Effect of Agitation on RNA Production by Bacillus mesentricus niger

Hara, Toshio
Laboratory of Microbial Technology, Faculty of Agriculture, Kyushu University

Ueda, Seinosuke
Laboratory of Microbial Technology, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23741

出版情報：九州大学大学院農学研究院紀要. 26 (2/3), pp.79-86, 1982-02. Kyushu University
バージョン：
権利関係：

J. Fac. Agr.. Kyushu Univ., 26 (2 . 3), 79-86 (1982)

Effect of Agitation on RNA Production by *Bacillus mesentricus niger*

Toshio Hara and Seinosuke Ueda

Laboratory of Microbial Technology, Faculty of Agriculture,
Kyushu University 46-09, Fukuoka 812

(Received August 28, 1981)

Sufficient oxygen supply was one of the most important culture conditions on RNA production by *Bacillus mesentricus niger No. 6021.* RNA productivity was markedly increased at a 850-rpm agitation speed and control of the broth-pa at around 7.0, and the maximum yield of RNA (5.7 mg/ml) was obtained with a 5-Z jar fermentor. However, insufficient oxygen supply markedly inhibited RNA accumulation. The decrease of the rate of oxygen supply caused the change in the amount and the kind of the products, and 93.6 % of the precipitate obtained under insufficient oxygen supply was found to be Gln.

INTRODUCTION

In 1961, culture methods of yeast were established from the sulfite pulp waste liquor (Miwa, 1952; Yamada *et al., 1953)* and phosphoesterase which degrade the yeast's RNA to 5' nucleotide were isolated from microorganisms (Kuninaka, 1961). In these days, RNA of *Saccharomyces cerevisiae* and *Candida utilis* has industrial uses as a source of 5'-nucleotides (5'-IMP and 5'-GMP) as flavor-enhancing materials and in the preparation of drugs and antibiotics (Naruse, 1975).

The accumulation of nucleotides or related compounds in the culture broth of various microorganisms has been demonstrated (Okabayashi and Masuo, 1960; Higuchi *et al., 1962;* Imada *et al.,* 1962; Ogata *et al.,* 1962; Arima *et al., 1963;* Demain *et al.,* 1964 a). However, little has been published on excretion of intact RNA by microorganisms. Only Demain *et al.* (1964 b, 1965) reported that RNA was excreted by *Bacillus subtilis* cells which had not undergone cell lysis.

In our previous paper (Hara and Ueda, 1981), we reported that B. *mesentricus niger No.* 6021 accumulated a large amount of RNA in the culture broth extracellularly.

Since the biosynthesis of nucleic acid related compounds from a carbon source requires much energy (Magasanik, 1962), sufficient oxygen must be supplied to fermentation broth in order to make the cell accumulate a large amount of such metabolites from a carbon source. The purpose of aeration and agitation in fermentors are, firstly to supply microorganisms with oxygen, and secondly, to mix fermentation broths in such a way that a uniform suspension of microbes is achieved and the mass-transfer rate of the metabolic

products accumulated. Therefore, studies on agitation are essential in this fermentation.

The present paper deals with the effects of agitation on RNA production by *B. mesentricus niger.*

MATERIALS AND METHODS

Microorganism

Bacillus mesentricus niger No. 6021 derived from IFO 3214 with 100 μg/ml of N-methyl-N'- nitro-N-nitrosoguanidine for 15 min at 37°C in Tris(hydroxymethyl)aminomethane-malate buffer at pH 6.0 was used throughout this study as a RNA-producing bacteria.

Medium and culture conditions

Medium was composed of 8 % maltose, 1.2 % sodium citrate•$2H_2O$, 2 % $(NH_4)_2HPO_4$, 0.15 % KCI, 0.05 % $MgSO_4$•$7H_2O$, 0.015 % $CaCl_2$•$2H_2O$, 0.015 % $CuSO_4$•$5H_2O$ and 0.0015% $MnSO_4$•$4H_2O$, and pH was adjusted to 8.0.

