Influence of the Soya Meal Fractions on Gibberellic Acid and Gibberellin A Production in Submerse Cultivation of *Gibberella fujikuroi*

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ABSTRACT

The relationship of different soya meal components to gibberellin (GA) production was studied. Fluorometric assay confirmed that under the given fermentation conditions, only gibberellic acid (GA_3) was synthesized on medium containing corn steep. On substituting soya flour for corn steep, the same amount of GA_3 was produced and in addition gibberellin A (GA_1) was formed. The $GA_3 : GA_1$ ratio was 1 : 1. The course of fermentation in media containing the soya meal protein fraction (fraction I), the soya meal amino acid complex, the corn steep amino acid complex and individual amino acids (γ-aminobutyric acid or tryptophane) was the same as in the control medium containing soya meal. The soya meal fraction II, which is characterized by a high cellulose and carbohydrate content, raised GA production by 25 % as compared with production in medium containling soya meal; it simultaneously stimulated GA_3 production, so that the final $GA_3 : GA_1$ ratio was 4 : 1.

According to some authors (Darken, Jensen & Shu, 1959; Řičicová *et al.*, 1960), corn steep is a suitable nitrogen source for gibberellic acid (GA_3) production by the fungus *Gibberella fujikuroi*. Fuska *et al.*, (1961), however, obtained an average increase of 100% in GA_3 production by substituting soya meal for corn steep. The composition of the fermentation medium influenced the composition, as well as the level, of gibberellin (GA) production (Kitamura *et al.*, 1953; Stodola *et al.*, 1955, Takahashi *et al.*, 1955a, 1957; Calam & Nixon, 1960; Kuhr *et al.*, 1961).

In the experiments submitted below, the authors studied the relationship of some of the components of soya flour to GA production, with special reference to GA_3, which is the biologically most active component (Brian, Hemming & Lowe, 1962).

MATERIALS AND METHODS

Strain and fermentation conditions. The fungus *Gibberella fujikuroi* strain U (Řičicová *et al.*, 1960) was used. Preparation of the fermentation medium was based on the basic medium of Fuska *et al.*, (1961), adding different nitrogen sources. The resultant media, the pH of which was adjusted to 30, were sterilized for 45 minutes at 120° C.

Vegetative inoculum was prepared according to Fuska *et al.*, (1961). For actual fermentation, 500-ml. flasks containing 80 ml. fermentation medium were used, on a rotating shaker apparatus (200 r.p.m., oscillation 5.5 cm.) at 26° C.

Fractionation of soya meal (Thornberry & Shanahan, 1951). Two fractions, I and II, were prepared from soya meal. Fraction I contained the protein components extracted into water at pH 8; these were precipitated from the aqueous solution by lowering the pH to 4.5 by adding 1N HCl and were separated by centrifugation. Fraction II was the fraction not soluble in water; it contained approximately 50% protein, 30% water--nonsoluble carbohydrates and 20% cellulose. When dried, 100 g. of the initial material yielded 33.5 g. fraction I and 31.5 g. fraction II.

Analytical methods. GA_3 and GA_1 were determined quantitatively by fluorometric assay (Theriault *et al.*, 1961) and on a Pulfrich photometer adapted for fluorescence (L-1 red filter, A-5 pre-filter). This method is based on the finding that at 0° C only GA_3 gives a reaction with H_2SO_4 fluorogen, while at 60° C GA_3 and GA_1 both have the same absorption; the value for GA_1 can therefore be obtained by subtracting the value for GA_3 at 0° C from the value for both GA at 60° C. Nitrogen was determined in the ingredient of the medium and in the fermentation medium by the method of Ma and Zuazaga (1942). Carbohydrates were determined as sucrose by Morris's method (1948) and starch and cellulose according to Viles and Silverman (1949). Amino acids were determined qualitatively in corn steep and soya meal according to Zelinka and Hudec (1958). Soya meal hydrolysates and their fractions were prepared by the method of Rackis *et al.*, (1961).

RESULTS AND DISCUSSION

Tab. 1 shows that only GA_3 was formed during fermentation in medium containing corn steep, while in medium containing soya meal a mixture of GA_3 and GA_1 was found after only two days. GA_3 production reached practically the same level in both media and was not influenced by soya meal as compared with GA_3 production in medium containing corn steep. The increase in GA production in medium containing soya meal was thus due entirely to GA_1 synthesis.

