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About the cause of the stimulative effect of humate in yeast cultures

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Abstract

Trials were undertaken to elucidate the stimulating effect of sodium humate on yeast multiplication and the intensity of their fermentation. This effect appears specifically at pH non-optimal for the medium. No correlation was found between this effect and the complex-forming properties of various natural and synthetic humate fractions or the concentration of phosphate and calcium ions in the medium. Application of cystein as reducing agent, aeration of the medium and addition of detergents to it did not substitute the effect of humate. Tannin and gibberellin, on the other hand, stimulated cell proliferation and fermentation at non-optimal pH, remaining almost without influence at optimal pH, similarly as does humate.

INTRODUCTION

In the preceding publication (Gumiński et al., 1977) results of comparative studies were presented concerning the influence of the particular fractions of natural and synthetic sodium humate on the growth of tomatoes in water cultures deficient in available iron. The corresponding fractions of both humates were found to exert a similar influence, and a common chemical basis of their biological activity was established: it consisted in the presence of carboxyl and phenol-hydroxyl groups on aromatic and quinoid structures and in their forming complexes with iron.

Badura (1965) demonstrated that the stimulating effect of sodium humate on the growth of the yeast population and the intensity of the fermentation caused by it occurs specifically at pH non-optimal for the medium. According to the above cited author this phenomenon cannot be explained by a facilitation of iron uptake. It was, therefore,

decided to attempt the elucidation of this problem by comparative experiments with natural and synthetic humate similarly as in the previous paper (Gumiński et al., 1977).

It was found (Badura, 1965) that the influence of humate does not consist in buffering the pH of the medium and that a similar stimulating effect may be achieved under unfavourable pH conditions of the medium by using resin ion exchangers (Badura, 1966). This indicated the possibility that stimulation is connected with regulation by humate of uptake or accumulation of mineral components (ions) other than iron, the sorption or penetration of which into the cells depends on the pH of the nutrient solution. The investigations were at first continued along these lines. Particular attention was devoted to the possibility of regulation of phosphate and calcium ions uptake by humate.

Since these studies gave no explanation of the studied phenomenon, other aspects were successively taken into account: the reducing--oxidating properties of humic compounds, their influence of the structure of the protoplasm and their action similar to that of some growth regulators. For this purpose comparative experiments were performed with cysteine as reducing agent and aeration of the medium. The influence of the detergent and tannin was investigated and the effects of auxin, gibberellin and of a bios set were compared with those of humate at various pH of the medium.

MATERIAL AND METHODS

The experiments were performed with yeast of the species *Saccharomyces cerevisiae*, strain D 4; the optimum pH for multiplication and fermentation of the yeast cultures was about 5.4 (according to Badura, 1965 and our trials).

Since Dzierzbicki (1909) was the first to describe the stimulating effect of humus compounds in yeast cultures, his medium was used for the experiments, composed of 100 g sucrose, 1.5 g asparagine, 1 g KCl 0.2 g $Ca(H_2PO_4)_2 \cdot H_2O$, 0.2 $MgSO_4 \cdot 7\ H_2O$ per 1 liter of bidistilled water and supplemented with two drops of 5 per cent $FeCl_3$ solution and 1 cm^3 of solution of Hoagland's microelements series no. 1 with Mo added according to series no. 2 of the same author (Ruhland, 1958).

The same medium was also used by Badura (1965) and we applied it in our earlier experiments (Gumiński, 1950; Gumiński and Sulej, 1967). For comparison, medium known as GO, modified according Badura (1965) was used. It consisted of: sucrose 100 g, 6 g $NH_4H_2PO_4$, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.1 g NaCl, 2 g $(NH_4)_2SO_4$, 1 g

KH_2PO_4, 0.1 g $CaCl_2$ per 1 l. of bidistilled water with 0.2 mg $FeCl_3$ added and microelements — H_3BO_3 0.5 mg, $CuSO_4 \cdot 5\ H_2O$ 0.04 mg, KJ 0.1 mg, $MnSO_4 \cdot 5\ H_2O$ 0.4 mg, $Na_2MoO_4 \cdot 2\ H_2O$ 0.2 mg and $ZnSO_4 \cdot 7\ H_2O$ 0.4 mg.

