The Influence of Oxygen, Glucose and Nitrate upon the Formation of Nitrate Reductase and the Respiratory System in *Bacillus licheniformis*

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SUMMARY

When a culture of *Bacillus licheniformis* was shifted from aerobic to anaerobic conditions, lysis occurred unless a fermentable carbon source or a system for nitrate respiration was present. Nitrate reductase was primarily induced by partial or complete anaerobiosis and partially repressed by glucose. The enzyme was repressed and inactivated by high oxygen concentrations. Respiration of bacteria grown anaerobically was 30 to 40 % of that of bacteria grown aerobically. Glucose decreased respiration of other substrates in both aerobically and anaerobically grown organisms. No cytochrome a_1 was present after anaerobic growth. Cytochrome c_1 was repressed by glucose; under anaerobic conditions this repression was antagonized by nitrate.

INTRODUCTION

Most of the work in the regulation of nitrate reductase has been done with coliform bacteria, in which it is induced by anaerobiosis in the presence of nitrate. The enzyme is repressed, inhibited and inactivated by oxygen (Pichinoty, 1965; Van't Riet, Stouthamer & Planta, 1968; De Groot & Stouthamer, 1970a)

Hackenthal (1966) found parallel induction of nitrate and nitrite reductase by nitrate, nitrite and some other monovalent anions in *Bacillus cereus*. In *B. stearothermophilis* nitrate reductase was induced by nitrate and repressed and inactivated by oxygen. When cells were adapted to nitrate, oxygen respiration decreased by 50 to 70 %; the main change in the cytochrome spectrum was the disappearance of cytochrome a_3 (Downey, 1966; Downey & Nuner, 1967; Downey & Kiskiss, 1969; Downey, Kiskiss & Nuner, 1969).

The *Bacillus* species in which nitrate reductase formation has been studied so far are obligate organisms. This paper describes a study of the regulation of nitrate reductase formation and of the influence of nitrate, glucose and oxygen on respiratory activity and cytochrome content in the facultative organism *Bacillus licheniformis*.

METHODS

Organism: *Bacillus licheniformis* s244 (originally designated *B. licheniformis* 430) was obtained from Dr J. W. Woldendorp, Agricultural University, Wageningen, The Netherlands. It was maintained on slopes of Oxoid brain heart infusion agar and stored at 4°.

Media. NBY medium contained Oxoid nutrient broth no. 2, 8 g./l., and Oxoid yeast extract, 3 g./l. NBYG was made by adding glucose (5 g./l.); NBYGNO₃ by adding glucose (5 g./l.) and KNO₃ (2 g./l.); and NBYNO₃ by adding KNO₃ (10 g./l.). Glucose and nitrate were added from sterile stock solutions. The pH of all media was 7.0.

Cultural conditions. Bacteria were grown at 37° in a New Brunswick MicroFerm Laboratory fermentor in a 7 l. jar containing 3 l. of medium either with vigorous aeration (10 l. air/min., agitation 650 rev./min.), with weak aeration (2 l. air/min., agitation 200 rev./min.) or anaerobically (nitrogen, agitation 100 rev./min.). The medium in the jar was inoculated with 2 × 100 ml. of culture in NBY, shaken overnight at 250 rev./min. at 37° in a 500 ml. bottle. If required, the pH of the medium was maintained at pH 7 by a New Brunswick automatic pH controller using 0.5 N-NaOH. Dissolved oxygen concentration was measured with a New Brunswick oxygen electrode and is expressed as percentage of the dissolved oxygen concentration in the oxygen-saturated medium. Bacterial density was determined by measuring the extinction at 660 nm. (E_{660}) in a Unicam SP 600 spectrophotometer.

Harvesting of bacteria and preparation of cell-free extracts. Bacteria were harvested by centrifugation in an MSE 18 high speed centrifuge at 10,000g at 2° for 15 min. For cell-free extracts, they were washed twice with cold phosphate buffer, 0.065 M, pH 7, resuspended in this buffer and sonicated in an MSE ultrasonic disintegrator, 60 W, 20 kc./sec. for 10 min. at 0°. Cellular debris was not removed, as this caused fluctuations in nitrate reductase activity.

