Translated from *Uspekhi Khimii* **60** 2362–2372 (1991) U.D.C. 579.24.242

Membranotropic autoregulatory factors in methane oxidising bacteria

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ABSTRACT. It is possible that the methane oxidising bacteria *M. capsulatus* synthesise in the course of their development and secrete into the culture liquid extracellular metabolites—membranotropic autoregulatory factors d_1 and d_2 the synthesis of which had been observed earlier in other groups of microorganisms. It is shown that the physiological effect of the factor d_2 is due to the influence of the fraction comprising free aliphatic acids and containing mainly palmitic palmitoleic acids. One of the manifestations of the effect of the specific autoregulatory factor d_1 as its concentration increases in the culture medium is the transition of the vegatative cells to a hypometabolic state. The active principle of the factor d_1 is the lactone of 3-deoxypentonic acid. A practical use for the chemical analogues of the autoregulatory factors d_1 and d_2 is suggested.

The bibliogrphy includes 25 references.

The increased ecological requirements as regards the safety of the technologies used in the national economy necessitates the development of new or an improvement of the efficiency of the already available technological solutions subject to the obligatory condition of their ecological cleanliness. One of the most vigorously developing branches of world economic practice is biotechnology, which has acquired the status of one of the priority fields in developed countries. The recognition of the leading role of biotechnology is not fortuitous. The intensification of agriculture and industries satisfying man's immediate needs is associated with the employment of biotechnological processes. Furthermore, the recovery of the environment subjected to anthropogenic influences should not only involve specific solutions based on biotechnological processes but also solutions based on the fundamental knowledge of the reactions and turnover of substances effected with participation of natural biological factors. A special role is attributed in this connection to microorganisms whose activity is associated with conversion cycles of the elements and whose use in numerous industrial processes can provide a basis for the ecologically safe preparation of the required products. The discovery of the characteristics of the vital activity of microorganisms and of the general physiological principles of microbial synthesis and the study of their trophic relations and their resistance and adaptation to environmental factors are therefore of fundamental importance.

Studies of the regulation of the growth and development of methane oxidising bacteria constitute one of the examples emphasising the necessity for extensive study of the characteristics of the vital activity of microorganisms. Methane oxidising bacteria are widely distributed in natural media, they play a major role in the transformations of carbon in nature,[1] and they participate in the formation of hydrogen sulphide in the petroleum stratum waters as a result of the trophic links between methane oxidising bacteria and sulphate reducing bacteria.[2] In national economic practice, methane oxidising bacteria are treated as a promising object for the ecologically clean manufacture of bacterial biomass from natural gas in order to employ it as feed additives, for the isolation of biologically active components from the biomass,[3] for use in mine shafts in order to reduce the concentration of methane in air to diminish the risk of explosion,[4] and as part of the composition of technolgenic biocenoses used in various biological methods for increasing the extraction of petroleum from the given geological stratum.[5] However, one should note that, despite the fairly widescale investigation of the physiology of the bacteria of this group, the characteristics of their development have been little investigated, which creates difficulties in obtaining them for large scale industrial processes. It is known that under unfavourable conditions certain methanotrophic organisms give rise to resting forms: exospores, lipid, cysts, and ripe and unripe cysts of the *Azotobacter* type.[6] For other methanotropic organisms, the resting forms are unknown. The causes of the uncontrolled change in metabolic activity under the conditions of periodic and chemosatic cultivation of methane oxidising bacteria have not been investigated. The instability of the process is frequently observed in the continuous cultivation of *M. capsulatus*, which is accompanied either by a spontaneous increase in the cell population, or a decrease in metabolic activity, or processes involving the lysis of the culture. However, the factors which determine the direction of the processes towards the transition of the cells to a hypometabolic (anabiotic) state or the state of cell autolysis remain unelucidated.