Natural and synthetic sodium humate and the particular fractions of these substances were prepared by the method described in earlier papers published in the same journal (Gumiński and Sulej, 1967; Gumiński et al., 1977). Natural humate was obtained from leaf compost, and the synthetic one from p-benzoquinone. The particular humic substances were added to the medium in the amount of 1 g dry weight per 1 l. on the basis of the results of Badura (1965) and our own trials. Various doses of phosphates, calcium salts and cysteine (0.02 g/l.) were applied. The medium was aerated by shaking the flasks. Detergent was added to the medium (ethoxylated nonylphenol 0.5 and 5 mg/l.), tannin (so-called Chinese) was added in several different doses, auxin (IAA) — 20 mg/l., gibberellin (GA_3) — 100 mg/l. also bios, that is a set of vitamins of group B was added. The vitamins composition (after Badura, 1965) was as follows: thiamine hydrochloride 4 mg, m-inositol 4 mg, nicotinic acid 1 mg, pantothenic acid 4 mg, pyridoxin 4 mg, biotin 0.08 mg, riboflavin 8 mg per 1 l of medium. Dosage of auxin and gibberellin was based on the paper by Yaganishima and Shimoda (1973), of detergent on the experiments of Gumiński et al. (1972), of cysteine also on our own trials (Gumiński, 1950).

The yeast was incubated in 100 cm^3 of medium in Erlenmayer flasks of 200 cm^3 volume. Into each flask 1 cm^3 of suspension of yeast cultured previously for 24 h in Dierzbicki's medium was introduced. After inoculation the experimental culture was run for 48 or 72 h in a thermostat at 28°C. The results were recorded in terms of the number of cells counted in a Fuchs-Rosenthal chamber after their killing with a formalin-ethanol mixture. The released amount of carbon dioxide was determined gravimetrically. The flasks had rubber valves protecting from water evaporation but letting out CO_2. The method of measurement was thus the same as that used by Badura (1965).

As a rule 5 simultaneous replications were run for each experimental combination. The results are listed as mean values for each combination after calculation of experimental error according to the formula:

$$\text{mean error} = \pm \sqrt{\frac{\Sigma(x_1 - \bar{x})^2}{n-(n-1)}}$$

where x_1 denotes the mean for the combination, $\bar{x}$ — the particular result and n — the number of replications.

RESULTS

I. Influence of particular natural and synthetic humate fractions at pH = 6.5

Comparison of cultures with various natural and synthetic humate fractions showed that all these substances stimulate to a lesser or greater degree yeast cell proliferation. In contrast to the test with tomato seedlings in water cultures with deficient iron (Gumiński and Sulej, 1967; Gumiński et al., 1977), the test with yeast at raised pH of medium demonstrated the stimulating also action of those fractions whis were adsorbed on aluminium oxide. The results of these experiments are listed in tables 1 and 2.

Table 1

Efect of natural humate and its fractions. Number of yeast cells per Fuchs-Rosenthal 1 chamber. Mean of 5 replications

Humic substances concentration 0.1%	After 24 h	After 48 h
Whole substance	11.438 ± 381	34.216 ± 843
Fraction 1	10.482 ± 394	30.520 ± 342
Fraction 2	12.300 ± 305	40.136 ± 882
Fraction 3	2.040 ± 82	11.537 ± 384
Fraction 4 adsorbed on Al_2O_3	3.345 ± 65	19.742 ± 494
Fraction 5	5.544 ± 245	13.477 ± 663
Control	3.628 ± 48	6.518 ± 123