The data in the literature (Zelinka &

Table 1. GA_3 and GA_1 production (μg./ml.) in media containing corn steep or soya meal

Added nitrogen source	Time of cultivation (days)					
	2		5		8	
	GA_3	GA_1	GA_3	GA_1	GA_3	GA_1
Corn steep (2%)	44	—	99	—	185	—
Soya meal (1%)	47	41	107	115	187	189

Table 2. GA_3 and GA_1 production (μg./ml.) in basic medium with different nitrogen sources

Added nitrogen source*)	Time of cultivation (days)					
	2		5		8	
	GA_3	GA_1	GA_3	GA_1	GA_3	GA_1
γ-Aminobutyric acid (1%)	32	45	108	102	172	189
Tryptophane (0.5%)	55	—	101	95	188	171
Corn steep amino acid complex (1%)**)	45	47	112	99	182	200
Soya meal amino acid complex (1%)***)	55	52	102	115	178	189
1% soya meal (control)	41	40	109	100	188	179

*) Concentration corresponding to the nitrogen concentration in corn steep (3.9%) or in soya meal (8.05%)
**) Prepared according to Cardinal and Hedrick (1948) and Zelinka and Hudec (1958)
***) Prepared according to Rackis *et al.*, (1961)

Hudec, 1958) and the results of the authors' analyses show that corn steep, as distinct from soya meal, contains γ-aminobutyric acid, while soya meal contains tryptophane (Rackis *et al.*, 1961), which is not found in corn steep. These differences were used as the basis for further experiments, the results of which are given in Tab. 2.

It can be seen from this table that a mixture of GA_1 and GA_3 was obtained in all media containing the given nitrogen sources, amount being approximately the same as that obtained in the control medium containing soya meal. A GA mixture was thus produced both in media containing γ-aminobutyric acid and in media containing the corn steep amino acid complex, i.e. substances contained in corn steep, which itself leads to GA_3 production when contained in the medium (Tab. 1).

These results do not permit definite conclusions to be formed on the relationship of the nitrogen source to formation of the individual GA, but they indicate that the relationship between the amino acid or protein component and the rest of the complex nitrogen source plays a role in the given biosynthesis processes.

There were no significant differences between the proportion of amino acids in the soya meal fractions I and II and in whole soya meal. Fraction II differed from the other nitrogen sources by the presence of water-nonsoluble carbohydrates (34%) and cellulose (19.8%). Fraction I and corn steep did not contained these substances. Soya flour contained roughly half the proportion of these carbohydrates (18.2%) and its cellulose content was much lower (5.2%).

Fig. 1 shows the concentrations of the various nitrogen sources which gave maximum GA production. It can be seen that fraction I gave approximately the same production as soya flour. The GA_3 : : GA_1 ratio likewise did not change and in both cases was 1 : 1. On adding fraction II, however, GA production in the fermentation medium was 25% higher than in the control medium containing soya flour, while the GA_3 : GA_1 ratio underwent a marked change (4 : 1, Fig. 1).

Since the ratio of the carbon source to the nitrogen source in the production phase plays a significant role in GA_3 synthesis (Borrow, Jefferys & Nixon,

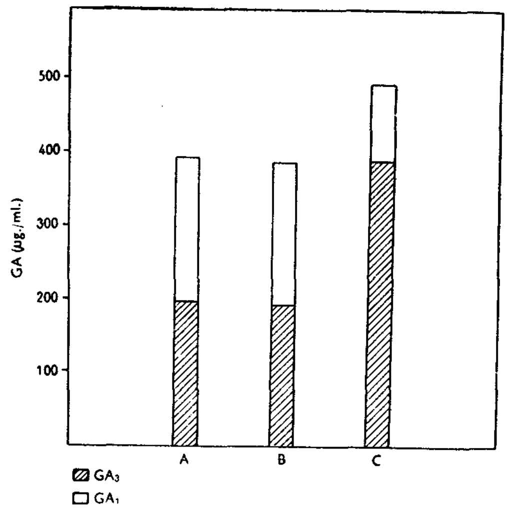

Fig. 1. Maximum GA, GA_3 and GA_1 production on media containing soya meal or fractions I and II. A — soya meal (1%), B — fraction I (0.4%), C — fraction II (1.2%).