In modern theoretical microbiology, there exists a concept according to which a developing microbial culture represents a self-regulating system, its behaviour being determined both by the action of external factors and by the influence of autoregulatory metabolites, which control the rate and the stages in the develoment of the culture.[7]

In recent years, the elucidation of the role of the autoregulatory metabolites involved in the control of the physiological states of the cells of microorganisms and in the development of their cultures has become a vigorously investigated field. In the most general definition, the autoregulatory factors are understood as physiologically active compounds of different chemical nature, secreted by the microbial population or its components into the surrounding medium, not used for the purposes of constructive and energetic metabolism, and playing in the cell community the role of a signal in relation to the alteration of the quantitative (rate of growth) or qualitative (cytodifferentiation) state of the microbial culture.[8] The principal biological function of the extracellular autoregulatory metabolites is to ensure the occurrence of the intercellular interactions and also the interactions of the microorganisms with the medium, directed towards the active growth of the population and the self-preservation of the species.

The extensive data obtained in the study of low-molecular-weight autoregulatory metabolites, synthesised by organisms at different

levels of development (prokaryotes and eukaryotes and unicellular and multicellular organisms), made it possible to formulate this field of knowledge as a separate science—chemical ecology.

We have discovered[8,9] membranotropic autoregulatory factors participating in the control of the structural state and physiological activity of membranes and hence regulating the metabolic activity of the cell, which is expressed by a change in the intensity of cell proliferation processes and/or the differentiation of the microbial cell. In conformity with the last activity among those listed above, they have been called factor d.[8] It has been shown that microorganisms belonging to various taxonomic groups synthesise in the course of the development of their periodic or non-periodic cultures and secrete into the surrounding (incubation) medium autoregulatory metabolites—factors d_1 and d_2 differing in their chemical nature and physiological activity.[9]

This review presents data concerning the discovery of a specific autoregulatory mechanism of the development of the methane oxidising bacterium *M. capsulatus*, effected with the aid of the membranotropic factors d_1 and d_2, and the study of the chemical nature and biological activity, which is important both for the characterisation of the growth and development of the methane oxidising bacteria in natural populations and for ensuring the stability and improving the productivity of the processes involved in their continuous cultivation.

The following biological tests have been adopted in the isolation and purification of the factors d: (1) the change in the intensity of the respiration of the intact cells and isolated membranes (index of membranotropic activity); (2) the change in the rate of growth of the proliferating culture of the producer (integral index of the metabolic activity of the cells); (3) stimulation of the growth of the resting forms of the producer; (4) induction of the autolysis of the cells and (biological activity of the factor d_2); (5) induction of the transition of the vegetative cells to a hypometabolic or anabiotic state (biological activity of the factor d_1).

The isolation, purification, and identification of the factors d_1 and d_2 are based on the methods of modern lipidology:[10] extraction of the autregulators from the culture liquid by lipophilic solvents, their partitioning in a solvent system as a function of the polarity of the molecules, fractionation on columns and plates with adsorbents using selective eluents and solvent systems, rechromatography for the preparative isolation of chromatographically homogeneous fractions, and gas-chromatographic separation for the determination of the component composition and possible identification.[8,11–13]

The membranotropic autoregulatory factors were isolated from the culture liquid separated from the cells by extraction with 1-butanol (2:1) and the residue, remaining after distilling off the solvent, was separated into two fractions in which the activity of the factor d_1 or the factor d_2 predominated. After the separation on a silica gel column of the "crude" preparation of the factor d_2 (chloroform–alcohol fraction in the chloroform–methanol–water two-phase system in proportions of 2:2:1), the neutral lipids, capable of being eluted with chloroform, exhibited the activity of the factor d_2. On separation of the active fraction by preparative thin-layer chromatography, the activity was observed mainly in the fraction comprising free aliphatic acids (FAA) (Table 1), which was inferred from the ability of the preparation both to inhibit the respiration of the membranes and cells and to induce the autolysis of the latter. The FAA fraction of the factor d_2 as well as the "crude" preparation of this autoregulator stimulated at low concentrations and inhibited at high concentrations the respiration of the producer cells. The degree of inhibition of the cell respiration and of the oxidase activities of the isolated membranes increased with increase in the concentration of the FAA introduced, with decrease in the pH of the incubation medium, and with decrease in the amount of the biological material in the measuring cell. It developed as a function of time and also depended on the physiological age of the culture (Figs. 1 and 2).

Table 1. The change in the rate of the endogenic respiration of the cells under the influence of fractions of the extracellular *M. capsulatus* lipids.

