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SHORT COMMUNICATIONS

New Evidence for the Ability of Methylobacteria and Methanotrophs to Synthesize Auxins

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Indole-3-acetic acid (IAA) is one of the most widespread auxins, a group of plant hormones that are derivatives of indole. The formation of indole and its derivatives from L-tryptophan is a taxonomically valuable characteristic of aerobic gram-negative bacteria [1]. According to the identification criteria of Bergey's Manual, methylobacteria are unable to produce indoles [2]. However, our previous study [3] showed that four species of methylobacteria, *Methylobacterium mesophilicum*, *Aminobacter aminovorans*, *Methylovorus mays*, and *Paracoccus kondratievae*, are able to produce IAA. This ability was established using the Salkowski and van Urk reagents, which allow an indole concentration as low as 2 μg/ml to be detected. At the same time, the Ehrlich and Kovacs reagents, which are used in routine microbiological tests, failed to detect indoles in the methylobacterial cultures because of the low sensitivity of these reagents (40 μg/ml). IAA synthesis in the methylobacteria studied was found to be inhibited by ammonium ions present in the growth medium. This finding prompted us to investigate the ability of a wider range of methylotrophic bacteria to synthesize indole derivatives from L-tryptophan.

Experiments were carried out using methylobacteria and methanotrophs from the All-Russia Collection of Microorganisms (VKM). The collection strains were grown in mineral media with 10 g/l KNO_3 as the source of nitrogen and 0.5 vol % methanol or 0.3 wt % methylamine as the source of carbon and energy for methylobacteria and 20 vol % methane in the gas phase as the source of carbon and energy for methanotrophs [3, 4]. Halophilic and alkaliphilic methylotrophic bacteria were grown in the media described previously [5, 6].

The growth media were supplemented with 1 mM L-tryptophan as the auxin precursor. In the late logarithmic growth phase, bacterial cells were removed by centrifugation at 10000 *g* for 20 min and the culture supernatant was analyzed for auxins as described by Gordon and Weber [7]. Specifically, one volume of the culture supernatant was mixed with 0.5 volume of the Salkowski reagent (0.05 M $FeCl_3$ in 35% $HClO_4$) and the mixture was incubated for 1 h, after which its optical density was measured at 540 nm in a Spekol-221 spectrophotometer (Germany). The concentration of auxins was determined from the calibration curve constructed using standard IAA solutions. Auxins, extracted from the culture supernatant as described earlier [3], were identified by the methods of thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and mass spectrometry (for this purpose, a Finnigan MAT 3430 mass spectrometer was used). IAA, indole-3-lactic acid, indole-3-pyruvic acid, indole-3-acetamide, indole-3-acetaldehyde, and indole-3-methanol purchased from Sigma (United States) were used as authentic samples.

As can be seen from the table, all of the methylotrophic bacteria tested are able to produce IAA in different amounts (from 2 to 100 μg/ml culture liquid) and indole-3-lactic acid in trace amounts. The concentration of IAA peaked in the early stationary growth phase. We failed to reveal any correlation between the amount of the IAA synthesized by a given strain and the type of its methylotrophy (either obligate or facultative) and the pathway of its C1-metabolism. The presence of ammonium ions in the growth medium considerably inhibited IAA synthesis. For instance, the bacterium *H. zavarzinii* grown in the presence of KNO_3 and $(NH_4)_2SO_4$ synthesized 21 and 3 μg/ml IAA, respectively. For *M. extorquens*, these quantities were 19 and 4 μg/ml, respectively.

As was shown previously, methylobacteria and methanotrophs are able to produce cytokinins [8]. The data presented in this paper demonstrate that aerobic methylobacteria of the genera *Hyphomicrobium*, *Methylobacterium*, *Methylopila*, *Methylarcula*, *Methylophilus*, *Methylobacillus*, *Methylovorus*, *Methylophaga*, *Methylobrevibacter*, *Paracoccus*, *Xanthobacter*, *Albibacter*, and *Ancylobacter* and methanotrophs of the genera *Methylomonas*, *Methylosinus*, *Methylocystis*, *Methylobacter*, and *Methylomicrobium* can also synthesize auxins (predominantly, IAA) from exogenous L-tryptophan. Facultative methylotrophic bacteria can also synthesize small amounts of IAA in media with peptone as the source of carbon and nitrogen. Taking into account that L-tryptophan is a plant exometabolite, the ability of aerobic methylotrophs to synthesize cytokinins and auxins confirms the existence of a metabolic relationship between these bacteria and plants.

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Synthesis of IAA from L-tryptophan by aerobic methylotrophic bacteria with different pathways of C_1-metabolism

Methylotrophic bacteria	IAA concentration in the medium, μg/ml
Ribulose monophosphate pathway	
Methylobacillus glycogenes VKM B-2060 (= ATCC 29475)	6.2
Methylovorus glucosetrophus VKM B-1745 (= ATCC 49758)	5.3
Methylophilus leisingerii VKM B-2013	18.2
Mp. methylotrophus VKM B-1623 (= NCIMB 10515)	30.3
Methylobrevibacter suomicum F-31 VKM B-2238	27.9
Methylophaga marina VKM B-2056 (= ATCC 35842)	8.1
Methylomonas methanica S_1 VKM B-2110	6.8
Methylobacter chroococcum 90 VKM B-2115	4.7
Mb. vinelandii 30 VKM B-2113	7.8
Mb. bovis 98 VKM B-2112	10.0
Methylomicrobium alcaliphilum 20Z VKM B-2133	6.1
Mm. buryatense 5b VKM B-2245	4.2
Mm. buryatense 7G	2.4
Serine pathway	
Methylobacterium organophilum XX VKM B-2066 (= NCIMB 11278)	1.4
M. radiotolerans VKM B-2144 (= JCM 2831)	3.0
M. rhodesianum VKM B-2141 (= JCM 2810)	13.6
M. zatmanii VKM B-2161 (= NCIMB 12243)	6.2
M. extorquens VKM B-2064 (NCIMB 9399)	8.7
M. dichloromethanicum VKM B-2191 (= DSMZ 6343)	5.5
M. rhodinum VKM B-2065 (= ATCC 14821)	14.6
Methylopila capsulata VKM B-1606	23.3
M. helvetica VKM B-2189	26.1
Hyphomicrobium zavarzini ZV-622 VKM B-2174	16.0
Methylarcula marina VKM B-2159	20.9
Methylorhabdus multivorans VKM B-2030 (= ATCC 51890)	4.8
Methylocystis minimus 42 VKM B-2124	28.4
Mcs. pyriformis 14 VKM B-2127	5.8
Mcs. echinoides 2 VKM B-2128	30.3
Methylosinus sporium 35 VKM B-2123	95.7
Ms. trichosporium OB3b VKM B-2117	22.9
Ribulose bisphosphate pathway	
Paracoccus aminovorus VKM B-2140 (= JCM 7685)	2.5
P. aminophilus VKM B-2141 (= JCM 7686)	12.8
P. denitrificans ATCC 17441	49.7
Xanthobacter autotrophicum VKM B-1393	7.0
X. viscosus VKM B-139	5.7
Albibacter methylovorans VKM B-2236	61.6
Ancylobacter vacuolatum DSMZ 1277	50.0
A. natronum VKM B-2242	24.2

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