Effect of Concentrations of Limiting Substrates on the Linear Growth Rates of *Streptomyces Levoris* Colonies

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Abstract The relationship between linear growth rates of *S levoris* zone-forming colonies and concentrations of different limiting energy and carbon sources in the nutrient medium has been studied A formula has been derived satisfactorily describing this relationship The utilization of the substrates in the source of vegetative and reproductive processes as well as possible mechanisms controlling the periodicity in the life activities of streptomyces colonies are discussed

Key words Relationship — Linear growth rate — Zone-forming colonies — Concentrations — Limiting energy and carbon sources

Introduction

Zone-forming first described by Lieske (1921) in colonies of streptomyces and fungi has attracted the attention of many scientists (Jerebzoff 1965, Bourret and Lincoln 1963, Esser 1969, Krassilnikov 1970, Sharkova 1970, Lysek and Esser 1971, Boltyanskaya et al 1972, Douglas and Bisset 1976, Nemchinov et al 1976) Although numerous hypotheses have been developed and attempts have been made to gain some insight into this phenomenon, its mechanism still remains obscure

Earlier we have shown (Savelyev et al 1976, Savelyev and Akoev 1982) that the periodical formation of concentric zones of sporogenous aerial mycelium (zone-formation) on the surface of *S levoris* colonies is due to the differentiation of substrate mycelium hyphae It was assumed that the space-time localization of this process is due to periodical switching of the energy metabolism controlling the live activity of the cells

A better understanding of the mechanism of zone-formation requires the study of the effects of concentrations of different energy metabolism substrates on the colony linear growth rates

Materials and Methods

Colonies of *S levoris* were grown on the following solid nutrient media 1) agar, concentrations of 0 5 to 2 %, phosphate buffer 100 mmol l^{-1}, pH 7 0 Salts (g/l) $CaCl_2$ 0.025, NaCl 1, $MgCl_2$ 0 25, NH_4Cl 1 (Akhmadieva et al 1976), 2) as sub (1) but with 1 % agar and 0 05 to 0.03 % yeast extract, 3) medium as sub (2) but with 0 005 % yeast extract Glucose, succinate, pyruvate, β-oxybutyrate and glycerol (2 to 40 mmol l^{-1}) were used as limiting carbon and energy sources In each experiment, at least 300 colonies were inoculated in series, 10 colonies each, and cultured at 27 °C in dark Every 2—3 days the colonies of one series were measured and photographed These colonies were not used in further experiments Linear growth rates were calculated on the linear section of the $R_{max}(t)$ plot by the least square method Statistical processing of the data was carried out using the beta-distribution (Hastings and Picock 1980) Our experimental results with mean square deviations not exceeding 10 % of the statistical distribution width were taken The parameters of the concentration dependence of the linear growth rate were calculated by a parameter optimization program (Raich and Vassilchikov 1979) on a M-4030 digital computer

Results and Discussion

The radius of a *S. levoris* colony growing on a solid nutrient medium increases linearly with time, almost throughout the life of the colony in a limited volume of the nutrient medium, beginning from the 24th up to the 600th—700th hour of growth (25—30 days). When other conditions (illumination, temperature, water-salt composition of the medium, pH, buffer capacity, volume of the nutrient medium, size of Petri dishes, etc.) are constant, the linear colony growth rate described by (Kozhevina 1977):

$$K = (R_{max} - R_0)/(t - t_0) \quad (1)$$

would depend on the concentrations of the limiting energy and carbon sources in the nutrient medium. Microorganisms growing on poor agar (0.5 – 2 %), show a hyperbolic increase in the growth rate from 17 $\mu m \cdot h^{-1}$ to 23 $\mu m \cdot h^{-1}$ (Fig. 1 *A*). This latter relationship follows the equation analogous to that of Monod (Pirt 1975):