No. of fraction	50 μl ml^{-1} fraction	TLC, R_f^*	Rate of consumption of oxygen			OD of cell suspension, % relative to control***
			Control μmol O_2 min^{-1}	expt.** μmol O_2 min^{-1}	expt.** % relative to control	
1	Diglycerides	0.12	0.93	0.86	92.5	88
2	Unidentified	0.16	0.90	1.08	120.0	92
3	Aliphatic acids	0.37	0.96	0.42	43.8	55
4	Unidentified	0.49	0.97	0.81	83.5	97
5	Triglycerides	0.47	0.92	1.03	112.0	88

* The spots on the chromatogram were identified by analogy with mono-, di-, tri-glyceride oleates.

** Data obtained by the polarographic method; the measurements were performed 3 min after introducing the preparation into the measuring cell.

*** OD = optical density of the cell suspension determined 3 h after introducing the preparations of the fractions into the culture.

Gas-chromatographic analysis of the composition of the FAA fraction (of the factor d_2), isolated from the culture liquid in the course of the stable cultivation of *M. capsulatus* in a chemostatic regime, made it possible to determine a wide range of aliphatic acids: with an even and odd number of carbon atoms ranging from C_{12} to C_{18}, saturated acids, and mono-, di-, tri-, and poly-unsaturated acids (Table 2).[14] Palmitic and palmitoleic acids, whose contents reached 36 and 25% respectively, were the main components. Other components present were 6% of stearic acid, 8% of oleic acid, and 6.5% of linoleic acid. Biological tests of the commercial preparations of the individual aliphatic acids, forming part of the composition of the factor d_2 in dominant amounts, established that unsaturated aliphatic acids are responsible for the physiological activity of this autoregulator (Table 3).

Model experiments designed to determine the biological activity of the components of the factor d_2 showed that oleic and palmitoleic acids induce the active lysis of the cells of the culture *M. capsulatus* in the linear growth phase. Thus in the presence of oleic acid, considered as a chemical analogue of the factor d_2, introduced into the cell suspension at a concentration of $(4-70) \times 10^{-2}$ μg ml^{-1}, vigorous lysis of the culture (70%) was observed after only 1 h of incubation, reaching virtually 100% with increase in the incubation time to 4 h (the autolysis of the cell was inferred from the fall in the optical density of the cell suspension and also from the amount of

soluble protein released from the cells into the incubation medium, the level of which amounted to 37% in the given instance). This conclusion agrees with the earlier results of the study of the chemical nature of the factor d_2 in the bacteria *Pseudomonas carboxydoflava* and *Bacillus cereus*.[11]

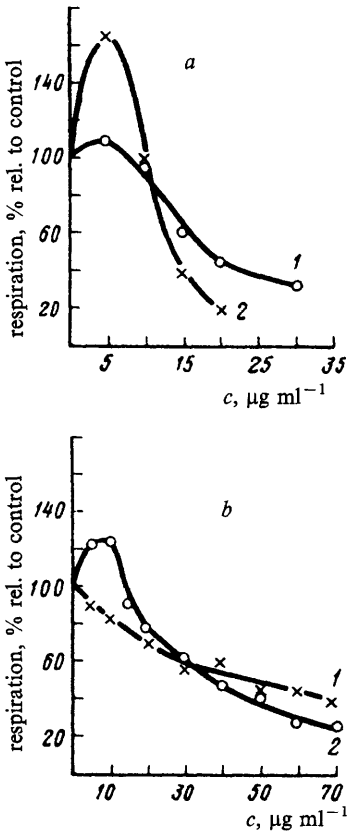


Figure 1. The influence of the factors d_1 (*a*) and d_2 (*b*) of a culture *M. capsulatus* on the endogenic respiration of the producer cells as a function of the concentration *c* of the autoregulator and the physiological state of the cells in the exponential (curve *1*) and linear (curve *2*) growth phases.[13]

