SUMMARY

The mating type factor FP (pseudomonad fertility factor) controls mating and recombination in *Pseudomonas aeruginosa*. FP can be transferred to strains lacking (FP^-) from those possessing it (FP^+), with varying efficiency depending on the particular FP^+ strain used. Unlike the analogous agent F in *Escherichia coli*, FP is refractory to acridines and metal salts and has not been shown to mutate to a stable Hfr form. The segregation of FP in crosses has been studied and its influence on the segregation of other markers demonstrated. Linkage of a number of such markers has been demonstrated and seven have been located onto one linkage group.

REFERENCES

ADAMS, M. H. 1950 Methods of study of bacterial viruses. In *Methods in medical research*, vol. 2, pp. 1–73. Year Book Publishers, Chicago.

ALBERT, A., S. D. RUBBO, R. J. GOLDACRE, M. E. DAVEY, AND J. D. STONE 1945 The influence of chemical constitution on antibacterial activity. II. A general survey of the acridine series. Brit. J. Exptl. Pathol., **26**, 160–192.

BERNSTEIN, H. L. 1958 Fertility factors in *Escherichia coli*. Symposia Soc. Exptl. Biol., **12**, 93–103.

CAVALLI, L. L. 1950 La sessualita nei batteri. Boll. ist. sieroterap. milan., **29**, 281.

CAVALLI-SFORZA, L. L. 1958 Recombination in bacteria. In *Recent progress in microbiology*. Proc. Intern. Congr. Microbiol., 7th Meeting, pp. 40–50.

CAVALLI, L. L., J. LEDERBERG, AND E. M. LEDERBERG 1953 An infective factor controlling sex compatibility in *Bacterium coli*. J. Gen. Microbiol., **8**, 89–103.

DAVIS, B. D., AND E. S. MINGIOLI 1948 Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol., **60**, 17–28.

HAYES, W. 1953 The mechanism of genetic recombination in *Escherichia coli*. Cold Spring Harbor Symposia Quant. Biol., **18**, 75–93.

HIROTA, Y. 1956 Artificial elimination of the F factor in *Bacterium coli* K12. Nature, **178**, 92–93.

HIROTA, Y., AND T. IIJIMA 1957 Acriflavine as an effective agent for eliminating F factor in *Escherichia coli* K12. Nature, **180**, 655–656.

HOLLOWAY, B. W. 1955 Genetic recombination in *Pseudomonas aeruginosa*. J. Gen. Microbiol., **13**, 572–581.

HOLLOWAY, B. W. 1958 Genetic recombination in *Pseudomonas aeruginosa*. In *Proc. Intern. Congr. Genet. 10th Meeting (Montreal)*, vol. 2, p. 123. Univ. of Toronto Press, Toronto, Ontario, Canada.

HOLLOWAY, B. W., AND P. A. JENNINGS 1958 An infectious fertility factor for *Pseudomonas aeruginosa*. Nature, **181**, 855–856.

JACOB, F., AND E. L. WOLLMAN 1956 Recombinaison genetique et mutants de fertilite chez *Escherichia coli*. Compt. rend. acad. sci., **242**, 303–306.

LEDERBERG, J. 1950 Isolation and characterization of biochemical mutants of bacteria. In *Methods in medical research*, vol. 3, pp. 5–22. Year Book Publishers, Chicago.

RICHTER, A. A. 1958 Recombination analysis of mating type in *Escherichia coli* K12. In *Proc. Intern. Congr. Genet., 10th Meeting*, vol. 2, p. 232. Univ. of Toronto Press, Toronto, Ontario, Canada.

CELL DIVISION IN A SPECIES OF *ERWINIA*[1, 2]

I. Initial Observations Relating to Nutritional Dependency

E. A. GRULA

Department of Bacteriology, Oklahoma State University, Stillwater, Oklahoma

Received for publication February 3, 1960

Cells of an *Erwinia* species when cultured on a proteose peptone-casitone-glucose agar medium, grow to a length of 10 to 20 μ or longer after 24 hr at 25 C. Cells from the same inoculum, when cultured on heart infusion agar grow only to a length of 1 to 2 μ.

