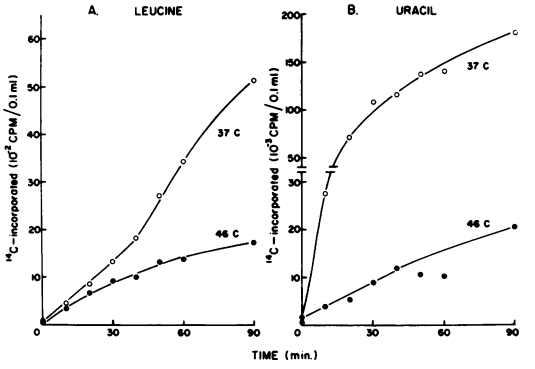

FIG. 7. *Incorporation of [2-^{14}C]leucine and [2-^{14}C]uracil into P. aeruginosa grown at 37 C and 46 C. (A) The culture was grown at 37 C to 0.55 absorbance unit. Twenty milliliters of the culture was centrifuged, and the cells were resuspended into 20 ml of fresh medium and divided into equal parts in two 50-ml flasks. Cultures were equilibrated at 37 and 46 C for 10 min, and 40 μl of [2-^{14}C]leucine (specific activity 0.5 mCi/mmol) was added. At the indicated times, 0.1-ml samples were withdrawn into 5 ml of cold trichloroacetic acid (10%) and filtered, and the precipitated radioactivity was then determined. (B) Same as A, but 0.2 ml of [2-^{14}C]uracil (specific activity, 56.6 mCi/mmol) was added to each flask at the respective temperatures.*

DISCUSSION

P. aeruginosa forms long, nonmotile filaments when cultivated at 46 C. This nonmotile characteristic of the filaments may be attributed to inactivation or deformation of flagella at 46 C, since elevated temperatures prevent synthesis of flagella in many strains of enteric bacteria (1). The outer cell wall and the cytoplasmic membrane appear to remain intact and functional during filament formation. The integrity of these structures is further substantiated because large spheroplasts are obtained from filaments, indicating that the cell wall and cytoplasmic membrane are continuous within the filament. This conclusion is further supported by examination of the thin section and freeze-fractured preparations. Phase-dark material appears to be localized at more or less regular intervals within the filament, and this observation is also evident in this section. The phase-dark material remains oriented towards the cytoplasmic membrane, even in spheroplasts obtained from large filaments. This regular occurrence of material may indicate the attachment of the chromosome to the cytoplasmic membrane.

Certain *E. coli* temperature-sensitive mutants divide at the restrictive temperature if NaCl is added to the cultures, and it was concluded that NaCl repaired a membrane lesion that results at the nonpermissive temperature (16, 24). Evidence obtained in the present study with *P. aeruginosa* indicates that the integrity of the cytoplasmic membrane remains intact in filaments. This conclusion is strengthened by the fact that NaCl does not induce filament fragmentation and deoxycholate, a reagent that lyses cells with membrane lesions (11, 16), has no visible effect upon the filaments. Stone (26) reported that NaCl permits the *E. coli fil ts* temperature-sensitive mutant to divide at 43 C and suggested that a defective envelope is responsible for its inability to divide under restrictive conditions. Similar observations have been reported for *Salmonella typhimurium* (6) and *E. coli* (22).

The results obtained in the present study by freeze-fracture and thin section examination and spheroplast formation indicate that the cell envelope of *P. aeruginosa* grown at 46 C is not defective. In addition, cultures that are shifted from 46 to 37 C begin to fragment and grow without an appreciable lag. The possibility exists that proteins of the periplasmic space of *P. aeruginosa* are affected by growth at 46 C. APase, for example, is located in the periplasm of *P. aeruginosa* (4) and its synthesis is almost completely inhibited by growth at 46 C. This is surprising, since the purified enzyme is ex-

tremely stable to heat (8), and these results are substantiated because enzyme preformed at 37 C is not destroyed by a shift to 46 C. Another enzyme of the periplasm, 5'-nucleotidase, is also affected by growth of *P. aeruginosa* at 46 C (data not presented). The specific activity of this enzyme is reduced 20-fold when compared with the control culture grown at 37 C. The enzyme level is fully restored after transfer of the 46 C grown culture to 37 C. When the 46 C culture is shifted to 37 C, the synthesis and appearance of active APase are almost immediate, indicating that the enzyme may be present in the periplasm as an inactive species. Although the rate of incorporation of [^{14}C]uracil into the ribonucleic acid and [^{14}C]leucine into the protein of filaments grown at 37 C and incubated at 46 C is decreased as compared with the 37 C control, the rates are not depressed to such a level as to indicate severe metabolic alteration of these cytoplasmic functions at 46 C.

Filaments obtained at 46 C are nonmotile, and complete flagella have never been observed in these preparations. When the filaments are shifted to 37 C, fragmentation begins at one polar end after 30 min, and this terminal cell becomes more physically active until it separates and is motile. The process is then repeated at the opposite pole and is repeated until the filament is completely fragmented to single, motile rods. The lack of flagella at 46 C may also indicate impairment of protein assembly in the outer cell envelope of *P. aeruginosa*.

The importance of a protein (or proteins) in the cell division cycle is not a new concept. Fan and Beckman (12) reported that the growth rate of *Bacillus subtilus ts* is greatly stimulated by the addition of lysozyme or *B. subtilus* autolysin. Smith and Pardee (25) also suggested that heat shock inactivates a protein necessary for cell division in *E. coli*, and the results of Ron and Davis (24) suggest that the decreased activity of homoserine *trans*-succinylase is associated with a decreased growth rate of *E. coli* when the culture is shifted from 37 to 45 C. In the case of *P. aeruginosa*, it is only under conditions where APase is not present or synthesized that cells form filaments. It is obvious that preexisting or added, purified APase is stable to 46 C, whereas cultures grown initially at 46 C possess little or no enzyme activity, but activity appears almost immediately after a shift to 37 C. The results suggest that APase monomer (5) is synthesized at 46 C but, because of its instability (5), it cannot dimerize to active APase. Since the APase activity appears almost immediately after the shift to 37 C, the view that impairment is due to the inhibition of protein assembly is further substantiated. The fact that a product of the APase reaction, P_i, duplicates the effects observed when the enzyme is present suggests that under P_i-limiting conditions the enzyme participates in the terminal stages of cell division.

Whether APase plays a direct or indirect role in cell division of *P. aeruginosa* is not completely obvious from the data presented. It is definitive, however, that the presence of the enzyme, or its product, P_i, alleviates the inhibition of cell division at 46 C. One possible role for APase maybe to supply P_i for another enzyme, or enzymes, which are involved in the cell division process. Although the proteose peptone is limited in its concentration of P_i, it can supply organic phosphate, which is mobilized by APase. This interpretation is strengthened by the observed lack of growth response of *P. aeruginosa* to 1 mM P_i at 37 C; i.e., the culture is not limited by total phosphate as long as APase is present. One such enzyme, or series of enzymes, which may require the P_i mobilized by APase are those involved in phospholipid, mucopeptide, teichoic acid, or deoxyribonucleic acid synthesis. Under conditions such as these, the role of APase is indirect, since the enzyme supplies substrate for other enzyme reactions. Regardless of the indirect or direct role of the enzyme, it is apparent that under conditions of P_i limitation APase is necessary for cell division. The examination of a more precise role of this enzyme and the effect of elevated temperatures on the synthesis and activity of other periplasm-located enzymes and proteins are under investigation.

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