$$K_r = K_r^{max} S/(K_s + S) \quad (2)$$

where $K_r^{max} = 28.736\,\mu m \cdot h^{-1}$ is the maximal (theoretical) growth rate, S is the agar concentration (in %), $K_s = 0.345$ % is the saturation constant analogous to the Michaelis-Menten constant, corresponding to an agar concentration (S) at which the growth rate is 0.5 K_r^{max}. In nutrient medium 2 the agar concentration was 1 %. Colonies grew on agars with a similar concentration with a rate of $21.56 \pm 1.2\,\mu m \cdot h^{-1}$. The addition of yeast extract (0.005 to 0.03 %) decreased the growth rate from 24.5 $\mu m \cdot h^{-1}$ to 13 $\mu m \cdot h^{-1}$ (Fig. 1 *B*). As shown by our computations, such a "humped" curve with a maximum at $S_{cr}^2 = K_s/K_i$ is well approximated by the equation for substrate inhibition (Pirt 1975):

$$K_r = K_r^{max} S/(K_s + S + S^2 K_i) \quad (3)$$

where K_r^{max} is the maximal growth rate, K_s is the concentration at which $K_r = 0.5\,K_r^{max}$, K_i is the inhibition constant.

This general equation (3) describes a curve starting at $S = 0$, $K_r = 0$. The experiments have shown that as the yeast extract concentration decreased to minimum, the growth rate of the colony did not approach zero: in our experiments it was maintained at approximately 20 $\mu m \cdot h^{-1}$. The curve described by equation (3) should therefore be shifted to the left by the value of $-S_0$ to yield K_r^o, the growth rate of the colony at zero concentration of the yeast extract.

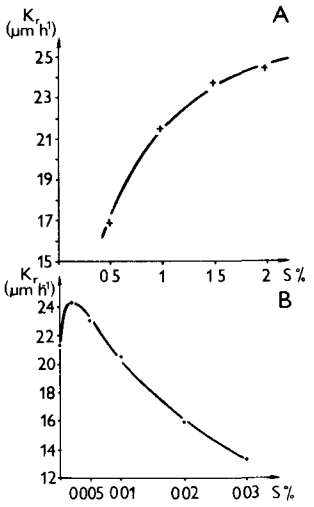

Fig. 1. Dependence of the linear growth rate (K_r) on C-source concentration (S) in the nutrient medium. *A* — agar, *B* — yeast extract (1 % agar concentration)

Thus, after simple transformations, equation (3) takes the form:

$$\tilde{K}_r = \frac{K_r}{K_r^o} = \frac{S + S_o}{S_o} \times \frac{(S_{cr} + S_o)^2 K_i + S_o + S_o^2 K_i}{(S_{cr} + S_o)K_i + (S + S_o) + (S + S_o)^2 K_i} \qquad (4)$$

where $\tilde{K}$ is the dimensionless growth rate.

The experiments with *S. levoris* grown on different media with different carbon sources showed that the curves of the linear growth rate are well approximated by relationship (4) over a concentration range from 0 to 40 $mmol \cdot l^{-1}$. Using the parameter optimization program, the following five constants were computed: K_r^{max}, S_{cr}, K_i, K_s, S_o. The results of these computations are given in Table 1. The values for the media with 1 % agar are given in numerator and those for the media with 1 % agar and 0.005 % yeast extract in denominator.

Variations of glucose concentration from 2 to 40 $mmol \cdot l^{-1}$ had no substantial effect on the growth rate of colonies grown on medium with 1 % agar

Table 1. Parameters of the function $\bar{K}_r = f(S)$, computed by (4) (see text)

C-source	$\bar{K}_r^{max}$	S_{cr} (mmol l^{-1})	K_s (mmol l^{-1})	K_i (mmol l^{-1})	S_o (mmol l^{-1})	$\pm Q$**
Pyruvate	1 207	1 8	0 028	0 0075	0 136	0 00021
	1 0	—	—	0 1632	—	0 005
Succinate	1 326	2 2	0 0682	0 0117	0 21	0 00821
	1 0		—	0 1022	—	0 0008
Glucose	1 061	10 0	0 1761	0 00103	3 0433	0 00621
	1 0	—	—	0 0229	—	0 0006
β-oxybutyrate	1 629	1 8	4 124	0 0234	11 461	0 00244
	1 0	—	—	0 033	—	0 01078
Glycerol	*	*	*	*	*	*
	1 0	—	—	0 0376	—	0 03083

