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Review

A review on microbial synthesis of hydrocarbons

N. Ladygina, E.G. Dedyukhina *, M.B. Vainshtein

Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, 142290 Russia

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Abstract

Review summarizes comparative data on the intracellular hydrocarbons of different microorganisms (cyanobacteria, aerobic and anaerobic bacteria, yeasts, and mycelial fungi). Certain systematic groups of microorganisms are characterized by specific composition of intracellular hydrocarbons, in particular, cyanobacteria are unique in their ability to produce 7- and 8-methylheptadecanes; photosynthetic bacteria are distinguished by the synthesis of cyclic hydrocarbons (pristane and phytane), whereas in fungi, long-chain hydrocarbons are predominant. The synthesis of hydrocarbons by microorganisms depends considerably on the growth conditions that provides a way for its physiological regulation. The processes for microbiological production of extracellular aliphatic and volatile non-methane hydrocarbons are exemplified. Pathways for the biosynthesis of straight chain-, branched-, volatile non-methane hydrocarbons, and isoprenoids are described. Mechanisms of the hydrocarbon synthesis appear to be different in various microorganisms. The role of hydrocarbons in microorganisms is discussed.

Keywords: Microbial hydrocarbons; Intracellular hydrocarbons; Extracellular hydrocarbons; Volatile hydrocarbons; Biosynthesis; Biofuel

Contents

1. Introduction

Hydrocarbons are considered to be the most stable group of naturally occurring compounds and are likely to retain much of their original architecture over very long periods of time. Hydrocarbon biomarkers are used to constrain the age of the ancient bacteria, archaea, and eukaryotes [1,2]. As the oldest

* Corresponding author.
E-mail address: dedeg@rambler.ru (E.G. Dedyukhina).

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fossils are of bacterial and algal origin, some more than 3 billion years old, the first studies on the isolation of the hydrocarbon-like substances from the cells of marine bacteria and algae were carried out with the aim to elucidate the role of microorganisms in the genesis of petroleum and formation of organic substances in marine sediments.

Extensive investigations of microbial hydrocarbons were greatly enhanced by the development of new analytical techniques, in particular, gas–liquid chromatography. By the 60th years of the last century, a lot of information appeared on the intracellular hydrocarbons of the representatives of different systematic groups of microorganisms and mechanisms of the hydrocarbon biosynthesis (see reviews [3–7]). In contrast to higher organisms, microbial forms could be cultivated in reactors that allows the industrial production of hydrocarbons to be developed. In recent years, microbial synthesis of extracellular aliphatic and volatile non-methane hydrocarbons attracted considerable interest in view of the development of effective and environmentally safe methods for the biofuel production [8–10].

Bacterial methane production has been reviewed at length [11] and falls outside the scope of this paper.

2. Intracellular hydrocarbons of microorganisms

The first report on the production of C_{10}–C_{25} aliphatic hydrocarbons by sulfate-reducing bacteria grown in the seawater-containing media supplemented with organic- or fatty acids was made in 1944 [12]. In 1952 Stone and ZoBell isolated hydrocarbon fractions from marine bacteria *Serratia marinorubrum* and *Vibrio ponticus* grown on the seawater–peptone media (0.25 and 0.03% of dry biomass, respectively) [13].

Historically, the development of gas–liquid chromatography initiated extensive studies of microbial hydrocarbons. From an experimental viewpoint, the biggest continuing problem was the exclusion of adventitious hydrocarbon contaminations that demanded the application of defined media and reagents of high purity. By using the labeled growth substrates, the hydrocarbon biosynthesis was evidenced for various groups of microorganisms including bacteria [14,15], yeasts [16–18], and fungi [3].

The comparative data on the intracellular hydrocarbons of microorganisms are summarized in Table 1. The hydrocarbon content of different systematic groups of microorganisms varied over a wide range, in particular, in bacteria, intracellular hydrocarbons ranged from 0.005 to 2.69% of dry biomass. It can be stated that certain systematic and, accordingly, physiological groups of microorganisms are characterized by specific composition of intracellular hydrocarbons. For instance, a peculiar feature of aerobic photosynthetic cyanobacteria is their ability to synthesize branched C_{18}-hydrocarbons (7- and 8-methylheptadecanes) in a ratio of 1:1; the hydrocarbon fractions were dominated by *n*-C_{17} (68–98% of the total hydrocarbons) [19–21]. Recent studies revealed that the hydrocarbon fraction of soil cyanobacterium *Microcoleus vaginatus* contained more than 60 branched alkanes, mainly 6- and 7-methylheptadecanes [22]. The synthesis of the complex of mono-, di-, and trimethylheptadecanes was demonstrated in filamentous cyanobacterium *Calothrix scopulorum* [23]. It was suggested that branched alkanes with the chain lengths from 17 to 20 carbon atoms could be used as biomarkers of cyanobacterial contributions to the sedimentary organic matter [2,23].

A distinctive feature of anaerobic phototrophic bacteria of the genera *Rhodopseudomonas*, *Rhodospirillum*, *Rhodomicrobium*, and *Chlorobium* is the synthesis of isoprenoid hydrocarbons (pristane and phytane). Some representatives of these genera synthesized squalene and higher cyclic hydrocarbons up to 95% of the total hydrocarbons [20,24]. It should be mentioned that the ability of prokaryotes to synthesize squalene has been questionable for a long time.

As seen from Table 1, Gram-positive bacteria of the genus *Clostridium*, which growth is based on the fermentative process, produced intracellular hydrocarbons from C_{11} to C_{35} with the predominance of middle-chain *n*-alkanes (C_{18}–C_{27}) [20] or long-chain *n*-alkanes (C_{25}–C_{35}) [25]. Surprisingly, in the hydrocarbon fractions of *Clostridium tetanomorphum* and *Clostridium acidiurici*, pristane and phytane, which are typical of phototrophic bacteria, were revealed [20]. In contrast, Gram-negative anaerobic bacteria *Desulfovibrio desulfuricans* provide their growth by using another metabolic mechanism based on the anaerobic sulfate respiration (sulfate reduction). Nevertheless, they also produced C_{11}–C_{35} hydrocarbons with the predominance of C_{25}–C_{35} *n*-alkanes (up to 80% of the total hydrocarbons) [10,26–28].

Quite different composition of the hydrocarbon fraction was observed in facultatively anaerobic Gram-negative bacteria of the genus *Vibrio*, in which *n*-heptadecane reached 80% [13,29]. Recently, a halotolerant bacterium *Vibrio furnissii* was isolated, which produced intracellular and extracellular hydrocarbons (C_{15}–C_{24}) in sum up to 60% of dry biomass [30,31]. Since the profile of the synthesized hydrocarbons was similar to that of kerosene and light oil, authors proposed the application of this strain for the fuel production from several organic substrates including wastes.

Aerobic Gram-positive bacteria, whose hydrocarbon composition has been most thoroughly investigated, belonged to the genera *Micrococcus* and *Sarcina*. The hydrocacbons of *Sarcina lutea* were mainly monounsaturated (C_{23}–C_{30}) with a double bond located near the middle of the chain; the odd-chain lengths prevailed over the even-chain hydrocarbons; the hydrocarbon chains terminated normally or with either *iso*- or *anteiso*-methyl branches [15,30–32].

A considerable body of information is available concerning hydrocarbons of the yeast cells. The synthesis of hydrocarbons by yeasts *Debaryomyces hansenii* [16], *Candida tropicalis*, *Candida guilliermondii* [18], and *Saccharomyces cerevisiae* [17] was proved with the use of radiolabeled glucose or acetate as the growth substrates.

