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Establishment of the Culture Conditions for the Ribonuclease F1 Production by *Fusarium moniliforme*

(ribonuclease／culture conditions／*Fusarium moniliforme*)

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Culture conditions suitable for the ribonuclease F1 production by the fungus, *Fusarium moniliforme,* were established. The composition of the medium was: 3% (w/v) glucose, 0.1% ammonium sulfate and 0.1% yeast extract in 50 mM sodium citrate buffer pH 5.8. When cultured in this medium at 25℃ for 1 week, the fungus secreted ribonuclease F1 up to a level of 150 units/ml corresponding to approximately 3 μg/ml.

Introduction

Fusarium moniliforme, a phytopathogenic fungus, secretes two nucleic acid degrading enzymes into culture medium: PDM phosphatase and ribonuclease (RNase) F1. We have been studying the structure and function of these enzymes. As for RNase F1, we purified the enzyme from a commercial enzyme source "Toyocelase" (1) and clarified some of its basic properties such as the primary structure (2). However, further studies were hampered by the low content of the enzyme in the starting material. In order to fully develop physicochemical studies, some 100 mg of the enzyme is required. Therefore, it is indispensable to establish culture conditions under which the fungus produces a good amount of the enzyme. With such an aim, we started searching for culture conditions suitable for the RNase production. Here, we report the satisfactory conditions which we could establish finally.

Materials and Methods

Chemicals——All reagents of the highest grade available were from commercial sources. Yeast extract and *Torula* yeast RNA were the products of Difco and Calbiochem, respectively.

Culture——The provenance and maintenance of the *F. moniliforme* strain were described previously (3). Potato-dextrose medium was prepared by a standard method as follows. Potato (200g) that had been peeled and cut into small cubes was immersed in 1 l of distilled water and boiled gently for 1 h with occasional supply of water. The resulting soup was filtered through a layer of cotton cloth. The broth obtained was supplemented with 10 g of glucose (dextrose) and autoclaved at 120℃ for 15 min after the volume was adjusted to 1 l with water. Other culture media were prepared as specified, then sterilized in the same way. When polypeptone was used as a nitrogen source, glucose was dissolved and sterilized separately from other components.

A culture experiment was carried out as follows. About 10 ml of 0.01% Tween 80 solution was added to a slant of fully grown *F. moniliforme* and the tube was shaken well. The solution was sucked out, filtered through two layers of gauze, added with 15% (v/v) glycerol and stored at -20℃ until use. The conidia suspension thus obtained was inoculated with a loop to 2 ml of a medium, which was then shaken at 25℃ for 2 days. The whole preculture suspension was poured into 100 ml of the same medium in a 500-ml Sakaguchi flask and the culture was carried out at 25℃ except otherwise stated under reciprocal shaking at a frequency of 120 cycles/min.

Determination of Dry Weight and RNase Activity——The growth was followed by the measurement of dry weight of fungal mycelia. At indicated times, a 3-ml portion of the culture was withdrawn and filtered through a membrane filter. Then, the dry weight of fungal mycelia remaining on the filter was measured as described previously (3). The filtrate was assayed for RNase activity by the method of Yoshida and Hanazawa (4) and expressed in units (U) as defined by them.

Results and Discussion

First, we searched for a medium which could sustain the RNase production. We tested two media commonly used for the culture of fungi. One was potato-dextrose (PD) medium which is potato broth enriched with 1% (w/v) glucose. The

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other is yeast extract-sucrose (YS) medium consisting of 3% sucrose, 0.1% ammonium sulfate and 1% yeast extract in 50 mM sodium citrate buffer pH 5.8. We also tested a synthetic medium reported by Arima et al. for the production of RNases by *Ustilago sphaerogena* (5), whose composition was 2% glucose, 0.2% glycine, 0.05% KH_2PO_4, 0.05% K_2HPO_4, 0.01% $MgSO_4$, 0.01% KCl and 0.01% $CaCl_2$. Those authors reported that the RNase production was greatly enhanced by the addition of 0.1% RNA instead of the phosphate salts.