MATERIALS AND METHODS

Formulation of the proteose peptone-casitone-glucose agar medium: proteose peptone no. 3, 1.5 per cent; casitone, 0.5 per cent; yeast extract, 0.1 per cent; glucose, 0.5 per cent; sodium chloride, 0.5 per cent; and agar to 2 per cent. Cells were always incubated at a constant temperature of 25 C unless otherwise indicated. When shaking conditions were employed during growth, tubes (150 by 17 mm) containing 5 ml of culture medium were aerated by placing them on a shaker having a rotational speed of 180 rpm.

The organism used was a stock culture of a soft-rot producing Erwinia that had been transferred routinely at least 7 years on either nutrient agar or nutrient agar containing 1 per cent glucose, and grown at room temperature. On the basis of biochemical tests, it has not been possible to identify accurately the organism, although it most nearly resembles *Erwinia carotovora*. Mushin, Naylor, and Lahovary (1959) point out that the soft-rot group has too much biochemical variability to allow accurate identification of species. Their statement is in agreement with Dowson (1957) who suggests that the 3 major species in the soft-rot group be united into one species.

[1] This investigation was supported by the Research Foundation, Oklahoma State University, project no. 91 and a National Science Foundation grant G9848.

[2] Portions of this study were presented at the 59th General Meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 10 to 15, 1959.

When washed suspensions were employed, 22- to 24-hr cultures were twice sedimented by centrifugation at room temperature in cold physiological saline and resuspended in cold saline to an optical density of approximately 0.4. One drop of this suspension was used as inoculum. All measurements for optical density were performed using a Bausch and Lomb spectronic 20 photocolorimeter adjusted to 540 mμ.

Routine observation of cells was done using oil immersion after staining heat-fixed cells with 1 per cent crystal violet for 1 min although Gram staining was periodically employed. Cells were also observed in wet mount preparations using both dark-field and phase microscopy. The RCA-EMU-2D electron microscope was also employed.

RESULTS

When the observation was first made, contamination or a culture mislabeling was suspected. Therefore, the culture was purified by 8 consecutive streak purifications of well isolated colonies (24-hr intervals), on heart infusion agar. After the 8th purification, one colony was picked, transferred to a heart infusion agar slant and grown for 24 hr at 25 C. This culture was used to streak another heart infusion agar plate and a plate containing proteose peptone-casitone-glucose agar medium. Observation after 24 hr again showed that the cells growing on heart infusion agar were small coccoid rods, whereas cells from the proteose peptone-casitone-glucose agar were long rods. It was noted that all colonies on heart infusion agar were typically smooth, whereas all colonies on the proteose peptone-casitone-glucose agar medium were typically rough. Although, with many bacteria, rough colonies are produced by nonencapsulated strains, in this case the rough-smooth colony variation is a reflection of cell size, since no prominent capsule exists on either the long or the short cells.

