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SHORT COMMUNICATIONS

Inhibition of Growth and Methane Consumption in *Methylocapsa acidiphila* by Mineral Salts

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Methanotrophs are unique and ubiquitous bacteria that use methane as the carbon and energy source [1]. Boreal acidic *Sphagnum* bogs, in which CH_4 oxidation is driven by acidophilic methanotrophs, are one of the major sources of atmospheric methane [2]. The currently recognized acidophilic methanotrophs have been assigned to two new genera and species: *Methylocella palustris* and *Methylocapsa acidiphila* [3, 4]. They prefer media with low salt concentrations (100–500 mg/l) and fail to grow on conventional media with a salt content of 1.5–3 g/l [1]. It is worth noting that *Methylocella* isolates were obtained on a medium with nitrate, whereas *Methylocapsa acidiphila* was isolated on a nitrogen-free medium. The growth of *M. acidiphila* does not depend on the availability of bound nitrogen, which fact, in conjunction with acidophily, allows it to thrive in acidic low-temperature habitats with a low content of available nitrogen, such as bogs and acidic soils of the boreal zone.

Our studies on the influence of nitrates, nitrites, NH_4^+, and other mineral salts on the methane-oxidizing activity of peat samples revealed that nitrates and chlorides caused maximum inhibition, whereas phosphates and sulfates were almost without effect, regardless of the cations involved [5]. The mechanism of the inhibitory effect of a number of mineral salts on the methane-oxidizing activity of peat and soil samples has not yet been elucidated. NH_4^+ is known to inhibit the key enzyme of methanotrophic metabolism, methane monooxygenase, which catalyzes methane oxidation to methanol [6]. However, in our studies with peat samples, we demonstrated that ammonium does not produce a stronger effect on methane consumption than other cations, K^+ in particular [5].

The present study on the effects of various salts on the growth of the acidophilic methanotroph *Methylocapsa acidiphila* B2 was aimed at elucidating the mechanism of the inhibitory effect of minerals on methane consumption.

M. acidiphila B2 (DSM 13967^T = NCIMB 13765^T) was cultivated on a minimal nitrogen-free medium [4] at 25°C on a shaker (120 rpm) in hermetically closed 500-ml serum flasks containing 100 ml of medium. The culture purity was tested using phase-contrast microscopy and media that promote the growth of heterotrophs [4]. Methane was introduced to a final concentration of 55 mg CH_4-C/l by means of a syringe through a bacterial filter with a pore size of 0.22 μm. Mineral salts (KNO_3, KCl, KBr, KI, LiCl, $SrCl_2$, $BaCl_2$, $AlCl_3$, and K_2SO_4) were added to the medium at concentrations of 1–10 mM. The control system was *M. acidiphila* B2 grown on the minimal medium without the above salts. Culture growth was estimated from the optical density of bacterial suspension samples taken at regular intervals. The optical density was measured with a SPEKOL 10 (Germany) spectrophotometer at 410 nm, and the results obtained were used to calculate biomass values based on carbon balance data [7]. CH_4 consumption and CO_2 production were measured with an INFRALYT-4 IR (Germany) gas analyzer. The specific growth rate (μ) was determined from the dynamics of OD_{410} during the exponential growth phase. The growth yield ($Y = dx/ds$) was estimated from the mass balance, taking into account the amount of methane consumed and CO_2 produced [7].

The table contains our data on the influence of a number of mineral salts on methane consumption, specific growth rate (μ), and growth yield (Y). $AlCl_3$ and KI were the strongest inhibitors; they suppressed culture growth at a concentration of 1 mM. Low KNO_3 concentrations (1 mM) stimulated methane consumption and increased the specific growth rate of *M. acidiphila*, but the bacterium virtually failed to grow during the first four days of cultivation if higher nitrate concentrations were used (3, 5, and 10 mM; see Fig. 1). The lag phase duration increased with an increase in the salt concentration. The addition of 1 mM $BaCl_2$ caused a lag phase of 4 days; 3 mM $BaCl_2$ completely inhibited growth. $SrCl_2$ and LiCl decreased the μ value but produced virtually no effect on the growth yield. The inhibition of the culture growth was enhanced by increasing KCl concentration, whereas the yield drastically decreased only at a KCl concentration of 10 mM. KBr produced an intermediate inhibitory effect in comparison to KCl

