

# Production of Gibberellic Acid by Fermentation

MARJORIE A. DARKEN, A. L. JENSEN, AND P. SHU

*Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York*

Received for publication February 20, 1959

The elongation of rice plants induced by the application of cell-free culture filtrates from the liquid culture of *Fusarium moniliforme* was first demonstrated in 1926 by Kurosawa. Twelve years later, Yabuta and Sumiki (1938) were able to isolate in crystalline form two growth-promoting compounds, gibberellins A and B, from filtrates of this organism; gibberellin A was the main substance obtained. In 1954, Curtis and Cross reported the discovery of a third metabolite, gibberellic acid, from a culture filtrate of *Gibberella fujikuroi*, the perfect stage of *F. moniliforme*. This finding was confirmed the following year by Stodola *et al.* (1955) and Takahashi *et al.* (1955). Brian and Grove (1957) have written an excellent comprehensive review of the gibberellins with emphasis placed upon gibberellic acid. It is this compound with which we are concerned at this time. A complete literature review has been prepared by Stodola (1958).

The strain of *Fusarium moniliforme* strain Kew no. 917, which we used for these investigational studies, was the one reported by Borrow *et al.* (1955) to give the highest yield of gibberellic acid. With this culture and a medium composed of glucose, 40 g per L; ammonium tartrate, 9.5 g per L; monopotassium phosphate, 2.0 g per L; potassium sulfate, 0.6 g per L; and magnesium sulfate, 0.2 g per L; they obtained 180 mg per L gibberellic acid. The yields obtained by Borrow *et al.* in a medium used by Yabuta, Sumiki, and Uno (1939) consisting of glycerol or glucose, ammonium chloride, and monopotassium phosphate were negligible with all of the Kew cultures.

The former value of 180 mg per L gibberellic acid is substantially greater than that of 22 mg per L reported by Stodola *et al.* (1955) in a medium consisting of glucose, 15 g per L; monopotassium phosphate, 3 g per L; ammonium chloride, 3 g per L; and magnesium sulfate, 3 g per L. Using the Kew no. 917 culture we obtained gibberellic acid yields of 880 mg per L in shaker flasks and 650 mg per L in 1000-gallon fermentors.

## EXPERIMENTAL METHODS

### *Fermentation*

The stock strain of *Fusarium moniliforme* was obtained from the Commonwealth Mycological Institute at Kew as no. 917. Subcultures were carried at 28 C on potato-glucose agar slants and transfers were made directly to the inoculum flasks. The inoculum flasks

contained 100 ml sterile medium of the following composition:

Corn steep liquor.....	15 g per L
Sucrose.....	30 g per L
Ammonium sulfate.....	2 g per L
Calcium carbonate.....	7 g per L

The inoculated flasks were incubated at 28 C for 24 to 48 hr on a reciprocating shaker with 2-in. stroke at 104 cycles per min. Four per cent by volume of the inoculum was used in flask experiments and 1 per cent two-stage inoculum was used for tank studies.

The basal fermentation medium contained 25 g of corn steep liquor, 1 g ammonium sulfate, and 0.5 g potassium dihydrogen phosphate per L. Flask fermentations were conducted in 250-ml Erlenmeyer flasks containing 50 ml medium at 28 C on a rotary Gump<sup>1</sup> shaker running at 240 rpm. In general, the fermentations were extended for a 7-day period.

Deep tank fermentations were carried out in 50-gallon, 650-gallon, and 1000-gallon fermentors with an appropriate degree of aeration and agitation. The temperature was kept at 30 C throughout the 6- or 7-day fermentation period.

## CHEMICAL ANALYSES

*Gibberellic acid.* Whole broth samples were centrifuged at 2000 rpm for 10 min, and photofluorometric assay determinations were carried out in accordance with the following unpublished method of Dr. J. Pierce of these laboratories. (A similar method has been reported by Theriault *et al.* (1958).)