We assessed the fungal growth visually and measured the RNase activity every day for up to 1 week (data not shown). PD medium sustained good growth but no RNase production, whereas YS medium induced good RNase production with fair growth. In our experiment, the synthetic medium gave poor growth with no RNase production even in the presence of RNA.

From these results, we chose YS medium and investigated further for conditions for more RNase production. Tests for a few energy (carbon) sources (Fig. 1A) revealed that sucrose and glucose were equally well for both growth and RNase production, while glycerol could not sustain the RNase production. Also tests for nitrogen sources (Fig. 1B) showed superiority of ammonium sulfate over others from the viewpoint of RNase production.

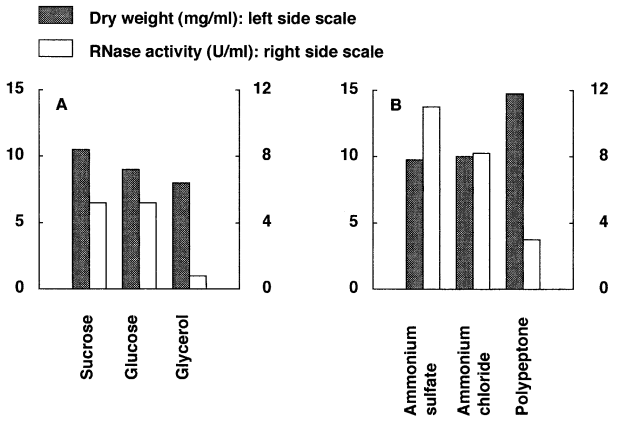

Fig. 1. Effect of changing energy (carbon) sources (A) and nitrogen sources (B) on the growth and RNase production. The results at day 6 are shown.

Therefore, we fixed the energy (carbon) source to glucose and the nitrogen source to ammonium sulfate. Then, we examined the effect of yeast extract concentration. Unexpectedly, 1% concentration usually recommended for the culture of fungi turned out to be not good for the RNase production, which reached a maximum at 0.1-0.2% and declined rapidly at higher concentrations (Fig. 2).

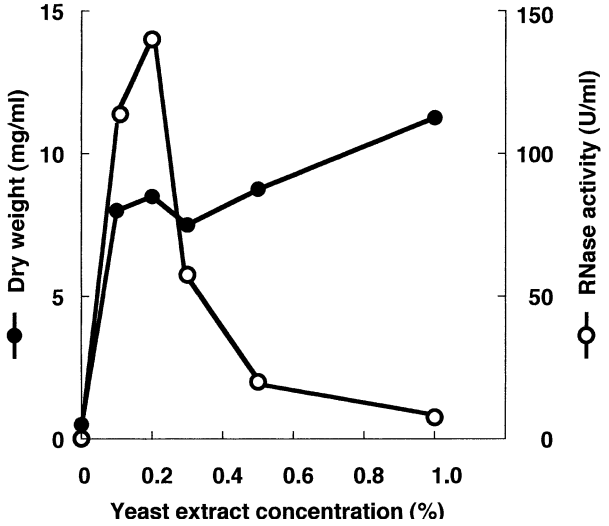

Fig. 2. Effect of the concentration of yeast extract on the growth and RNase production. The results at day 6 are shown.

Upon these investigations, we established the culture conditions as follows: 3% glucose, 0.1% ammonium sulfate and 0.1% yeast extract in 50 mM sodium citrate buffer pH 5.8. The time course of the growth and RNase production in this medium was examined (Fig. 3). The fungus entered into the exponential phase at day 1 and reached the stationary phase at day 5. The RNase activity increased in concert with the growth but continued to increase slowly even in the stationary phase until day 7, by then the RNase concentration reached a level of 150 U/ml corresponding to approximately 3 μg/ml.

