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Formation and physiological role of biosurfactants produced by hydrocarbon-utilizing microorganisms

Biosurfactants in hydrocarbon utilization

Rolf K. Hommel
Bereich Biochemie, Sektion Biowissenschaften, Karl-Marx-Universität, Talstr. 33, Leipzig, 0-7010, Germany

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Abstract

Microbial growth on water-insoluble carbon sources such as hydrocarbons is accompanied by metabolic and structural alterations of the cell. The appearance of surface-active compounds (biosurfactants) in the culture medium or attached to the cell boundaries is often regarded as a prerequisite for initial interactions of hydrocarbons with the microbial cell. Under this point of view, biosurfactants produced by hydrocarbon-utilizing microorganisms, their structures and physico-chemical properties are reviewed. The production of such compounds is mostly connected with growth limitation in the late logarithmic and the stationary growth phase, in which specific enzymes are induced or derepressed. Addition of purified biosurfactants to microbial cultures resulted in inhibitory as well as in stimulatory effects on growth. Therefore, a more differentiated view of microbial production of surface-active compounds is proposed. Biosurfactants should not only be regarded as prerequisites of hydrocarbon uptake, but also as secondary metabolic products.

Introduction

One of the major problems to be overcome by microorganisms in hydrocarbon metabolization is to make the hydrophobic carbon source accessible to the cell. The high insolubility of alkanes in aqueous media necessitates specific uptake mechanisms. Mainly three different typs have been proposted:

- uptake of monodispered dissolved alkanes,
- direct contact of cells with large oil drops, and
- contact with fine oil droplets (pseudosolubilized alkane).

Whether mechanism is involved, alterations to the cell are required that will enable it to adhere to oil droplets and, in the latter case, to recognize the substrate to be solubilized. Einsele (1983) showed alterations of the cell surrounding of the yeast *Candida lipolytica* grown on n-hexadecane was accompanied by a stronger affinity to lipophilic carbon sources. Other examples were summarized by Boulton & Ratledge (1984).

Very small oil droplets (microemulsions) may be generated by mechanical means such as stirring, however those droplets will continously coalesce with each other. Stable submicron, pseudosolubilized alkane droplets may only be formed in presence of interfacial active substances. As reviewed by Haferburg et al. (1986) surfactants may promote growth of microorganisms on lipophilic substrates by improvement of hydrocarbon transport. Good growth was correlated in most cases with conditions which provide greater interfacial area. However, the results vary from strain to strain and depend on which surfactants are used. Hisatsuka et al. (1971) and Itoh et al. (1971, 1972) reported strong stimulatory effects of extracellular rhamnose lipid added in trace amounts to cultivations of the producing organism itself, *Pseudomonas aeruginosa*, growing on hydrocarbons. The ability of a rhamnolipid-negative mutant strain to grow on hydrocarbons was only restored after addition of rhamnoplipid (Itoh & Suzuki 1972). Based on this, the capacity of microorganisms able to utilize n-alkanes or other oils has been recognized mainly for production of interfacial active agents.

It has therefore been concluded that alkane-utilizing microorganisms produce such surfactant compounds to disperse the growth substrate into oil-in-water emulsions in order to increase the interfacial area and thereby enhance the availability of carbon source. In most recent reviews, the presence of biosurfactants has been regarded as indispensable for the microbial growth on water-immiscible substrates (Cooper & Zajic 1980; Boulton & Ratledge 1984; Lang & Wagner 1987; Syldatk et al. 1984; Syldatk & Wagner 1987, e.g.). From this point of view, this paper considers only biosurfactants, which are synthesized by bacteria and yeasts able to utilize hydrophobic carbon sources, together with some of their properties and conditions of product formation.

Types of biosurfactants

The presence of both a hydrophilic and a lipophilic moiety within the biosurfactant molecule creates the typical property of surfactant: depending on this concentration and conditions, surfactants may aggregate to form micelles or reversed micelles, and accumulate at liquid/liquid, liquid/gaseous and liquid/solid interfaces. The term 'biosurfactant' has been often used loosely to refer to other compounds like biopolymers which generally do not reduce interfacial tension but may prevent oil droplets from coalescing. The most representative bioemulsifier, Emulsan, recreted by *Acinetobacter calcoaceticus* growing on ethanol or alkanes has been the main subject of recent reviews of Gutnick & Shabtai (1987) and Gutnick & Minas (1987). Excretion of other polymeric bacterial bioemulsifiers were also reported from other bacteria growing not only on non-hydrocarbon substrates (Banerjee et al. 1983; Cooper & Goldenberg 1987, e.g.). The bioemulsifier from *C. lipolytica*, Liposan, appeared only in the stationary phase of growth on long-chain alkanes (Cirigliano & Carman 1985). Its composition, a mannoprotein, was similar to that isolated from *Saccaromyces cerevisiae*, and strains of other genera including *C. lipolytica* (Cameron et al. 1988). Bioemulsifiers in general displayed similar composition to the cell wall or capsules.