Long cells were observed in the bright-field

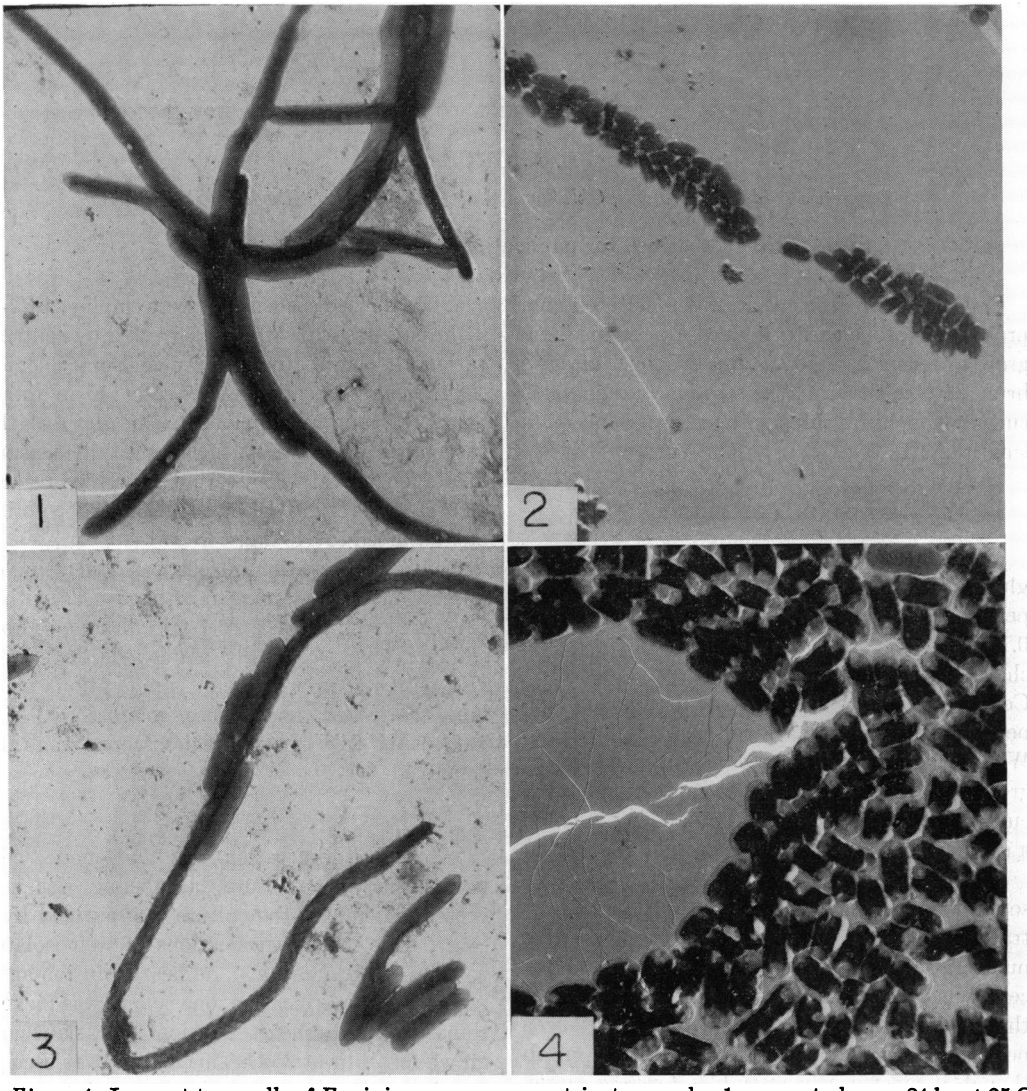

Figure 1. Longest type cells of *Erwinia* sp. grown on nutrient agar plus 1 per cent glucose 24 hr at 25 C. No cross walls are apparent. ×2600.

Figure 2. Short type cells of *Erwinia* sp. grown on nutrient agar without glucose 24 hr at 25 C. ×2600.

Figure 3. A long partially autolyzed cell of *Erwinia* sp. The other cells are also considered long and occur with greater frequency than the longest type cells shown in figure 1. Grown on nutrient agar plus 1 per cent glucose 24 hr at 25 C. ×2600.

Figure 4. Short type cells grown on nutrient agar without glucose 24 hr at 25 C. Shown at greater magnification (×4540) to demonstrate flagella.

Figures 1–4 are reduced about 22 per cent for reproduction here.

phase, dark-field, and electron microscopes to determine if the long cells were chains of short cells. Electron micrographs are shown in figures 1 to 4. The longer type cells are not chains of small cells.

Mutation has also been ruled out since all cells inoculated from the same suspension develop into colonies which are either rough and contain only long cells when grown on the proteose peptone-casitone-glucose agar medium, or colonies which are smooth and contain only short cells when grown on heart infusion agar within a 24-hr incubation period.

The observation was then made that cells

grown on nutrient agar were similar to those grown on heart infusion agar in that the cells were small coccoid rods and the colonies smooth. To determine what was causing the cells to become long (the small coccoid rod was accepted as "normal"), each constituent of the proteose peptone-casitone-glucose agar medium was individually added to tubes containing nutrient agar so that final concentration of each constituent in nutrient agar was the same as it was in the proteose peptone-casitone-glucose medium. After a 24-hr growth period, microscopic examination of all cultures revealed that glucose was the compound responsible for cell elongation. Cells grown on nutrient agar containing 1 per cent glucose were long and the colonies rough as expected. The reverse experiments were also done wherein individual mixtures of meat extract, peptone, and sodium chloride were added to the proteose peptone-casitone-glucose agar medium. In all these experiments, cells were always of the long type. Thereafter, cells were cultured on either nutrient agar or nutrient agar containing 1 per cent glucose to obtain short or long cells during further experimentation.