Table 2

Efect of synthetic humate and its fraction. Number of yeast cells per Fuchs-Rosenthal 1 chamber. Mean of 5 replications

Humic substances concentration 0.1%	After 24 h	After 48 h	After 72 h
Whole substance	6.680 ± 85	13.420 ± 76	23.000 ± 128
Fraction 2	6.540 ± 36	12.100 ± 98	19.000 ± 152
Fraction 3	54.040 ± 185	66.360 ± 257	125.440 ± 585
Fraction 4 adsorbed on	18.080 ± 220	20.680 ± 153	49.280 ± 568
Fraction 5 Al_2O_3	52.880 ± 352	68.800 ± 435	98.390 ± 295
Control	3.912 ± 36	9.140 ± 48	20.280 ± 336

II. Various phosphate doses at pH = 6.4

Comparison of yeast proliferation and intensity of fermentation in media with normal, reduced to one half and doubled phosphate ion dose did not reveal any influence of these ions on the stimulative

effect of humate. It was, moreover, found that the yeast did not react at all to rather wide differences in phosphate content in the medium (Table 3).

Table 3

Various phosphate doses at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	10.240 ± 256	0.150 ± 0.007
1/2 PO_4^{-3}	10.032 ± 345	0.155 ± 0.008
2 × PO_4^{-3}	10.580 ± 217	0.145 ± 0.006
Humate	40.480 ± 685	1.480 ± 0.012
1/2 PO_4^{-3} + humate	41.920 ± 716	1.445 ± 0.031
2 × PO_4^{-3} + humate	40.760 ± 567	1.407 ± 0.021

III. Various calcium doses at pH = 6.4

Reduction of the calcium amount in the medium to 1/5 did not affect the yeast culture, whereas a fivefold dose in the form of chloride inhibited almost by one half cell multiplication. However, as shown by the comparative combination with sodium chloride, this effect was rather due to chloride than to calcium ions. The stimulating influence of humate was distinctly reduced when the calcium level in the medium was lowered, and it was slightly weaker with increased $CaCl_2$ doses and when NaCl was applied (Table 4). The reduction of the calcium

Table 4

Various calcium doses at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	10.776 ± 614	0.152 ± 0.005
1/5 Ca^{+2}	10.840 ± 425	0.160 ± 0.006
5 × Ca^{+2} ($CaCl_2$)	6.845 ± 245	0.145 ± 0.004
NaCl *	6.600 ± 476	0.139 ± 0.003
Humate	59.440 ± 1246	1.760 ± 0.041
1/5 Ca^{+2} + humate	46.575 ± 1265	1.445 ± 0.025
5 × Ca^{+2} ($CaCl_2$) + humate	52.786 ± 1380	1.387 ± 0.016
NaCl * + humate	54.750 ± 1533	1.520 ± 0.031

* NaCl — Cl ions in amount corresponding to 5 × $CaCl_2$.

content in the medium to 1/10 and still more to 1/100 markedly inhibited yeast proliferation and stimulation of cell multiplication by humate. The effect of humate on fermentation was less pronounced (Table 5).

Table 5

Reduced calcium doses at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	8.330 ± 415	0.016 ±0.006
1/10 Ca^{+2}	7.462 ± 275	0.110 ±0.002
1/100 Ca^{+2}	5.424 ± 253	0.090 ±0.001
Humate	70.780 ±1752	1.735 ±0.032
1/10 Ca^{+2}+humate	45.304 ±1657	1.575 ±0.043
1/100 Ca^{+2}+humate	24.672 ± 479	1.290 ±0.038

IV. Comparison of action of natural humate and cystein at pH = 6.4

The use of cysteine in the amount of 0.02 g/l. medium did not affect cell multiplication or the intensity of fermentation, whereas humate strongly stimulated both these processes. Joint application of humate and cystein gave the same effect as treatment with humate alone (numerical data listed in table 6).

