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RELATIONSHIPS BETWEEN QUALITY OF THE PLANT RAW MATERIALS AND BIOTECHNOLOGY CREATION OF ANTIBIOTICS

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SUMMARY

In the biosynthesis of antibiotics problems regarding the uses of plant raw materials very often arise. Due to their large quantities - from 50 % in Tetracycline to 90 % in Penicillin, it is important that the most reliable plant raw material for processing is used. A pilot installation was created with which the relations between quality of maize hybrids, their corn steep liquor and the results of their use in biosynthesis of Tetracycline was studied. Full scale research as per the standards, and additional investigations for amino acids in maize and corn steep liquor has been conducted. Our results show that it is possible to obtain about 70 % higher activity in the process of biotechnological manufacture of Tetracycline in comparison with the control sample obtained from the most widespread Bulgarian corn steep liquor.

KEY WORDS

plant raw material, biosynthesis of antibiotics, corn steep liquor, maize hybrids

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INTRODUCTION

In the manufacture of biosynthetic antibiotics large quantities of plant raw material are used /1/. According to our research, these quantities for penicillin reach 90 % where as tetracycline uses approximately 50 %/2/. The most largely used maize-corn derivative, for the manufacture of biosynthetic antibiotics is corn extract. Many studies have been conducted in which means of improvement or replacements with synthetic medium have been researched. These studies have shown that as raw material for biosynthetic processing, corn extract has a big future since it has a commercially lower cost and contains a large spectrum of substances necessary for biosynthesis /3/. The basic draw-back appears in its inconsistent composition which creates many difficulties for the technologist leading to the discredition of the entire process of biosynthesis.

The object of the investigation was to find out the possibilities for quality optimisation of the corn maize extract from the point of view of the biosynthetic processes. The main idea was to discover the reasons for the deviations in the composition of the extracts and if possible, their removal. Similar studies have been carried out by Egyptian workers but they have linked the quality of the extract with the countries from which they are obtained, i.e. the extracts were categorised as Russian, French, British, American and others /4/. Our model for investigation was based upon an entirely different concept. In the first instance, the reasons for the different qualities of the extract were subdivided into 3 subgroups - factors related to the raw material from which it is obtained, factors related to the technological process for the obtaining of the extract, and those related to the storage of the extract. In our investigation a multidimensional coverage was made to study the reasons for the deviation from the quality of the raw material.

MATERIALS AND APPARATUS

11 Bulgarian and one American maize hybrids were studied. They cover all the most widespread hybrids obtained in Bulgaria (Table 1). A detailed analysis of the hybrids was conducted including the content of micro-elements, amino acids, moisture and the quantity of starch. For this object a laboratory was specially set up. The technical processing of the maize into the corn steep liquor was performed using different hybrids for obtaining different extracts. The experimental equipment for the vacuum evaporation of the biomixes was constructed of glass apparatus from the company "Simax" (Chekhia). The influence of the temperature changes during the processing was studied at selected temperatures, from 40°C - 60°C (+/- 5°C). The evaporation temperature was maintained with steam generator of our indigenous construction. The vacuum regimen 0.3 to 0.8 mPa was generated using water pump (Polski zaklady - Katovice).

METHODS

50 kg maize corn from selected hybrids was taken into the extractor. 100 l water at temperature 40 °C was used for swelling the corn mixing with the pump 12 E 60 F (Bulgarian). The water contains about 0.18 (+/-0.02) % H_2SO_3. The extract develops about 10 -12 % dried substance after 24 hours. This extract was finally concentrated to 50 % dry residue /5/. The different obtained extracts were studied for the determination of the content of amino acids and other characteristics which are not included as existing working standards.

The determination of the amino acids was conducted after the hydrolysis of the samples. 100 mg (+/- 0.2 mg) air dried material (size reduced to 0.5 mm) was transferred into an ampoule for hydrolysis and was wetted with 3 drops of C_2H_5OH to which was added 10 ml 6 N HCl. The probes were sealed while

passing N_2 (202 kPa). Each of the probes were left at 103° - 105°C for 24 hours after which the ampoules were broken open and the contents transferred to a porcelain dish. The ampoules were washed twice with 5 ml portions of distilled water and the washings added to the contents of the porcelain dish. The porcelain dishes were warmed on a water bath at 60°C until dryness. To these hydrolysates 10 ml each of buffer solution was added. The resultant solutions were filtered, left to cool in refrigerator and subsequently examined by the apparatus "Technikon".

The obtained extracts were used as the nutrition medium for the biosynthesis of tetracycline, with the preservation of the values of the remaining elements of the nutrition medium and the type of strain producer.

