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EXPERIMENTAL ARTICLES

Isoprenoid Pigments in Representatives of the Family *Microbacteriaceae*

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Abstract—By using fosmidomycin and mevinolin (inhibitors of the synthesis of isoprenoid pigments), spectrophotometry, and mass spectrometry, the presence of isoprenoid pigments is shown in 71 of the 78 strains under study. All of these strains belong to 11 genera of the family *Microbacteriaceae.* Yellow, orange, and red pigments are found to have absorption spectra typical of C_{40}-carotenoids. Eight out of the sixteen strains of the genus *Microbacterium* are able to synthesize neurosporene, a precursor of lycopene and β-carotene. The biosynthesis of carotenoids in some representatives of the genera *Agromyces, Leifsonia*, and *Microbacterium* is induced by light. Inhibition of the biosynthesis of isoprenoid pigments by fosmidomycin suggests that they are synthesized via the nonmevalonate pathway. Twelve strains are found to exhibit both the nonmevalonate and mevalonate pathways of isoprenoid synthesis. These data, together with the difference in the inhibitory concentration of fosmidomycin, can be used for differentiating various taxa within the family *Microbacteriaceae.*

Key words: isoprenoids, fosmidomycin, mevinolin, chemotaxonomy, *Microbacteriaceae*, absorption spectra, mass spectrometry.

Chemotaxonomic characteristics, which reflect the chemical structure of certain microbial polymers and other molecules, can be used (along with morphological characteristics) to differentiate actinobacterial genera on a phenotypic level. The isolation of many new organisms in recent years has revealed the limitations of actinobacterial systematics, as there is a shortage of phenotypic characteristics that can be used for differentiating groups of microorganisms differing phylogenetically at the generic and species levels. Nevertheless, many cellular compounds of actinobacteria, including pigments, have not been adequately studied from a taxonomic perspective, although the color of colonies is widely used as a systematic trait for the differentiation of actinobacterial species [1].

The chemical nature of pigments has been investigated only in some taxonomic groups [2, 3]. In particular, little is known about the *Microbacteriaceae* pigments. The bacterium *Clavibacter michiganensis* subsp. *insidiosus* has been found to contain iodinine (a derivative of phenazine) [4, 5]. Carotenoids (compounds of an isoprenoid nature) have been detected in *Agromyces ramosus* [5] and two *Leifsonia* sensu lato species [6], which are phylogenetically close to *Rhodoglobus.* Most members of the *Microbacteriaceae* family exhibit a yellow, orange, or red color of varying intensity and tint, the color being determined by isoprenoids (carotenoids), quinones, prodigiosin and anthracyclinone derivatives, phenazines, and other heterocyclic compounds [7, 8].

Isoprenoids are synthesized via the nonmevalonate or mevalonate pathways, which are inhibited by fosmidomycin and mevinolin, respectively [9, 10]. The suppression of pigmentation by these inhibitors at concentrations lower than those inhibitory to growth may serve as an indication (along with the results of chemical analysis) that the pigmentation is due to isoprenoids. Fosmidomycin is of great independent interest because it can be used for the production of a new-generation of antibiotics [11, 12].

The aim of this work was to investigate the nature of pigments in different genera and species of the family *Microbacteriaceae*, study the synthesis of isoprenoids using fosmidomycin and mevinolin as research tools, and evaluate the taxonomic significance of the relevant characteristics found.