The hydrocarbon content of the yeast cells usually ranged from 0.01 to 1.6% of dry weight under aerobic growth [6,30,33] and reached 10.2% of dry biomass in anaerobically grown *Saccharomyces* [34] (Table 1). Yeasts are able to synthesize a wide range of hydrocarbons from C_{10} to C_{34}, which include not only *n*-alkanes but also unsaturated and branched components.

Table 1
Intracellular hydrocarbons of microorganisms

Microorganisms	% of dry biomass	Hydrocarbon profile	Major hydrocarbons (% of the total hydrocarbons)	References
Cyanobacteria				
N. muscorum, *Anacystis nidulans*, *Phormidium luridum*, *Chlorogloea fritschii*	0.025–0.12	*n*-C_{15}–C_{18}; 7- and 8-methylheptadecanes	*n*-C_{17} (68–90)	[19–21]
Trichodesmium erythaeum, *Oscillatoria williamsii*, *Microcoleus chthonoplaseis*	0.05–0.12	*n*-C_{15}–C_{18}	*n*-C_{17} (91–95)	[21]
Coccochloris elabens, *Agmenellum quadruplicatum*	0.05–0.12	*n*-C_{18};$C_{17:1}$;$C_{19:1}$; $C_{19:2}$	$C_{19:1}$ (85–98)	[21]
Plectonema terebrans	0.05–0.12	*n*-C_{15}–C_{18}; $C_{17:1}$	*n*-C_{17} (90)	[21]
M. vaginatus	ND	*n*-C_{17}; $C_{17:1}$; 2-methylhexadecane; 6-, and 7-methylheptadecanes	$C_{17:1}$; 6- and 7-methylheptadecanes	[22]
C. scopulorum	ND	1-, 2-, and 3-methylheptadecanes	ω-4,5-methylheptadecanes	[23]
Anaerobic phototrophic bacteria				
Rhodopseudomonas spheroids Chlorobium sp.	0.006	*n*-C_{15}–C_{20}; pristane; phytane	*n*-C_{17} (43–50)	[20,24]
Rhodospirillum rubrum, *Rhodomicrobium vannielii*	0.005	*n*-C_{15}–C_{21}; pristane; squalene; higher cyclic hydrocarbons	squalene; higher cyclic hydrocarbons (93–95)	[20,24]
Gram-negative anaerobic sulfate-reducing bacteria				
D. desulfuricans	0.8–2.25	*n*-C_{11}–C_{35}	*n*-C_{25}–C_{35}	[10,26–28]
Gram-negative facultatively anaerobic bacteria				
V. ponticus, *V. marinus*	0.03	*n*-C_{15}–C_{18}	*n*-C_{17} (80)	[13,29]
V. furnissii	60.0	*n*-C_{15}–C_{24}	*n*-C_{22}, C_{24}	[30,31]
E. coli	0.0035	C_{13}–C_{23}	*n*-C_{16}–C_{18} (22–28)	[20,24]
Gram-positive anaerobic bacteria				
C. tetanomorphum	ND	C_{15}–C_{28}; pristane; phytane	*n*-C_{18}–C_{27} (59)	[20]
C. acidiurici	ND	C_{15}–C_{26}; pristane; phytane	*n*-C_{17} (50)	[20]
C. pasteurianum	ND	C_{11}–C_{35}	*n*-C_{25}–C_{35} (50–54)	[25]
Gram-positive aerobic bacteria (eubacteria)				
Bacillus sp.	0.33	C_{14}–C_{34}	*n*-C_{27}–C_{29} (61)	[30]
S. lutea	0.4	$C_{23:1}$–$C_{30:1}$	$C_{29:1}$ (43–79)	[31]
Arthrobacter sp.	0.93	C_{15}–C_{34}	*n*-C_{21}, *n*-C_{22} (33)	[30]
Micrococcus sp.	0.68	C_{17}–C_{30}	*n*-C_{27}, *n*-C_{28} (78)	[30]
M. lysodeikticus	0.075	$C_{24:1}$–$C_{29:1}$	$C_{29:1}$ (50–84)	[31]
Corynebacterium sp.	0.17	C_{15}–C_{33}	*n*-C_{27} (18)	[30]
Mycobacterium sp.	2.69	C_{17}–C_{31}	*n*-C_{25}–C_{29} (70)	[30]
Yeasts				
Saccharomyces sp.	0.04	C_{17}–C_{34}	*n*-C_{27}–C_{30} (69)	[30]
S. oviformis	1.6	C_{10}–C_{31}	*n*-C_{10}–C_{19}	[34]
S. oviformis, *Saccharomyces ludwigii* (anaerobically grown)	3.6–10.2	C_{10}–C_{31}; squalene	*n*-C_{10}–C_{19}	[34]
C. tropicalis (glucose- or ethanol-grown)	0.006–0.013	C_{14}–C_{25}	*n*-C_{16}–C_{19} (50–59)	[6,18]
C. tropicalis (acetate- or glycerol-grown)	0,012–0.031	C_{16}–C_{26}	*n*-C_{22}–C_{25} (49–66)	[6]
C. tropicalis (propionate-grown)	0.015	C_{16}–C_{28}	*n*-C_{15}–C_{19} (86)	[6]
Fungi (mycelium)				
Penicillium sp., *Aspergillus* sp., *Trichoderma virida*	0.06–0.70	C_{15}–C_{36}	*n*-C_{27}–C_{30} (47–62)	[30]
C. resinae (glucose-grown)	0.1	C_{7}–C_{36}; pristane	*n*-C_{16}	[33]
C. resinae (glutamate-grown)	0.12	C_{7}–C_{23}, pristane	*n*-C_{13}, *n*-C_{14}, *n*-C_{16},	[33]
D. hansenii	ND	C_{16}–C_{39}	*n*-C_{22}	[41]
Pullularia pullulans	ND	C_{16}–C_{28}	C_{19}–C_{22}	[42]
Fungi (spores)				
Ustilago maydis	0.004	C_{19}–C_{33}	*n*-C_{27}, C_{29} (33–36)	[36,38]
U. nuda	0.006	C_{25}–C_{37}	C_{35} (18)	[36]
Urocystis agropyri	0.004–0.015	C_{19}–C_{31}	*n*-C_{29} (46)	[38]
Sphacelotheca reiliana	0.015	C_{23}–C_{33}	C_{29} (34)	[36,38]
Tilletia foetida, *T. caries*, *T. controversa*	0.004–0.015	C_{21}–C_{33}	*n*-C_{29} (26–35)	[37,38]

Note: ND stands for ''no data''.

The hydrocarbon synthesis by yeasts depended considerably on the growth conditions that provides a way for physiological regulation of this process. For instance, anaerobic conditions promoted the increased production of intracellular hydrocarbons by *Saccharomyces oviformis* and induced the synthesis of squalene, which was not revealed under aerobic growth [34].The synthesis of individual hydrocarbons varied significantly in dependence on the carbon substrate applied. In particular, middle-chain alkanes (C_{16}–C_{19}) prevailed in *C. tropicalis* grown in the glucose- or ethanol-containing media, whereas long-chain (C_{22}–C_{25}) alkanes amounted to 49–66% of the total hydrocarbons in the acetate- or glycerol-grown yeasts [6]. Unusual predominance of odd-numbered alkanes was revealed in propionate-grown *C. tropicalis* [6].