In all experiments to this point, glucose had always been added prior to autoclaving of the media. To determine if autoclaving of glucose was necessary, glucose, sterilized by passage through a sintered glass filter, was added to nutrient agar. Cells grown in the presence of the filtered glucose were always long; therefore, autoclaving of glucose is not needed to produce the effect.

Because glucose is an excellent carbon-energy source and because a theory might be advanced for growth of longer cells in the presence of abundant amounts of carbon and energy, other compounds were tested in nutrient broth to determine if other utilizable carbon-energy sources also caused long cell development. The results of these tests are presented in table 1.

Other compounds which permitted some elongation included mannitol, arabinose, rhamnose, levulose, sucrose, lactose, and possibly

TABLE 1

*Response of small type cells of Erwinia sp. to different carbon-energy sources in nutrient broth**

Carbon-Energy Compound†	Chemical Nature	OD‡	Final pH	Cell Size (μ)
None added	—	1.2	7.8	1–2
D-Glucose (dextrose)	Aldohexose	0.85	4.8	10–20; 5% to 30
D-Galactose	Aldohexose	1.7	5.3	2.0; 10% to 10
D-Mannose	Aldohexose	1.45	4.85	2–3; 1% to 10
Mannitol	6-Carbon sugar alcohol	1.1	4.9	4–5; 5% to 10
D-Glucuronic acid	6-Carbon uronic acid	1.3	7.5	1–2; 1% to 10
Potassium D-gluconate	6-Carbon onic acid	1.9	7.5	1–2; 10% to 4
D-Ribose	Aldopentose	1.4	5.2	2–3; 1% to 10
L-Arabinose	Aldopentose	1.3	4.9	4.0; 10% to 10
D-Xylose	Aldopentose	1.5	5.2	1–2
Rhamnose	6-Deoxy-L-mannose	1.25	4.8	4–5
D-Fructose (levulose)	Ketohexose	1.4	4.8	95% 2–5; 5% to 20
D-Ribitol (adonitol)	5-Carbon sugar alcohol	1.15	7.85	1–2; 5% 3–4
Sucrose	Disaccharide (glucose + fructose)	1.0	4.8	5–10
Lactose	Disaccharide (glucose + galactose)	1.35	4.8	4–5; 10% to 10
Maltose	Disaccharide of D-glucose	1.15	7.8	1–2
Raffinose	Trisaccharide (glucose + fructose + galactose)	1.4	4.9	90% 2–5; 10% to 10
Inulin	Polysaccharide of fructose	—	7.8	1–2
Starch	Polysaccharide of glucose	—	7.8	1–2

* Cells grown 22 hr at 25 C on a rotary shaker.

† Compound present at a level of 5 mg/ml; autoclaved separately at 10 lb for 10 min and added aseptically.

‡ Optical density measured at 540 mμ against a nutrient broth blank.

raffinose. From a comparison of the open-chain formulas of the compounds, there does not appear to be any feature common to either the sugars that produce elongation, or those that do not. This was surprising since many carbohydrates are considered to be "metabolically equivalent" compounds and all of the compounds, with the exception of starch, inulin, adonitol, and maltose, were metabolized as judged by pH measurements. Of the 3 aldopentoses tested, arabinose caused good elongation whereas cells were extremely short in the presence of xylose. Of the 3 aldohexoses tested, only glucose caused elongation. With reference to the sugar alcohols, mannitol (6-carbon) caused elongation whereas adonitol (5-carbon) did not. Elongation by lactose and sucrose can be attributed to their D-glucose and, in the case of sucrose, D-fructose content. Although glucose inhibited growth (optical density of 0.85 vs. 1.2 in the control tube), no obvious correlation exists between inhibition and cell elongation with reference to the other carbon-energy sources. The problem of growth inhibition will be considered in greater detail in forthcoming publications.

Because one of the effects of sugar metabolism is to lower pH, it appeared possible that a lowered pH was causing some enzymatic change which, in turn, allowed development of the long type cells. From the data in table 1, it is apparent that utilization of most of the carbon-energy sources resulted in a lowered pH; however, cell elongation did not occur in all cases, particularly in the presence of mannose or xylose. Further experiments using glucose nutrient broth buffered with phosphate revealed that elongation still occurred in the pH range 6.5 to 7.0. Also, the organism was still small after it had been subcultured for a period of 14 days at 24-hr intervals on nutrient agar containing 1 per cent xylose.