MATERIALS AND METHODS

The strains used in this study (Table 1) were obtained from the All-Russia Collection of Microor-

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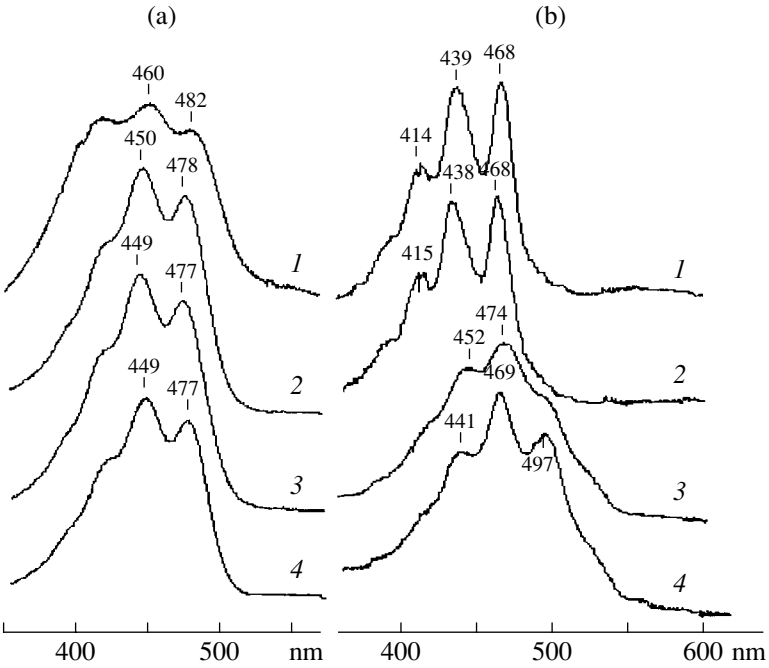

Fig. 1. (a) The absorption spectra of pigments in (*1*) the cells and (*2*) the hexane extract of *Plantibacter* sp. Ac-1787, (*3*) *Okibacterium fritillariae* Ac-2059 (the hexane extract), and (*4*) *Agreia* sp. Ac-1783 (the hexane extract). (b) The absorption spectra of the hexane extracts of (*1*) *Microbacterium* sp. Ac-2053, (*2*) *Microbacterium liquefaciens* Ac-1018, (*3*) *Curtobacterium flaccumfaciens* pv. *poinsettiae* Ac-1924, and (*4*) *Agrococcus jenensis* Ac-1839.

ganisms (VKM). The strains were grown as described earlier [13] on corynebacterial agar (CBA) containing (g/l) casein peptone, 10; glucose, 5; yeast extract, 5; NaCl, 5; and agar, 15 (pH 7.0) or in a YPD medium containing (g/l) yeast extract, 3; peptone, 5; glucose, 5; and K_2HPO_4, 0.2 (pH 7.2). Cells were grown up to the exponential growth phase (18 h of cultivation), harvested by centrifugation at 5000 *g* for 15 min (4°C), and washed with 0.1 M NaCl. A portion of the wet biomass was used to extract pigments, and the rest was used to evaluate the pigment content (the latter was expressed in OD/mg dry wt. cells).

The pigments were extracted from the wet biomass with a hexane–methanol (1 : 2) mixture, and this extract was then dried. The dry residue was dissolved in a minimal volume of hexane and subjected to analysis by TLC on Silufol plates (Czech Republic) in a nonpolar benzene–acetone (3 : 1) system. Chromatographic spots were eluted with methanol and analyzed by using a Finnigan MAT model 8430 mass spectrometer (Germany). Ionization was performed by electron impact (70 eV). The absorption spectra of the pigments in whole cells and in solvent extracts were recorded at room temperature with a Shimadzu UV-160 spectrophotometer (Japan).

The concentrations of fosmidomycin (Jomaa Pharmaka GmbH) necessary to inhibit pigment synthesis and the growth of actinobacteria were determined using a method of serial dilutions onto CBA containing fosmidomycin at concentrations of 1, 0.5, 0.3, 0.1 and 0.05 mg/ml. The agar plates were incubated for 3 days, after which the growth of actinobacteria was evaluated semiquantitatively on a five-grade scale. The effect of mevinolin (Sigma) on pigment synthesis and the growth of actinobacteria was studied according to a similar method. The concentration of mevinolin was 0.5 mg/ml. All the experiments were performed in 2 to 8 replicates.