Because of the low content of intracellular hydrocarbons, it is difficult to suppose that they play the role of the carbon and energy supply, most probably, hydrocarbons fulfill physiological functions, such as the regulation of the cell membrane permeability. This assumption was supported by the findings that the composition of the hydrocarbon fraction depended considerably on its localization within the yeast cells. In the case of *Candida utilis* grown anaerobically, the hydrocarbon fraction isolated from the cell walls was represented by hexatriene (82%); hydrocarbons of cytoplasmic membrane contained mainly squalene (51%), whereas in the hydrocarbon fraction localized in cytoplasm, *n*-alkanes were predominant [35].

In fungi, the hydrocarbons accumulated in mycelia or in spores differ significantly in their amount and composition. As seen from Table 1, the hydrocarbon content ranged from 0.06 to 0.7% of dry mass in mycelia [30,33] and was sufficiently lower in spores (0.004–0.015%) [36–38]. The hydrocarbon profile of fungal spores was similar to that of higher plants and was dominated by the odd-numbered *n*-alkanes (C_{27}, C_{29}, and C_{35}) [36–40]. Therefore, the origin of alkanes in spores remains doubtful. Information on the hydrocarbon composition of fungal mycelia seems to be more substantial since fungi were cultivated under controlled conditions. Mycelial hydrocarbons contained generally *n*-alkanes from C_{15} to C_{36} with the predominance of long-chain C_{19}–C_{30} hydrocarbons [30,41,42]. Unusual composition of the hydrocarbon fraction was revealed in *Cladosporium resinae* grown on the glucose- or glutamate-containing media [33]; it includes pristane, which is typical of photosynthetic bacteria. Recently, the production of diterpene hydrocarbons was revealed in the fungi *Hericium erinaceum* and *Phoma betae* [43,44].

Thus, a great bulk of information concerning microbial hydrocarbons demonstrates that the ability to synthesize hydrocarbons is widespread among microorganisms. Certain systematic groups of microorganisms are characterized by specific composition of the hydrocarbon fractions, for instance, cyanobacteria are unique in their ability to produce 7- and 8-methylheptadecanes; photosynthetic bacteria are distinguished by the synthesis of cyclic hydrocarbons (pristane and phytane), whereas in fungi, long-chain hydrocarbons are predominant. It was assumed that the hydrocarbon composition of microorganisms could be used as a chemotaxonomic criterion [45]. The occurrence of branched hydrocarbons in organic matter is usually used as a biomarker of bacterial origin of marine sediments [2,23,46].

It should be noted that microbial cells are usually characterized by the low content of hydrocarbons (no more than 10% of dry mass); the only exception is bacterium *V. furnissii* [30,31]. Until recently, the only promising source of hydrocarbons was considered to be green microalga *Botryococcus braunii*, which accumulated hydrocarbons up to 75% of dry mass [47,48]. The attempts were made to clone the *B. braunii* genes encoding the key enzymes of the hydrocarbon biosynthesis into bacterial cells to increase the rate of the hydrocarbon production and to reduce the problems associated with a high viscosity of the *B. braunii* broth [48]. Thus, the main biotechnological problem consists in the determination of the number of metabolic steps (enzymes) involved in the process of the hydrocarbon synthesis. If the transfer of one or two enzymes can activate the hydrocarbon synthesis, the application of recombinant strains for the biotechnological production of renewable fuel appeared to have great potential in the near future.

3. Extracellular hydrocarbons of microorganisms

3.1. Long-chain hydrocarbons

Microbial production of extracellular long-chain hydrocarbons received considerable interest in the last years in connection with their role in the petroleum formation and the possibility of their industrial application as modern energy resources.

Bacteria of the genus *Desulfovibrio* and *Clostridium* were shown to produce extracellular long-chain hydrocarbons (up to 19 and 30 mg/l, respectively) [25,49–51]. Unlike intracellular hydrocarbons, which included mainly long-chain C_{25}–C_{35} *n*-alkanes (50–90%), extracellular fractions were represented by hydrocarbons with the lower chain lengths (C_{19}–C_{21} and C_{16}–C_{18} in *Clostridium pasteurianum* and *D. desulfuricans*, respectively) and contained 2.6-fold increased amount of *iso*-forms [25]. The release of hydrocarbons from the *C. pasteurianum* cells was suggested to be connected with the functioning of cytoplasmic membrane: the hydrocarbons of low molecular weight passed through the membrane into the culture broth, whereas the retained hydrocarbons were elongated [25].

The synthesis of extracellular saturated C_{21}–C_{33} hydrocarbons (10–15 μg/l) was revealed in *Pseudomonas fluorescens* grown in the glucose-containing defined medium [52]. It was assumed that extracellular hydrocarbons had the role of antiadhesins and, therefore, promoted the cell aggregation [52,53].

The process for the production of extracellular hydrocarbons was developed; it involved the cultivation of sulfate-reducing bacteria *D. desulfuricans* in the medium containing mineral salts and calcium lactate under anaerobic conditions in an atmosphere of $CO_2 + H_2$ (1:20). The isolated hydrocarbon fraction reached 30 mg/l; it consisted of aliphatic C_{14}–C_{25} hydrocarbons of normal and *iso*-structure [9].

Recently, extensive production of extracellular hydrocarbons (up to 50% of dry biomass) by bacterium *V. furnissii* was revealed; since the composition of hydrocarbons was similar to that of kerosene and light oil, this strain was considered to be a promising producer of renewable fuel [30,31].

The application of sulfate-reducing bacterium *D. desulfuricans* in a biofuel cell was supposed to offer a new opportunity for the power engineering [54].

3.2. Volatile non-methane hydrocarbons

The hydrocarbon gases are ubiquitous in the atmosphere and oceanic sediments worldwide [55]. Scant information concerning microbial production of the non-methane hydrocarbons (NMHCs) evoked geochemists to formulate criteria for distinguishing thermogenic or biogenic origin of volatile hydrocarbons. The production of significant amounts of C_2–C_4 alkanes is generally believed to require thermocatalytic reactions at 60–180 °C [56,57], whereas methane and alkenes ($C_{2:1}$ and $C_{3:1}$) are formed biologically [58]. Criteria for determining the origin of hydrocarbon gases were suggested to be the ratios of $C_1/(C_2 + C_3)$ [59] and $C_2/C_{2:1}$ [60]. According to these criteria, a seep gas from a biogenic source was characterized by a $C_1/(C_2 + C_3)$ ratio of over 1000 and a $C_2/C_{2:1}$ ratio significantly <1, on the other hand, a seep gas from a thermogenic source had a $C_1/(C_2 + C_3)$ ratio <50 and a $C_2/C_{2:1}$ ratio significantly >1 [58,61,62].

Bacterial origin of the NMHCs in marine sediments was proved by direct chemical analysis of isolated samples [61,63], by the incorporation of radiolabeled carbon into the gases [64], and by revealing the hydrocarbon production in the growth medium inoculated with sediment [65]. Modern biogenic origin of ethane in marine sediments was confirmed by the analyses of the carbon stable isotopes [66].

The volatile hydrocarbon isoprene (2-methyl-1,3-butadiene) was shown to be produced by actinomycetes belonging to the genera *Pseudonocardia*, *Saccharomonospora*, *Streptomyces*, and *Thermomonospora* [67] and by various bacterial species, both Gram-negative (*Acinetobacter*, *Agrobacterium*, *Escherichia*, *Erwinia*, *Pseudomonas*) and Gram-positive (*Bacillus*, *Micrococcus*, *Rhodococcus*) [68]. The representatives of the genus *Bacillus* were indicated as the most active isoprene producers; the rate of isoprene production by *Bacillus subtilis* was at least 100-fold higher than it was observed in other cultures [69,70]. It was stated that the relative rates of isoprene synthesis by bacteria and by plant leaves were comparable (8.5 and 14 nmol/(g h), respectively) [68].