A few trial experiments had shown that nutrient broth cultures not aerated (by shaking) developed into intermediate sized cells (3 to 5μ) either in the presence or absence of glucose. These experiments revealed that good aeration was necessary for development of the longest cells in the presence of glucose or the shortest type cells in its absence. Experiments designed to demonstrate an effect of oxidation-reduction potential on cell size by addition of cystine or cysteine (2×10^{-3} to 2.4×10^{-5} M final concentration in the medium), to standing cultures in the absence of glucose, resulted in the formation of small to intermediate sized cells. The mechanism of the effect of aeration is a subject for further investigation.

To delineate more accurately the time period wherein glucose was exerting its effect on cell division and growth, washed cells from nutrient agar were inoculated into nutrient broth and nutrient broth containing 1 per cent glucose. Growth was followed by plate counts of the cells using nutrient agar, staining, and determining dry weight (100 C at 24 hr) of samples of cells removed from the growth medium. Results of the plate counts and dry weight determinations are shown in figures 5 and 6.

Between the 10th and 12th hr, cells which were growing in nutrient broth plus 1 per cent glucose appeared to stop dividing, whereas cells growing in nutrient broth without glucose continued to divide for several more hours. Although the glucose-grown cells stopped dividing, individual cell growth continued since dry weight of the culture continued to increase. Mass, on a dry weight basis, of the long type cell has varied from 7.5 to 20.5 times greater per cell than the short type cell, depending on which experiment and set of figures are used in the calculations. Microscopic data revealed that cells in the glucose medium became long at about the same time, i.e., a difference in size of cells in either culture medium

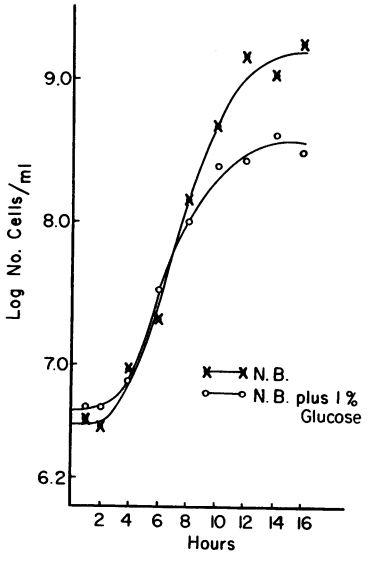

Figure 5. Comparative growth curves for cells growing in the presence and absence of glucose.

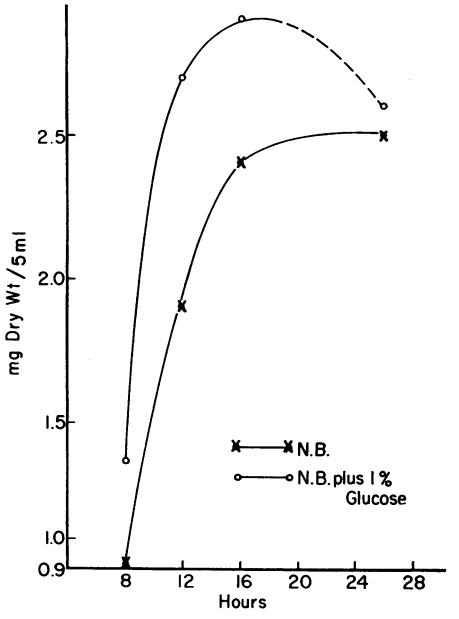

Figure 6. Comparative dry weight curves for cells growing in the presence and absence of glucose.

was not very obvious until between the 10th and 12th hr of incubation, after which cells growing in the presence of glucose definitely became longer and easily distinguishable from the cells growing in nutrient broth. At 26 hr, some lysis of cells in the glucose broth occurred.