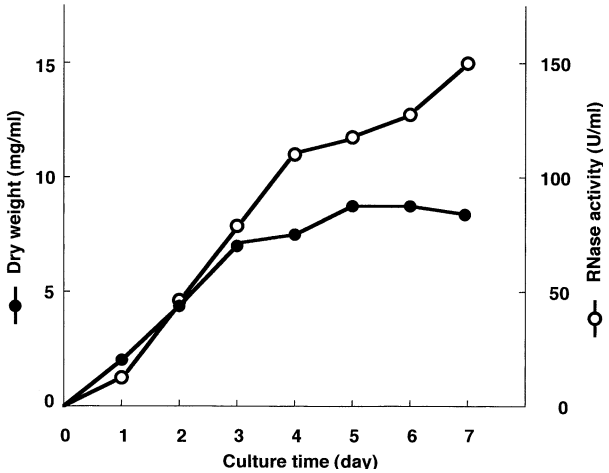

Fig. 3. Time course of the growth and RNase production under the standard conditions.

Finally, we examined effects of some other factors (Fig. 4). Addition of 0.1% RNA to the medium inhibited the RNase production severely without much effect on the growth.

Therefore, RNA does not seem to induce the RNase production by *F. moniliforme*, contrary to the finding made by Arima et al. with U. *sphaerogena* (5). Shift of the pH of the medium to pH 7.0 did not exert much effect on both growth and RNase production. However, shift of the incubation temperature to 37°C had inverse effects on the growth (inhibition) and the RNase production (slight increase).

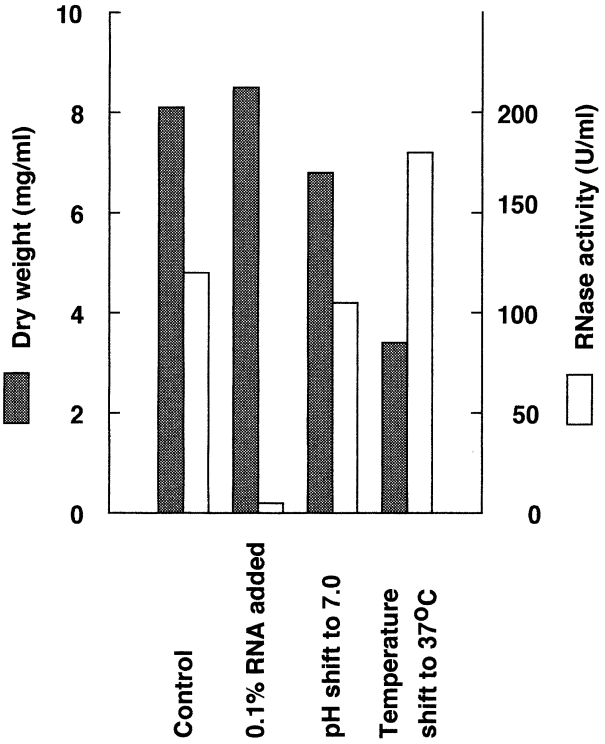

Fig. 4. Effect of some other factors on the growth and RNase production. The culture was performed under the standard conditions (control) except otherwise specified. For pH change, 50 mM Tris-HCl buffer pH 7.0 was used. The results at day 6 are shown.

In any case, we could not find conditions significantly superior in viewpoint of the RNase production. Practically, the established conditions met our expectation. Thus, we scaled up the culture to 10 l, obtained RNase F1 in several tens of milligrams of amount, and supplied the RNase to physicochemical studies such as Raman spectroscopy (6), NMR study (7) and X-ray study (8).

We reported previously the effect of culture conditions on the PDM phosphatase production (3). Chronologically, however, part of the present study was conducted earlier. So, the previous study was based on the present one. Comparing the RNase production with that of PDM phosphatase, we can point out the followings. (a) The optimal yeast extract concentration (about 0.1%) was the same for the production of both the enzymes. (b) RNase F1 was secreted prior to PDM phosphatase. The former began to increase as soon as the fungus entered the exponential phase, whereas the latter began to increase only after the fungus reached the stationary phase. (c) As for pH, pH 5.8 was better than pH 7.0 for the PDM phosphatase production, whereas not much difference was observed for the RNase production. (d) As for temperature, 25°C was better than 37°C for the PDM phosphatase production, whereas 37°C was slightly better than 25°C for the RNase production. It is of interest to know what component(s) of yeast extract causes secretion of PDM phosphatase and RNase F1. Further studies will be needed for elucidation of the mechanism of the induction.

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