# SELECTION OF STRAIN AND OPTIMIZATION OF MUTANASE PRODUCTION IN SUBMERGED CULTURES OF *TRICHODERMA HARZIANUM*

A. WIATER and J. SZCZODRAK*

Department of Industrial Microbiology, Maria Curie-Skłodowska University,
Akademicka 19, 20-033 Lublin, Poland

Nineteen fungal strains belonging to different genera were tested for extracellular mutanase production in shaken flasks. The optimal enzymatic activity was achieved by *Trichoderma harzianum* F-470, a strain for which the mutanase productivity has not yet been published. Some of factors affecting the enzyme production in shaken flasks and aerated fermenter cultures have been standardized. Mandels mineral medium with initial pH 5.3, containing 0.25% mutan and inoculated with 10% of the 48-h mycelium, was the best for enzyme production. A slight mutanolytic activity was also found when sucrose, raffinose, lactose and melibiose were carbon sources. Application of optimized medium and cultural conditions, as well as use of a fermenter with automatic pH control set at pH 6.0 enabled to obtain a high mutanase yield (0.33 U/ml, 2.5 U/mg protein) in a short time (2–3 days). The enzyme in crude state was stable over a pH range of 4.5–6.0, and at temperatures up to 35 °C; its maximum activity was at 40 °C and at pH 5.5.

*Keywords: Trichoderma harzianum* – mutanase production – shaken flask cultures – fermenter cultures – optimization

## INTRODUCTION

As matrix compounds of dental plaque, extracellular water-insoluble glucans (the so-called mutans) of streptococcal origin seem to play an important role in the etiology of dental caries. They have a branched structure in which $\alpha$-1,3 glycosidic bonds predominate, while a higher content of $\alpha$-1,3 linkages is associated with greater insolubility. Mutans act as a framework for dental plaque, where they mediate attachment of bacteria to the tooth surface and to other bacteria, stabilizing thus the plaque biofilm [6, 9, 21].

Given the importance of mutans in plaque, the mutanases ($\alpha$-1,3 glucan 3-glucanohydrolase, EC 3.2.1.59) were tested for their potential as a caries preventive agent, due to their ability to remove the biofilms created by oral bacteria *in vitro* [19] and to reduce the plaque formation *in vivo* [5]. Active ingredients in dentifrice prepa-

* Corresponding author; e-mail: szczo@biotop.umcs.lublin.pl

rations and chewing gum, such as enzymes, could become a useful supplement to mechanical cleaning of teeth and dentures. However, this technology had little a commercial success, due to the unavailability of mutanase preparations acceptable for oral applications. Mutanases from various bacteria and filamentous fungi have been investigated; yet, in most cases the enzyme activity is relatively low, and cultural conditions for mutanase production have not been thoroughly established [3, 4, 8]. Also, the search for additional mutanase producers among wild-type fungi is necessary to find potentially new sources with different characteristics. Moreover, to induce mutanase synthesis and secretion, it was necessary to prepare first mutan *in vitro* for use as the sole carbon source in microbial cultures containing sucrose and glucosyltransferase enzymes prepared from mutans streptococci. This type of water-insoluble glucan has not yet been available as a commercial product. Therefore, identification of alternative, soluble and inexpensive carbon sources to support the induction of mutanase would be useful to facilitate the enzyme production on a larger scale, and at relatively low costs.

The purpose of these investigations was to select the best mutanase-producing fungus, to find the best operating conditions for efficient production of fungal mutanase in shaken flasks and fermenter cultures, to search for alternative inducers of enzyme synthesis, and to determine some of mutanase physicochemical properties.

## MATERIALS AND METHODS

### *Preparation of mutan*

Mutan was synthesized from sucrose by a mixture of streptococcal glucosyltransferases. Stock cultures of *Streptococcus mutans* strain 20381 (provided by the German Collection of Microorganisms, Braunschweig, Germany) were stored at –20 °C in 50% glycerol and routinely transferred on Todd-Hewitt agar slants (pH 7.8). Bacteria were grown aerobically in a glucose-containing brain-heart infusion (BHI) medium. All bacterial media were obtained from the Baltimore Biological Laboratory, Cockeysville, MD, USA.