In contrast, some biosurfactants are of a definite structure (see Fig. 1). The lipophilic portion is usually the hydrocarbon (alkyl) tail of one or more fatty acids which may be saturated, unsaturated, hydroxylated or branched. This fatty acid is linked to the hydrophilic group by a glycosidic, ester or amide bond.

Based on the character of the hydrophilic moiety, biosurfactants may be grouped into the following classes: (1) glycolipids, (2) lipopeptides, (3) fatty acids, (4) phospholipids, (5) neutral lipids.

Most biosurfactants are either neutral or negatively charged. The anionic character is due to carboxylate groups. A small number of cationic biosurfactants contain amine functions. Table 1 (and see also Fig. 1) gives a survey on most important biosurfactants and their properties. Detailed structures of biosurfactants were extensively described by Haferburg et al. (1986), Lang & Wagner (1987) and Gutnick & Minas (1987). In most cultivations of some *Actinomycetes*, mixtures of different surface-active compounds were described which were mostly, but not completely characterized.

The most important group of biosurfactants produced by hydrocarbon-utilizing microorganisms are glycolipids. In the trehalose lipids of *Actinomycetes* and related bacteria, a-branched β-hydroxy fatty acids, known as corynomycolic acids, with 20 to 40 carbon atoms are esterified (mono- and diesters) with the sugar (e.g., Rapp et al. 1979; Kretschmer et al. 1982). Tetraesters of trehalose with succinate and fatty acids reflecting the carbon skeleton of the hydrocarbon substrate in 2,3,4,2′ positions are known from *Rhodococcus erytropolis* (Ristau & Wagner 1983). A pentasaccharide esterified with seven fatty acids and succinate was synthesized by *Nocardia corynebacteroides* SM 1 (Powalla et al. 1989). The carbohydrate backbone contains one α,α-trehalose unit which is substituted at the same position as the trehalose tetraester of *R. erytropolis*. This trehalose lipid was therefore, considered to be biosynthetic precursor of the oligosaccharide lipid. Trehalose moieties of mycolates may be replaced by other sugars (Göbbert et al. 1988; Itoh & Suzuki 1974; Li et al. 1984).

In rhamnolipids, rhamnose or a di-rhamnosyl unit (α-1,2 linked) is glycosidically linked with β-hydroxy decanoic acid, which itself is esterified with a second β-hydroxy decanoic acid. Syldatk et al. (1985a) isolated also two rhamnolipids with only one fatty acid. Rhamnolipids in which an additional decenoyl moiety was linked in 2′ and 2″ position with the mono- and di-rhamnosyl unit, respectively, were described by Yamaguchi et al. (1976).

In the sophorose lipids of *Candida* (*Torulopis*) yeasts, sophorose is glycosidically linked with a long chain (ω − 1)-hydroxy fatty acid which mostly reflects the backbone of the water-insoluble carbon source (Tulloch 1976). The free carboxylic group may be lactonized with the 4″ hydroxy group. Additionally, ω-hydroxy fatty acids were reported in crystalline lipids from *Torulopsis apicola* (Weber et al. 1990). The lipids are, in general, mixtures of different sophorose lipids which exhibit different degrees of acetylation in 6′ and 6″ position or possess unsaturated fatty acids. The main components account up to 60% (Asmer et al. 1988) or even more than 80% (Stüwer et al. 1987). Additional types of lactonization of 17-hydroxy octadecanoic acid, 1,6′ lactone and 1,6″ lactone, respectively were reported by Asmer et al. (1988). The extracellular sophorose lipid of *Candida bogoriensis* was characterized as 13-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]docosanoic acid 6′, 6″-diacetate (Cutler & Light 1979).

In the mannosyl-erythritol lipids of *Candida* sp. B-7, the mannosyl moiety of the carbohydrate backbone was reported to be esterified manly in 2′ and 6′ position by a mixture of C_7 to C_{17} fatty acid (Kawashima et al. 1983). In 2′, 3′, 4′ and 6′ position the acetylation and acylation (C_8 to C_{14} fatty acids) was reported in the lipid from *Candida antarctica* (Kitamoto et al. 1990a).