RESULTS AND DISCUSSION

Seventy-one of the 78 strains belonging to 11 genera of the family *Microbacteriaceae* exhibited a yellow, orange, or red color of different intensity and tint. In *Leifsonia aquatica, Leifsonia poae, Microbacterium* sp. VKM Ac-2047, and five *Agromyces* sp. strains, the yellow pigment was synthesized only under continuous illumination (Table 1). The induction of the synthesis of pigments by light has previously been observed for carotenoids but in the representatives of other taxonomic groups [5, 7, 8]. Carotenoids can easily be detected in cells and cell extracts due to their specific absorption spectra [8]. Spectral analysis of the wet biomass, lyophilized cells, and pigment extracts showed that their spectra are typical of C_{40}-carotenoids (Table 1 and Fig. 1). The spectra of four *Plantibacter* and two *Okibacterium* strains were virtually identical (Fig. 1a, spectrum *1*). The spectra of three *Agreia* strains were similar (within the instrumental accuracy) to those of *C. michiganensis* subsp. *michiganensis.* The 1-day-old *Curtobacterium*

Table 1. The effect of fosmidomycin on the pigmentation and growth of bacteria belonging to 11 genera of the family *Microbacteriaceae*

Strain	Growth and color of colonies in the presence of fosmidomycin (mg/ml)						Biosynthetic pathway	Spectra
	0.00	0.05	0.10	0.30	0.50	1.00		
1	2	3	4	5	6	7	8	9
Agreia bicolorata VKM Ac-1804^T	4o	3o	3o	2o	2	1	nm	(a_1^{**})
A. bicolorata VKM Ac-1803	4o	3o	3o	2o	2	1	nm	(a_1^{**})
Agreia sp. VKM Ac-1783	4y	4y	3y	2y	2	1	nm	$a_{2–4}$
Agreia sp. VKM Ac-2052	4y	2	2	2	<2	1	nm	(a_{2-4})
Agreia sp. VKM Ac-1806	4y	2	2	1	1	<1	nm	(a_1)
A. pratensis VKM Ac-2510^T	4y	0	1	<1	1	0	nm	a_{2-4}
Agrococcus jenensis VKM Ac-1839^T	4y	3y	3	2	1	0	nm	b_4
Agromyces rhizospherae VKM Ac-2086^T	4y	–	2y	3	0	0	nm	(a_1)
A. bracchium VKM Ac-2088^T	4y	–	3y	3	3	2	nm	(a_1)