RESULTS

The investigation of the amino acid contents in the hybrids and the extracts show that the some of the amino acids remain in the same quantity, as in the hybrids (e.g. Phenylalanine), whereas in others like Leucine the quantity decreases after the evaporation (Table 2). Since most of the biologically active substances depend upon the type of the hybrids, it follows that there exists a direct correlation between the hybrids and the quantity of the amino acid in extracts. This factor leads to about 80% variation in the quality of extract, where as the factors related to the technology of the hybrid cultivation and the environmental factors have a much smaller influence. (Table 3)

The results of the experimental biosynthesis of Tetracyclin is shown in the Table 4.

DISCUSSION

It is important here to point out the difference between the studies of specifications of the cultivated raw material. Factors like, higher maize yields per

TABLE 1

Studied corn hybrids

N	Hybrid	Code number	FAO group
1	Kneja - 180	K - 180	1
2	Kneja - 430	K - 430	2
3	Kneja - 510	K - 510	3
4	Kneja - 530	K - 530	4
5	Kneja - 566	K - 566	4
6	Kneja - 611	K - 611	4
7	Kneja - 641	K - 641	4
8	H - 708	H - 708	4
9	Kneja - 47	K - 47	4
10	Kneja - 50	K- 50	4
11	Kneja - 32	K - 32	4
12	Kneja - 614	K - 614	4

TABLE 2

Amino acid contens in hybrids and their extracts

Number		*Hybrids*			Extracts		
	Amino acids g/100g	***N - 4 K-530***	***N- 6 K-611***	***N - 8 H-708***	N - 4 K-530	N - 6 K-611	N - 8 H-708
1.	Tyrosine	***0,315***	***0,208***	***0,285***	0,22	0,19	0,23
2.	Phenylalanine	***0,451***	***0,413***	***0,442***	0,47	0,40	0,53
3.	Lysine	***0,304***	***0,274***	***0,343***	1,53	1,00	1,01
4.	Histhidine	***0,391***	***0,325***	***0,312***	0,42	0,33	0,55
5.	Arginine	***0,467***	***0,457***	***0,628***	0,49	0,47	0,78
6.	Aspartic acid	***0,672***	***0,412***	***0,572***	0,96	1,04	1,26
7.	Glutamic acid	***1,605***	***1,793***	***2,150***	2,07	2,19	2,36
8.	Threonine	***0,320***	***0,334***	***0,380***	0,64	0,57	0,72
9.	Serine	***0,361***	***0,357***	***0,594***	0,67	0,65	0,74
10.	Proline	***0,706***	***0,762***	***0,881***	1,66	1,31	1,74
11.	Alanine	***0,604***	***0,570***	***0,494***	1,50	1,16	1,47
12.	Glycine	***0,309***	***0,462***	***0,415***	0,86	0,75	0,91
13.	Valine	***0,391***	***0,430***	***0,437***	0,84	0,70	0,90
14.	Cystine	***0,090***	***0,166***	***0,153***	0,25	0,21	0,18
15.	Methionine	***0,090***	***0,057***	***0,056***	0,34	0,36	0,41
16.	Isoleucine	***0,423***	***0,374***	***0,361***	0,49	0,45	0,53
17.	Leucine	***1,743***	***1,239***	***1,689***	1,29	1,19	1,28
18.	Tryptophan	***x***	***x***	***x***	0,04	0,05	0,05

TABLE 3

Variation of the corn kernel in relation to the hybrid and conditions of cultivation

Influence of :	Size of kernel	Mass	Volume
Hybrid	50,5	86,5	72,5
Soil	6,1	1,7	2,43

TABLE 4

Tetracyclin activity in culture liquid

/%/

No of Probes	K-530	K - 611	H-708	Control
1	182,3	143,26	119,15	100
2	167,8	135,57	108,05	100
3	182,2	153,19	109,93	100
4	179,9	171,64	125,37	100
5	163,1	140,27	108,05	100
6	177,3	153,2	109,92	100
$\bar{x}$ average	174,4	149,35	113,41	100
σ	7,46	11,49	6,55	

decare in the conditions of irrigation do not relate to significant changes in the biochemical composition, have to be considered. The most important factor for the quality of the extract is the presence of the amino acids. However, facts related to the dependence of biosynthesis on a specific amino acid or a particular group of amino acids have not been investigated so far. Our results have shown the possibility for decreesing the quantity of the antibiotics.

CONCLUSIONS

It was established by our studies that the fundamental factor for the quality of the extract is the type of maize hybrid. To introduce simpflications in the manufacturing process of the antibiotics, a new standard has been created which takes into account the requirements of the type of the maize hybrid from which the extract is obtained. In this way the corn maize extract manufactured in

different countries, and by different manufacturers from the same maize hybrid would be with approximately the similar composition.

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