Lipopeptides usually appear as mixtures of closely related compounds which show slight varia-

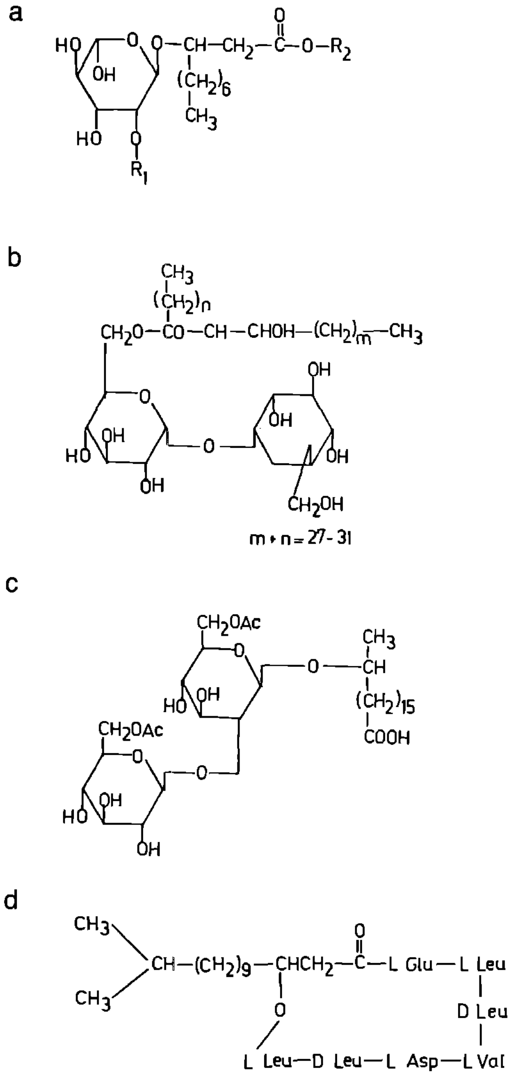

Fig. 1. Structures of selected biosurfactants.

(*a*) Rhamnolipids of *Pseudomonas aeruginosa*.

	I	II	III	IV
R_1	L-α-Rhamnopyranosyl	H	L-α-Rhamnopyranosyl	H
R_2	β-Hydroxydecanoic acid	β-Hydroxydecanoic acid	H	H

(*b*) Trehalose-6-monocorynomycolate of *Rhodococcus erytropolis*.

(*c*) Sophoroselipid (acid form) of *Torulopsis bombicola*.

(*d*) Surfactin of *Bacillus subtilis*.

tions in their amino acid composition and/or lipid portion which is mostly a hydroxy fatty acid. The lipopetides like surfactin from *Bacillus subtilis* (Vater 1986), viscosin of *Pseudomonas fluorescence* (Neu et al. 1990) or cyclodepsipeptides of *Serratia marcescens* (Matsuyama et al. 1986), represent a family of cyclic petides with consist of 8 to 17 amino acids.

Growth and biosurfactant formation

Mixtures of different biosurfactants

Mixtures of surface-active lipids have been seen to appear during growth of corynebacteria and related bacteria on hydrocarbons and/or kerosene (Gerson & Zajic 1979; MacDonald et al. 1981; Duvnjak & Kosarik 1985, e.g.). In some cases, the surface tension of broth was lowered before cell growth started (Cooper et al. 1979; MacDonald et al. 1981). Distinct maxima of surface activity of the culture broth during alkane cultivations were attributed to the sequentional appearance of free corynomycolic acids, lipopetides, phospholipids, neutral lipids and fatty alcohols. The corynomycolic acids were mainly constituents of some of more complex compounds such as lipopeptides (Cooper et al. 1979). Highest surfactant concentrations were measured in the medium of hexadecane growing on cultures (Atkit et al. 1981). Supplementation of glucose reduced biosurfactant yields. Duvnjak & Kosaric (1985) noted biosurfactant production by *Corynebacterium lepus* also on glucose, however, the surfactant remained cell-bound and could be released by treatment of cells with n-alkanes. It was concluded that the direct contact between cells and hexadecane was important for hydrocarbon transport. Similar observations have been made with *C. lepus* and with *Arthrobacter paraffineus* (Duvnjak et al. 1982). The excretion a peptidotrehaloselipid by the coryneform bacterium species H13A, was accompanied with the stationary phase of growth on alkanes (Singer 1985). On water-

Table 1. Selected properties of some structurally elucidated biosurfactants.