Seed culture in a 500-ml shaking flask containing 50ml of medium was incubated on a reciprocal shaker (120 strokes/min; pitch, 50mm) for 2 days at 30°C and transferred into the fermentation culture at a ratio of 5 %. Cultures were carried out in a 5-*l* jar fermentor containing 3 liters of medium at an aeration rate of 1.0 vvm and agitation speed of 200 to 1,000 rpm at 37°C for 3 days, depending on the experiments.

Analytical methods

(1) Bacterial growth was monitored by measuring the optical density (OD) at 660nm of appropriately diluted culture broth with water. (2) Samples of precipitates obtained by adding three volumes of 95 % ethanol to the supernatant solution of culture broth were subjected to RNA assay by orcinol (Mejbaum, 1939) with yeast RNA as standard. (3) Sugars were estimated by the method of Morris (1948). (4) Ultraviolet (UV) absorbancy at 260 nm were measured with a Hitachi 124 spectrophotometer with l-cm light path.

Thin-layer chromatography of the hydrolyzates

Samples of precipitates obtained by adding three volumes of 95 % ethanol to the supernatant solution of culture broth were hydrolyzed with 6N HCl for 1 hr at 100°C. Thin-layer chromatography of the hydrolyzates was carried out by ascending technique at room temperature on cellulose plate (Avicel, Funakoshi Chemical Co.) for 2.5 hr. The solvent system used was *n*-butanol-acetic acid-water (12 : 3 : 5). After development and drying, the spots were estimated by spraying with methanol containing 0.4 % ninhydrin. The quantity of amino acids was measured by using Densitol DMU-33C (Tokyo Science Co.).

Procedure of spore formation

Culture broth was heated at 100°C for 10 min or 30min to kill vegitable cells, and plated by spreading on nutrient agar plates and then incubated

overnight at 37°C.

RESULTS

Effect of aeration in shaking culture

An experiment was designed to study the relation between the aeration as a culture condition and the productivity of RNA. The experiments were carried out at several aeration levels in a 500-ml shaking flask with varied working volume of the medium. Table 1 shows that a high level of aeration resulted in a good growth and a high yield of RNA. It was evident that the optimum volume of the medium was 25 ml per 500-ml shaking flask within the range of this experiment.

Effect of agitation in a jar fermentor

In the previous studies (Hara and Ueda, 1981), it was observed that strain No. 6021 produced a considerable amount of extracellular RNA in a shaking culture, and that aeration was one of the most important culture conditions on RNA production. The effect of agitation on RNA production were examined with a 5-*l* jar fermentor to establish the more efficient production methods.

As shown in Fig. 1, bacterial growth and RNA productivity increased with

Table 1. Effect of aeration on RNA production. Cultivation was carried out in a 500-ml shaking flask under the conditions as described in Materials and Methods.

Volume in flask	OD_{660}	OD_{260}	RNA (mg/ml)
25ml	76.0	103.4	3.a
50			2.7
75	64.5	86.0 86.0	2.4
	50.3		2.5
100	46.4	94.0	2.3

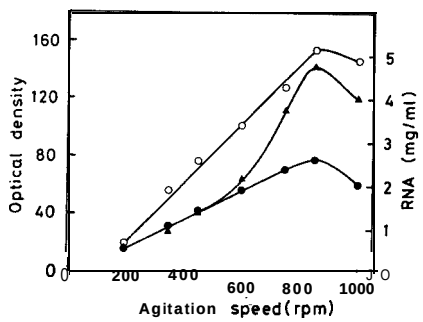

Fig. **1.** Effect of agitation speed on RNA production. Cultivation was carried out at various agitation speeds for 4 days at 37°C with a 5-liter jar fermentor. Symbols : ●, OD_{660}; ○, OD_{260}; ▲, RNA.

increasing agitation speed. Bacterial growth increased up to 850rpm linearly and RNA productivity was also observed hasty rise similarly. It is concluded that a 850-rpm agitation speed is appropriate for the production of RNA. Figure 2 shows the time courses of bacterial growth and RNA production at different agitation speeds of 200rpm and 850 rpm, respectively. At 200 rpm (Fig. 2--A), bacterial growth was repressed and consequently RNA productivity was very low. As shown in Fig. 2-B, bacterial growth and RNA productivity at agitation speed of 850rpm were increased several times as compared with