A. luteolus VKM Ac-2085^T	4y	–	–	–	3	2	nm	(a_1)
Agromyces sp. VKM Ac-1802	4y	4y	4y	4y	3	2	nm	(a_1)
A. cerinus subsp. *cerinus* VKM Ac-1340^T	4y*	3	2	2	2	2	nm	
A. fucosus VKM Ac-1345^T	4y*	4	2	2	3	2	nm	(a_1)
A. cerinus subsp. *nitratus* VKM Ac-1351^T	4y*	4	2	2	3	2	nm	
A. hippuratus VKM Ac-1352^T	4y*	4	2	2	3	2	nm	
A. albus VKM Ac-1800^T	4	4	2	2	2	2	nm	tr.
A. mediolanus VKM Ac-1388^T	4y*	4	2	2	3	2	nm	
Clavibacter michiganensis subsp. *michiganensis* VKM Ac-1403^T	4y	2	1	0	0	0	nm	(a_1)
C. michiganensis subsp. *nebraskensis* VKM Ac-1404^T	4o	2	3	1	<2	0	nm	(a_1)
C. michiganensis subsp. *tessellarius* VKM Ac-1406^T	4p	3	3	3	<2	<2	nm	(b_3) a_2
Clavibacter sp. VKM Ac-1371	4p	–	–	2	2	0	nm	tr.
Clavibacter sp. VKM Ac-2060	4y	3	2	0	0	0	nm	tr.
C. michiganensis subsp. *insidiosus* VKM Ac-1402^T	4	2	0	0	0	0	nm	(a_1)
C. michiganensis subsp. *insidiosus* VKM Ac-1416	4	4	–	–	0	0	nm	
C. *michiganensis* subsp. *sepedonicus* VKM Ac-1405^T	4	0	–	–	0	0	nm	
Curtobacterium flaccumfaciens pv. *flaccumfaciens* VKM Ac-1923	4y	4y	3y	4y	2p	2p	nm	b_3
C. flaccumfaciens pv. *poinsettiae* VKM Ac-1924	4o–p	–	4y	4y	+3p	+3p	m, nm	b_3
C. flaccumfaciens pv. *betae* VKM Ac-1925	4y	4y	3y	4y	4p	3p	m, nm	b_3
C. citreum VKM Ac-2187^T	4y	–	3o	3o	+3p	+3p	m, nm	(b_3)
C. pussilum VKM Ac- 2099^T	4y	4	3	3	3p	3p	m, nm	(b_3)
"*C. calamagrosticola*" VKM Ac-1811	4y	–	2	2	3p	3p	m, nm	b_3
Curtobacterium sp. VKM Ac-1395	4y	4y	4y	4y	4y	3y	m, nm	(b_3)
Curtobacterium sp. VKM Ac-1376	4y	4y	4y	4y	4y	<3y	m, nm	b_3
C. herbarum VKM Ac-2512^T	4p	–	3y	3p	4p	4p	m, nm	(b_3)
Curtobacterium sp. VKM Ac-1809	4y	2y	3p	2y	3o	3p	m, nm	b_3
C. betae VKM B-1206^T	4	–	>3	>2	>2	0	nm	(b_3) tr.
C. luteum VKM Ac-2188^T	4y	–	2	1	0	0	nm	(b_3)
"*Frigoribacterium mesophilum*" VKM Ac-1396	4y	–	3y	3y	0	0	nm	$a_{2–4}$
Leifsonia poae VKM Ac-1401^T	4y*	4	4	4	3	2	nm	(a_1)
L. aquatica VKM Ac-1400^T	4y*	4	4	4	3	2	nm	(a_1)