The BHI medium (pH 7.4) in 5-l flasks, 4410 ml each, was autoclaved for 30 min at 120 °C. A precultured broth (24-h-old, 90 ml) of bacteria grown at 37 °C in the same medium was used as seed for flask inoculation. Batch cultures were run at 37 °C for 24 h under stationary conditions. The formed biomass was separated by centrifugation at $12,000 \times g$ for 30 min. A clear supernatant fluid, containing glucosyltransferases, was allowed to react with sucrose (final concentration of 3%) in the presence of 0.05% sodium azide as a preservative. After incubation at 37 °C for 48 h, the formed water-insoluble glucan was collected by centrifugation at $12,000 \times g$ for 30 min, washed thoroughly with deionized water, freeze-dried, ground to a powder and stored at –20 °C. The linkage structure of mutan showed itself to be a mixed-linkage $(1 \rightarrow 3)$, $(1 \rightarrow 6)$ polymer with greater proportion of 1,3 to 1,6 linkages (59.1

and 24.4 mol%, respectively) and major branch points of 3,6-linked glucose [20]. Hydrolysis of the glucan with mutanase, prepared in our laboratory from *Trichoderma harzianum*, released only glucose as visualized by thin layer chromatography.

### *Fungal strain, media and culture conditions*

Stock cultures of *Trichoderma harzianum* F-470 (Czech Collection of Microorganisms, Brno, Czech Republic), maintained at 4 °C on potato dextrose agar slants, were used for inoculations. The liquid mineral medium C (pH 5.3), as by Mandels et al. [7], supported by 0.25% mutan and 0.1% Tween 80 was used for mutanase production. This medium was optimized during experiments with respect to carbon source, initial pH and medium volume. The media listed in Table 2 were also studied. Shake cultures were conducted in 500 ml conical flasks containing 100 ml of sterile medium. Unless otherwise stated, the flasks were seeded with conidia to a final concentration of about $2 \times 10^5$ conidia/ml and placed on orbital rotary shaker at 220 rpm and 28 °C for 3 d.

For enzyme production on a larger scale, the strain was grown in an optimized Mandels medium in 2 liter batches in a 3 l glass fermenter (BioFlo III, New Brunswick Scientific, Edison, NY, USA). The medium was autoclaved for 20 min at 117 °C and inoculated with 10% (v/v) of the 48 h mycelium of *T. harzianum* grown in shaken flasks in the same medium but with 0.5% glucose. The fermenter culture was run at 30 °C for 7 d at aeration rate of 2 l air/min and with stirrer speed of 450 rpm (if not otherwise stated). The pH was not regulated, except for cases when after 24 h incubation, it was automatically maintained at a value of 6.0. Antifoam B emulsion (Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) was used for breaking the foam. The mycelium from flasks and fermenter cultures was separated by centrifugation (20 min at $6,000 \times g$) and the clarified supernatant was used as enzyme solution for various tests.

### *Assays*

The standard mutanase assay mixture contained 0.5 ml of 0.4% mutan in 0.2 M sodium acetate buffer (pH 5.5) and 0.5 ml of suitably diluted enzyme solution. After 1-h incubation at 40°, the released reducing sugars were quantified by Somogyi–Nelson method [10, 16]. Appropriate substrate and enzyme blanks were included to correct any free reducing group not emanating from the mutan. One unit of mutanase activity (U) was defined as an amount of enzyme hydrolyzing mutan to yield reducing sugars equivalent to 1 μmol of glucose/min, and expressed as units per ml of culture (U/ml). Specific activity was defined as mutanase units per mg of protein (U/mg protein). 1 U corresponds to 16.67 nkat. Dextranase activity was determined according to the method described by Szczodrak et al. [17]. Protein concentration was mea-

sured by the method of Schacterle and Pollack [14] using crystalline bovine serum albumin as a standard.

The influence of pH on mutanase activity was examined in standard assay mixture, except that 0.2 M phosphate-citrate (McIlvaine) buffers (pH 3.0–8.0) were used instead of acetate buffer. pH stability was determined in a similar way, apart from the fact that after preincubation of the enzyme solution at various pH values and at 40° for 24 h, aliquots were removed, and the remaining activities were assayed by the standard method at the optimum pH of 5.5. The effect of temperature on the enzyme activity was estimated in standard assay mixture containing acetate buffer, but the reaction temperature was changed gradually from 20 to 60 °C and the activity was measured at optimum pH value. Thermal stability was measured in the same way as the temperature optimization. The enzyme solution was incubated without mutan at different temperatures for 1 h under optimum pH conditions. Then it was cooled, and the residual activity was assayed by the standard method at the optimum pH of 5.5 and temperature 40 °C. Relative activity at each pH and temperature was expressed as a percentage of the maximum activity.