Miscellaneous observations. (1) Many of the small coccoid rod forms of this organism are motile whereas relatively few of the large cells are motile. When motility is present in the long type cells, the cells usually move with a pronounced undulatory motion. When 2 or 3 cells exist in a chain, the last cell in the chain appears to propel the other cells by constant "whiplike" movements. Examination with the electron microscope has revealed that significantly fewer flagella are present on the long cells. Whether synthesis of flagella is inhibited to some degree in long cells is not definitely known. (2) Long type cells will settle nearly to the bottom of standing tubes during a 24-hr period, whereas the short type cells show little or no settling during the same time period. (The longer the cells, the more quickly they settle.) (3) Long cells are thermoagglutinable (less than 1 min at 100 C in physiological saline), whereas small cells show no thermoagglutinability. Preliminary studies of the somatic antigenic structure of the 2 types of cells (formalin-killed) have revealed that although cross agglutination does occur, the long type cells elicit a lower titer than short cells when tested against either homologous or heterologous sera. (4) After extended growth, long cells show a pronounced loss of "basophilia" in portions of the cells or throughout an entire cell length. This phenomenon is very disconcerting since assay is based on the appearance of stained cells and one cell can appear to be 2 or 3 or more cells in stained preparations. When doubt existed regarding cell size, cells were examined using phase microscopy. (5) In one experiment, cells grown at 38 C on nutrient agar in the absence of glucose became very long; however, growth was extremely poor even after 90 hr incubation. Further attempts to verify this result at temperatures up to 42 C were unsuccessful.

DISCUSSION

Wahlin and Almaden (1939) reported that glucose was one of the compounds that could cause the formation of "megalomorphic" forms of *Salmonella schottmulleri* after 6 to 9 days incubation in a defined medium. When yeast extract (4 to 8 per cent) was added to the medium to aid growth, other organisms (*Aerobacter aerogenes*, *Salmonella paratyphi*, and *Klebsiella ozaenae*), developed megalomorphic forms in from 3 to 8 days. Because these investigators were primarily concerned with bacterial "life-cycles," they were more interested in "bulb" forms of the bacteria than long cells; however, their pictorial data reveal that long cells existed in their cultures. Long forms of several bacteria can be produced by employing many diverse chemical or physical treatments (*see* review by Hughes, 1956). However, the formation of long cells and retardation of bacterial cell division by sugars in the growth medium is not well documented.

The experiments reported here reveal that some, but not all, carbon-energy sources utilized for growth exert a specific control by greatly retarding cell division. Sugars, such as galactose and glucose, which are generally considered to be "metabolically equivalent" in that both can be converted to glucose 6-phosphate and then metabolized either by the Embden-Myerhoff or the hexose monophosphate shunt pathway prior to final oxidation, may be different in their effect on cell size. It is usually not thought that one

carbohydrate will influence a process as fundamental as cell division in a manner different from the other, even though the end products of partial oxidation may differ qualitatively or quantitatively. Apparently, the sugar being utilized exerts a control on cell division at the same point in its metabolic life either because of the synthesis of some product(s) detrimental to the division process or to the lack of synthesis of some product(s), or both.

ACKNOWLEDGMENT

The writer wishes to acknowledge the aid of Mr. Fred Rowe of the Research and Development Department, Continental Oil Company, Ponca City, Oklahoma, in preparation of the electron micrographs.

SUMMARY

Cell division can be retarded in a species of *Erwinia* under what may be considered "normal" nutritional conditions. Glucose, lactose, sucrose, levulose, arabinose, rhamnose, mannitol, and possibly raffinose all retard division of cells growing in nutrient broth. Cell division is not retarded by galactose, mannose, maltose, glucuronic acid, potassium gluconate, ribose, xylose, adonitol, starch, or inulin. There does not appear to be any structural feature common to either the compounds that produce elongation, or those that do not. Changes in either pH or oxidation reduction potential of the medium do not appear to be the primary causes for the retardation of division although shaking conditions appear to aid formation of the long cells in the presence of the appropriate carbon-energy source.

REFERENCES

DOWSON, W. J. 1957 *Plant diseases due to bacteria*, 2nd ed. Cambridge University Press, New York.

HUGHES, W. H. 1956 Bacterial anatomy. The structure and development of the induced long forms of bacteria. Symposium Soc. Gen. Microbiol., **6**, 341–360.

MUSHIN, R., J. NAYLOR, AND N. LAHOVARY 1959 Studies on plant pathogenic bacteria. I. Cultural and biochemical characters. Australian J. Biol. Sci., **12**, 223–232.

WAHLIN, J. G., AND P. J. ALMADEN 1939 Megalomorphic phase of bacteria. J. Infectious Diseases, **65**, 147–155.