Table 1. (Contd.)

Strain	Growth and color of colonies in the presence of fosmidomycin (mg/ml)						Biosynthetic pathway	Spectra
	0.00	0.05	0.10	0.30	0.50	1.00		
1	2	3	4	5	6	7	8	9
Leucobacter komagatae VKM Ac-2073^T	4	–	4	4	2	2	nm	
Microbacterium hominis VKM Ac-2081^T	4y	4y	4	4	1	0	nm	$b_{(1,2)}$
M. aurum VKM Ac-1950^T	4y	–	–	–	1	0	nm	$b_{(1,2)}$
M. terrae VKM Ac-1945^T	4y	2	2	2	1	0	nm	$b_{1,2}$
Microbacterium sp. VKM Ac-2053	4y	2	2	2	1	0	nm	$b_{1,2}$
M. arborescens VKM Ac-1944^T	4p	2p	2p	2p	1p	1p	m, nm	$b_{1,2}$
M. laevaniformans VKM Ac-1138^T	4	3	3	3	1	1	nm	$b_{(1,2)}$
Microbacterium sp. VKM Ac-2049	4y	3	2	2	2	1	nm	$b_{(1,2)}$
M. dextranolyticum VKM Ac-1940^T	4y	4	2	2	2	2	nm	$b_{(1,2)}$
M. liquefaciens VKM Ac-1018^T	4y	3y	3y	3y	2	1	nm	$b_{1,2}$
Microbacterium sp. VKM Ac-2050	4y	4	4	3	2	1	nm	$b_{(1,2)}$
M. luteolum VKM Ac-1922^T	4y	3	3	3	2	1	nm	$b_{(1,2)}$
M. aurantiacum VKM Ac-2075^T	4o	–	3o	3o	3o	1p	nm	$b_{1,2}$
M. chocolatum VKM Ac-2078^T	4o	–	3o	3o	3o	2p	m, nm	$b_{(1,2)}$
M. arabinogalactanolyticum VKM Ac-1943^T	4	–	>3	>3	>3	>2	m, nm	$b_{(1,2)}$
M. esteraromaticum VKM Ac-1942^T	4y	4y	4y	4y	4	2	nm	$b_{1,2}$
M. oxydans VKM Ac-2116^T	4y	4	4	4	3	2	nm	$b_{(1,2)}$
M. barkeri VKM Ac-1020^T	4y	4y	4y	4	3	1	nm	$b_{(1,2)}$
Microbacterium sp. VKM Ac-2047	4y*	3	3	4	3	2	nm	$b_{(1,2)}$
Okibacterium fritillariae VKM Ac-2059^T	4y	3y	3	2	1	<1	nm	a_{2-4}
O. fritillariae VKM Ac-2062	4y	3y	3	2	1	<1	nm	a_{2-4}
Plantibacter flavus VKM Ac-2504^T	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. cousiniae*" VKM Ac-1787	4y	4y	4	4	4	4	nm	a_{2-4}
"*P. agrosticola*" VKM Ac-1380	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. agrosticola*" VKM Ac-1379	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. agrosticola*" VKM Ac-1381	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. agrosticola*" VKM Ac-1789	4y	–	–	–	4	4	nm	(a_{2-4})
"*P. elymi*" VKM Ac-1784	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. elymi*" VKM Ac-1382	4y	4y	4	4	4	4	nm	(a_{2-4})
"*P. elymi*" VKM Ac-1385	4y	4y	4	4	4	4	nm	(a_{2-4})
Rathayibacter rathayi VKM Ac-1601^T	4y	3y	3	3	2	1	nm	(a_1)
R. iranicus VKM Ac-1602^T	4y	–	–	–	2	1	nm	(a_1)
R. tritici VKM Ac-1603^T	4y	4y	3y	3	2	1	nm	(a_1)
R. festucae VKM Ac-1390^T	4o	4o	3o	3	2	2	nm	(a_1)
R. caricis VKM Ac-1799^T	4y	–	3y	3	2	2	nm	(a_1)

Note: The asterisk * indicates that pigmentation was induced by illumination. The 3- to 4-day-old cells of *Clavibacter michiganensis* subsp. *insidiosus* VKM Ac-1402^T and *C. michiganensis* subsp. *insidiosus* VKM Ac-1416 contained lilac crystals. The numerals 0–4 indicate the growth rate of bacteria with respect to the control without fosmidomycin (4). The sign "+" indicates the stimulation of growth by fosmidomycin. The sign "–" stands for not determined. The letters y, o, and p denote the yellow, orange, and pink color of the colonies. The absence of any letter implies the absence of pigmentation. The abbreviation tr. stands for traces. The letters a and b with subscripts denote the spectra of carotenoid pigments (shown in Fig. 1) in the cell extracts: a_1^{**} denotes the spectrum of a pigment localized in the cell walls and extracellular polysaccharide; a_1 denotes the spectra of pigments localized in whole cells (the position of the spectral maxima is typical of carotenoids); b_3 denotes the spectra of pigments localized in whole cells (the position of the spectral maxima is typical of spectrum b_3 in the figure); $b_{1,2}$ denotes the spectra of some microbacteria typical of neurosporene; and $b_{(1,2)}$ denotes the spectra of pigments in trace amounts similar to that of neurosporene.

Table 2. The values R_f (TLC analysis) and *m/z* (mass spectrometry analysis) of the pigments extracted from bacteria of the family *Microbacteriaceae*