1. Dilute centrifuged broths with water to obtain an estimated concentration of 5 to 10  $\mu$ g per ml.

2. Add 0.1 ml of diluted broths to test tubes and dry under vacuum.

3. Add 0.5 ml of 83.3 per cent sulfuric acid in absolute alcohol, and mix thoroughly by rolling.

4. Heat tubes in 75 C water bath for 1 min, cool in tap water.

5. Add 10 ml of 28.6 per cent sulfuric acid and mix vigorously.

6. Read in Coleman<sup>2</sup> photofluorometer Model No. 12A after standing a minimum of 10 min.

<sup>1</sup> B. F. Gump Co., Chicago, Illinois.

<sup>2</sup> Coleman Instruments, Inc., Maywood, Illinois.

7. The standard curve may be prepared from a sample of pure gibberellic acid. For routine work, a fluorometrically equivalent amount of quinine solution prepared in 0.1 N sulfuric acid may be used in place of the gibberellic acid standard.

*Total carbohydrate.* The centrifuged broth samples were diluted, and the total carbohydrates were determined by the anthrone method of Morris (1948).

### RESULTS

Our medium for the fermentation of gibberellic acid differs from that of Borrow *et al.* (1955) in the addition of a slowly utilized carbon source and corn steep liquor. Although the organism grows rapidly, the production of gibberellic acid is relatively slow with maximum yields obtained in 7 to 8 days with the media studied. The slowly utilized carbon source apparently permits more effective production of gibberellic acid after the maximum rate of growth has been obtained with the rapidly utilized glucose, sucrose, or corn starch. The yields obtained in shaker flasks with various carbon sources are presented in table 1. This table shows that the highest amount of gibberellic acid was produced with glycerol, 20 g per L; glucose, 10 g per L; and lactose, 20 g per L. Using these carbon sources, yields in shaker flasks averaged 880 mg per L.

Pilot tank fermentations were comparable to those in shaker flasks, and media which had proved superior in the laboratory were also found to give better yields in the fermentors. The carbon source which permitted

TABLE 1  
*Gibberellic acid yields in shaker flasks*

Carbon Source				Avg Gibberellic Acid Yield
Starch	Glycerol	Glucose	Lactose	
g/L				mg/L
30	20	0	0	590
0	20	0	0	220
0	30	0	0	360
0	60	0	0	695
0	20	50	0	540
0	20	0	50	330
0	20	20	30	805
0	20	10	20	880

TABLE 2  
*Gibberellic acid yields in shaker flasks and tanks*

Carbon Source				Avg Flask Yield	Avg Tank Yield
Starch	Glycerol	Glucose	Lactose		
g/L				mg/L	mg/L
30	0	0	0	400	380
30	20	0	0	590	515
30	30	0	0	780	650
0	20	30	20	805	570

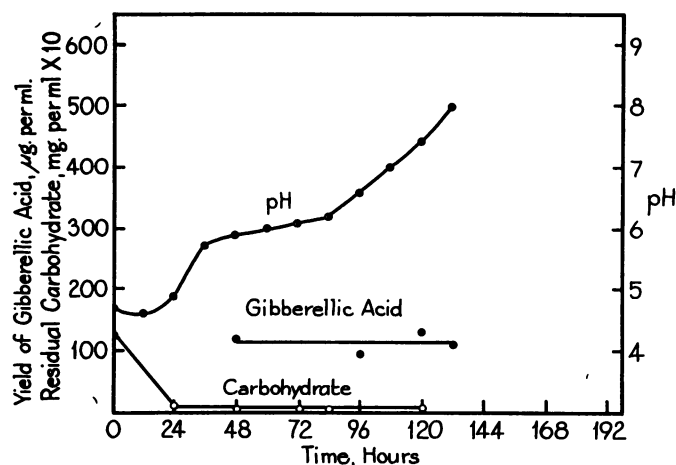


Figure 1. Chemical changes in the control medium. The control medium contained 25 g corn steep liquor, 1 g ammonium sulfate, 0.5 g potassium dihydrogen phosphate, and 20 g glucose per L.