Soil bacteria and fungi are known to be significant producers of other volatile hydrocarbons, such as ethane, ethylene, propane, and propene [71–73]. In particular, ethylene is usually produced by a wide variety of soil microorganisms including bacterial and fungal plant pathogens [73–79]. Biological formation of ethylene has attracted increasing attention due to its role as a plant growth regulator; ethylene is implicated in the inhibition of the root elongation, the development of disease symptoms, such as petiolar epinasty and abscission, and in the promoting of early ripening of fruits [78,80]. The capability for the synthesis of ethylene (from 0.18 to over 500 ppm) was revealed in 58 fungal strains out of 228 species tested [74]. The production of ethylene by the most active species *Aspergillus clavatus* reached 514 ppm [74]. According to Fukuda et al. [73], out of 166 strains of bacteria, yeasts, and fungi examined, the ethylene production was revealed in 49 strains; the most active producers, *Penicillium digitatum*, *Schizosaccharomyces octosporus*, *Penicillium urticae*, and *Cryptococcus laurentii*, synthesized ethylene at the rates of 1394, 204, 114, and 54.6 nl/ml/h, respectively.

The synthesis of ethylene by many microorganisms was shown to be a methionine-required process. In particular, a strong dependence of ethylene synthesis on the presence of methionine in the media was revealed in experiments with the fungi *Cenococcum geophilum*, *Hebeloma crustuliniforme*, *Laccaria laccata*, *Fusarium oxysporum* [78], and *Botrytis cinerea* [79], yeasts *Cryptococcus albidus* [81], and bacteria belonging to the genera *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Arthrobacter*, *Erwinia*, *Citrobacter*, *Enterobacter*, *Serratia* [82], and *Escherichia coli* [83]. On the other hand, the ethylene production by *P. digitatum* and *Pseudomonas syringae* was dependent on the presence of 2-oxoglutarate in the media [84,85]. These findings imply the functioning of different pathways for the ethylene synthesis in microorganisms.

Another widespread volatile hydrocarbon, ethane, was found to be produced by 49 strains (out of 166 strains tested) belonging to fungi, yeasts, bacteria, and actinomycetes [73]. The rates of ethane synthesis by yeasts *Rhodotorula glutinis*, *Brettanomyces bruxellensis*, *S. octosporus*, and *Schizosaccharomyces pombe* reached 21.5, 15,8, 12.1, and 11.4 nl/ml/h, respectively [73].

Many microbial species were able to produce C_3-hydrocarbons such as propane and propylene, under aerobic conditions [86,87]. When tested 178 strains of fungi, yeasts, bacteria and actinomycetes, the synthesis of propane and propylene was revealed in 87 and 37 strains, respectively [86]. The rates of propane synthesis by the most active producers, *Brevibacterium ammoniagenes* and *C. albidus*, reached 8.6 and 6.5 nl/ml/h, respectively. The most active propylene-producers, *Gliocladium roseum* and *S. octosporus*, synthesized propylene at the rates of 3.0 and 1.2 nl/ml/h, respectively [86]. Thus, it can be assumed that the production of C_3-hydrocarbons was not taxonomically specific.

The producers of C_4-hydrocarbons were found to belong to different systematic groups of microorganisms (bacteria, yeasts, and fungi). When tested 178 cultures, the synthesis of *n*-butane, isobutene, isobutane, 1-butene, *trans*-2-butene, and *cis*-2-butene was revealed in 52, 37, 23, 14, 7, and 4 strains, respectively [86]. It is known that isobutene, unsaturated hydrocarbon with four carbon atoms, is widely used in the petrochemical industry. The rate of isobutene production by the most active strain *Rhodotorula minuta* var. *texenis* reached 16.4 nl/ml/h) [86].

The process for microbial production of the mixture of saturated and unsaturated C_2–C_5 hydrocarbons under aerobic conditions was developed [8]. It was found that certain amino acids had regulatory effect on the synthesis of individual

hydrocarbons by microorganisms. In particular, the synthesis of isobutene was stimulated by the addition of L-leucine into the growth medium; under these conditions, aromatic amino acids (L-phenylalanine, L-tryptophane, and L-tyrosine) as well as benzene compounds (benzoic acid, L-phenylglycine, L-phenylpyruvic acid, etc.) had a synergistic effect on the isobutene production by yeasts [88]. It was suggested that L-phenylalanine induced the synthesis of some enzymes involved in the formation of isobutene [89,90]. The production of *trans*-2-butene and *cis*-2-butene was stimulated by L-isoleucine; in this case, L-phenylalanine had synergistic effect on the isobutene synthesis. The ethylene synthesis was stimulated by L-methionine; on the other hand, the formation of saturated hydrocarbons such as ethane, propane, *n*-butane, isobutene, and *n*-pentane, was stimulated by L-cysteine [8]. The involvement of methionine in the ethylene synthesis is discussed below (Section 4.3.1), whereas the role of other amino acids in the hydrocarbon synthesis remains unclear.

4. Pathways for the hydrocarbon synthesis by microorganisms

4.1. Biosynthesis of straight-chain hydrocarbons

Aliphatic hydrocarbons were presumed to derive from fatty acids. Two routes for the biosynthesis of straight-chain hydrocarbons have been widely discussed: the ''elongation–decarboxylation'' and the ''head-to-head condensation'' pathways. The best known of them, the ''elongation–decarboxylation'' pathway envisages de novo synthesis of long-chain fatty acids (e.g. C_{16} and C_{18}), which are elongated by the continuous addition of a C_2 unit derived from malonyl-CoA with the subsequent decarboxylation. This route was first discovered in plant tissues [91,92]. Similar mechanism of the hydrocarbon synthesis was also suggested in *E. coli* [93] and in cyanobacterium *Nostoc muscorum* [94].

The other widely considered pathway for the alkane biosynthesis involves the head-to-head condensation of two fatty acids (or suitable derivatives) with the subsequent decarboxylation of one of them. This route was investigated in detail in bacteria *S. lutea* [3,4,15,95].

The most intriguing step in these pathways is the reaction of decarboxylation. It is considered that the direct loss of the carboxyl carbon of a fatty acid is not mechanistically feasible without an electron-withdrawing group adjacent to the α-carbon [96]. It has been well documented that the elimination of CO_2 from carboxylic acids requires high energy and, therefore, has to be activated by a β-substituent able to stabilize the negative charge generated by CO_2 release (cited from [7]). Accordingly, it has generally been thought that the activated fatty acid derivatives are the intermediates in the decarboxylation reaction leading to hydrocarbons. Experimental results suggested that an aldehyde could be the immediate precursor of a hydrocarbon. The fatty acyl-CoA reductase activity responsible for converting fatty acids to aldehydes has been identified in a variety of organisms from bacteria to animals [7,97–99]. The gene encoding this enzyme has been recently cloned from the plant seeds and γ-proteobacteria (*Acinetobacter calcoaceticus* and *Photobacterium leiognathi*) [100–102].

The discovery of the decarbonylation reaction in the microsomal fraction of green alga *B. braunii* suggested a possible mechanism for the conversion of aldehydes to hydrocarbons. The purified aldehyde decarbonylase containing the cobalt–porphyrin complex catalyzed the decarbonylation of octadecanal to heptadecane and CO with near a 1:1 stoichiometry [96,103]. The role of metal ion involved in the enzymatic decarbonylation remains unclear. The metal ion-binding chelators were found to inhibit the conversion of aldehydes to hydrocarbons in microsomal or solubilized and purified preparations from the animal-, insect-, plant-, and algal sources [104].