Type	Charge	Location	σ_s (mNm^{-1})	c.m.c.* (mg l^{-1})	σ_i (mNm^{-1})	Reference
Trehalose mycolates						Kretschmer et al. (1982)
mono-	nonionic	c.b.[1]	32	2	16	
di-	nonionic	c.b.	36	4	17	
Sugar esters of mycolates						Li et al. (1984)
Mannose	nonionic	c.b.	40	5	19	
Glucose	nonionic	c.b.	40	10	9	
Maltose	nonionic	c.b.	33	1	1	
Rhamnolipids						Syldatk et al. (1985a)
R I	anionic	c.f.[2]	31	20	<1	
R II	anionic	c.f.	25	200	<1	
R III	anionic	c.f.	31	20	3	
R IV	anionic	c.f.	30	200	<1	
Sophorolipids						
Lactone	nonionic	c.f.	35	60	9	Stüwer et al. (1987)
Mixture	nonionic	c.f.	25		<0.9	Hommel et al. (1987)
acidic	anionic	c.f.			3	Lang et al. (1984)
Surfactin	nonionic	c.f.	27	5	1	Cooper & Zajic (1980)

[1] Cell-bound

[2] Cell-free (extracellular)

* cmc = critical micelle concentration, σ_i = interfacial tension, σ_s = surface tension.

soluble carbon sources the glycolipid remained cell-associated.

Trehalose lipids and sugar mycolates

R. erythropolis growing on hydrocarbon displayed a biphasic growth pattern (Rapp et al. 1979). In the first phase, cells grew in the alkane droplets and cell-bound trehalose mycolates were produced at a constant rate – only 10% of the total trehalose dimycolates were released into the culture medium. After phase inversion at a precise ratio of cells/glycolipid/alkane, the hydrophobized cells aggregated in the aqueous phase which caused a limitation of oxygen transfer. Growth limitation coincided with the end of biosurfactant production.

Similar growth patterns were observed for the biosurfactant production of *Rhodococcus aurantiacus* (Ramsay et al. 1988). The production of glycolipids was accompanied with initial growth. After the exponential phase, linear growth, which was attributed to limitations in hydrocarbon transport, was observed without changes in the oxygen uptake rate or in biosurfactant production. Synthesis of trehalose tetraester by *R. erythropolis* started after depletion of nitrogen in the stationary growth phase (Ristau & Wagner 1983). Limitations of multivalent metal ions or the lowering of specific growth rate by temperature shifts may also initiate tetraester biosynthesis.

Low concentrations of other mycolates have also been recorded during fermentation. The production of the pentasaccharide ester by *N. corynebacteroides* SM 1 was 4-fold higher with cells growing on alkanes than with glucose (Powalla et al. 1989). Minor components synthesized were dicorynomycolate and monocorynomycolate. The production of glycolipids continued into the stationary phase after the nitrogen source was exhausted. The substitution of trehalose in trehalose mycolates by other sugars was obtained with strains of *Arthrobacter, Corynebacteria, Brevibacteria* and *Nocardia* isolated as hydrocarbon-utilizing bacteria (Itoh & Suzuki 1974; Suzuki et al. 1974) after growth on the respective sugar. The same replacement of the sugar moiety has also been described by Li et al. (1984) with resting cells of *Arthrobacter* sp. DSM 2567 which provided higher yields than in batch cultures. The corynomycolic acids remained unchanged. Its synthesis did not demand a supply of hydrocarbons. This reaction is catalysed by a highly regioselective enzyme of the particulate cell fraction which accepted mono-, oligo-saccarides, sugar alcohols and *p*-nitrophenyl derivatives as substrates (Göbbert et al. 1988).

Rhamnose lipids

Rhamnolipids of *P. aeruginosa* are formed from cultures growing on either hydrocarbons (Hisatsuka et al. 1971; Itoh et al. 1971; Syldatk et al. 1985a, b) or on water-soluble carbon sources (Haferburg et al. 1989; Guerra-Santos 1984, 1986; Reiling et al. 1986; Syldatk et al. 1985a, b). Limitations of one component of the growth medium, e.g. nitrogen, phosphorus or sulphur, were prerequisites for biosynthesis to commence. The C : N ratio of the medium constituents was mostly above 20. High concentrations of bivalent cations inhibited biosynthesis of the surfactant. Limitation of growth by iron caused a further increase in rhamnolipid production (Guerra-Santos et al. 1986; Haferburg et al. 1989; Syldatk et al. 1985a). A direct correlation of rhamnolipid production and specific activity of glutamine synthetase was established (Mulligan & Gibbs 1989). Ammonium and glutamine repressed both the non-growth associated rhamnolipid production and the glutamine synthase activity in the wild-type strain and also an overproducing chloramphenicol tolerant mutant (Mulligan et al. 1989). The enzyme was at maximum activity at the end of exponential phase, the time at which nitrogen and phosphate sources had been exhausted, and remained high throughout rhamnolipid production. Rhamnolipid production occurred in both batch cultivations and with resting cells (Syldatk et al. 1985a). Neither rhamnose nor β-hydroxy decanoic acid could be replaced indicating a *de novo* synthesis of this lipid.