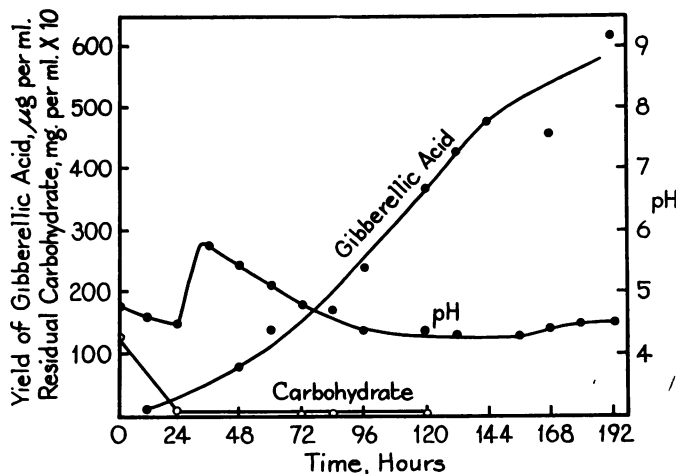


Figure 2. Chemical changes in the control medium with the addition of glucose at a rate of 0.02 per cent per hr commencing at 24 hr.

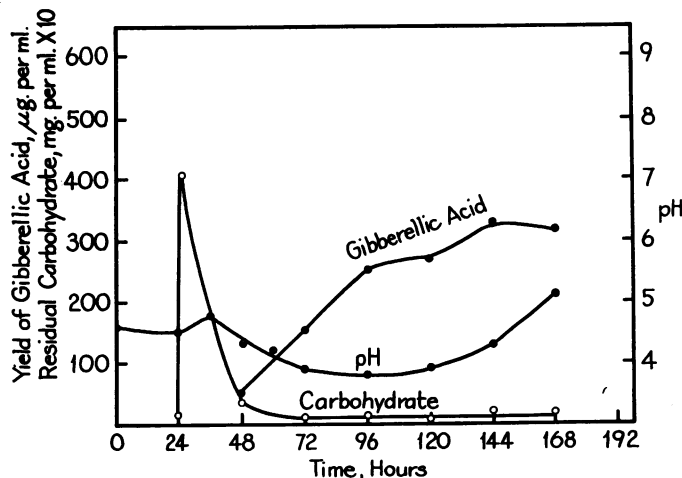


Figure 3. Chemical changes in the control medium with 3 per cent glucose added after 24 hr.

the highest yield of gibberellic acid under our conditions of tank fermentation was glycerol, 30 g per L, and starch, 30 g per L. Table 2 presents comparative data obtained with various media in shaker flasks and in 650- or 1000-gallon fermentors.

To establish the beneficial effect of a slowly utilized or constantly available carbon source, glucose-feed experiments were carried out in 50-gallon fermentors. A basic corn steep liquor-ammonium sulfate-monopotassium phosphate medium with 2 per cent glucose was used in one tank; in the second tank, glucose was fed to this base medium at the rate of 0.02 per cent per hr commencing at 24 hr; and in the third tank, 3 per cent glucose was added to the basic medium at 24 hr (this amount is equivalent to that fed during the slow-feed run). The chemical changes during these tank fermentations are presented in figures 1, 2, and 3. It can be seen from these figures that the slow-feed experimental fermentation produced more gibberellic acid (620 mg per L) than did either of the other two (130 and 320 mg per L).