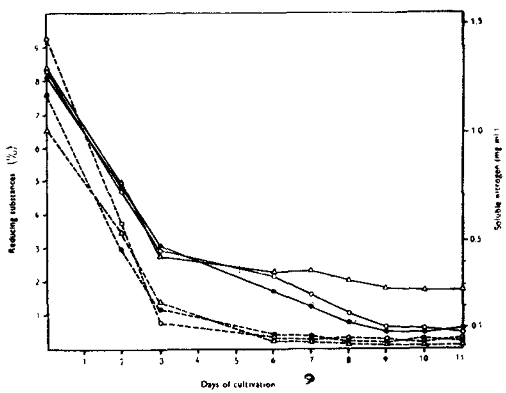

Fig. 2. Levels of reducing substances (unbroken lines) and soluble nitrogen (broken lines) in media containing different nitrogen sources, ○—○ — medium with soya meal, ●—● — medium with fraction I, △—△ — medium with fraction II.

1959), it was assumed that cellulose and carbohydrates, as slowly assimilated carbon sources, maintained a relatively higher level of reducing substances during fermentation. According to the results illustrated in Fig. 2, the concentration of reducing substances in medium containing fraction II remained practically unchanged during the phase decisive for GA production, i.e. from the 6th to 10th day of fermentation, while in the other media a permanent decrease in reducing substances was noted. A constant C : N ratio with a suitable concentration of reducing substances was thus maintained only in medium containing fraction II (Fig. 2). According to the results of Darken *et al.*, (1959), GA_3 synthesis can be stimulated by maintaining the concentration of reducing substances at a given level (about 2%), e.g. by adding sucrose to the medium during fermentation. This provides the possibility of comparing the course of fermentation in medium containing fraction II with the course of fermentation characterized by the addition of sucrose (bearing in mind the differences in regulation of the concentration of reducing substances). The results indicate that the high cellulose and carbohydrate concentration in fraction II was largely responsible for stimulation of GA_3 production, by maintaining an optimal level of reducing substances in the medium.

In corn steep or soya meal media in which the concentration of reducing substances was artificially maintained at about 2% by adding sucrose, GA_3 production (both alone and together with GA_1) was stimulated (Fig. 3). The increase in GA_3 production was not so pronounced, however, as in medium containing fraction II.

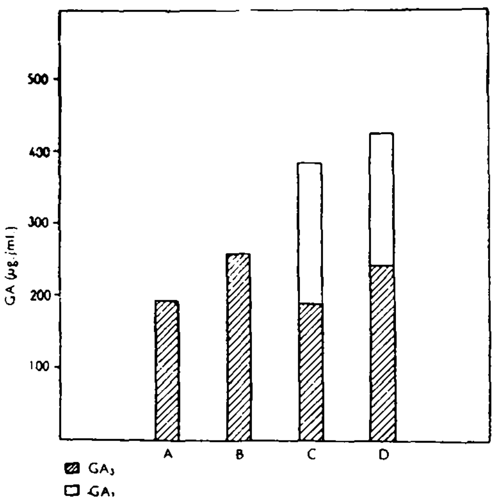

Fig. 3. Maximum GA, GA_3 and GA_1 production on media containing corn steep or soya meal, with continuous maintenance of the sucrose concentration (2%). A — corn steep (2%), B — corn steep (2%) + maintained sucrose concentration, C — soya meal (1%), D — soya meal (1%) + maintained sucrose concentration.

References

Borrow, A., Jefferys, E. G., Nixon, I. S.: *Process of producing gibberellic acid by cultivation of Gibberella fujikuroi.* U.S.A. Patent 2906671, 1959.

Brian, P. W., Hemming, H. G., Lowe, D.: *Relative activity of the gibberellins.* Nature 193 : 946, 1962.

Calam, Ch. T., Nixon, I. S.: *A process for the manufacture of gibberellic acid.* Brit. Patent 839652, 1960.

Cardinal, E. V., Hedrick, L. R.: *Microbiological assay of corn steep liquor for amino acid content.* J. biol. Chem. 172 : 609, 1948.

Darken, Marjorie, A., Jensen, A. L., Shu, P.: *Production of gibberellic acid by fermentation.* Appl. Microbiol. 7 : 301, 1959.

Fuska, J., Kuhr, I., Podojil, M., Ševčík, V.: *The influence of the nitrogen source on the production of gibberellic acid in submerse cultivation of Gibberella fujikuroi.* Fol. microbiol. 6 : 18, 1961.