Suspensions in phosphate buffer lysed rapidly at 37° and failed to respire. Lysis was prevented by adding MgSO₄ · 7H₂O (10 %, w/v). Bacterial suspensions were therefore washed and resuspended in MgSO₄ · 7H₂O (100 g./l.), tris (1.21 g./l.), KCl (2.47 g./l.), adjusted to pH 7 with HCl. Throughout harvesting, chloramphenicol (30 µg./ml.) was present.

Measurement of acid formation. Cultures were grown anaerobically in NBYG or NBYGNO₃ to $E_{660} \approx 1.4$. Four hundred ml. of culture was chilled by addition of ice. The bacteria were harvested by centrifugation and resuspended in 200 ml. of fresh cold NBYG medium with chloramphenicol (30 µg./ml.). Samples (50 ml.) in a beaker were gently stirred and bubbled with nitrogen in a 37° water bath. The pH was measured continuously with a Philips pH meter model PR 9403/01 and at 2 min. intervals adjusted to pH 7 with NaOH (c. 0.1 N) previously titrated with 0.1 N-oxalic acid. At zero time 0.5 ml. of distilled water or 20 % KNO₃ solution was added. Samples (0.5 ml.) were taken for glucose determinations at 5 min. intervals.

Cytochromes. Cytochromes were measured in dithionite-reduced suspensions using a Unicam SP 800 spectrophotometer. Cytochrome c_1 content was expressed as the difference in extinction between the peak at 552 nm. and the line connecting 540 and 570 nm.; cytochrome a_1 content as the difference in extinction between the peak at 595 nm. and the line connecting 580 and 610 nm. Specific cytochrome content was expressed as E_{552} or $E_{595}/10$ mg. of protein.

Other assays. Oxygen uptake by whole bacteria was measured by standard Warburg techniques.

Glucose content of the medium was determined with the glucose oxidase (Boehringer, Mannheim, Germany).

Nitrite content of the medium was assayed as described by Van't Riet, Stouthamer & Planta (1968).

Nitrate reductase activity was measured according to Lowe & Evans (1964); the assay mixtures were incubated for 15 min. at 37°.

Protein was estimated according to Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

Effect of anaerobiosis, induction of nitrate reductase and lysis

In vigorously aerated cultures, no nitrate reductase could be demonstrated as long as the cell density was not too high. When a NBYNO₃ culture grown with vigorous aeration was shifted to anaerobic conditions, rapid lysis occurs (Fig. 1) and no nitrite was formed.

Fig. 1

Fig. 2

Fig. 1. Lysis of *Bacillus licheniformis* s244 in NBYNO₃ medium after a shift from vigorous aeration to anaerobiosis.

Fig. 2. Growth, nitrite formation and nitrate reductase synthesis of *Bacillus licheniformis* s244 in NBYNO₃ medium during weak aeration, followed by a shift to anaerobiosis. ●, E_{660} ; ○, nitrate reductase specific activity; ▲, nitrate reductase total activity; ×, nitrite content of medium.

When grown with weak aeration in NBYNO₃, the oxygen consumption by the bacteria soon exceeded the oxygen supply and the oxygen tension in the medium was reduced. Nitrate reductase was induced and nitrite was produced. During this period, oxygen and nitrate respiration were occurring simultaneously, and change to anaerobic conditions did not cause lysis until the nitrate was exhausted (Fig. 2).

In glucose-containing media, shifts from vigorous aeration to anaerobic conditions never caused lysis, but acid was produced immediately. In cultures without pH regulation, growth stopped when pH 5.7 was reached (Fig. 3*a*). The pH was regulated in all further experiments in glucose-containing media. Nitrate reductase was formed under anaerobic conditions in NBYG, in spite of the absence of nitrate (Fig. 3*a*).

Addition of nitrate caused a further 2.5-fold increase in specific activity (Fig. 3*b*). Specific activity in NBYNO₃ was considerably higher than in glucose-containing media: glucose repressed nitrate reductase. More nitrite was formed in NBYNO₃ than in NBYGNO₃, in spite of the greater total nitrate reductase activity in the latter medium. Apparently, in the presence of glucose the transfer of electrons to the nitrate reductase was less effective.