The pH patterns encountered in these three tank fermentations differed sharply. With glucose added initially, the pH rose from 4.5 to values above 5.5 in 32 hr when sugar could no longer be detected in the fermentation broth; a pH of 8.0 was reached in 132 hr. With the slow feed, the pH rise was comparable for the first 32 hr but subsequently there was a gradual pH drop to 4.5. When the extra glucose was added at 24 hr, the pH did not rise early in the cycle but remained at 4.5 with a gradual drop to 3.8 at 100 hr; this in turn was followed by a gradual rise to 5.0 at 168 hr. Sugar was not detected after 70 hr in this fermentation. With the glycerol medium, the pH started around 4.8 and gradually dropped to 4.0 at the end of the fermentation, thus giving a pH pattern resembling, in general, that obtained with the slow feeding of a rapidly utilized carbon source.

#### DISCUSSION

The main objectives of the present study have been attained, namely, the development of an improved medium composed of readily available materials and the establishment of fermentation conditions which would give consistently high yields of gibberellic acid.

The results of these studies offer further evidence for the importance of preferential carbon sources, as well as of other required nutrients, in the production

of gibberellic acid. Laboratory investigations were readily scaled up to tank fermentations.

#### ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Messrs. Thomas C. Boyd and Lawrence Wolcott.

#### SUMMARY

Details of the fermentation of gibberellic acid with *Fusarium moniliforme* have been presented and yields of 650 mg per L have been obtained in 1000-gallon fermentors. Experimental results indicate that a slowly utilizable carbon source is desirable for the production of high yields of gibberellic acid.

#### REFERENCES

- BORROW, A., BRIAN, P. W., CHESTER, V. E., CURTIS, P. J., HEMMING, H. G., HENEHAN, C., JEFFREYS, E. G., LLOYD, P. B., NIXON, I. S., NORRIS, G. L. F., AND RADLEY, M. 1955 Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*: some observations on its production and isolation. *J. Sci. Food Agr.*, **6**, 340-348.
- BRIAN, P. W. AND GROVE, J. F. 1957 Gibberellic acid. *Endeavour*, **16**, 161-171.
- CURTIS, P. J. AND CROSS, B. E. 1954 Gibberellic acid. A new metabolite from the culture filtrates of *Gibberella fujikuroi*. *Chem. & Ind.*, p. 1066.
- KUROSAWA, E. 1926 Experimental studies on the nature of the substance excreted by "bakanae" fungus. *Trans. Nat. Hist. Soc. Formosa*, **16**, 213-227.
- MORRIS, D. L. 1948 Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science*, **107**, 254-255.
- STODOLA, F. H. 1958 *Source book on gibberellin* ARS-71-11. Agricultural Research Service, U. S. Department of Agriculture, pp. 1827-1957.
- STODOLA, F. H., RAPER, K. B., FENNEL, D. I., CONWAY, H. F., SOHNS, V. E., LANGFORD, C. T., AND JACKSON, R. W. 1955 The microbiological production of gibberellins A and X. *Arch. Biochem. & Biophysics*, **54**, 240-245.
- TAKAHASHI, N., KITAMURA, H., KAWARADA, A., SETA, Y., TAKAI, M., TAMURA, S., AND SUMIKI, Y. 1955 Biochemical studies on "bakanae" fungus. Part XXXIV. Isolation of gibberellins and their properties. *Bull. Agr. Chem. Soc., Japan*, **19**, 267-277.
- THERIAULT, R. J., FRIEDLAND, W. C., PETERSON, M. H., AND SYLVESTER, J. C. 1958 A fluorometric assay for gibberellic acid. *Abst. of papers, 134th Meeting American Chemical Society, Chicago, Illinois*.
- YABUTA, T. AND SUMIKI, Y. 1938 The crystallization of gibberellins A and B. *J. Agr. Chem. Soc., Japan*, **14**, 1526.
- YABUTA, T., SUMIKI, Y., AND UNO, S. 1939 The biochemistry of the "bakanae fungus". IV. Cultural conditions of *Gibberella fujikuroi* for the production of gibberellin and fusaric acid. *J. Agr. Chem. Soc., Japan*, **15**, 1209-1220.