Rhamnolipids may be produced in continuous culture (e.g. Guerra-Santos et al. 1984; Reiling et al. 1986) and also by alginate immobilized cells on glycerol (Müller-Hurtig et al. 1987) under specific limitations. In batch cultures, production was not growth-associated using either n-alkanes or water-soluble substrates (Syldatk et al. 1984). In contrast to this, Koronelli et al. (1983) reported the appearence of a peptidoglycolipid containing rhamnose by *P. aeruginosa* P-20 was strongly connected with the beginning and to the middle of exponential growth phase on hexadecane.

Sophorose lipids

Formation of extracellular sophorolipids by yeasts of the genus *Candida* and *Torulopsis* has been reviewed by Tulloch (1976) and Spencer et al. (1979). The latter genus has been recently reclassified. Producing strains like *Torulopsis magnoliae* (Gorin et al. 1961), *T. apicola* (Tulloch & Spencer 1968), *Torulopsis gropengiesseri* (Jones 1967) and *Torulopsis bombicloa* (Spencer et al. 1970) now belong to the genus *Candida*.

In presence of glucose, methylesters of saturated and unsaturated fatty acids in the range between C_{13} to C_{20} were directly incorporated into the surfactant molecule (Tulloch 1976). Fatty alcohols also served as substrates (Spencer et al. 1979). Ito et al. (1980) isolated a lactonic sophorose lipid after growth of *T. bombicola* KSM-36 on safflower oil and glucose. Under these conditions anionic glycolipids were also produced (Ito & Inoue 1982). With hydrocarbons as sole source of carbon, glycolipid production did not occur (Ito & Inoue 1982). Maximum production of an anionic glycolipid mixture of sophorose lipids was obtained with *T. bombicola* ATCC 22214 on a mixture of carbohydrate and vegetable oils or by subsequent addition of one of the principal carbon sources (Cooper & Paddock 1984). Under both conditions, most of the glycolipids appeared in the late exponential phase of growth. *T. bombicola* ATCC 22214 cultivated both on a mixture of glucose and oleic acid and sole on oleic acid produced a mixture of several different sophorosides of 17-hydroxy octadecanoic and 17-hydroxy octadecenoic acid (Asmer et al. 1988). In broths of logarithmicalty growing cells, only traces of glycolipid were detected. Glycolipid production started in the late exponential phase.

A mixture of water-soluble non-ionic glycolipids was obtained with *T. apicola* IMET 43734 (Hommel et al. 1987) with higher yields on hexadecane than on glucose. Biosurfactant concentration increased with increasing hexadecane concentrations. Excretion started in the middle of exponential growth phase and rose suddenly and very markedly in the late exponential phase. On a mixture of glucose and alkanes or vegetable oils, the same strain produced a crystalline glycolipid (Stüwer et al. 1987). This lipid was only obtained in fermentations in which organic acids (preferably citrate) were added which themselves did not permit growth. Glycolipid excretion started with the beginning stationary phase and continued at a constant rate. With resting cells of *T. bombicola* ATCC 22214 Göbbert et al. (1984) tried but failed to change the sugar moiety. 17-Hydroxy octadecenoic acid was the lipophilic constituent independently whether shorter-chain (C_{14}, C_{15}) alkanes were used as carbon sources.

The decreasing susceptibility of growth and glycolipid production of *T. apicola* towards cerulenin (an inhibitor of fatty acid biosynthesis) during growth on glucose and on mixed carbon source suggested that glucose is the growth substrate in mixed substrate cultivation in spite of synchronic disappearence of both glucose and hexadecane (Hommel et al. 1990). Whole cells of *Torulopsis* sp. strain 319–67 hydroxylated stereospecifically octadecenoic acid to the 17-L-hydroxy acid (Heinz et al. 1969). This reaction was carried out by a mixed-function oxidase of a particulate fraction of cells grown on a mixture of glucose and oil (Heinz et al. 1970). In *T. apicola* IMET 43747 cytochrome P-450 was detected after growth on glucose as well as on hexadecane and on a mixture of both appearing in the late exponential phase which coincided with the beginning of glycolipid production (Fig. 2) (Kleber et al. 1989). No repression of the cytochrome P-450 occurred in presence of glucose which is different to inducible monooxygenases involved in alkane hydroxylation

