of ppApp (R_f=0.51) but larger than that of pppApp (R_f=0.34) and its identity was unknown. Works are carried out at present to identify spots a and b. Preliminary data also suggested that there is a correlation between the rate of instantaneous RNA synthesis in the mutant as well as the wild type cultures with the appearance of HPN and their sporulation frequencies. Experiments to refine these date are in progress.

In conclusion, we have presented evidence in support of the involvement of ppApp in the initiation of sporulation in *Bacillus subtilis*. Since ppApp is known to alter transcriptional selectivity of *Escherichia coli* RNA polymerase[17] and it restores the sporulation capacity of the conditional asporogenous rifampin-resistant mutant of *B. subtilis* to the same extent as 4 amino acids, this system is useful in the possible illumination of the involvement of amino acids in the transcriptional control of sporulation[18].

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Biosynthesis of roquefortine and 3,12-dihydroroquefortine by the culture *Penicillium farinosum*

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Summary. A new culture *Penicillium farinosum* synthesizing roquefortine and 3,12-dihydroroquefortine was found. Unlike *P. roqueforti*, a known producer of these compounds, the culture under study does not synthesize clavine alkaloids. The maximal roquefortine content was observed in the late logarithmic and early stationary growth phases while the maximal 3,12-dihydroroquefortine content was obtained in the beginning of the stationary phase.

Roquefortine and 3,12-dihydroroquefortine are basic components of alkaloid fractions of several strains of the fungus *Penicillium roqueforti* used in the production of many varieties of blue cheese[2-5]. Besides the compounds mentioned, this culture synthesizes an appreciable amount of clavine alkaloids such as isofumigaclavine A and isofumigaclavine B, found earlier in *Claviceps*[6] and *Aspergillus fumigatus*[7].

We found that the culture of *Penicillium farinosum* also performs the biosynthesis of roquefortine and 3,12-dihydroroquefortine. This culture was obtained from the All-Union Collection of Microorganisms.

Methods. The cultures were grown in shaking flasks in medium containing mannitol and succinic acid[2]. Extraction of alkaloids from the mycelium and filtrate of the culture liquid, chromatographic separation and analysis were performed as described earlier[5]. With the aim of determining the quantity of roquefortine and 3,12-dihydroroquefortine, a sample, dissolved in chloroform, was banded on a Silufol plate (UF-254, ČSSR) washed beforehand with methanol. After development, the UV-absorbing regions, corresponding to roquefortine and 3,12-dihydroroquefortine, were scraped off and eluted with ethanol. Concentrations of substances were determined spectrophotometrically at the wavelength of 328 and 301 nm for roquefortine and 3,12-dihydroroquefortine, respectively, using calibrating straight lines constructed with the aid of individual compounds isolated earlier from *P. roqueforti*. Identification of substances was performed by the TLC method[5] with the help of UV-, IR-spectroscopy and mass-spectrometry.

Results and discussion. Dynamics of changes in the contents of roquefortine and 3,12-dihydroroquefortine in the culture under study are given in figures 1 and 2. The maximal intracellular concentration of these compounds was observed on the 4th-5th day of the growth. During the stationary growth phase, their contents diminish and are minimal by the 12th day. The maximal contents of roquefortine and 3,12-dihydroroquefortine in the culture liquid are 24 mg/l, and 2.0 mg/l, respectively. The maximal

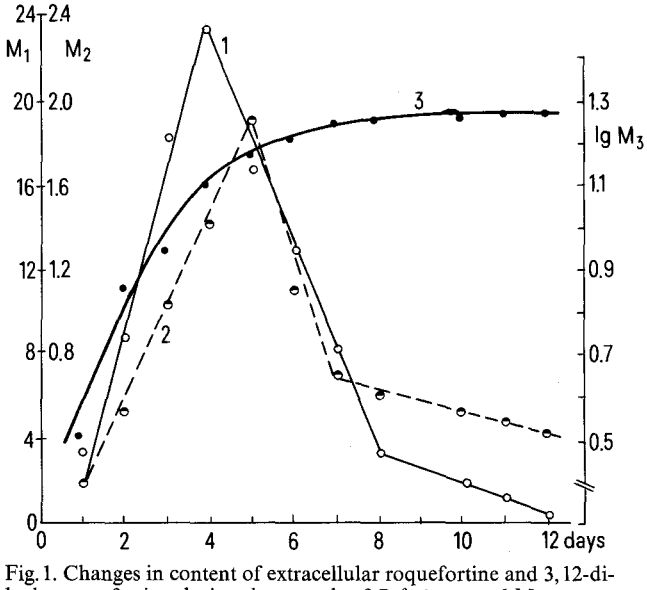

Fig. 1. Changes in content of extracellular roquefortine and 3,12-dihydroroquefortine during the growth of *P. farinosum*. *1* M_1, roquefortine (mg/l); *2* M_2, 3,12-dihydroroquefortine (mg/l); *3* M_3, biomass (g dry mycelium/l).

intracellular concentrations of roquefortine and 3,12-dihydroroquefortine make up 300 µg/g and 30 µg/g of dry biomass, respectively.