Submerged cultures were performed in triplicate, and analyses were carried out at least in duplicate. The values given here are means of all the independent measurements. Mean standard error of the mutanase estimate was $\pm 7.6 \times 10^{-3}$ U and ranged from $\pm 6.9 \times 10^{-5}$ to $\pm 1.3 \times 10^{-2}$. Mean standard error in protein determination was $\pm 4.8 \times 10^{-3}$ mg and ranged from $\pm 5.7 \times 10^{-5}$ to $\pm 9.1 \times 10^{-3}$. Other methodological details are given in tables and figures.

# RESULTS AND DISCUSSION

## *Choice of mutanase producer*

A total of 19 fungal cultures, including representatives of the genera *Aspergillus, Penicillium*, and *Trichoderma* were examined for extracellular mutanase activity. However, given a high dextranolytic activity, *Penicillium* strains were excluded from further screening procedure. Differentiation between mutanase and dextranase activities is very difficult, the more so the obtained mutan is a mixed-linkage (α-1,3), (α-1,6) polymer. Other fungi we tested produced only mutanase (none of those produced dextranase on dextran), and their enzyme productivities in shaken flasks are presented in Table 1.

The highest mutanase yield per culture filtrate (0.397 U/ml), as well as per mg of soluble protein (2.05 U/mg), was reached by the strain *Trichoderma harzianum* F-470. The selected strain produced the enzyme in a relatively short time (3 days), and alkalized the medium slightly, resulting in a pH rise from 5.3 to above 7.0. Also, our further study was confined to this strain, and the most suitable culture conditions for mutanase production with the selected strain were established.

## *Optimization of mutanase production*

In further study, the mineral medium C, applied for mutanase production by the selected strain, was compared with others described in the literature for mutanase or dextranase biosynthesis. All tested media were supported by 0.5% mutan.

Table 2 shows that the medium C we selected (its composition was given earlier by Mandels et al. [7]) was most effective with respect to mutanase productivity. The enrichment of this medium in peptone proteose shortened the cultivation time by one day, but decreased (about 15%) mutanase activity. Other media gave smaller effects, and required longer periods of incubation (5–6 days). Also, a simple medium reported by Szczodrak et al. [17] for effective dextranase production was found to be useless for mutanase synthesis. Hence, the mineral medium C was used in our further studies.

During initial experiments the concentration of mutan in the culture medium was 0.5%. To ensure suitable conditions for mutanase production, we studied its activity at mutan concentrations between 0.1% and 1.0 %. It follows clearly from the data in Table 3 that a fourfold increase in the mutan amount from 0.25% to 1.0% increased the enzyme activity by only 43%. Thus using such a high mutan concentration is not economically justified, the less as this polymer is not yet available as a commercial product. Basing in Table 3, we concluded that the substrate of 0.25% giving a rela-

*Table 1*

Extracellular mutanase production by selected fungal strains in shaken flask cultures
on mineral medium with mutan[a]

| Organism | Origin[b] | Days[c] | Mutanase activity | | Final pH[d] |
|---|---|---|---|---|---|
| | | | U/ml | U/mg protein | |
| *Trichoderma harzianum* 55A5 | DEM | 3 | 0.002 | 0.040 | 6.5 |
| *Trichoderma harzianum* 70B5 | DEM | 4 | 0.008 | 0.103 | 7.4 |
| *Trichoderma harzianum* 32A3 | DEM | 5 | 0.012 | 0.083 | 7.4 |
| *Trichoderma harzianum* F-340 | CCM | 4 | 0.315 | 1.781 | 7.2 |
| *Trichoderma harzianum* F-470 | CCM | 3 | 0.397 | 2.048 | 7.1 |
| *Trichoderma harzianum* 53059 | GCM | 3 | 0.001 | 0.014 | 5.7 |
| *Trichoderma reesei* F-560 | CCM | 5 | 0.008 | 0.111 | 6.7 |
| *Trichoderma reesei* M-7 | FT | 3 | 0.250 | 0.949 | 6.6 |
| *Aspergillus nidulans* 1 | IB | 7 | 0.133 | 0.534 | 6.3 |
| *Aspergillus niger* C | DIM | 4 | trace | trace | 6.7 |

[a] The medium C of Mandels et al. [7] with 0.5% mutan was used.