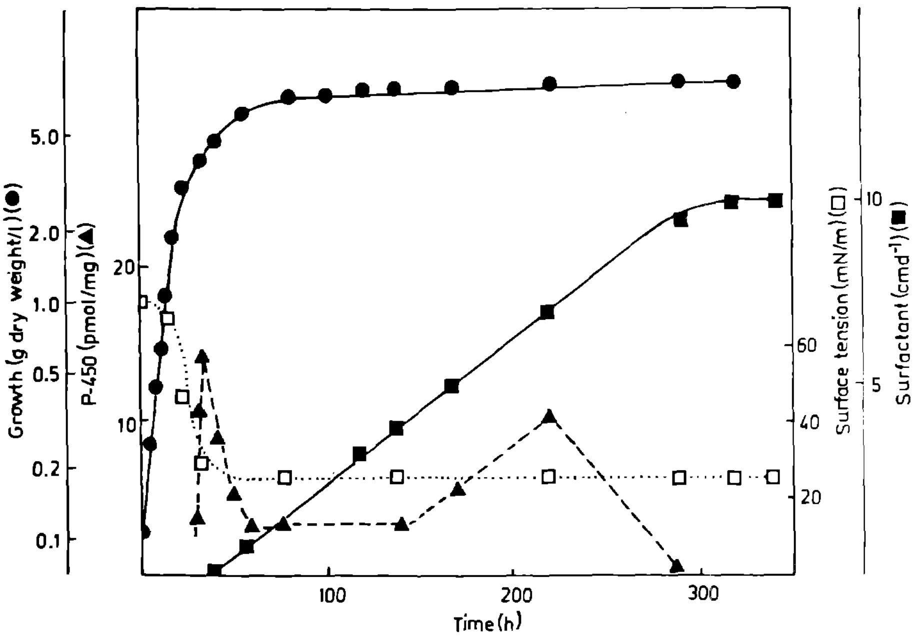

Fig. 2. Glycolipid production (critical micelle dilution, cmd^{-1}) and content of cytochrome P-450 during cultiviation of *Torulopsis apicola* IMET 43747 on glucose (100 g l^{-1}) (according to Kleber et al. 1989).

(Käppeli 1986). In the same context the fatty alcohol oxidase of *T. bombicola* ATCC 22214 was not repressed by glucose. The two constitutive enzymes of stationary cells (Hommel & Ratledge 1990) oxidized fatty alcohols and diols but ω-hydroxy fatty acids.

A sophoroside of a slightly different structure is formed by *C. bogoriensis* growing on glucose (Tulloch et al. 1968; Esders & Light 1972). The relative amount of glucose and yeast extract regulated the productivity: high concentrations of glucose promoted glycolipid biosynthesis (Cutler & Light 1979). The production was not growth associated and continued into the stationary phase of growth. Two different glycosyltransferases induced in the late exponential growth phase catalysed the stepwise transfer of UDP-glucose units to the hydroxy fatty acid and to 13-glucopyranosyloxydocosanoic acid, respectively (Breithaupt & Light 1982).

Mannosyl-erythritol lipids

The formation of an acylated mannosyl-erythritol lipid by *Candida* sp. B-7 is closely connected with the metabolism of n-alkanes and triacylglycerol (Kawashima et al. 1983). The addition of this lipid strongly increased the growth rate. *Candida* sp. KSM-1529, which is also able to produce this lipid, produced only non-acylated mannosyl-erithritol when grown on glucose and this probably serves as the direct precursor of the lipid (Kobayashi et al. 1987). Recently isolated strains of *Candida antarctica* were able to produce large amounts of a structurally modified mannosyl-erythritol lipid when grown on vegetable oils but not on alkanes (Kitamoto et al. 1990a). Supplementation of glucose did not affect the production. The addition of glycerol and erythritol caused significant increases in yields (Kitamoto et al. 1990b).

Lipopeptides

B. subtilis is only able to synthesize surfactin if grown on water-soluble carbon sources. The biosynthesis of peptide moiety of surfactin occurres non-ribosomally. Supplementation of hydrocarbons repressed surfactin synthesis whereas growth was not affected (Cooper 1984). The production of surfactin-like lipopeptides is not restricted just to *B. subtilis*. Similar componds have been described from other microorganisms which also belong to *Cyanobacteria* and *Actinomycetes* (Vater 1986). Production of surfactin started in the logarithmic growth phase and continued in the stationary phase (Cooper 1984; Vater 1986). Excessive production did not appear to be associated with actively growing cells; production was inversly correlated to biomass. Under both aerobic and anaerobic conditions, *Bacillus licheniformis* JF-2 produced a biosurfactant with properties similar to surfactin (Javaheri et al. 1985). The formation of further types of lipopeptides, such as the ornithine- and/or lysine-containing lipids and other types of biosurfactants, was reviewed by Haferburg et al. (1986) and Lang & Wagner (1987).