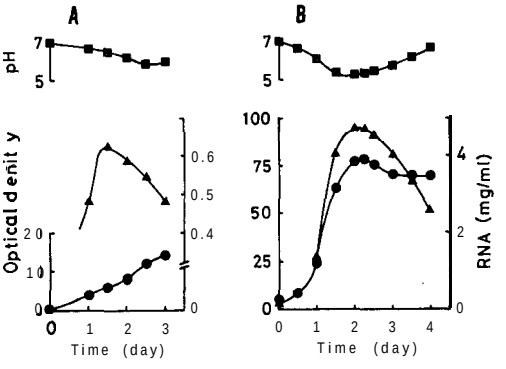

Fig. 2. Time course of RNA production by strain No. 6021. Cultivation was carried out in a 5-liter jar fermentor at 200-rpm agitation speed (A) or at 850-rpm (B) for 4 days at 37°C. Symbols: ●, OD_{660}; ▲, RNA; ■ , pH.

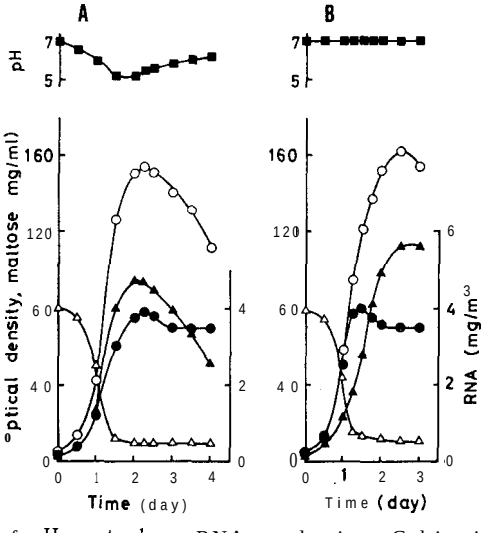

Fig. 3. Effect of *pH-control* on RNA production. Cultivation was carried out by a jar fermentor (aeration rate 1.0 vvm, agitation speed 850 rpm) at 37°C for 4 days. Symbols: (A) pH not controlled; (B) pH maintained at 7 with 4N NaOH or 4N H_2SO_4; ●, OD_{660}; ○, OD_{260}; ▲, RNA; △, maltose; ■ , pH.

those at 200 rpm, respectively. The yield of RNA at 850rpm reached to 4.8 mg per ml of culture broth for 48 hr.

Time course of RNA production with pH-control

The pH of the culture broth decreased to around 5 accompanying bacterial growth and owing to exhaustion of available sugar, and then cell lysis occurred. Excretion of the materials absorbing at 260 nm preceeded the growth of cell closely, and RNA production was also parallel with cell growth. The pH of this culture gradually increased to about 6. The productivity of RNA decreased with increasing the pH of the culture (Fig. 3-A).

When the pH of such a culture was maintained throughout at around 7 with 4 N NaOH or 4 N H_2SO_4, there was no significant cell growth. But, rate of bacterial growth (0. 135 hr^{-1}) took place more rapidly as compared with that (0.085 hr^{-1}) of pH non-control. However, RNA production was allowed to continue for 72 hr after cell lysis and the amount of RNA produced was 5.7 mg/ml (Fig. 3-B).

Conversion of microbial products at different agitation speeds

The yield of RNA at agitation speed of 200rpm was one-seventh as compared with that of 850 rpm, as shown in Fig. 1, but this culture broth was found to become sticky. On fhe other hand, this phenomenon of stickness was not observed at high-speed agitation ranges. In this fermentation, the biosynthesis of RNA from maltose requires ATP yielded by energy metabolism, which is sensitive to oxygen deficiency (Table 1 and Fig. 1). Therefore, some intermediate metabolites of maltose degradation might be implied to be accumulated under insufficient oxygen supply.

We examined spore formation at different agitation speeds, as shown in Table 2. Strain No. 6021 formed so many spores during the fermentation was delayed and RNA was less produced. The sticky materials obtained from culture broth were subjected to thin-layer chromatography, and the Rf values of hydrolyzates of isolated materials is shown in Table 3. Twelve spots were detected from the hydrolyzate at 850rpm and among them, especially, alanine mounted up to about 21.4%. But, at 200 rpm, only two spots corresponding to glutamine and glutamic acid were detected, and the amount of glutamine reached to about 93.6 %.