In spite of the obvious generality of the pathway involving the decarbonylation of aldehydes to hydrocarbons, alternative reactions of the hydrocarbon biosynthesis have been revealed recently. In particular, both decarbonylation and decarboxylation of octanal was suggested in the pathway for the *n*-heptane synthesis in plant tissues [105]. In archaea, the acetyl-CoA decarbonylase–synthase complex was shown to contain five different subunits (cited from [7]).

A new pathway for the synthesis of long-chain alkanes via 1-alcohols was proposed in *V. furnissii* [106]. It was assumed that 1-hexadecanal derived from 1-hexadecanoic acid was oxidized to 1-hexanol, and, finally, converted to hexadecane. This pathway explains the occurrence of similar levels of even- and odd-numbered alkanes in bacteria, while the even-numbered fatty acids were predominant [106].

The synthesis of extracellular hydrocarbons by sulfate-reducing bacteria *D. desulfuricans* grown heterotrophically in the lactate-containing medium under the atmosphere of $H_2 + CO_2$ was studied by using labeling substrates [10]. It was revealed that ^{14}C from bicarbonate was actively incorporated into formate and acetate (37 and 41%, respectively). The synthesis of formate was suggested to proceed via the direct reduction of CO_2. The other electron acceptors (sulfate and nitrate) were less suitable for the hydrocarbon formation than CO_2. When labeled formate was added into the medium, about 78–79% of ^{14}C was found in acetate that indicates the involvement of formate in the acetate synthesis by *D. desulfuricans*. In the presence of labeled acetate, ^{14}C derived from methyl group was incorporated into hydrocarbons more actively than the label derived from carboxylic group that testifies the involvement of the decarboxylation reaction in this process. By using tritium-labeled water, it was revealed that protons derived from water were actively incorporated into extracellular hydrocarbons by sulfate-reducing bacteria.

On the basis of the results obtained, it was suggested that the hydrocarbon synthesis by sulfate-reducing bacteria involves formation of acetate and formate from CO_2 with the subsequent reduction of these acids to aldehydes, which, in turn, undergo aldol condensation with the chain elongation to produce hydrocarbons [10] (Fig. 1). The involvement of formaldehyde in the condensation process may explain the production of both even- and odd-numbered hydrocarbons. It is not excluded that

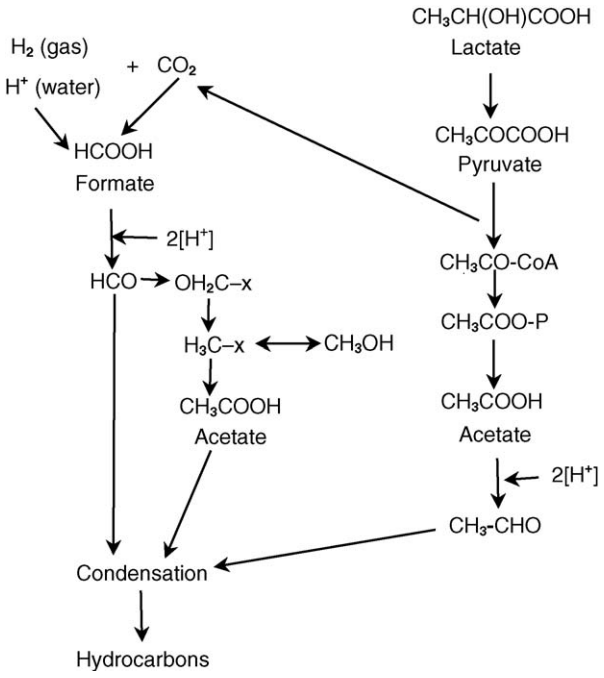

Fig. 1. Pathway for the hydrocarbon biosynthesis by sulfate-reducing bacteria (adapted from [10]).

formaldehyde and acetaldehyde can interact with other compounds, such as derivatives of lactate, pyruvate, and acetate, to form alcohols and then hydrocarbons [10] (Fig. 1).

It was shown that the synthesis of extracellular hydrocarbons by sulfate-reducing bacteria depended considerably on the medium composition and the ratio of H_2 and CO_2 in a gas phase that allows the physiological regulation of this process to be performed [51].

4.2. Biosynthesis of branched hydrocarbons

The synthesis of branched hydrocarbons was studied in detail in bacteria *S. lutea*, whose the most abundant hydrocarbons contained methyl groups on both ends of the molecule and a double bond near the center. The number of the carbon atoms in the hydrocarbon molecule was equal to one less than two times the average number of the carbon atoms in the most abundant fatty acids [95]. The distribution of the carbon chains of isoleucine, valine, and acetate in fatty acids and hydrocarbons synthesized in vivo is consistent with the ''head-to-head condensation'' pathway of the hydrocarbon synthesis [4,95]. Labeling studies showed that *anteiso* aliphatic groups of fatty acids and hydrocarbons were derived from five terminal carbons of isoleucine and the remaining carbons originated from acetate, whereas valine contributed *iso*-aliphatic groups [15]. The observed variations in the relative proportions of the members of each alkene family in *S. lutea* was assumed to depend on the availability of isoleucine and valine in the growth media [3].

The mixture of 7- and 8-methylheptadecanes, which is typical of cyanobacteria was shown to derive from *cis*-vaccenic acid (11-octadecenoic acid) by the addition of methyl-^{14}C group of methionine to a double bond with the subsequent reduction and decarboxylation of the fatty acids [3,107].

4.3. Biosynthesis of volatile non-methane hydrocarbons

4.3.1. Ethylene biosynthesis

At present, three pathways for the ethylene biosynthesis are known (Fig. 2). In higher plants, the most acceptable pathway for the ethylene biosynthesis proceeds via amino-cyclopropane-1-carboxylic acid (ACC) and is termed ''the ACC pathway'' [79,108–110]. According to this pathway (Fig. 2A), at the first stage, the intermediate *S*-adenosyl methionine (SAM) is produced from methionine by SAM synthase and then it is converted to ACC and 5′-methylthioadenosine (MTA) by ACC synthase. MTA is then recycled to methionine through a number of intermediates (5′-methylthioribose (MTR), 5′-methylthioribose-1-phosphate (MTR-1-P), and 2-keto-4-methylthiobutyric acid (KMBA)) that allows the level of L-methionine to remain relatively unchanged even at high rates of ethylene production. The final step is the conversion of ACC to ethylene by ACC oxidase (Fig. 2A).

The ACC pathway is of rare occurrence in microorganisms; it has been described only in the slime mold *Dictyostelium mucoroides* [111] and in the fungus *Penicillium citrinum* grown in methionine-containing medium; from the latter, ACC synthase was purified and characterized [112].

The pathway for the ethylene synthesis, which is common in microorganisms, is termed ''the KMBA pathway'' (Fig. 2B). It assumes the generation of ethylene from methionine that is in agreement with the well-known methionine requirement for the ethylene synthesis in a great number of microorganisms [78,79,81–83]. This pathway involves the transaminase-catalyzed conversion of methionine to KMBA, which is then oxidized to ethylene in the presence of hydroxyl radicals (Fig. 2B). The transaminase activity requires α-ketoacid as a co-substrate and results in the formation of KMBA and the amino acid [3,113]. The oxidation of KMBA to ethylene can proceed either enzymatically (with the involvement of peroxidase or oxidoreductase) or photochemically. In particular, when *B. cinerea* was cultivated in light or in the dark, the KMBA was accumulated in the culture broth during the first hours of the hyphae growth and then it was converted to ethylene either enzymatically (in the dark) or photochemically (in light). The addition of peroxidase to the dark-grown cultures induced ethylene synthesis [114]. Fukuda et al. [81] isolated NADH–Fe(III)EDTA oxidoreductase involved in the conversion of KMBA to ethylene in *C. albidus*. This enzyme catalyzed the release of hydroxyl radicals, which reacted with KMBA to produce ethanol. Similar enzyme, which required NADH, Fe(III)EDTA, and oxygen as cofactors to produce ethylene from KMBA, was isolated from the cell-free extracts of *E. coli* [83].