Note upper values refer to C-sources supplemented with 1 % agar ($K_r^o = 21\ 36\ \mu m\ h^{-1}$), lower values refer to C-sources containing 1 % agar + 0 005 % yeast extract ($K_r^o = 23\ \mu m\ h^{-1}$)

* — data lacking

** — mean absolute deviation per point $Q = \pm(X_{exp} - X_{ther})$

(Fig. 2 *A*, curve 4). β-oxybutyrate as a limiting energy and carbon source in concentrations of 5—10 mmol . l^{-1} inhibited the growth. The linear growth rate of colonies on a medium with succinate (curve 2) reached the maximum at a succinate concentration of 2.2 mmol . l^{-1}; higher concentrations reduced the growth rate. Curve 1, characterizing the dynamics of the growth rate on a medium with pyruvate, has the same shape.

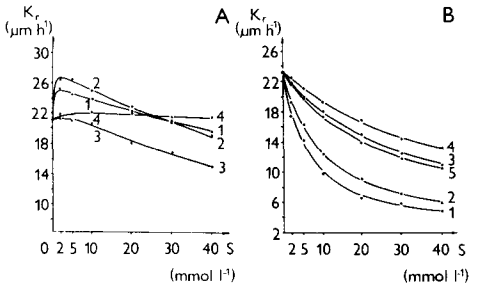

Fig. 2. Dependence of the linear growth rate (K_r) on C-source concentration in the nutrient medium: *A* — 1 % agar + C-source; *B* — 1 % agar + 0.005 % yeast extract + C-source. 1 — pyruvate, 2 — succinate, 3 — β-oxybutyrate, 4 — glucose, 5 — glycerol.

The addition of 0.005 % yeast extract significantly altered the kinetics of $K_r(S)$ (Fig. 2 *B*): the curve shifted sharply to the left and decreased monotonically. The inhibitory effect was most pronounced when concentrations of pyruvate and succinate were increased (Fig. 2 *B*, curves 1 and 2, respectively) (Table 1). Upon increasing the concentrations of β-oxybutyrate and glycerol a decrease of the growth rate was observed (Fig. 2 *B*, curves 3 and 5), the values of K_i in both cases were very similar (Table 1).

A comparative analysis of the data (Table 1) and the plots allows the assumption that both succinate and pyruvate are utilized through the same pathway, the TCA-cycle, whereas glycerol and β-oxybutyrate probably follow the β-oxidation pathway.

It is known that the concentration dependence of the linear growth rate, K_r, characterizes the vegetative growth of microorganisms, and that the frequency of zone-forming to some extent reflects (Savelyev and Akoev 1982; Savelyev 1984) the process of differentiation. Comparing the kinetics of these two processes, it should be noted that: 1. the shapes of curves $K_r(S)$ and $\nu(S)$ practically coincide for glucose and β-oxybutyrate; 2. the kinetics of growth and zone-forming on pyruvate and succinate are essentially different: at concentrations between 5 and 40 mmol . l^{-1}, the linear growth rate decreases whereas the frequency of zone-forming increases. An increase in succinate and pyruvate concentrations in the medium containing the yeast extract inhibited both the vegetative and the reproductive process; however, an initial slight increase in the zone-forming frequency could be observed at a concentration of about

5 mmol l^{-1} The kinetics of vegetative growth on media with yeast extract and different concentrations of glucose and β-oxybutyrate is entirely different from that of the zone forming frequency (Savelyev 1984) with increasing concentrations of the carbon source, the growth becomes inhibited, whereas the rhythm of zone-formation becomes accelerated to reach some steady level at concentrations exceeding 10 mmol l^{-1}

The differences observed in the kinetics between the differentiation and the vegetative growth allow the conclusion that these processes are relatively independent of each other Based on the Selkov's hypothesis (Selkov 1978, 1983), it can be assumed that exogenous sources of energy and carbon are utilized during the vegetative growth, a portion of the source being expended directly for plastic processes, the other one being stored in form of granules of reserve substances The transition from growth to processes of differentiation can presumably be explained by the action of low-molecular substances such as "A-factor" (Kalakoutskii and Agre 1977, Khokhlov 1979) which switch the energetic metabolism from exogenous to endogenous energy sources The capacity of endogenous sources probably depends on the concentrations of the exogenous carbon sources in the nutrient medium

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