Table 2. Effect of agitation speed on spore formation. Samples were plated on nutrient agar plates with or without heating culture broth. Time indicates heating period of culture broth.

Colony former	Colony/ml	
	Agitation speed	
	200 rpm	850 rpm
Viable cell	4.5×10^8	2.8×10^9
Spore, 10 min	4.1×10^2	2.7×10^2
30 min	1.7×10^2	1.0×10^2

Table 3. Rf value of amino acids from hydrolyzates of culture broth at two different agitation speeds. Hydrolyzates obtained by adding 95% ethanol to the culture broth after growing the organism at 85C rpm agitation speed (A) and at 200rpm (B). Hydrolysis was performed with 6 N HCl for 1 hr at 100°C.

Amino acid	Rf value[a]		
	Hydrolyzate A	Hydrolyzate B	Standard
cys-cys	0.21 (8.4)	N. D.[b]	0.19
Lys	0.27 (7.4)	N. D.	0.26
Arg	N. D.	N. D.	0.30
Asn	N. D.	N. D.	0.31
Gln	0.32 (11.9)	0.32 (93.6)	0.32
His	N. D.	N. D.	0.33
Gly	N. D.	N. D.	0.35
Asp	N. D.	N. D.	0.39
Ser	N. D.	N. D.	0.39
Hyp	0.41 (0.9)	N. D.	0.41
Glu	N. D.	0.44 (5.9)	0.44
Thr	0.48 (8.7)	N. D.	0.45
Ala	0.55 (21.4)	N. D.	0.56
Pro	N. D.	N. D.	0.58
Tyr	0.65 (3.8)	N. D.	0.66
Trp	N. D.	N. D.	0.71
Val	0.74 (2.0)	N. D.	0.74
Met	N. D.	N. D.	0.75
Phe	0.82 (5.4)	N. D.	0.84
Ile	0.87 (11.1)		
Leu	0.94 (4.8)	N. D.	0.86 0.90

[a] The numeral in parenthesis indicates the percentage present in hydrolyzate
[b] Not detected.

DISCUSSION

The influential factors in the production of RNA were investigated by using strain No. 6021 derived from ***B. mesentricus niger*** IFO 3214. Production of RNA was markedly increased when the fermentation was performed at 850-rpm agitation speed. But, at 54 hr of cultivation, RNA production suddenly stopped and the productivity of RNA began decrease. This was the point where carbon source was exhausted and pH began to rise sharply. However, the replenishment of carbon source did not increase RNA production anymore (data not shown).

This result suggested that the control of pH was important to enhance RNA productivity. Therefore, the broth-pH was tried to maintain at around 7 with 4 N NaOH or 4 N H_2SO_4. The abrupt diminution of RNA productivity was prevented, and the maximum yield of RNA (5.7 mg/ml) was obtained. From the above findings, the presence of active RNase was suspected in the culture broth at around 5.

This amount of RNA produced by strain No. 6021 is 4.5 times higher than that in the case of ***B. subtilis*** reported by Demain ***et*** al. (1965). However, UV-absorbancy was decreased with degragation of excreted RNA after cell lysis.

This suggested that nucleotides were not accumulated as the degradation products of RNA, which is mentioned by Demain *et al.* (1964 b), and in the case of strain No. 6021, probably bases themselves were degraded.

Insufficient oxygen supply markedly inhibited RNA accumulation, even though maltose in the medium was almost assimilated (data not shown). This fact suggests that some other product might be excreted in place of RNA. In glutamic acid fermentation, the decrease of the rate of oxygen supply caused the change in the amount and the kind of the products (Okada *et al.*, 1961). In this fermentation, some intermediate metabolite of maltose degradation was implied to be accumulated under insufficient oxygen supply.

The biosynthesis of RNA resulted in the sensitivity of the productivity to oxygen deficiency since energy metabolism to yield ATP is extremely labile to anaerobic condition. Strain No. 6021 possessed sporulating property. Because of this property, this strain formed so many spores during the fermentation that the fermentation was delayed and RNA was less produced. The sporulation was strictly depended on the oxygen supply condition during fermentation and its causes decrease of RNA yield.