Table 6

Comparison of effect of natural humate and cysteine (0.02 g/l) at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	7.660 ±234	0.112 ±0.002
Cysteine	7.250 ±315	0,118 ±0.005
Humate	32.420 ±786	1.245 ±0.052
Cysteine+humate	31.850 ±654	1.205 ±0.046

V. Aeration and stirring of cultures and the effect of the natural humate at pH = 6.4

Aeration and stirring the flasks by a circular movement of the hand several times daily did not affect either cell multiplication or fermentation intensity. Humate exerted the same stimulating action both on unshaken (control) and aerated cultures (Table 7).

VI. Detergent and natural humate, pH = 6.4

The non-ionic detergent (ethoxylated nonylphenol) in both 0.5 and 5 mg/l. concentration in the medium did not produce any effect, where-

as humate as usual stimulated growth and fermentation. The simultaneous presence of detergent in the medium did not affect the activity of humate (Table 8).

Table 7

Comparison of aeration and of humate at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	8.335 ± 415	0.150 ± 0.005
Aeration	7.972 ± 275	0.135 ± 0.004
Humate	70.780 ± 1752	1.730 ± 0.032
Aeration + humate	69.580 ± 1645	1.662 ± 0.023

Table 8

Comparison of effect of detergent ENF (mg of active substance per 1 l.) and of humate at pH 6.4 in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
Control	8.679 ± 354	0.185 ± 0.007
ENF 0.5 mg/l	9.055 ± 218	0.154 ± 0.008
ENF 5 mg/l	8.894 ± 654	0.192 ± 0.006
Humate	69.635 ± 1673	1.558 ± 0.062
Humate + ENF 0.5 mg/l	70.100 ± 1260	1.630 ± 0.072
Humate + ENF 5 mg/l	69.150 ± 942	1.550 ± 0.026

VII. Influence of tannin and natural humate in dependence on pH of the medium

It was established in preliminary experiments that tannin stimulates yeast proliferation and fermentation at pH = 6.4, the optimal concentration of the solution being 1g/1 l. of medium. A comparative

Table 9

Comparison of effect of humate (1 g/l) and of tannin (1 g/l) at various pH in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
pH = 5.3		
Control	60.192 ± 846	1.84 ± 0.042
Tannin	80.600 ± 1755	2.43 ± 0.051
Humate	64.470 ± 1120	2.37 ± 0.054
pH = 6.4		
Control	6.340 ± 307	0.16 ± 0.004
Tannin	75.520 ± 1585	2.47 ± 0.053
Humate	63.140 ± 934	2.20 ± 0.062

experiment with tannin and humate at pH = 5.3 and pH = 6.4 demonstrated that both tannin and humate enhance strongly cell multiplication and fermentation at pH = 6.4. On the other hand, at pH = 5.3 they hardly exert any influence (Table 9). The action of both these substances, thus proved to be identical.

VIII. Influence of gibberellin and auxin in dependence on pH of the medium

Addition of 0.1 g GA_3/1 l. to the medium with pH = 5.3 stimulated yeast proliferation by about 40 per cent and CO_2 release by about 100 per cent, whereas at unfavourable pH = 6.4 the stimulation of

Table 10

Comparison of effect of GA and IAA at various pH in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
pH=5.3		
Control	10.680 ±402	0.284 ±0.004
GA — 100 mg/l	14.776 ±415	0.546 ±0.011
IAA — 20 mg/l	10.102 ±325	0.224 ±0.006
GA+IAA	14.816 ±435	0.580 ±0.008
pH=6.4		
Control	2.092 ± 80	0.123 ±0.005
GA — 100 mg/l	15.148 ±205	0.572 ±0.006
IAA — 20 mg/l	2.004 ± 67	0.192 ±0.003
GA+IAA	15.040 ±386	0.514 ±0.011

Table 11

Comparison of effect of humate (1 g/l) and of gibberellin (0.1 g/l) at various pH in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
pH=5.0		
Control	5.711 ±247	0.194 ±0.004
Gibberellin	8.768 ±216	0.284 ±0.005
Humate	22.658 ±428	0.878 ±0.007
pH=6.4		
Control	2.120 ±112	0.101 ±0.001
Gibberellin	8.204 ±428	0.305 ±0.002
Humate	20.712 ±270	0.785 ±0.008

these processes was sevenfold and fivefold, respectively. Indolyl-3-acetic acid applied in a 20 mg/l. concentration did not affect these processes (Table 10).