The mechanism of the action of FAA (the factor d_2) on cells is based on their ability to be incorporated in membranes without the participation of acyl-transferring proteins.[15] The stimulation of the respiration of the cells of ageing cultures under the influence of low FAA concentrations, which has been demonstrated, can be accounted for by the ability of unsaturated aliphatic acids to increase the liquidity of the membrane lipids, which increases the activity of the electron-transport chain enzymes integrated in the membrane.[16] The stimulation of respiration by saturated aliphatic acids is probably based on their ability to uncouple respiration and oxidative phosphorylation.[17] This effect is more pronounced when the medium is acidified, whereupon the FAA are fully protonated. The introduction of high concentrations of the factor d_2 (20–80 μg ml^{-1}) or of its active principle—unsaturated aliphatic acids, in the given instance oleic and palmitoleic acids (200 nm ml)—into the profilerating bacterial culture leads to a supercritical increase in the liquidity of the membranes, which induces the inhibition of the respiration of the cells and stimulates the autolysis of the cells. This conclusion does not conflict with the available literature data showing that saturated aliphatic acids exert a destabilising influence on membranes.[18]

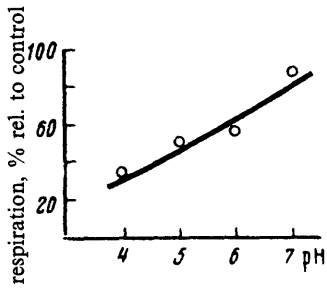


Figure 2. The influence of the factor d_2 of a culture of *M. capsulatus* (20 μg ml^{-1}) on the endogenic respiration of the linear growth phase cells suspended in 0.15 M phosphate-citrate buffer as a function of the pH.

Table 2. The aliphatic acid composition of the extracellular lipids of a *M. capsulatus* culture.[14]

Acids	Content oflipids under chemostatic conditions (D* = =0.21 h^{-1}, *t*=41 °C)%	Content of lipids under lysis conditions, %
12:0	0.63	0.22
13:0	1.07	0.75
14:0	1.45	1.59
14:1	2.32	2.97
15:0	2.70	4.18
15:1	Сл.**	См.***
16:0	36.75	29.21
16:1	24.37	15.71
17:0	0.38	См.***
16:2	3.19	6.86
17:1	0.39	См.***
18:0	6.59	4.54
18:1	8.12	6.73
18:2	6.51	8.87
18:3	1.77	15.51
18:4	3.76	2.82
	$\sum_{unsat}$=50.43	$\sum_{unsat}$=59.47

* D = flow rate, h^{-1}

** The acid was detected in trace amounts (less than 0.01%).

*** The acid was not detected.

It is essential to note that the autolytic degradation of microorganisms induced by the influence of high concentrations of the factor d_2 or its chemical analogue, which has been chosen as the

biological test, represents the response of the cells to the extreme effect of autoregulators. In the course of the development of cultures of microorganisms, the change in the structure and functional activity of membranes under the influence of physiological FAA concentrations ensures that the metabolic processes (katabolic and anabolic), which play an important role throughout the entire life cycle of the microorganisms, remain in balance.

Table 3. The influence of aliphatic acids on the endogenic respiration of the cells of a *M. capsulatus* culture.

Acid concentration nmol ml^{-1}	Cell respiration (% relative to 100% in the control 3 min after the introduction of the acid)			
	palmitic	palmitoleic	stearic	oleic
20	140	150	120	160
200	110	5	115	5
400	85	5	120	5

Thus the biological role of the factor d_2 or microorganisms consists, in the case of the methane oxidising bacteria, in maintaining the liquidity of the cell membrane lipids within the limits ensuring the optimum and necessary lipid–protein interactions through which the functional activity of the membrane is controlled. At the limiting permissible concentration of the factor d_2 in the culture, the latter destabilises the membranes, which induces the development of autolytic processes.

These data can be used to develop procedures for the autolysis of methane oxidising bacterial cells with the aid of the factor d_2 or its chemical analogue in order to obtain the cell protein in a soluble form, which increases significantly the assimilability and digestability of the microbial biomass used as a protein feed additive in protein-balanced feed for agricultural animals and birds.