Strain	R_f	Major fragments in mass spectra, *m/z* (%)
Agreia sp. VKM Ac-1783	0.58	314(60), 299(59), 285(15), 253(100), 211(30)
"*Plantibacter cousiniae*" VKM Ac-1787	0.56	314(60), 299(59), 285(15), 253(100), 211(30)
Okibacterium fritillariae VKM Ac-2059^T	0.6	*m/z* 550, 522, 314(20), 299(100), 285(60), 253(15), 239(30)
Curtobacterium flaccumfaciens pv. *poinsettiae* VKM Ac-1924 (18 h)	0.54	*m/z* 550, 522, 314(60), 299(59), 285(15), 253(100), 211(30)
	0.58	*m/z* 550, 522, 314(20), 299(100), 285(60), 253(15), 239(30)
Curtobacterium flaccumfaciens pv. *poinsettiae* VKM Ac-1924 (48 h)	0.54	314(60), 299(59), 285(15), 253(100), 211(30)
	0.58	314(20), 299(100), 285(60), 253(15), 239(30)
Microbacterium sp. VKM-Ac-2053	0.54	314(20), 299(100), 285(60), 253(15), 239(30)

and *Frigoribacterium* cultures also had similar spectra, although the spectra of the 2- to 3-day-old *Curtobacterium faccumfaciens* pv. *faccumfaciens, C. faccumfaciens* pv. *betae*, and *C. faccumfaciens* pv. *poinsettiae* cells exhibited an elevated absorption in the region 510–530 nm. In members of the genera *Agromyces, Leifsonia*, and *Microbacterium*, the absorption spectra of the pigments were typical of carotenoids (Table 1).

Except for members of the genera *Clavibacter* and *Rathayibacter*, the pigments could easily be extracted from the cells of almost all the strains under study. Unlike the yellow pigment, the pink pigment could only be partially extracted, suggesting that the latter may form complexes with cell-wall components. The extracted pigments were localized in the hexane phase, which is typical of carotenoids containing no oxygen atoms. The TLC analysis of the yellow extracts showed that they contained only one pigment. At the same time, the pink extract of *C. faccumfaciens* pv. *poinsettiae* VKM-1924 was found to contain two pigments with different mobilities and spectra (Table 2). The spectra of the pigments extracted from the members of the genera *Plantibacter, Okibacterium*, and *Agreia*, as well as *"Frigoribacterium mesophilum"* strain VKM Ac-1396 (Fig. 1a, spectra *2–4*; Fig. 1b, spectra *3, 4*), were typical of C_{40}-carotenoids. The spectra of the pigments extracted from bacteria of the genus *Microbacterium* (Fig. 1b, spectra *1*, *2*) differed in the position of their spectral maxima and minima and in a number of other details. These pigments were identified as neurosporene [7]. In general, the spectral characteristics of pigments can be used to differentiate the members of the genus *Microbacterium* from the other genera of the family *Microbacteriaceae*.

The carotenoid nature of the pigments of four strains was confirmed by the mass spectrometry data (Table 2). At the evaporation temperature 180–220°C, the chromatographically pure pigments gave rise to ion peaks with equal mass-to-charge ratios (Table 2), although the intensities of the ion peaks slightly differed. The mass spectra of the pigments extracted from *Okibacterium fritillariae* and *C. flaccumfaciens* pv. *poinsettiae* had low-intensity high-molecular-weight peaks with *m/z* = 550 and 522. Presumably, the yellow and pink pigments of *C. flaccumfaciens* pv. *poinsettiae* have a similar chemical composition but different stereoconfigurations. However, this suggestion needs further investigation.

The biosynthesis of C_{40}-carotenoids is a complex multistage process in which phytoene sequentially transforms into phytofluene; ζ-carotene; neurosporene; lycopene; and, as a result of cyclization, β-carotene. In most eubacteria, the first three reactions are catalyzed by the same enzyme, Crt1-type phytoene desaturase; the fourth reaction is catalyzed by the second desaturase; and the fifth reaction, by lycopene cyclase [14]. The accumulation of neurosporene in some bacteria of the genus *Microbacterium* suggests that the Crt1-type desaturase alone is present in these bacteria, as in the case of the photosynthesizing bacteria of the genus *Rhodobacter* [14]. In *Microbacterium* sp. VKM Ac-2047 and in some bacteria of the genera *Agromyces* and *Leifsonia*, which produce carotenoids only under illumination, illumination is likely to induce the synthesis of desaturase(s). The spectral and mass spectroscopic analyses (Table 2, Fig. 1) show that the pigmented bacteria of the family *Microbacteriaceae*, except for those of the genus *Microbacterium*, transform neurosporene into compounds with a larger molecular mass than that of β-carotene. The lack of phytoene synthase is the most probable reason for the absence of isoprenoid pigments in *M. laevaniformans, Clavibacter michiganensis* subsp. *insidiosus* (VKM Ac-1402^T and VKM Ac-1416), *C. michiganensis* subsp. *sepedonicus*, and *Leucobacter komagatae.*