The KMBA pathway for the ethylene synthesis was described in various microorganisms, such as *E. coli* [83], *C. albidus* [81], *B. cinerea* [79,114], and in the fungi isolated from soil rhizosphere [115].

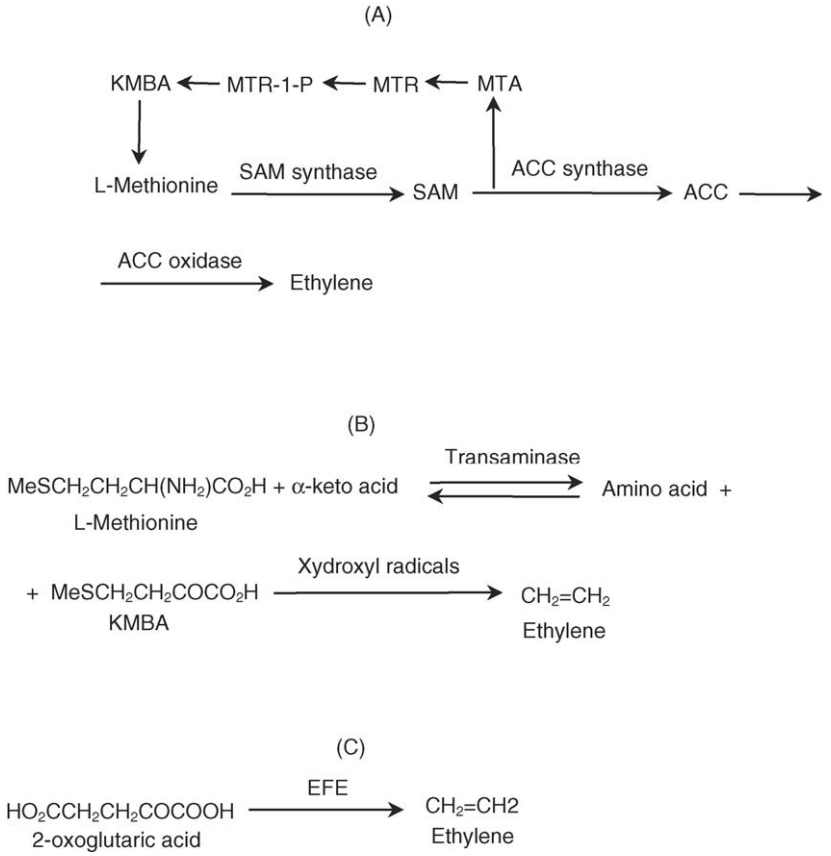

Fig. 2. Pathways for the ethylene biosynthesis: (A) the ACC pathway (adapted from [108]); (B) the KMBA pathway [3,115]; (C) the pathway involving 2-oxoglutarate (adapted from [122]). Abbrevations: ACC, aminocyclopropane-1-carboxylic acid; SAM, S-adenosylmethionine; MTA, 5′-methylthioadenosine; MTR, 5′-methylthioribose; MTR-1-P, 5′-methylthioribose-1-phosphate; KMBA, 2-keto-4-methylthiobutyric acid.

The third known pathway for the ethylene biosynthesis involves 2-oxoglutaric acid as a precursor and the multifunction enzyme termed ''ethylene-forming enzyme'' (EFE) (Fig. 2C). This pathway was described in fungi *Penicillium cyclopium* [116], *P. digitatum* [117,118], *F. oxisporum* [119], and in bacterium *P. syringae* [85,120]. The EFE was purified from *P. digitatum*, its activity was stimulated by L-arginine and ferrous ions under reduced conditions [118]. Recently, successful attempts were made to introduce the gene encoding EFE from *P. syringae* to *E. coli* [121]. Photosynthetic conversion of carbon dioxide to ethylene was demonstrated in the recombinant cyanobacterial strain *Synechococcus* sp. PCC 7942, which harbors the EFE-encoding gene from *P. syringae* [122]. Thus, the application of genetic engineering appears to be perspective for the construction of recombinant strains capable of intensive ethylene production.

4.3.2. Isobutene biosynthesis

The synthesis of isobutene was studied using the selected strain *R. minuta* [88,90]. It was found that the isobutene production was increased by the addition of L-leucine to the growth medium or to the suspension of the resting cells; L-phenyalanine had a synergistic effect on the production of isobutene in the presence of L-leucine [88]. It was assumed that isobutene was formed by the decarboxylation of 3-methylcrotonyl CoA in the catabolic pathway of the L-leucine turnover [88]:

l-leucine → α-ketoisocaproic acid →
isovaleryl-CoA → 3-methylcrotonyl-CoA → isobutene

It is noteworthy that L-phenylalanine showed a synergistic effect on the isobutene synthesis not only in the presence of leucine, but also with α-ketoisocaproic and isovaleric acids [88]. Isovaleric acid was the best substrate for the isobutene synthesis by the cell-free system obtained from *R. minuta* [90]. It still remains obscure the mechanisms of synergistic effects of amino acids on the synthesis of C_2–C_5 hydrocarbons.

4.3.3. Isoprenoid biosynthesis

For a long time, it had remained unclear whether there is a universal mechanism for the isoprenoid formation in bacteria, plants, and humans. At present, it is assumed that fundamental units of the isoprenoid biosynthesis, such as isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), can be produced via two completely different pathways, which are termed the mevalonate pathway (MVA) and the methylerythritol phosphate pathway (MEP) (Fig. 3). The MVA pathway had long been known, whereas the reactions of the MEP pathway were determined much later [123–128]. The MVA