The results obtained in the determination of the chemical structure and biological activity of the factor d_2 have been used to analyse the data on the stability of the technological process for the preparation of the methanotrophic bacterial biomass under experimental-industrial conditions. In elucidating the causes of the spontaneous autolysis of a chemostatic culture of *M. capsulatus*, we began with the following hypothesis. It may be that under conditions considered the synthesis of the extracellular factor d_2 was increased and hence the balance between the activities of the factors d_1 and d_2 was disturbed in favour of the latter, which stimulated the developing autolysis of the cell. Comparative analysis of the level of the factor d_2 in the versions involving a stably growing chemostatic culture and an autolysing culture showed that in the latter case there was a significant increase both in the activity of the factor d_2 (12.3 units litre^{-1} for the stable culture and 19.6 units litre^{-1} for the autolysing culture) and in the specific content of unsaturated aliphatic acids (Table 1). It is noteworthy that the increase in the sum of unsaturated aliphatic acids in the autolysing culture compared with the stably growing culture is not correlated with the corresponding increase in the activity of the factor d_2. However, the observed fact can be explained by taking into account data showing that the greater destabilising effect of polyunsaturated aliphatic acids on membranes compared with monounsaturated acids. It follows from Table 1 that significant amounts of C_{18} polyunsaturated acids appear in the autolysing culture. Thus the instability of the production of the *M. capsulatus* biomass associated with the autolysis of the cells in the chemostatic culture may be caused by the increase in the level of activity of the factor d_2. On this basis, a possible practical recommendation involves the necessity to achieve balanced levels of the activities of the factors d_1 and d_2, which can be achieved by the artificial introduction of a chemical analogue of the factor d_1.

The question arises what is the autoregulatory factor d_1 of *M. capsulatus*? As shown above, the autoregulatory factors d_1 detected in certain bacteria[9] exhibit a membranotropic activity, a test for which involes a change in the endogenic respiration of cells and in the exogenically stimulated respiration of the membranes. The biological activity of the factors d_1 is manifested by their ability to induce the transition of vegetative cells to the anabiotic state, a test for which involves the formation of refractile resting forms of microorganisms under the influence of the factor d_1.[19]

In the course of the purification of the factor d_1 of a culture of *M. capsulatus*, it was established that the sought type of activity was exhibited by the acetone fraction obtained in the separation of the "crude" preparation of the factor d_1 (aqueous alcohol fraction in the chloroform–methanol–water two-phase system in proportions of 2:2:1) on a silica gel column. Subsequent separation of this fraction by thin layer chromatography in the chloroform–methanol–acetic acid–ethyl ether solvent system (in proportions of 35:5:5:45) revealed that the activity of the factor d_1 was observed in the fraction with R_f = 0.67. After rechromatography, a chromatographically homogeneous preparation was obtained, its individuality being demonstrated by chromatography on Silufol and Merck plates in the initial solvent system and also in the chloroform–acetone–methanol (12:4:1) and the chloroform–methanol (85:15) systems. The chromatographically individual fraction of the factor d_1 had all the properties of the autoregulators of this type described previously. At concentrations of 7–40 μg mol^{-1}, the preparation inhibited the endogenic respiration of the cells and the respiration of the isolated membranes stimulated by NADH and malate or succinate. The degree of inhibition of the respiration depended on the concentration of the factor introduced and the pH of the medium, developing as a function of time (Figs. 3 and 4). The individual isolated fraction of the factor d_1 of a culture of *M. capsulatus* did not exhibit species specificity in relation to both other species of methane oxidising bacteria and bacteria of other genera, but the extent of their effect on the cells was different. The specific biological activity of the factor d_1 of a *M. capsulatus* culture was similar to the activity of the autoregulators of this type in previously investigated bacteria.[8,9] In doses of 20–80 μg ml^{-1}, the factor d_1 induced the formation of refractile anabiotic forms of *M. capsulatus*. It follows from the adopted definition of the resting forms of microorganisms, that specialised cells, whose formation is a natural event in the cycle of the development of microbial cultures, may be included among them.[20] On the basis of the foregoing, conditions were selected for the cultivation of *M. capsulatus*, where provision was made for the possible increase in the synthesis of the factor d_1 in the culture, as a consequence of which the cycle of its development terminated with the formation of refractile forms similar to those obtained when the bacterial cells were acted upon by the pure preparation of the factor d_1. The refractile forms of *M. capsulatus*, both obtained on treatment with the factor d_1 and formed in the

natural cycle of the development of these bacteria, exhibited all the characteristics of the resting forms of the microorganisms: the absence of an experimentally determined metabolic activity, an increased refraction of light by the organisms as a consequence of the dehydration of the cell protoplast, prolonged survival, and the characteristic features of their ultrastructural organisation which are different from those of vegetative cells.