Biosurfactant additions in hydrocarbon utilization

The reports of Hisatsuka et al. (1971) and Itoh et al. (1971, 1972) demonstrated the stimulation of hydrocarbons metabolism by *P. aeruginosa* in presence of added rhamnolipid. *T. bombicola* ATCC 2217 and strain KSM-36 utilized a number of alkanes which were normally not assimilated in presence of lactonic sophorosides, methyl sophorose lipid or a mixture of sophorose lipids which had been produced during growth on glucose and safflower oil (Ito & Inoue 1982). Additionally, enhanced rates and yields of biomass were obtained. Synthetic surfactants could not replace the glycolipids. However, stimulation of growth by sophorose lipids was restricted to sophoroside-producing *Torulopsis* strains. Safflower lipid, lactonic sophoroside and also methyl sophoroside inhibited in general growth of other typical alkane-utilizing yeasts tested such as strains of *Candida* and *Pichia* on hexadexane (Ito & Inoue 1982; Ito et al. 1980). Similar results were obtained with the water-soluble, non-ionic glycolipid fraction of *T. apicola* IMET 43747 (Hommel et al. 1987). Its addition shorten the lag phase of particular alkane-utilizing bacteria and yeasts but displayed a decreasing effect on growth rate for strains tested.

Table 2 shows that the inhibitory effect of the lactonic sophoroside of *T. apicola* on different strains of *Candida* is restricted when they are using alkanes (Hommel et al. 1988). The low solubility of the lactonic sophoroside, which is also a main constituent of the safflower lipid, was judged to be responsible for the inhibitory effect because it exhibited no ability to emulsify hydrocarbons (Ito et al. 1980). Cooper & Paddock (1984) excluded this glycolipid as a promoter of alkane uptake because it did not stabilize alkane-in-water emulsions in

Table 2. Effect of sophoroselipid ($1\,g\,l^{-1}$) on growth (A_{600}) of yeasts on glucose and on hexadecane (according to Hommel et al. 1988).

Strain	Carbon source			
	Glucose[1]		Hexadecane[2]	
	without Sophoroside	with Sophoroside	without Sophoroside	with Sophoroside
Candida				
C. boidinii H127	5.0	4.6	0.32	0.11
C. catenulata H173	4.1	3.7	1.8	0.15
C. maltosa H47	5.2	5.3	4.5	0.12
C. melinii H48	4.4	4.2	0.55	0.15
C. membranefaciens H93	4.6	4.6	0.60	0.09
C. mycoderma H259	3.5	3.8	3.8	0.16
C. paralopsilosis H260	2.8	2.6	2.5	2.4
C. pseudotropicalis H261	3.2	n.d.[3]	0.4	0.28
C. rugos H263	3.9	n.d.	0.52	0.32
C. sake H58	4.2	4.0	5.5	3.8
C. vini H96	4.6	4.0	4.9	2.9
Torulopsis				
T. apicola IMET 43747	6.0	5.9	4.8	4.6
T. bombicola ATCC 22214	5.2	5.5	4.1	4.8
T. candia Y127	8.2	7.2	5.5	0.2
T. famata H158	7.6	7.2	7.8	0.28
T. glabrata IMET 43548	7.4	6.8	5.9	0.58

[1] Glucose concentration: $50\,g\,l^{-1}$
[2] Hexadecane concentration: $20\,g\,l^{-1}$
[3] n.d. = not determined

vitro. However, Lang et al. (1984), Hommel et al. (1987) and Stüwer et al. (1987) reported strong interfacial activities with n-alkanes. This property is the basis of appearence of fine, dispersed non-coalesing alkane droplets in cultivation broths of *T. apicola* IMET 43747 and *T. bombicola* ATCC 22214.

Macroscopic emulsions may be considered as a possibility to facilitate uptake (and metabolism) of water-immiscible carbon sources. Each microorganism itself has developed individual mechanisms of environmental adaption. Microorganisms such as *C. lipolytica* or *A. calcoaceticus* which excrete bioemulsifiers were strongly inhibited by the addition of sophorosides (Ito & Inoue 1982; Hommel et al. 1987).

Syldatk et al. (1984) concluded that there were different mechanisms of emulsification with charged and neutral biosurfactants. Ionic biosurfactants, like the rhamnolipids or sophorolipids, emulsify (pseudosolubilize) the alkane which increases the surface area. Non-ionic biosurfactants (trehalose lipids) render the charged cell surface hydrophobic which should then facilitate the attachment and subsequent passive transport of alkanes into the cell (Ramsay et al. 1988; Rapp et al. 1979).