[b] DEM, Department of Environmental Microbiology, Maria Curie-Skłodowska University, Lublin, Poland; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; GCM, German Collection of Microorganisms, Braunschweig, Germany; FT, Department of Food Technology, Agricultural University, Lublin, Poland; IB, Institute of Biotechnology, Warsaw, Poland; DIM, Department of Industrial Microbiology, Maria Curie-Skłodowska University, Lublin, Poland.

[c] Incubation period for maximum activity.

[d] Initial pH 5.3.

*Table 2*

Effect of different mineral media on mutanase production
by *T. harzianum* F-470 in shaken flask cultures

| Medium[a] | Days[b] | Mutanase activity | |
|---|---|---|---|
| | | U/ml | U/mg protein |
| Mandels et al. [7] – medium C | 3 | 0.410 | 2.09 |
| Mandels et al. [7] – medium A[c] | 2 | 0.350 | 1.60 |
| Saunders et al. [13] | 6 | 0.317 | 1.95 |
| Szczodrak et al. [17] | 5 | 0.013 | 0.05 |

[a] All media (pH 5.3) were supported by 0.5% mutan.

[b] Incubation period for maximum activity.

[c] The same as C but with the addition of peptone proteose (0.1%).

*Table 3*

Culturing factors affecting mutanase production by *T. harzianum* F-470
in Mandels medium C[a]: effect of initial pH, medium quantity, and some medium constituents

| Serial No. | Factor varied | Concentration, % | Mutanase activity[b] | |
|---|---|---|---|---|
| | | | U/ml | U/mg protein |
| 1. | Mutan | 0.10 | 0.106 | 1.700 |
| | | 0.25 | 0.326 | 2.315 |
| | | 0.50 | 0.429 | 1.977 |
| | | 0.75 | 0.458 | 1.761 |
| | | 1.00 | 0.467 | 1.636 |
| 2. | Initial pH of the medium[c] | | | |
| | 4.0 | | 0.113 | 1.801 |
| | 5.0 | | 0.239 | 2.189 |
| | 5.3 | | 0.318 | 2.381 |
| | 5.5 | | 0.226 | 2.315 |
| | 6.0 | | 0.095 | 1.561 |
| 3. | Volume of optimized medium taken (ml)[d] | | | |
| | 50 | | 0.264 | 2.569 |
| | 100 | | 0.331 | 2.691 |
| | 150 | | 0.278 | 2.611 |
| | 200 | | 0.266 | 2.263 |

[a] Composition of the medium was the same as that of the original one, except for the factor or its concentration that varied as indicated.

[b] Enzyme activity in culture filtrates was measured after 3 days of submerged cultivation in shaken flask cultures.

[c] Concentration of mutan was 0.25%.

[d] Concentration of mutan was 0.25%; pH was 5.3.

tively high enzyme activity would be an adequate substrate concentration for mutanase production. Also, the initial medium pH of 5.3, and its volume of 100 ml were adopted as the best conditions, yielding in shaken flask cultures about 0.32–0.33 units of mutanase per ml of culture broth after 3 days of cultivation (Table 3).

The use of conidia as inoculum extends the cultivation time by a stage indispensable to form physiologically active mycelium. Therefore, an attempt was made to replace conidia of *T. harzianum* by a suspension of its vegetative mycelium to shorten the cultivation time on the selected medium. The use of 10% of the 48-h mycelium as inoculum shortened the incubation time by one day, as compared to the use of conidial suspension, maintaining mutanase activity on a level slightly higher than that obtained in control runs (Table 4). Similar results with respect to cellulases were reported by Szczodrak et al. [18] using 20 h suspension of *Aspergillus terreus* mycelium. Shukla et al. [15] observed the highest formation of dextranase by *Penicillium aculeatum, P. purpurogenum*, and *P. funiculosum* with the use of an inoculum containing 4% of 48 h mycelium.

*Table 4*
Effect of the kind and amount of inoculum from *T. harzianum* F-470
on mutanase activity in shaken flask cultures[a]

| Inoculum quantity, v/v% | Mutanase activity | |
| --- | --- | --- |
| | U/ml | U/mg protein |
| 2[b] | 0.309 | 2.485 |
| 2[c] | 0.189 | 1.765 |
| 5[c] | 0.252 | 2.194 |
| 10[c] | 0.387 | 2.787 |
| 20[c] | 0.231 | 1.882 |

[a] Mandels medium C with 0.25% mutan was used.

[b] Control inoculated with aqueous suspension of conidia of *T. harzianum* ($2\times10^5$/ml); enzyme activity for the control test was determined after 3 days of culture.