Kitamura, H., Kawarada, A., Seta, Y., Takahashi, N., Otsuki, T., Sumiki, Y.: *The biochemistry of bakanae fungus. Part XXVII. The production of gibberellin by submerged culture (III).* J. Agric. chem. Soc. Japan 27 : 545, 1953.

Kuhr, I., Fuska, J., Podojil, M., Ševčík, V.: *Factors affecting the production of various gibberellins during submerge cultivation of Gibberella fujikuroi.* Fol. microbiol. 6 : 179, 1961.

Ma, T. S., Zuazaga, G.: *Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method.* Ind. Eng. Chem., Anal. Ed. 14 : 280, 1942.

Morris, D. L.: *Quantitative determination of carbo-*

hydrates with Dreywood's anthrone reagent. Science 107 : 254, 1948.

Rackis, J. J., Anderson, R. L., Sasame, H. A., Smith, A. K., Van Etten, C. H.: *Amino acids in soybean hulls and oil meal fraction.* J. Agric. Food Chem. 9 : 409, 1961.

Řičicová Alena, Podojil, M., Musílek, V., Ševčík, V.: *Laboratory fermentation of gibberellic acid.* Fol. microbiol. 5 : 181, 1960.

Stodola, F. H., Raper, K. B., Fennell, D. I., Conway, H. F., Sohns, V. E., Langford, C. T., Jackson, R. W.: *The microbiological production of gibberellins A and X.* Arch. Biochem. Biophys. 54 : 240, 1955.

Takahashi, N., Kitamura, H., Kawarada, A., Seta, Y., Takai, M., Tamura, S., Sumiki, Y.: *Biochemical studies on bakanae fungus. Part XXXIV. Isolation of gibberellins and their properties.* Bull. agric. chem. Soc. Japan 19 : 267, 1955.

Takahashi, N., Seta, Y., Kitamura, H., Sumiki, Y.: *Biochemical studies on the bakanae fungus. Part XLII. A new gibberellin, gibberellin A_4.* Bull. agric. chem. Soc. Japan 21 : 396, 1957.

Theriault, R. J., Friedland, W. C., Peterson, M. H., Sylvester, J. C.: *Fluorimetric assay for gibberellic acid.* J. Agric. Food Chem. 9 : 21, 1961.

Thornberry, H. H., Shanahan, A. J.: *Streptomycin production by Streptomyces griseus from fractions of peanuts and soybeans.* Arch. Biochem. Biophys. 33 : 459, 1951.

Viles, F. J., Silverman, L.: *Determination of starch and cellulose with anthrone.* Analyt. Chem. 21 : : 950, 1949.

Zelinka, J., Hudec, M.: *K otázke aminokyselín vo fermentačných pôdach (I). Identifikacia kyseliny γ-aminomaslovej v kukuričnom výluhu.* Chem. zvesti 12 : 620, 1958.

ВЛИЯНИЕ ФРАКЦИЙ СОЕВОЙ МУКИ НА ОБРАЗОВАНИЕ GA_3 И GA_1 ПРИ ГЛУБИНОЙ КУЛЬТИВАЦИИ GIBBERELLA FUJIKUROI

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Контролировалось отношение различных компонентов соевой муки к образованию гиббереллинов (GA). Флуорометрическим методом было подтверждено, что при данных условиях ферментации в среде с кукурузным экстрактом возникает только гибберелловая кислота (GA_3). Если же кукурузный экстракт заменяли соевой мукой, образовались такие же количества GA_3, как в среде с кукурузным экстрактом, и сверх того еще гиббереллин A (GA_1). Соотношение $GA_3 : GA_1$ равнялось 1 : 1. Ферментация в среде с белковой фракцией соевой муки (фракция I), с комплексом аминокислот соевой муки или кукурузного экстракта и с отдельными аминокислотами, т. е. с аминомасляной кислотой или триптофаном, проходила так же, как и в контрольной среде с соевой мукой. Фракция II из соевой муки, отличающаяся высоким содержанием целлюлозы и углеводов, повышала образование GA по сравнению с его образованием в среде с соевой мукой на 25% при одновременном повышении образования GA_3, так что окончательное соотношение $GA_3 : GA_1$ равнялось 4 : 1.