Evidence for the isoprenoid nature of pigments can come not only from chemical studies but also from inhibition analysis of isoprenoid synthesis [9, 10]. This analysis showed that, at concentrations of 0.05 to 1 mg/ml, fosmidomycin (the inhibitor of the nonmevalonate pathway of isoprenoid synthesis) inhibited both the growth and pigment formation in most of the bacterial strains studied (Table 1). As a rule, pigment formation was inhibited by fosmidomycin concentrations lower than those that inhibited bacterial growth. For instance, 0.05 mg/ml fosmidomycin inhibited pigment formation in 20 strains, indicating the presence of the nonmevalonate pathway of isoprenoid synthesis (Table 1). Thirteen strains of *Microbacterium aurantiacum, M. arborescens*, and *M. chocolatum*, as well as ten

strains of the genus *Curtobacterium*, exhibited the presence of both mevalonate and nonmevalonate biosynthetic pathways. In the case of the *Microbacterium* strains, the presence of these pathways is evident from the following findings: (1) The cultivation of *M. aurantiacum* in the presence of mevinolin was accompanied by a depigmentation of the cells and an increase in their mass. (2) Cultivation in the presence of fosmidomycin was accompanied by a decrease in the cell mass, although the cells remained pigmented (Table 1). (3) cultivation in the presence of the two inhibitors at the same time was accompanied by both cell depigmentation and growth inhibition. The difference in the pathways used for the synthesis of isoprenoid pigments and the isoprenoids necessary for bacterial growth has previously been demonstrated for a *Streptomyces* species [15]. Bacteria belonging to the genus *Curtobacterium* synthesize isoprenoid pigments through both the mevalonate and nonmevalonate pathways, as is evident from the ability of fosmidomycin to change the color of the bacterial cells from yellow (or orange) to pink, enhance the formation of more deeply colored yellow pigments, and suppress the synthesis of yellow pigments in the Ac-2099 and Ac-1811 strains at low concentrations (the synthesis of pink pigments was not inhibited by fosmidomycin even at high concentrations).

In general, the members of the genus *Clavibacter*, especially those in which photoinduced synthesis of the pigments occurs, were fairly susceptible to fosmidomycin (their growth and pigment synthesis were inhibited by 0.1 and 0.05 mg/ml fosmidomycin, respectively). In the case of the *Plantibacter* bacteria, at a concentration of 0.1 mg/ml, fosmidomycin arrested the pigment synthesis, whereas, even at a concentration of 1 mg/ml, it did not inhibit the growth of these bacteria (Table 1). In the case of the *Okibacterium* and *Agrococcus* bacteria, fosmidomycin inhibited not only the synthesis of pigments but also the growth of these bacteria in a concentration-dependent manner. The relative tolerance of the *Plantibacter* bacteria to fosmidomycin makes it possible to differentiate them from the phylogenetically close *Okibacterium* species and phenotypically similar *Rathayibacter* species (Table 1).

In the case of the other genera of the family, the concentration of fosmidomycin needed to inhibit pigmentation was species-dependent. For instance, two strains of the genus *Curtobacterium* were highly susceptible to fosmidomycin, whereas ten strains of this genus were almost completely insensitive to this antibiotic. Among the microbacteria studied, the clinical isolates *M. hominis* and *M. oxydans* were the most resistant to fosmidomycin (Table 1).

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