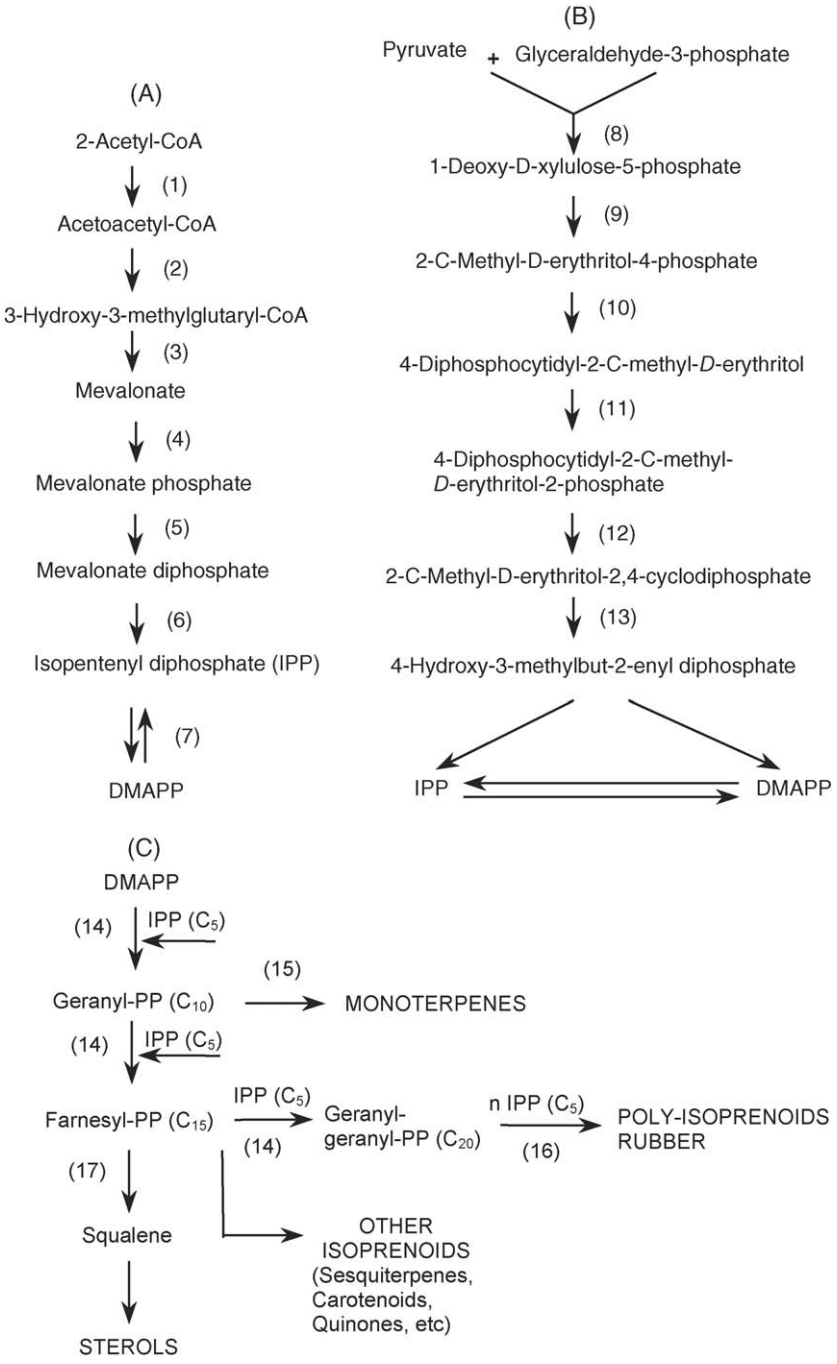

Fig. 3. Pathways for the isoprenoid biosynthesis: (A) the MVA pathway (adapted from [128]); (B) the MEP pathway (adapted from [128]); (C) interrelationship between the syntheses of monoterpenes, sterols, and isoprenoids (adapted from [128,134]). Abbrevations: IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate. Enzymes: (1) β-ketothiolase; (2) 3-hydroxy-3-methylglutaryl-CoA synthase; (3) 3-hydroxy-3-methylglutaryl-CoA reductase; (4) mevalonate kinase; (5) 5-phosphomevalonate kinase; (6) 5-diphosphomevalonate decarboxylase; (7) isopentenyl-diphosphate isomerase; (8) 1-deoxyxylulose-5-phosphate synthase; (9) 1-deoxyxylulose-5-phosphate reductoisomerase; (10) 4-diphosphocytidyl-2-C-methylerythritol synthetase; (11) 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; (12) 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; (13) (E)-4-hydroxy-3-methylbut-2-methyl-D-erythritol-2,4-cyclodiphosphate synthase; (14) prenyl transferase; (15) enzymes of the limonene pathway; (16) rubber transferase; (17) squalene synthase, SQS (ERG9).

pathway starts from acetyl-CoA; the key intermediate is mevalonate phosphate formed from 3-hydroxy-3-methylglutaryl-CoA (Fig. 3A). The MEP pathway (Fig. 3B) starts from pyruvate and glyceraldehydes-3-phosphate, which are converted into 1-deoxy-D-xylulose-5-phosphate (DXP); the main intermediates are 2-C-methyl-D-erythritol-4-phosphate, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate, and 4-hydroxy-3-methylbut-2-enyl diphosphate. The genes encoding enzymes involved in the MEP pathway have been recently identified [128].

The comparative studies on the efficiency of the MVA and the MEP pathways revealed that they are quite different

with respect to stoichiometry, energy consumption, and the oxidation/reduction balance [128]. On assuming that glucose is used as a carbon source and metabolized through the fructose 1,6-bisphosphate pathway, the overall stoichiometry of the conversion of glucose to IPP is as follows [128]:

$$\text{The MVA pathway}: 1.5\text{glucose} \rightarrow \text{IPP} + 4CO_2 + 8[H];$$

$$\text{The MEP pathway}: 1\text{glucose} + 3\text{ATP} + 2[H] \rightarrow \text{IPP} + CO_2 + 3\text{ADP}$$

Thus, from an energetic point of view, the MVA pathway is much more efficient since the synthesis of IPP requires no ATP and yields 8[H], which could be subsequently used for the ATP production in the course of respiration, whereas the conversion of glucose to IPP via the MEP pathway consumes 3 mol of ATP and 2[H].

It seems somewhat surprising that the MEP pathway being less energetically efficient than the MVA pathway is widespread among prokaryotes. It was stated that many bacteria, both Gram-negative and Gram-positive, were able to assemble the C-5 building blocks of the isoprenoid biosynthesis (IPP and DMAPP) through the MEP pathway rather than via the MVA pathway [123].

The study of the isoprene formation by *B. subtilis* from ^{13}C-labeling substrates revealed that it proceeds via the MEP pathway rather than through the classical MVA pathway [124]. The absence of the MVA pathway was also suggested by the finding that the *B. subtilis* genome lacked a gene for the key enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and the growth of bacteria was not blocked by simvastatin, a potent inhibitor of HMGR [124,129].

The MEP pathway was revealed in several highly pathogenic microorganisms, such as *Mycobacterium tuberculosis*, that makes it possible to use specific inhibitors of the MEP pathway (fosmidomycin and fluoropyruvate) as potent drugs to defeat pathogens. For example, fosmidomycin and fluoropyruvate were recently identified as inhibitors of enzymes involved in the MEP pathway (1-deoxy-D-xylulose-5-phosphate (DXP) reductoisomerase and DXP synthase) [128].

The occurrence of the MEP and MVA pathways in bacteria of different taxonomic groups was established by investigating the effect of inhibitors of the MEP and MVA pathways (fosmidomycin and mevinoline, respectively) on the growth of 34 anaerobic and 10 aerobic prokaryotic strains [130]. The MEP pathway was revealed in nine (out of 10) representatives of the family *Microbacteriaceae*, four (out of five) strains of the genus *Thermobacter*, and 11 (out of 12) strains of the genus *Clostridium*, whereas the MVA pathway was found in lactobacilli (*Carnobacterium*), methanogenic and sulfate-reducing bacteria. Thus, it can be assumed that the functioning of the MVA or the MEP pathways in microorganisms is a species-specific process. It is possible that in some bacteria, more than one pathway for the isoprenoid biosynthesis may function, as suggested in *Streptomyces aeriouvifer* [131].

The enzyme isoprene synthase, which catalyzes the Mg^{2+}-dependent conversion of DMAPP to isoprene and PPi was detected and partially purified from *B. subtilis* [132]. The isoprene synthase reaction involves simple elimination of pyrophosphate, probably through a transient carbon atom, which can rearrange to form isoprene. As in other isoprenoid synthases, divalent cation in this enzyme facilitates the departure of the pyrophosphate group [133].