In RNA fermentation, aeration and agitation were discussed from the following standingpoint; the maintenance of the dissolved oxygen above the critical level for the cell to satisfy their oxygen demand.

REFERENCES

Arima, K., T. Fukami. M. Fujiwara, K. Yokota and G. Tamura 1963 Accumulation of nucleic acid derivatives by microorganism Part I. Studies for the accumulation by strain of stock cultures and mutant strains. ***J. Agric. Chem. Soc. Japan, 37: 453-463***

Demain, A. L., R. W. Burg and D. Hendlin 1965 Excretion and degradation of ribonucleic acid by ***Bacillus subtilis. J. Bacterior., 89:*** 640-646

Demain, A. L., I. M. Miller and D. Hendlin 1964 a Production of extracellular guanosine 5′-monophsphate by ***Bacillus*** *subtilis.* ***J.*** *Bacteriol.*, ***88*** : 991-995

Demain, A. L., R. A. Vitali, B. L. Wilker, J. W. Rothrock and T. A. Jacob 1964 b Extracellular guanosine 5′-monophosphate and guanosine 5'-diphosphate in ***Bacillus subtilis*** broths. ***Biotechnol.*** Bioeng.. 6: 361-365

Hara, T. and S. Ueda 1981 RNA production by ***Bacillus mesentricus*** *niger.* ***J. Ferment. Technol., 59: 341-346***

Higuchi, M.. H. Tanaka and T. Uemura 1962 Secretion of nucleotides by yeast cells Part V. Release of ultraviolet absorbing materials from growing yeast cells in the medium containing citrate buffer. ***J. Agric. Chem.*** *Soc.* ***Japan, 36: 971-977***

Imada, A., Y. Nakao and K. Ogata 1962 Excretion of 5′-nucleotides by bacteria Part III. Degradation of ribonucleic acid in a ***Bacillus*** by its own polynucleotide phosphorylase. ***Agric. Biol.*** *Chem.*, 26: 611-623

Kuninaka, A. 1961 Production of nucleotides by degradation of ribonucleic acid. ***Shokuhinkogyo, 4: 9-14***

Magasanik, B. 1962 Biosynthesis of purine and pyrimidine nucleotides. *In* "The Bacteria," Vol. III. ed. by I. G. Gansalus and R. Y. Stainer, Academic Press Inc., New York, pp. 295-334

Mejbaum, W. 1939 Über die Bestimmung kleiner Pentosemengen, insbesondere in Derivaten der Adenylsäure. ***Z. Physiol. Chem., 258:*** 117-120

Miwa, M. 1952 Yeast production from sulfite pulp waste liquor. *J. Ferment. Technol.*, **30: 297-300**

Morris, D. L. 1948 Quantitative determination of carbohydrates with Dreywood's anthrone reagent. *Science*, 107: 254-255

Naruse, M. 1975 Application of nucleic acid related compounds for drugs. ***Amino Acid and Nucleic Acid, 32: 89-93***

Ogata, K., A. Imada and Y. Nakao 1962 Excretion of 5'-nucleotides by bacteria Part I. Accumulation of 5'-nucleotides in the culture fluid of a ***Bacillus*** during growth. ***Agric. Biol. Chem.*, 26: 586-595**

Okabayashi, T. and E. Masuo 1960 Occurrence of nucleotides in the culture fluid of microorganisms Part II. The nucleotides in the broth of ***Brevihacterium*** *liquefaciens* ***nov. sp. Chem. Pharm. Bull.. 8: 1089-1094***

Okada, H.. I. Kaneyama, S. Okumura and T. Tsunoda 1961 L-Glutamic acid and succinic acid fermentation by ***Brevibacterium*** *flavum* No. 1996. ***J. Gen. Appl. Microhiol., 7: 177-191***

Yamada, K., J. Takahashi and H. Okada 1953 Fundamental studies on the aerobic fermentation Part II. Determination of an empirical formula on the efficiency of oxygen supply of fermentor. ***J. Agric. Chem. Soc. Japan, 27: 704-708***