Fig. 3. Growth and nitrate reductase formation of *B. licheniformis* s 244 in (a) NBYG and (b) NBYGNO₃ media after a shift from vigorous aeration to anaerobiosis, with and without pH control. With pH control: ●, E_{660} ; ○, nitrate reductase specific activity; ▲, nitrate reductase total activity; ×, nitrite content of medium. Without pH control (applies to (a) only): □, E_{660} ; △, nitrate reductase specific activity; ■, pH of the medium.

Effect of aeration. *Bacillus licheniformis* was grown in NBY, NBYNO₃, NBYG and NBYGNO₃ media with weak aeration, and the dissolved oxygen content, nitrate reductase and nitrite formation were followed. Nitrate reductase formation in glucose-free and glucose-containing media started at 20 and 0% oxygen respectively (Fig. 4*a, b*). In NBY and NBYNO₃ nitrate reductase was produced at the same rate; the same applies to NBYG and NBYGNO₃. Also, in NBYNO₃ and NBYGNO₃, nitrite formation started at 20 and 0% oxygen respectively (Fig. 4). When an oxygen concentration of 20% was reached in NBY and NBYNO₃, the oxygen concentration did not decrease any further, and the growth rates dropped (Fig. 4*a*).

Inactivation of nitrate reductase by aeration. When an anaerobic NBYGNO₃ culture was aerated vigorously, the specific nitrate reductase activity dropped, but the total activity remained constant, and the nitrite content of the medium still increased. Therefore, in order to study inactivation of nitrate reductase by aeration, growth and oxygen consumption have to be decreased by suspending the bacteria in a buffer with chloramphenicol and without substrate (Fig. 5). The aerated suspension showed inactivation of nitrate reductase, whereas the nitrate reductase activity in the non-aerated suspension remained constant.

Fig. 4. Growth, oxygen consumption, nitrate reductase and nitrite formation in various media with weak aeration. Closed symbols: NBY, NBYG. Open symbols: NBYNO₃, NBYGNO₃. ○●, E_{660} ; △▲, nitrate reductase; □, oxygen concentration (for all media); ×, nitrite content of medium (NBYNO₃ and NBYGNO₃ only).

Fig. 5. Effect of aeration upon nitrate reductase in cells grown anaerobically in NBYGNO₃ to $E_{660} = 1.5$. Cells were washed and resuspended in Mg-tris-KCl medium to $E_{660} = 1.5$. 50 ml. was shaken in a 500 ml. flask at 37° in a water bath shaker at a speed of 250 strokes/min. (●). 50 ml. was left static at 37° in a 50 ml. flask (▲). At 5 min. intervals, 5 ml. samples were removed for determination of the residual nitrate reductase activity. During harvesting, washing and the inactivation experiment, 30 μg. of chloramphenicol/ml. was present.

Changes in the respiratory system, concomitant with anaerobiosis and nitrate respiration. Table 1 shows oxygen uptake by suspensions of washed bacteria, grown under various conditions. After anaerobic growth in NBYNO₃, respiration with all substrates was reduced to 30 to 40 % of the aerobic level, as was glucose respiration in glucose-containing media. The presence of glucose reduced respiration of all substrates except glucose and abolished that of acetate and citrate.

Table 1. *Effect of growth conditions on oxygen uptake*

Averages of two or three experiments are given. Correction has been made for endogenous oxygen uptake.

Substrate	Oxygen uptake (μ moles O ₂ /mg. protein/h.)					
	Aerobic			Anaerobic		
	NBY or NBYNO ₃	NBYGNO ₃	NBYG	NBYNO ₃	NBYGNO ₃	NBYG
Glucose	12.08	13.96	10.33	3.71	5.94	3.66
Pyruvate	10.52	5.22	3.42	3.25	2.13	2.53
Acetate	10.79	0	0	3.59	0	0
Citrate	2.16	0	0	0.91	0	0
Succinate	13.09	2.01	0.70	3.57	0.44	0

Table 2. *Effect of growth conditions on cytochrome content*

Medium	Cytochrome <i>c</i> ₁ <i>E</i> ₅₅₂ /10 mg. protein			Cytochrome <i>a</i> ₁ <i>E</i> ₅₉₅ /10 mg. protein		
	Vigorous aeration	Weak aeration	Anaerobic	Vigorous aeration	Weak aeration	Anaerobic
NBY	0.210	0.187	—	0.058	0.051	—
NBYNO ₃	0.196	0.143	0.189	0.064	0.061	0
NBYG	0.046	0.059	0.056	0.081	0.011	0
NBYGNO ₃	0.042	0.047	0.110	0.083	0.015	0

