# NEW FUNGAL STRAINS THAT PRODUCE A SPECIFIC INHIBITOR OF ADHESIVE GLUCAN SYNTHESIS BY *STREPTOCOCCUS MUTANS*

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Mutastein, an extracellular product of *Aspergillus terreus* M3328, is a specific inhibitor of adhesive, insoluble glucan synthesis by glucosyltransferases (GTase) of *Streptococcus mutans*[1,2]. It is effective in inhibiting the development of caries in rats inoculated with *S. mutans*[3]. Mutastein is heat-stable glycoprotein with a high molecular weight and it is being used commercially as a protective agent against caries development in humans.

In the present study, 1,677 fungal strains obtained from IFO (Institute for Fermentation, Osaka) (596 genera, 1,489 species) and newly isolates (about 1,000 strains) from soil samples were grown and tested for their ability to produce an inhibitor of adhesive, insoluble glucan synthesis by *S. mutans*. As a result, 10 strains were found to produce such an inhibitor with high molecular weight.

Fungal strains were grown aerobically at 25°C for 10 days in a medium containing glucose 3.5%, potato starch 1%, soybean meal 2%, meat extract 0.5%, Polypeptone 0.5%, NaCl 0.2%, $KH_2PO_4$ 0.05% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 5.8). The culture filtrates obtained were treated at 100°C and pH 7.0 for 5 minutes prior to the assay described below. *S. mutans* B13 (serotype *d*) was grown at 37°C for 18 hours in brain heart infusion broth (Nissui Medical Co., Tokyo) and cell-free GTase was prepared[1]. Inhibitory activity was determined by measuring inhibition of adhesive, insoluble glucan synthesis from sucrose by cell-free GTase[1]. The reaction mixture which contained, in a total volume of 1 ml, 50 mM potassium phosphate buffer, pH 6.5, 10 μg protein of cell-free GTase, 1% sucrose and 0.02% $NaN_3$ was incubated at 37°C for 16 hours. One unit of mutastein activity was defined as the amount that gave 50% inhibition of adhesive, insoluble glucan synthesis under the above assay conditions.

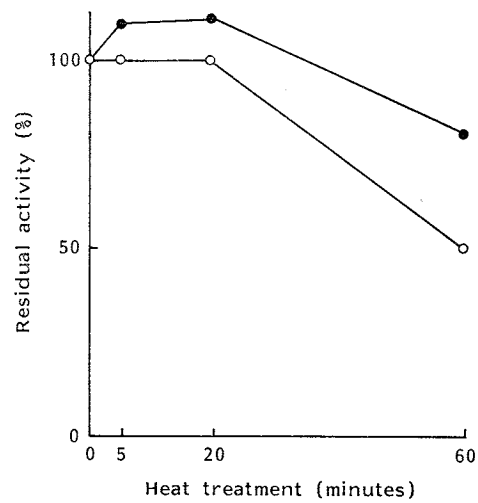
Of the 10 active strains, *Drechslera erythrospila* IFO 7378 was the most active. Inhibitory ac-

Table 1. Fungal strains active in the production of mutastein.

| Fungal strain | Activity (units/ml broth) |
|---|---|
| *Aspergillus terreus* M3328 | 1,500 |
| *Cercospora cruenta* IFO 6164 | 680 |
| *Cladospolium resinae* IFO 6367 | 680 |
| *Colletotrichum dematium* IFO 6704 | 400 |
| *Leptographium kitajimara* IFO 6908 | 670 |
| *Drechslera erythrospila* IFO 7378 | 6,700 |
| *Sporormiella intermedia* IFO 8392 | 500 |
| *Dactylaria purpurella* IFO 9336 | 200 |
| *Fennellia flavipes* IFO 9655 | 200 |
| *Stachybotrys microspora* IFO 30018 | 290 |
| *Monascus ruber* M6684 | 330 |

Fig. 1. Heat stability of *Drechslera erythrospila* mutastein.

Partially purified mutastein (0.75 μg/ml) was heated at 100°C in buffers of pH 3.0 (●) and 7.0 (○), respectively. After heat treatment for the indicated intervals, residual activity was determined at pH 6.5 as described in the text.

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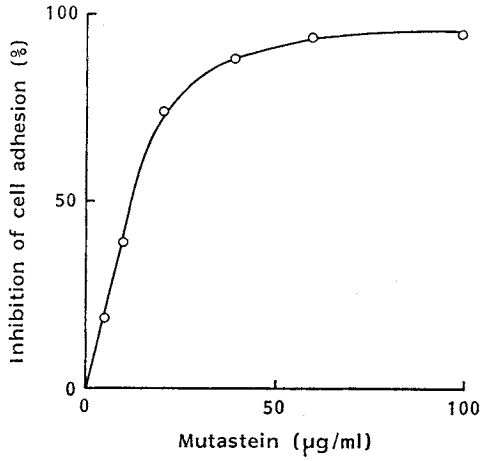
tivity of this strain was approximately 4-fold higher than that of *A. terreus*, an active strain isolated in the previous study[1]. Other 9 strains were far less active than *A. terreus* (Table 1).

To isolate a partially purified preparation of the inhibitor, *D. erythrospila* IFO 7378 was grown under the conditions described above. The culture filtrate obtained (100 ml) was adjusted to 50% saturation with ammonium sulfate and the resulting precipitate was collected by centrifugation. The pellet was dissolved in and dialyzed extensively against 25 mM potassium phosphate buffer, pH 7.0, followed by gel filtration in a column of Toyopearl HW-60 (Toyo Soda Manufacturing Co., Ltd.). The column (1.9×95 cm) was developed with the same buffer and the active fractions (10 ml) were pooled for the experiments described below. Specific activity of the partially purified preparation was 4,300 units/mg protein. Its purity and molecular weight was ~90% and 100,000, respectively, as judged from gel chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The partially purified inhibitor contained ~20% of carbohydrate, as determined by the phenol-sulfuric acid method.

After heat treatment at 100°C for 60 minutes, the residual activity was 80% at pH 3.0 and 50% at pH 7.0, respectively (Fig. 1). These data indicate that *D. erythrospila* inhibitor is a mutastein like substance. The *S. mutans* B13 cell adhesion to glass surface, which was assayed as described by KOGA *et al.*[2], was inhibited 50% at a concentration of 12 μg/ml of *D. erythrospila* inhibitor (Fig. 2).

The experiments in this study demonstrate that *D. erythrospila* is also useful in the commercial production of mutastein.

Fig. 2. Inhibition of the adhesion of resting *Streptococcus mutans* cells to glass surface by *Drechslera erythrospila* mutastein.




## References

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