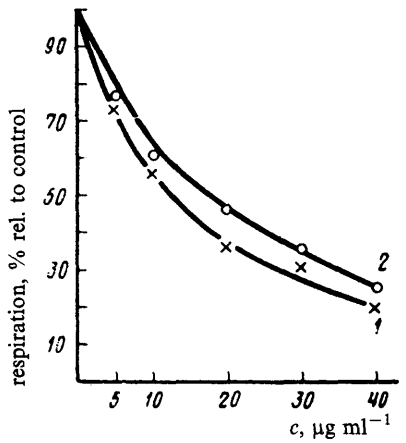


Figure 3. The suppression of the NADH-oxidase (curve *1* and malate oxidase (curve *2*) activities of the isolated membranes of *Micrococcus lysodeikticus* by the factor d_2/[13]

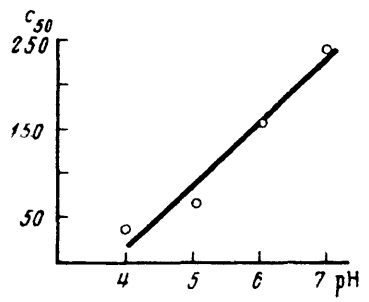


Figure 4. The influence of the pH of the incubation medium (0.2 M phosphate-citrate buffer) on the inhibition by the factor d_1 of the endogenic respiration of the *M. capsulatus* cells; c_{50} = amount of the factor d_1 ($\mu g\ ml^{-1}$) causing 50% inhibition of the respiration of the cells.

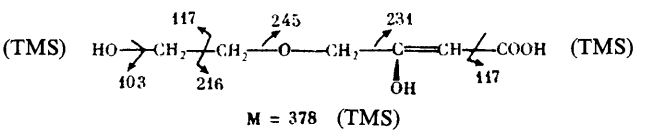
Thus the anabiotic cells, whose formation is induced by the activity of the factor d_1, can be classified as cyst-like anabiotic forms designed to preserve the bacterial species under conditions unfavourable for growth.

The authors suggest that the resting forms described for certain methane oxidising bacteria are apparently formed with participation of their factors d_1 and the differences, mentioned by Gal'chenko et al.[6] and Whittenburg and Dalton,[21] between the structures of their envelopes and their refractile properties are caused by the conditions which determine the activity of the above autoregulator and its concentration. The complete analogy in the manifestation of the biological activity of the factor d_1 of the *M. capsulatus* bacteria and the previously described factor d_1 of the *P. carboxydoflava* and *B. cereus* bacteria suggests also the identity of the mechanisms of their action on the structural organisation of the membranes.[22,23] The mechanism of the action of the factor d_1 on the lipid bilayer of the membrane is apparently based on the formation of a network of intermolecular hydrogen bonds between the functional groups of the autoregulator molecule and the lipid molecules, which leads to the immobilisation of the lipid matrix. A consequence of the structural reorganisation of the membrane (polycrystallisation) is a change in the functional activity of the enzymes integrated in it (in particular inhibition of the enzymes of the electron-transport chain), a change in the permeability of the membrane to univalent ions, and the associated dehydration of the cell (the increase in refractility). Thus the factors d_1 are capable of modifying the structure of the lipid phase of the membrane, increasing its microviscosity, in contrast to the action of the factors d_2, which increase the liquidity of the membrane.

Finally, one should note yet again that the regulatory function of the factors d_1 and d_2 of microorganisms depends not only on their concentration in the developing culture but also on the external cultivation conditions, in particular on the pH and ionic composition of the growth medium. In this connection, they can be regarded as mediators of the transmission of the external signal to the cell.

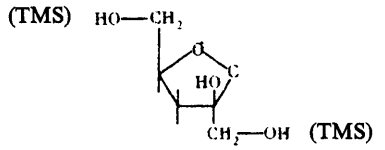
Having described the biological activity of the factor d_1 of a *M. capsulatus* culture, we shall turn to the problem of the identification of its chemical structure. The determination of the structure of the factors d_1 was based on the results of spectroscopic and mass-spectrometric analyses and proton magnetic resonance spectra. It had been shown previously[12] that, as regards their chemical nature, the factors d_1 of the *P. carboxydoflava* and *B. cereus* bacteria are alkoxybenzenes, which are present in the bacterial cultures in the form of a mixture of isomers or homologues differing in the positions of the hydroxy-groups in the ring and the configuration of the alkyl group.