The dynamics of changes in the concentration of roquefortine and of 3,12-dihydroroquefortine in *P. farinosum* differ from the dynamics of accumulation of these compounds in the culture of *P. roqueforti;* in the latter the maximal quantity of the metabolites under study was observed on the 5th–7th day of growth[2]. Evidently, the difference seen in the time-course of the accumulation of roquefortine and 3,12-dihydroroquefortine is conditioned by distinctive features of the regulation of the biosynthesis and metabolism of these compounds. Specifically, *P. roqueforti* synthesizes an appreciable amount of clavine alkaloids, which appear in the mycelium and culture liquid at the very early stages of cultivation, while in *P. farinosum* they were not found.

The fact that *P. farinosum* does not produce clavine alkaloids makes this culture a convenient object for studying the biosynthesis of roquefortine and related compounds. *P. roqueforti* is an attractive tool for investigations into the simultaneous synthesis of 2 different groups of alkaloids as well as into the possibility of regulation of the directed synthesis of these or other metabolites.

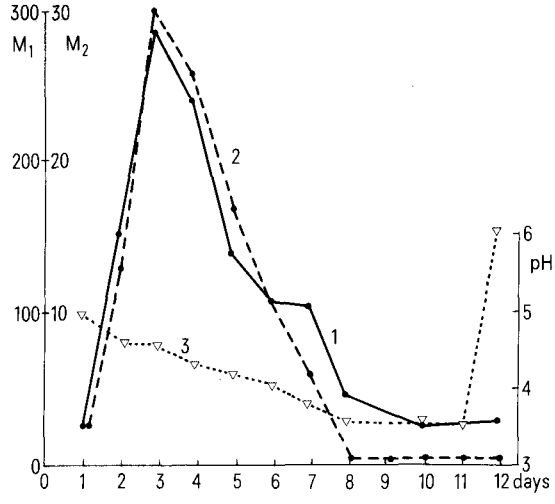

Fig. 2. Changes in contents of roquefortine and 3,12-dihydroroquefortine in *P. farinosum* mycelium and changes in pH during fermentation. *1* M_1, roquefortine (µg/g dry mycelium); *2* M_2, 3,12-dihydroroquefortine (µg/g dry mycelium); *3* changes in pH.

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Interaction of basic dyes with the thiamine transport system in *Saccharomyces cerevisiae*[1]

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Summary. Basic dyes such as methylene blue and triphenyltetrazolium chloride were found to inhibit thiamine transport in *Saccharomyces cerevisiae.* Conversely, the reduction of methylene blue and triphenyltetrazolium chloride by yeast cells was inhibited by thiamine. A thiamine transport mutant of *Saccharomyces cerevisiae* showed decreased utilization of these dyes. From the results, the possibility that the uptake of basic dyes may proceed via a membrane-bound thiamine-binding protein in the thiamine transport system of the yeast is discussed.

It has been demonstrated that the transport of thiamine is *Saccharomyces cerevisiae* occurs by a carrier-mediated active process which is specific for thiamine[2–5]. Experimental inhibition of thiamine transport by various analogs of thiamine suggested that at least the intact pyrimidine moiety of the thiamine molecule is necessary to bind to some components of the transport system[6,7]. Recently, however, evidence suggesting that uptake of dibenzyldimethylammonium (DDA) proceeds via the thiamine transport system of the yeast has been shown[8]. During the course of the study on structural specificity of the yeast thiamine transport system we found that methylene blue inhibits thiamine transport competitively and the dye is effective in abolishing the growth inhibition of *Saccharomyces cerevisiae* by pyrithiamine, which is known to be taken up by a common transport system for thiamine in yeast cells[9].

In this paper we show that basic dyes such as methylene blue and triphenyltetrazolium chloride (TTC) inhibit thiamine transport in *Saccharomyces cerevisiae,* and conversely their utilization by yeast cells is inhibited by thiamine and it is also decreased considerably in a thiamine transport mutant of *Saccharomyces cerevisiae.* Figure 1 shows inhibitory effects of methylene blue, TTC, rhodamine 6GO and safranin O, which are common to be basic dyes, on thiamine transport in *Saccharomyces cerevisiae.* The inhibition was strongest with safranin O among these dyes tested, whereas TTC was less inhibitory.

Among these, both methylene blue and TTC are known to be reduced in living yeast cells. Therefore the reduction of methylene blue during the growth of *Saccharomyces cerevisiae* was examined. During growth without shaking at 30 °C for 20 h, approximately 81% of the methylene blue added (10 µM) was reduced, whereas the presence of 10 µM thiamine in the growth medium was remarkably effective in preventing the reduction of the dye (table 1). Since dimethialium, an analog of thiamine which is also taken up by yeast cells but not converted to the coenzyme form in the cells, showed the same effect, the site of their action appeared to be on the surface of the cell membrane. On the other hand no preventive effect on the reduction of methylene blue was observed with oxythiamine, which is ineffective in inhibiting thiamine transport[3]. DDA at the concentration of 0.1 mM could partly inhibit the utilization of methylene blue by growing yeast.

These results strongly suggest that thiamine and the inhibitors of yeast thiamine transport have a common inhibitory