Comparison of the action of gibberellin and natural humate at pH = 5 (markedly lower than the optimal value) and at pH = 6.4 (much higher than optimal) revealed the similarity of action of both these substances, the effect of humate being much stronger (Table 11).

IX. Effect of bios

Comparison of the influence of substance of bios type with the effect of natural humate at various pH values showed a similar physiological activity of both these kinds of substances and a summation of their effects (Table 12).

Table 12

Effect of substances of bios type and of humate at various pH in medium. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
pH=5.3		
Control	77.340 ± 1784	1.10 ± 0.032
Bios	117.272 ± 1068	2.64 ± 0.045
Humate	95.770 ± 1140	2.01 ± 0.030
Humate + bios	143.050 ± 1785	3.70 ± 0.063
pH=6.4		
Control	41.984 ± 867	0.71 ± 0.021
Bios	72.220 ± 1067	1.34 ± 0.032
Humate	92.880 ± 1563	1.90 ± 0.041
Humate + bios	108.420 ± 1568	2.25 ± 0.042

X. Effect of pretreatment with humate

Yeast was cultured on the medium of Dzierzbicki with pH = 5.3 and on the same medium with addition of natural humate (as usual in 1 g/l. concentration). The cultures were 3 times passaged at 24-h intervals with and without humate respectively. Then they were inoculated on Dzierzbicki's medium without humate, adjusted to pH = 5.0 and pH = 6.4. The yeast not pretreated with humate grew and fermented much better at pH 5.0 than at pH 6.4, and that previously treated with humate grew and fermented almost equally at both pH values. Stimulation produced by pretreatment with humate was much higher at pH = 6.4 than at pH = 5.0. It was, moreover, observed that the effect was more pronounced on fermentation than on cell multiplication (Table 13).

Table 13

Effect of pretreatment with humate on yeast culture in control medium at various pH. Mean of 5 replications

Combination	No. of yeast cells	CO_2 released, g
pH=5.0		
Cells passaged without humate	8.850 ±345	0.325 ±0.005
Cells passaged with humate	10.240 ±487	0.503 ±0.004
pH=6.4		
Cells passaged without humate	5.250 ±186	0.174 ±0.006
Cells passaged with humate	9.680 ±314	0.385 ±0.007

XI. Effect of natural humate in Dzierzbicki's medium and GO. medium at pH = 6.4

Comparative experiments with these media indicated that in both cases humate stimulated fermentation. On Dzierzbicki's medium fermentation was first weaker than on GO. medium, but later the situation was reversed, the stimulating effect of humate both at an earlier and later time was stronger on Dzierzbicki's medium than on GO. medium (Table 14).

Table 14

Comparison of effect of humate on fermentation course in Dzierzbicki's medium and GO. medium at pH 6.4. Mean of 5 replications

Combination	CO_2 released, g after 48 h	CO_2 released, g after 72 h
Dzierzbicki's medium	0.143 ±0.003	0.275 ±0.002
Dzierzbicki's medium+humate	0.875 ±0.004	1.740 ±0.008
GO medium	0.175 ±0.003	0.245 ±0.001
GO medium+humate	0.247 ±0.004	0.787 ±0.003