Table 3. *Effect of nitrate in growth and test media upon rate of acid formation and glucose consumption*

Cultivation medium	Acid formation (μ moles/mg. protein/h.) in		Glucose consumption (μ moles/mg. protein/h.) in	
	NBYG	NBYGNO ₃	NBYG	NBYGNO ₃
NBYG	12.70	8.4	5.04	5.60
NBYGNO ₃	11.78	7.3	5.70	5.30

The cytochrome contents of bacteria grown under different conditions are given in Table 2. Although some *b*-type cytochrome was present, this could not be determined quantitatively from our spectra. Glucose strongly repressed cytochrome *c*₁ formation; under anaerobic conditions, this repression was somewhat released by nitrate.

After growth with vigorous aeration with or without glucose, cytochrome *a*₁ was present. In bacteria grown anaerobically it was completely absent. The amount of cytochrome *a*₁ in bacteria grown without glucose was the same whether they had been vigorously or weakly aerated. In cultures grown in glucose-containing media with weak aeration, however, considerably less cytochrome *a*₁ was found. This could be

explained by the fact that in glucose-free media with weak aeration 20 % of the dissolved oxygen is left, and in glucose-containing media nothing.

Effect of nitrate upon the rate of acid formation and glucose breakdown. The rates of acid formation and glucose consumption were measured in NBY and NBYGNO₃, after cultivation in these media, as described under Methods. Nitrate decreased acid formation by 40 %, but did not influence the rate of glucose breakdown (Table 3).

DISCUSSION

When aeration of cultures of *Bacillus subtilis* is interrupted, rapid lysis ('anaerobic lysis') occurs (Nomura, 1955). Similarly, lysis of *B. subtilis* during aerobic growth is observed after exhaustion of the energy source in the medium (Monod, 1942; Hadji-petrou & Stouthamer, 1963; Baillie & Holms, 1968). In this organism, lysis occurs when the generation of ATP is interrupted. *Bacillus licheniformis* behaves similarly in that anaerobic lysis can be prevented only by the presence of the nitrate-reducing system or a fermentable carbon source. *Bacillus subtilis* and *B. licheniformis* appear to be subject to autolysis in the absence of the continued synthesis of cell wall substance (Shockman *et al.* 1961), for which ATP is required. This autolytic activity interferes with the preparation of washed suspensions; 10 % MgSO₄·7H₂O protected cells of *B. licheniformis*. In this medium the bacteria retained their normal rod-like shape, perhaps because the autolytic enzymes were inhibited by the high concentration of Mg²⁺ ions.

The most striking feature of the regulation of nitrate reductase formation in *Bacillus licheniformis* is the induction by anaerobiosis, even in absence of nitrate. In *Escherichia coli* (Showe & De Moss, 1968), *Aerobacter aerogenes* (Van't Riet *et al.* 1968), *Proteus mirabilis* (De Groot & Stouthamer, 1970a) and *Haemophilus parainfluenzae* (Sinclair & White, 1970) nitrate reductase activity is low or absent during anaerobiosis in the absence of nitrate. Showe & De Moss (1968) have suggested that the formation of nitrate reductase in *E. coli* is regulated by two repressors, one of which is sensitive to nitrate, and a second which is sensitive to the intracellular redox potential. The presence of two repressors for the regulation of nitrate reductase formation has also been postulated for *H. parainfluenzae* (Sinclair & White, 1970) and for *P. mirabilis* (De Groot & Stouthamer, 1970a). In *B. licheniformis* the intracellular redox potential is of prime importance in the regulation of nitrate reductase formation, and there is no evidence for regulation by nitrate.