It has been demonstrated by chromatograph-linked mass-spectrometric analysis under the conditions indicated by Svetlichnyi et al.[11] that two substances enter into the composition of the active principle of the factor d_1 of a *M. capsulatus* culture. These substances cannot be identified by computer from the Wiley and NBS libraries. However, their retention times are in the region of linear C_{10} aliphatic acids, C_6 polyfunctional acids, and deoxypentoses. Mass-fragmentation analysis revealed that the spectra of both compounds contain common ions characteristic of carboxylic acids (the ions 117, 129, 135, and 145) and a hydroxy-group (alcohol group) (the ion 103). This shows that the above substances are related—isomers or molecules with small structural differences. The structures of the test molecules are the same as far as the ion 261. On the basis of a computer search catalogue, the following structure of the molecules, which explains the mass-spectrometric data for these compounds, can be put forward according to the details of the fragmentation of the molecules (under the influence of electron impact at 70 eV):



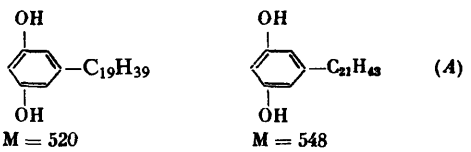
M = 378 (TMS)

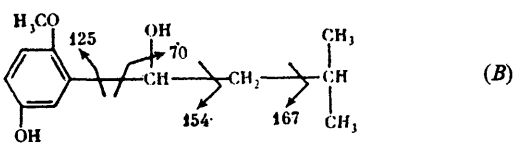
The closest version suggested by the computer in the identification process indicates that these substances are lactones of pentonic acids.[24] They give rise to many of the ions present in the alcoholic derivatives of the test substances, namely 103, 117, 129, 131, 133, 147, 217, 245, and 235. The lactone of 3-deoxypentonic acid (M = 378, trimethylsilyl derivative) is closest to the test substances:



M = 378 (TMS)

The chemical structure of the factors d_1 isolated from the *P. carboxydoflava (A)*, and *B. cereus (B)* cultures is as follows:[12]





M = 210

Thus investigations have shown that the chemical nature of the factor d_1 of a *M. capsulatus* culture, inducing the formation of anabiotic forms of the producer, differs from that established previously for the factor d_1 of the *P. carboxydoflava* and *B. cereus* bacteria. The very fact of the observation of differences between the chemical structures of compounds exhibiting the same type of biological activity is very interesting. Studies on the final identification of the chemical structure of the factor d_1 of a *M. capsulatus* culture as well as the determination of the nature of the factors d_1 of other microorganisms appears to be extremely significant. Firstly, these data can provide an idea about what general structure or functional groups are responsible for the biological activity associated with membrane polycrystallisation processes, the changes in the functional activity of the membranes, and the dehydration of cells in the course of the development of the anabiotic state. Secondly, the expansion of knowledge about the structures of compounds exhibiting the type of activities investigated is essential for the selection of cheap and readily available chemical analogues for their practical application in the development of technologies associated with the employment of stabilised cells in order to produce bacterial preparations for different purposes.

In conclusion, one must emphasise yet again that the data described constitute only a special example of the large field of biological knowledge concerning the nature and action of low-molecular-weight metabolites, whose function is to control the changes in the qualitative state of the organism, the physiological activity of the cells, and their interaction with one another and with the surrounding medium. One should note the necessity for the study of the biological activity of autoregulators strictly in relation to changes in the external conditions in the surrounding medium, in the given instance the conditions in the cultivation of microorganisms. The combination in one system of our ideas about the role of external and endogenic (autoregulatory) factors in cell proliferation processes (the growth of the culture) and cytodifferentiation will promote the acquisition of fundamental knowledge about the features of the vital activity of microorganisms in their environment. In addition progress in studies on the mechanisms of the regulation of the growth and development of microorganisms may serve as a basis for a theory of controlled cultivation and may help in the solution of specific biotechnological problems.

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