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Effect of mineral salts on methane consumption, specific growth rate, and biomass yield in *M. acidiphila* B2

Compound added	Concentration, mM	Inhibition of CH_4 consumption, %	Specific growth rate μ, h^{-1}	Growth yield *Y*, g C/g CH_4-C
Control	0	0.0	0.03	0.58
KNO_3	1	–62.9*	0.04	0.61
	3	28.0	0.03	0.39
	5	30.8	0.03	0.44
	10	20.9	0.02	0.60
LiCl	1	23.1	0.04	0.63
	3	61.9	0.01	0.52
KCl	1	8.2	0.04	0.63
	3	22.8	0.03	0.64
	10	38.9	0.02	0.41
KBr	1	11.7	0.04	0.62
	3	46.5	0.02	0.63
KI	1	64.1	0.01	0.40
	3	100	0	0
K_2SO_4	10	22.9	0.01	0.56
$SrCl_2$	1	24.2	0.02	0.61
$BaCl_2$	1	19.0	0.02	0.57
	3	100	0	0
$AlCl_3$	1	100	0	0

* Stimulation of CH_4 consumption.

and KI. K_2SO_4 influenced methane consumption and growth to a considerable extent only if applied at a concentration of 10 mM.

Thus, the inhibitory effect of chlorides increased with an increase in the charge and atomic weight of the cations involved: $K^+ < Li^+ < Sr^{2+} < Ba^{2+} < Al^{3+}$. As for potassium halides, the inhibitory effect increased in the order $Cl^- < Br^- < I^-$.

This work revealed that methanotrophic bacteria are susceptible to a strong inhibitory effect produced, apart

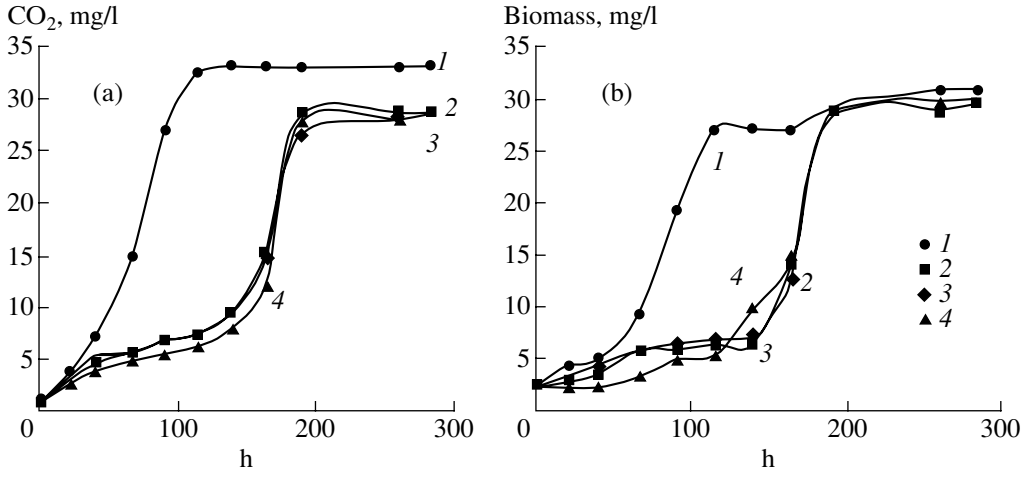

Fig. 1. Dynamics of (a) CO_2 evolution and (b) biomass accumulation in *M. acidiphila* B2 as dependent on the potassium nitrate concentration: (*1*) 0, (*2*) 3, (*3*) 5, and (*4*) 10 mM.

from ammonium salts, by a wide range of mineral salts as well. Based on a kinetic analysis of the inhibitory effect carried out for the microbial community of peat [5], we suggest that this phenomenon is a consequence of cytoplasm acidification by anions of strong acids. The results obtained can be used for optimizing methanotroph cultivation under laboratory conditions and in biotechnological setups. The high sensitivity of acidophilic methanotrophs to salts sheds light on the regulatory mechanisms of their in situ metabolic activity, which ultimately determines the scale of the methane flux to the atmosphere.

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