Azoulay, Puig & Couchaud-Beaumont (1969) have shown that redox enzymes in *Escherichia coli* chlorate-resistant mutants cannot be complexed in active membrane-bound form. Similarly, Azoulay, Puig & Martins Rosado de Sousa (1969) proposed that complexing of redox enzymes is also impossible during aerobic growth. De Groot & Stouthamer (1970b) have observed that in *Proteus mirabilis* the formation of redox enzymes is repressed when there is no electron flow to these enzymes, and suggested that such enzymes cannot be complexed in active membrane-bound form with other electron transport components without electron flow. Induction and glucose repression of nitrate reductase in *Bacillus licheniformis* can be explained in a similar way. With weak aeration, the oxygen supply is limiting and electrons can flow to nitrate reductase. In glucose-containing media there is hardly any electron flow to nitrate reductase from other substrates, because their oxidation is inhibited (Table 1). In

medium with glucose, active fermentation of glucose occurs and consequently the total electron flow to nitrate reductase is small. The possibility that glucose acts by catabolite repression cannot be ruled out.

Inactivation of nitrate reductase has been observed in *Proteus mirabilis* only when the electron flow is withdrawn from the enzyme (De Groot & Stouthamer, 1970a). In *Bacillus licheniformis* nitrate reductase is less easily inactivated and occurs only with vigorous aeration in the absence of a substrate that can be respired (Fig. 5). In a complete medium there is repression, no inactivation. It seems that inactivation requires complete withdrawal of the electron flow, whereas for repression a partial withdrawal is sufficient.

The effect of glucose inhibiting oxidation of other substrates by *Bacillus licheniformis* has also been shown in *Staphylococcus aureus* (Strasters & Winkler, 1963). This is surprising, as the effect of glucose upon the citric acid cycle enzymes in the closely related species *B. subtilis* and even in another strain of *B. licheniformis* is completely different. In these organisms the enzymes of the first part of the citric acid cycle are partially repressed by glucose and almost completely repressed by a mixture of glucose and glutamate. The enzymes of the second part of the citric acid cycle are not affected (Flechtner & Hanson, 1969). Repression of cytochrome *c* by glucose is also known from *S. aureus* (Strasters & Winkler, 1963), and from *Salmonella typhimurium* (Richmond & Maaløe, 1962).

The influence of anaerobiosis upon respiration has recently been reviewed by Wimpenny (1969). Aeration of cells grown anaerobically generally leads to a three- or four fold increase of overall respiratory activity. Thus *Bacillus licheniformis* fits well into the general pattern.

Increase of cytochrome-*c* content and complete disappearance of *a*-type cytochromes during nitrate respiration has been reported for *Micrococcus denitrificans* (Scholes & Smith, 1968) and for *Bacillus stearothermophilus* (Downey & Kiskiss, 1969). Downey & Kiskiss (1969) suppose that disappearance of *a*-type cytochromes is a common feature of denitrifying bacteria, and that it is caused by active nitrate respiration. When *B. licheniformis* is cultivated anaerobically in NBYG medium, however, nitrate reductase is formed, but no active nitrate respiration occurs because of the absence of nitrate. So we conclude that in *B. licheniformis* disappearance of *a*-type cytochrome is merely caused by anaerobiosis.

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REFERENCES

- AZOULAY, E., PUIG, J. & COUCHAUD-BEAUMONT, P. (1969). Etudes des mutants chlorate-résistants chez *Escherichia coli* K12. I. Réconstitution *in vitro* de l'activité nitrate-réductase particulaire chez *Escherichia coli* K12. *Biochimica et biophysica acta* **17**, 238-252.
- AZOULAY, E., PUIG, J. & MARTINS ROSADO DE SOUSA, M. L. (1969). Régulation de la synthèse de la nitrate-réductase chez *Escherichia coli* K12. *Annales de l'Institut Pasteur, Paris* **117**, 475-485.
- BAILLIE, R. D. & HOLMS, H. W. (1968). Loss of viability and respiration of *Bacillus subtilis* grown in peptone medium supplemented with glucose. *Biochimica et biophysica acta* **170**, 442-445.
- DE GROOT, G. N. & STOUTHAMER, A. H. (1970a). Regulation of reductase formation in *Proteus mirabilis*. II. Influence of growth with azide and of heme deficiency on nitrate reductase formation. *Biochimica et biophysica acta* **208**, 114-127.