[c] Experimental tests inoculated with a suspension of vegetative *T. harzianum* mycelium (48 h) cultured on Mandels medium with glucose (0.5%); enzyme activity was determined after 2 days of culture.

Production of mutanase by *T. harzianum* was also compared when the organism was grown in optimized medium containing various carbohydrates as the sole carbon source and inducer of mutanase synthesis. From 17 sugars tested, mutan was the most specific and effective inducer of enzyme synthesis (Table 5). A slight mutanolytic activity (0.01 to 0.03 U/ml) was also found when sucrose, raffinose, lactose, and melibiose were carbon sources. Other carbohydrates had no effect on enzyme induction. Raffinose-induced mutanase production was also reported by Quivey and Kriger [12] in shaken flask cultures of *T. harzianum* OMZ 779 strain.

*Table 5*

Effect of carbon source on mutanase production by *T. harzianum* in shaken flask cultures[a]

| Carbon source[b] | Main linkage(s) | Days[c] | Mutanase activity | | Final pH |
| | | | U/ml | U/ mg protein | |
|---|---|---|---|---|---|
| Mutan | α-1,3; α-1,6 | 3 | 0.310 | 2.27 | 6.40 |
| Sucrose | α,β-1,2 | 2 | 0.031 | 1.20 | 6.26 |
| Raffinose | α-1,6; α,β-1,2 | 4 | 0.020 | 0.42 | 6.53 |
| Lactose | β-1,4 | 3 | 0.016 | 0.70 | 4.15 |
| Melibiose | α-1,6 | 3 | 0.011 | 0.02 | 3.67 |
| Melizitose | α-1,3; β-1,2 | 5 | trace | trace | 6.23 |
| Inulin | β-1,2 | 2 | trace | trace | 5.95 |
| Esculin | β-glycosidic | 3 | trace | trace | 6.09 |

[a] Mandels medium C with 0.25% mutan or 1% other carbohydrates was used.

[b] Other sugars (dextran, starch, maltose, trehalose, sorbitol, mannose, galactose, fructose, glucose) supported only growth of the organism, but none activity of mutanase was detected in this set of carbohydrates.

[c] Incubation period for maximum activity.

The identification of alternative, soluble and inexpensive carbon sources (such as sucrose, raffinose or lactose) capable of inducing mutanase synthesis is useful to facilitate the enzyme production on a larger scale and at relatively low costs. Therefore, further experiments on mutagenic activation of fungal mutanase produced on soluble and accessible carbon sources are now in progress in our laboratory.

Figure 1 illustrates a typical culture time course of *T. harzianum* when the organism was grown for 5 d in shaken flasks on the optimized medium containing mutan.
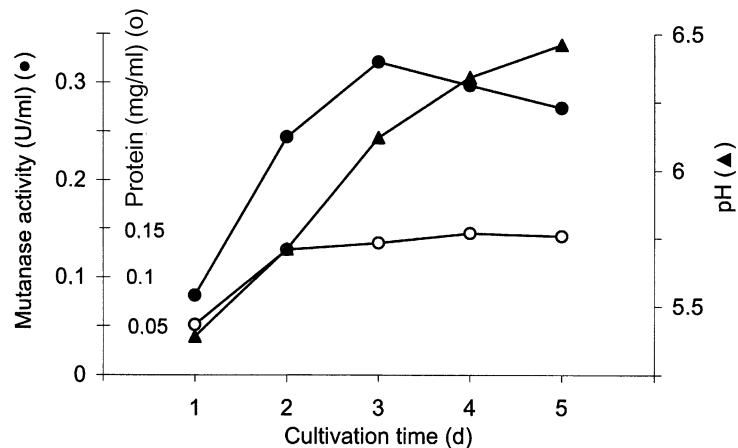


*Fig. 1.* Rates of mutanase (M) and protein production (F), and changes in pH ( ▲ ) during shaken flask cultures of *T. harzianum* on optimized medium containing mutan as the sole carbon source. Initial medium pH was 5.3

Mutanase activity increased gradually for 3 d, and then declined slowly. The maximum mutanase yield (0.32 U/ml) and extracellular protein accumulation (0.145 mg/ml) were measured on the third and fourth days of cultivation, respectively. The pH of the medium rose (from 5.4 to 6.5) over the whole period of incubation. Consequently, a 3-day period was assumed as the optimum time for the synthesis of mutanase by *T. harzianum* in shaken flask cultures.