DISCUSSION

The finding that the fractions of both humates adsorbing on aluminium oxide (fractions IV, V and VI) have a stimulating effect indicates that binding of metal cations cannot be assumed for explaining the favourable action of the humic compounds applied to yeast cultures at nonoptimum pH of the medium. This conclusion is based on the results of earlier studies (Gumiński and Sulej, 1967; Gumiński et al., 1977). In the light of this argumentation the nega-

tive result of experiments in which it was attempted to neutralise the influence of humate by raising or lowering the calcium dose is not surprising, although, in investigations on other organisms and under different conditions, ant antagonism was found between the effectiveness of humate and calcium (Gumiński et al., 1965; Skinder and Gumiński, 1976; Jurajda 1974). If the stimulating role of humate in yeast cultures were due to very strong binding of calcium, then, treatment with humate should have deepened the consequences of calcium deficit when the doses were reduced 10- and 100-fold. In these combinations, however, a stimulating effect of humate was observed. On the other hand, increased calcium doses did not neutralise the stimulation evoked by humate. Analysis of the results, taking into account the additional combination with NaCl, leads to the conclusion that the favourable effect of humate at too low medium acidity does not consist either in facilitating or impeding calcium ion uptake by yeast.

Yeast proved unsusceptible to changes in phosphate concentration within the limits of the experiment, and these changes did not affect the stimulation due to humate. Therefore, the cause of this stimulation cannot be searched for in the regulation of phosphate uptake at non-optimal pH.

Since the biochemical reductor — cysteine — did not affect the yeast cultures or change the effect of humate, it seems probable that this effect did not consist in a reducing action on humic compounds. It is true that only one concentration was applied (effective in experiments with *Rhizobium*, Gumiński, 1950). However, in view of the completely negative result, it seemed aimless to continue the tests.

The results of the experiment with medium aeration and simultaneous shaking of the sediment showed that the causes of the stimulation due to humate cannot be attributed to the substitution of humate for oxygen (as eventual hydrogen acceptor) or it counter action against the presumed influence of sediment deposition on the flask bottom, which was prevented by stirring.

Neither did the use of detergent for eventual increasing of the permability of the protoplasm, and elimination in this way the consequences of the unfavourable pH of the medium give any solution.

On the other hand, the finding of a distinct analogy between the effect of tannin and that of humate in dependence on the medium pH leads to the supposition that the action of humate compounds is tannin-like. This from the chemical viewpoint seems rather probable. It would seem, therefore, that combinations may be formed with protoplasmic protein. Since it results from the papers by Prát (1963) and Rypáček (1962) that high molecular weight humic compounds (humic and hymatomelanic acids) do not readily penetrate into the

cell, thus it may be supposed that the contact of these substances is limited to the plasmalemma, if protein is not present as well in the cell wall. This influence on the plasmalemma modifies essentially it permeability, this being manifested among other things in the course of plasmolysis (Rypáček, 1962). It is known, moreover, that these phenomena are characteristically influenced by hydrogen ions (Prát 1926).

Similar effect of hydrogen ions, humate, tannin, gibberellin and bios substances seems strange. However, the stimulating effect of gibberellin on yeast proliferation is known from the papers of Yaganishima and Shimoda (1973). According to these authors, the growth of some yeast strains is also stimulated by IAA (in certain cases after pretreatment with gibberellin). It was found at present that the effect of gibberellin is much more pronounced at too high than at optimal pH at which it almost disappears. The same was observed when applying humate or tannin. Taking into account the suggestion of Hager et al. (1971) and of Rayle and Cleland (1972) and also of Sakurai, Nevine and Masuda (1977), concerning the similarity of the growth effects produced by H^+ ions and auxins as well as the conclusions resulting from earlier studies by Strugger (1934) on the influence of pH on plant growth, the supposition may be advanced that in all these cases we are dealing with stimulation of the enzymes occurring in the plasmalemma structures and the cell wall, which are active in growth processes (proton pump or hydrolysis and synthesis of polysaccharides). The object in view in our investigations was the multiplication of cells, we did not, however, examine their "elongation" growth, and the effects were obtained with the use of gibberellin, not of auxin; however, the interrelations here are very strict (Yaganishima and Shimoda, 1973). It would seem, therefore, that both an appropriate hydrogen ion concentration and that of humate, tannin and gibberellin influence the enzymatic proteins directly or indirectly by changing the structure of the plasmalemma and the cell wall, and in this way they cause similar growth effects. It should be mentioned, however, that Green and Corcoran (1975) noted growth inhibition induced by various gibberellins when applying tannin substances including Chinese tannin. Their experiments were performed on pea seedlings and concerned "elongation" growth exclusively.