- DE GROOT, G. N. & STOUTHAMER, A. H. (1970b). Regulation of reductase formation in *Proteus mirabilis*. III. Influence of oxygen, nitrate and azide on thiosulphate reductase and tetrathionate reductase. *Archiv für Mikrobiologie* **74**, 340–349.
- DOWNEY, R. J. (1966). Nitrate reductase and respiratory adaptation in *Bacillus stearothermophilus*. *Journal of Bacteriology* **91**, 634–641.
- DOWNEY, R. J. & KISZKISS, D. F. (1969). Oxygen and nitrate induced modification of the electron transfer system of *Bacillus stearothermophilus*. *Microbios* **2**, 145–153.
- DOWNEY, R. J., KISZKISS, D. F. & NUNER, J. H. (1969). Influence of oxygen on development of nitrate respiration in *Bacillus stearothermophilus*. *Journal of Bacteriology* **98**, 1056–1062.
- DOWNEY, R. J. & NUNER, J. H. (1967). Induction of nitrate reductase under conditions of nitrogen depletion. *Life Sciences* **6**, 855–861.
- FLECHTNER, V. R. & HANSON, R. S. (1969). Coarse and fine control of citrate synthase from *Bacillus subtilis*. *Biochimica et biophysica acta* **184**, 252–262.
- HACKENTHAL, E. (1966). Die parallele Induktion von Nitratreduktase bei *Bacillus cereus* durch verschiedene Anionen. *Biochemical Pharmacology* **15**, 1119–1126.
- HADJIPETROU, L. P. & STOUTHAMER, A. H. (1963). Autolysis of *Bacillus subtilis* by glucose depletion. *Antonie van Leeuwenhoek* **29**, 256–260.
- LOWE, R. H. & EVANS, H. J. (1964). Preparation and some properties of a soluble nitrate reductase from *Rhizobium japonicum*. *Biochimica et biophysica acta* **85**, 377–389.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.
- MONOD, J. (1942). *Recherches sur la Croissance des Cultures Bactériennes*. Paris: Herman and Cie.
- NOMURA, M. (1955). Studies on the autolytic phenomenon of *Bacillus subtilis*. I. The anaerobic lysis of *Bacillus subtilis*. II. Discoveries of the killing factor and autolysis in the lysate and induced lysis of *Bacillus subtilis* with ultraviolet light. *Journal of the Agricultural Chemical Society of Japan* **29**, 674–682.
- PICHINOTY, F. (1965). L'effet d'oxygène de la biosynthèse des enzymes d'oxydoréduction bactériens. *Centre National de Recherche Scientifique Symposia* **124**, 507–522.
- RICHMOND, M. H. & MAALØE, O. (1962). The rate of growth of *Salmonella typhimurium* with individual carbon sources, related to glucose metabolism or the Krebs cycle. *Journal of General Microbiology* **27**, 285–297.
- SCHOLES, P. B. & SMITH, L. (1968). Composition and properties of the membrane-bound respiratory chain system of *Micrococcus denitrificans*. *Biochimica et biophysica acta* **153**, 363–375.
- SHOCKMAN, G. D., CONOVER, M. J., KOLB, J. J., PHILLIPS, P. M., RILEY, L. S. & TOENNIES, G. (1961). Lysis of *Streptococcus faecalis*. *Journal of Bacteriology* **83**, 106–111.
- SHOWE, M. K. & DE MOSS, J. A. (1968). Localization and regulation of nitrate reductase in *Escherichia coli*. *Journal of Bacteriology* **95**, 1305–1313.
- SINCLAIR, P. R. & WHITE, D. C. (1970). Effect of nitrate, fumarate and oxygen on the formation of membrane-bound electron transport system of *Haemophilus parainfluenzae*. *Journal of Bacteriology* **101**, 365–372.
- STRASTERS, K. C. & WINKLER, K. C. (1963). Carbohydrate metabolism of *Staphylococcus aureus*. *Journal of General Microbiology* **33**, 213–229.
- VAN'T RIET, J., STOUTHAMER, A. H. & PLANTA, R. J. (1968). Regulation of nitrate assimilation and nitrate respiration in *Aerobacter aerogenes*. *Journal of Bacteriology* **96**, 1455–1464.
- WIMPENNY, J. W. T. (1969). Oxygen and carbon dioxide as regulators of microbial growth and metabolism. *Symposia of the Society for General Microbiology* **19**, 161–197.