The isoprenoid synthesis involves the successive additions of IPP to DMAPP or to the condensation products catalyzed by prenyltransferases that results in the formation of geranyl diphosphate (C_{10}), farnesyl diphosphate (C_{15}), and geranyl-geranyl diphosphate (C_{20}). The interrelationship between the production of monoterpenes, sterols, and poly-isoprenoids is shown schematically in Fig. 3C [128,134]. Farnesyl diphosphate appeared to be a branching point in the synthesis of sterols, poly-isoprenoids (including rubber-like polymers), and other isoprenoids (sesquiterpenes, carotenoids, quinines, etc.).

Recent advances in the elucidation of the pathways for the isoprenoid and poly-isoprene biosynthesis have encouraged attempts to develop the production of the rubber-like polymers by microorganisms [128,134], particularly, by oleaginous yeasts (*Yarrowia lipolytica*, *Cryptococcus curvatus*, and others), which are able to accumulate lipids up to 50% of dry mass. The attempts are now initiated to re-direct the metabolic flux from acetyl-CoA in favor of the formation of poly-isoprenes by using multiple transgenesis [134]. To increase the precursor supplies for the rubber polymerase genes, which can be cloned from Brazilian rubber tree *Hevea brasiliensis* or guayule *Parthenium argentatum* in microbial cells, the following steps are suggested to be involved: the disruption of the *ACC* gene encoding acetyl-CoA carboxylase (a key enzyme of the fatty acid biosynthesis), the over-expression of *HMGR* gene encoding hydroxymethylglutaryl-CoA reductase, which is involved in the mevalonate synthesis, and the disruption of *SQS* gene encoding squalene synthase. As a first step for the metabolic engineering, the isolation of *SQS* gene from *Y. lipolytica* was reported [134].

It is implied that only biotechnological production of natural rubber by microorganisms will provide the full flexibility of this process and its independence of geographic, climatic, seasonal and other factors. Another advantage of microbe applications is high accessibility of microbial metabolism to genetic engineering and its great flexibility with regard to the flux of intermediates and pathways of engineering. It is also possible the establishment of microbial systems for the production of poly-isoprene molecules with modified chemical structures of defined molecular weights. In view of this, the strategy should be to screen for the poly-isoprenoid-synthesizing prokaryotes or to obtain recombinant bacteria capable of producing poly-isoprenoids with a chemical structure identical to natural rubber by using genetic and metabolic engineering [128].

5. Role of microbial hydrocarbons

It is known that in higher plants, alkanes are mainly involved in the synthesis of epicuticular wax layer, which primary function is to reduce the water loss through the epidermis; in addition, this outer layer has a major function in the plant

interactions with herbivorous insects and the plant pathogenic fungi; the composition of pollen wax was revealed to be important factor for the proper pollen–pistil interactions [135]. Isoprenoids of higher plants play a role in protecting plants against herbivores and pathogens, in attracting pollinators and the seed dispersing animals and serve as allelochemicals, which influence the competition between plant species (cited from [136]). Hydrocarbons of green microalga *Botryococcus* were shown to serve as the storage intermediates for the synthesis of epoxides and other lipids [47].

The role of hydrocarbons in microorganisms is still not completely understood. Hydrocarbons arranged at the wall surfaces of fungal spores appear to fulfill protective function [137]. This is important in the plant disease development because the spores became more resistant to desiccation and to fungicidal sprays. The functions of intracellular hydrocarbons of microorganisms remain unclear; because of their low content, it is difficult to suppose that they have the role of the carbon and energy supply; most probably, intracellular hydrocarbons play a part in the microbial cell walls and control physicochemical properties of cytoplasmic membranes; this is confirmed by the data on different composition of hydrocarbons located in the cell walls, cytoplasmic membranes, and cytoplasm of yeasts [35]. Hydrocarbons can also promote the intracellular accumulation of hydrophobic compounds (much like the consumption of water-insoluble elemental sulphur by thionic bacteria).

The biosynthesis of extracellular straight-chain hydrocarbons by sulfate-reducing bacteria and clostridia appeared to be associated with the formation of capsules on the cell surface, which protect bacteria from high concentrations of excreted acids [25]. It is suggested that extracellular saturated C_{21}–C_{33} hydrocarbons produced by bacteria *P. fluorescens* are involved in the autoregulation of the cell adhesion to a glass surface and promote the cell aggregation [52].

The production of ethylene by slime mold *D. mucoroides* was shown to induce the formation of macrocysts during the sexual cycle of the cell development, whereas the possible functions of ethylene in the asexual development were associated with the cell aggregation and differentiation [111].

Microbial production of gaseous hydrocarbons seemed to have important role in the plant–microorganism and interspecies interactions. Volatile unsaturated hydrocarbons have been implicated as possible causative agents of soil fungistasis [138]. Different functions of ethylene were suggested in symbiotic and in the pathogen–host interactions. Ethylene is known to exhibit the root growth promoting properties much like auxins. It was found that when a functional mycorrhizal association was formed, low level of ethylene was produced and the lateral root formation increased; on the other hand, high levels of ethylene associated with the disease development caused by *Fusarium* fungi may function in lowering the disease resistance of the host plant [78]. The ethylene production by soil microorganisms was shown to have detrimental effects on the growth of plants and microorganisms [82]. It was found that volatile hydrocarbons produced by *Bacillus* species were antagonistic to cyanobacteria and the plant pathogenic fungi [139–141]; in particular, isoprene was supposed to be an antimicrobial agent, perhaps acting synergistically with isoamyl alcohol [68].

There are hints that isoprene could serve as a volatile repellent of soil predators or as the growth inhibitor of competing microbes. For example, it has been reported that *Collembola* insects grazing bacteria and fungi in soil are repelled by isoprene [142]. It is known that *Bacillus* species are prevalent in the rhizosphere, where they can produce the plant growth stimulating factors and synthesize antifungal agents [143]; it is possible that isoprene could play a role in these extracellular events. In terms of the intercellular communication, it has been discovered that Gram-positive bacteria (for instance, *B. subtilis*) are able to use the cell density-dependent signaling events [144]; it has been suggested that some uncharacterized secondary metabolites may play a role in bacterial communications [145]. Isoprene might be such a secondary metabolite; its volatility would allow it to serve as a transient signaling molecule.

Thus, microbial hydrocarbons appear to regulate the cell development, act as causative agents in the plant–microorganism, predator–prey, and interspecies interactions, and play an important ecological and physiological role.

6. Conclusions

1. Based on the material reviewed, it can be stated that the capability for hydrocarbon synthesis is widespread among microorganisms (both prokaryotes and eukaryotes).
2. Certain systematic groups of microorganisms are characterized by specific composition of intracellular hydrocarbons, which may serve as a chemotaxonomic criterion. At present, hydrocarbon biomarkers are widely used to constrain the age of the ancient bacteria, archaea, and eukaryotes and determine the role of microorganisms in the genesis of petroleum and marine sediments. The synthesis of hydrocarbons by microorganisms varied in dependence on the growth conditions that provides a way for its physiological regulation.
3. The developed processes for microbial production of extracellular C_{14}–C_{25} hydrocarbons and volatile nonmethane hydrocarbons appear to be very promising. The hydrocarbon content of microbial cells is usually low (no more than 10% of dry mass) that gives no hope for the industrial production of the hydrocarbon-rich biomass with the use of native microorganisms. The construction of recombinant strains bearing genes of the hydrocarbon synthesis cloned from algae or plants seems rather tempting. Microbes possess significant potential advantages over photosynthetic organisms, such as the higher growth rate, the capability for the growth in large-scale fermentors, great metabolic flexibility and high accessibility to genetic engineering. Gains in the genetic and metabolic engineering will allow the establishment of microbial systems for the production of renewable fuel and valuable hydrocarbon compounds, such as the rubber-like polymers.

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