It cannot be established on the basis of the present investigations whether stimulation of growth processes or of fermentation is the primary effect of hydrogen ions, humate, tannin and gibberellin. As the cells multiply, the medium becomes highly acidic owing to CO_2 evolution. Since acidification of the medium with initial pH 6.4 improves the conditions for cell multiplication, it may be assumed that,

under the influence of the substances applied, fermentation is primarily stimulated, and this secondarily enhances growth, owing to the influence of hydrogen ions. This, however, is contradicted by the fact that humate, like gibberellin, stimulates yeast proliferation not only at too high but also at too low pH of the medium. Thus, it should rather be suggested that growth is primarily stimulated, while fermentation only secondarily or else both processes are enhanced simultaneously. In the case of bios substances, however, fermentation is apparently first stimulated. The fact that the joint growth and fermentation effect was similar in our experiments should not be surprising considering the metabolic connections between these two processes.

The detection of similar growth effects induced by an appropriate hydrogen ion concentration in the medium and application of gibberellin, tannin or humate, the latter substance being considered as incapable of penetrating into the cell, supplies a new argument in favour of the assertion that important growth regulation in plant cells occurs within the plasmolemma and cell wall. This argument is also supported by the fact demonstrated by Badura (1966) that an effect similar to that of humate stimulating yeast cultures may be obtained at unfavourable pH of the medium by application of resin ion exchangers which, obviously, cannot penetrate into the protoplasm.

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O przyczynie efektu stymulacyjnego humianu w kulturach drożdży

Streszczenie

W poprzedniej pracy (Gumiński, Augustyn i Sulejowa 1977) znaleziono zgodność efektywności biologicznej odpowiadających sobie frakcji humianu naturalnego i modelowego w kulturach wodnych pomidorów z niedostatkiem dostępnego żelaza. Wspólną podstawą chemiczną tej aktywności okazała się zdolność kompleksowania żelaza.

Obecnie dociekano przyczyny, dla której humian sodowy stymuluje namnażanie się drożdży i powodowaną przez nie fermentację przy nieoptymalnym pH środowiska, a nie jest aktywny przy pH optymalnym (Badura 1965). Okazało się, że stymulację powodują w kulturach drożdży także i te frakcje humianu

tak naturalnego, jak i modelowego, które w kulturach wodnych pomidorów nie wpływały korzystnie (nie były one zdolne do wiązania żelaza).

Nie znaleziono korelacji pomiędzy stężeniem jonów fosforanowych i wapniowych w pożywce a efektem stymulacyjnym humianu, co w połączeniu z wyżej podanym wynikiem skłoniło nas do odrzucenia hipotezy jakoby humian stymulował kultury drożdży przy niekorzystnym pH pożywki przez regulację pobierania jonów mineralnych.

Próby zastąpienia humianu przez stosowanie cysteiny jako reduktora, przez napowietrzanie pożywki i mieszanie osadu, a także przez dodatek detergentu do pożywki okazały się chybione.

Natomiast tanina (chińska) oraz giberelina (GA_3) stymulowały silnie namnażanie się komórek i fermentację przy nieoptymalnym pH pożywki, pozostając niemal bez wpływu przy pH optymalnym zupełnie podobnie jak humian.

Przedyskutowano zbieżność efektów wywoływanych przez optymalne stężenie jonów wodorowych, humian, taninę i giberelinę.