In this context, reports on effects of biosurfactants in oil polluted areas are valuable. Poremba et al. (1989) assumed the participation of cell-bound and microemulsion-forming extracellular biosurfactants in crude oil degradation by marine bacteria. Similar results have been reported for the biphasic degradation of a hydrocarbon mixture by a natural population of soil bacteria in a stirred reactor (Oberbremer & Müller-Hurtig 1989). Whereas in the first phase, water-soluble components were degraded, in the second phase the production of biosurfactants was observed which coincided with near complete exhaustion of alkanes. The glycolipids produced were trehalose tetraesters comparable to those reported by Ristau & Wagner (1983). Additionally, large amounts of a trehalose diester were produced which however remained cell-bound as described for *R. erythropolis* (Rapp et al. 1979). The addition of different biosurfactants to this model system caused a doubling of hydrocarbon degradation rate (Table 3) (Oberbremer et al. 1990). The adaption phases of the biphasic degradation were shorted and the extent of hydrocarbon degradation was enhanced. In the second phase, both the degradation of biosurfactants and the *de novo* synthesis of surface-active glycolipids was observed.

Conclusions

In contrast to the bulk number of alkane degrading microorganisms there is only a limited number of bacteria and yeasts known to produce interfacially active compounds. Most of them have been de-

Table 3. Effect of glycolipid addition on degradation efficiency in oil polluted soils (according to Oberbremer et al. 1990).

Cultivation	Hydrocarbon elemination		Degradation capacity [g hydrocarbon (kg soil)$^{-1}$d^{-1}]
	Duration [h]	Degree [%]	
With oxygen limitation			
Without	114	81	16.3
Trehalose-6,6′-dicorynomycolates	71	93	37.2
Sophorose lipids	75	97	39.0
Cellubiose lipids	79	99	32.3
Rhamnose lipids	77	94	28.2
Trehalose-2,3,4,2′-tetraesters	94	95	23.8
Without oxygen limitation			
Without	69	89	28.1
Sophorose lipids	57	95	46.5

scribed in connection with growth of microorganisms on hydrocarbons (Wagner et al. 1981; Zajic & Panchal 1976; Kosarik et al. 1983; Singer 1985). Effects of biosurfactants added to alkane cultivations have established that the function of these compounds in alkane emulsification or micelle formation is mainly restricted to the producing organism itself. The production of biosurfactants is, in general, not growth associated. However, already small amounts above critical micelle concentrations (c.m.c.) may allow the pseudosolubilization of such substrates. Indeed, the c.m.c. values reported (cf. Table 1 and Gutnick & Minas 1987) are generally very low. The substitution of trehalose in mycolic acid esters by other sugars suggests that alkanes do not induce their synthesis. In *Actinomycetes* their composition, partially or completely, corresponds to components of the cell wall or outer membrane. One effect of hydrocarbons on biosurfactant recovery is probably on extraction of lipids from the cell membranes (Cooper & Goldberg 1987; Duvnjak et al. 1982; Duvnjak & Kosaric 1985). Additionally, the same surface-active compounds are usually excreted by alkane-utilizing, biosurfactant producers like *P. aeruginosa* and strains of *Torulopsis* growing on water-soluble carbon sources. Biosurfactant production by *B. subtilis* is inhibited by alkanes (Cooper 1984) and is only connected with metabolism of water-soluble carbon sources. Similarly sophoroside production by *C. bogoriensis* is not linked to alkane utilization (Esders & Light 1972).

Common regulatory principles of biosurfactant synthesis are different and respond to different kinds of nutrient limitation (Syldatk & Wagner 1987). This indicates that enzymes involved in biosynthesis are normally repressed in actively, growing cells. The exhaustion, i.e. limitation, of either the carbon sources, or nitrogen or phosphorous is considered one factor leading to derepression of enzymes of secondary metabolism (Behal 1986; Malik 1982). The appearence and non-repression of cytochrome P-450 by glucose and the constitutive character of fatty alcohol oxidase in *T. apicola* and *T. bombicola* (Kleber et al. 1989; Hommel & Ratledge 1990), the time courses of glutamnine sythetase in *P. aeruginosa* (Mulligan & Gibbs 1989) and of the glycosyltransferases in *C. bogoriensis* (Breithaupt & Light 1982), suggests different regulatory mechanisms to those controlling primary metabolism.

In summary, a general consideration of biosurfactants as prerequiste of microbial alkane metabolism can not explain all the physiological aspects known so far. Some of biosurfactants, such as the sophorosides, rhamnolipids or peptidolipids, should be attributed to secondary metabolism, others, such as the trahalose esters or coynomycolic acids, are similar to existing cell wall constituents, and may be involved in cellular adaptation to hydrophobic growth substrates. Nevertheless, both types of surfactant may promote growth of natural mixed populations on hydrocarbon and some may also possess antimicrobial properties (Haferburg et al. 1986; Lang et al. 1989).

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