For mutanase production on a larger scale, *T. harzianum* strain was grown in a 3-l fermenter on the optimized medium with mutan. Some of parameters affecting the enzyme production have been standardized. The effect of increasing the stirrer speed from 200 to 450 rpm on mutanase production is shown in Figure 2a. In all cases, the enzyme activity increased gradually and attained its maximum (0.27 U/ml) after 5 d
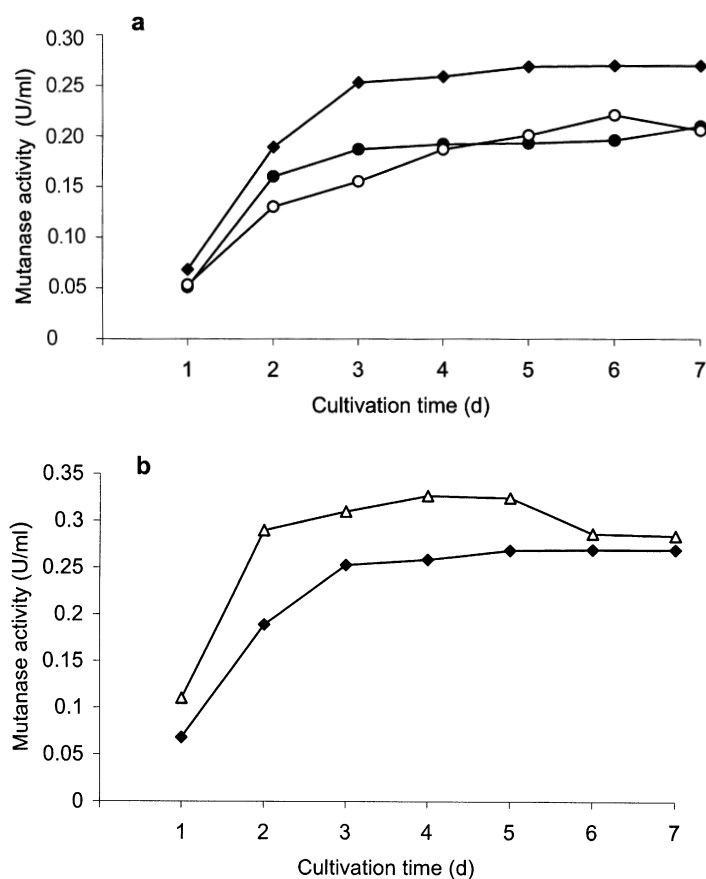


*Fig. 2.* Effects of stirring speed (a) and regulation of medium pH (b) on production of *T. harzianum* mutanase in 3-l glass fermenter on optimized culture medium containing mutan. (a) stirrer speed: M – 200 rpm, F – 300 rpm, ♦ – 450 rpm; the pH-value was not controlled (the initial medium pH was 5.3 and the final – 6.6). (b) ♦ – non-regulated pH, △ – regulated pH (automatically maintained at a value of 6.0 after 24 h incubation); stirrer speed was 450 rpm

of incubation at the stirrer speed of 450 rpm. Other stirrer speeds (200 and 300 rpm) gave smaller effects, and required longer periods of incubation (6–7 days). A further increase in impeller speed – from 450 to 600 rpm – brought a reduction in mutanase activity.

Observations under light microscope showed mycelial differences with varying speed rates. The cells grew almost exclusively in form of pellets at stirrer speed from 100 to 450 rpm. Increasing the stirrer speed from 450 to 600 rpm induced transformation of fungal biomass to less hairy pellets. After 7-d cultivation at 600 rpm, a part of biomass was mechanically disintegrated, exhibiting short tiny threads.

The use of a fermenter with automatic pH control set at pH 6.0 induced a growth of mutanase productivity (Fig. 2b). Under these conditions, the highest enzyme activity of 0.33 U/ml was reached after 4 d of incubation. It was by 26% higher as compared to that obtained within the same time by the fungus in fermenter culture with non-regulated pH-value. Moreover, an about 89% of enzyme activity had already been monitored on the second day of cultivation.

Application of optimized medium and culture conditions, as well as use of a fermenter maintaining pH-value on the same level enabled us to obtain a high mutanase yield (in average 0.33 U/ml, 2.5 U/mg protein) in a short time (2–3 days). For example, Guggenheim and Haller [3], testing the enzyme production of *T. harzianum* OMZ 779 in fermenter runs, obtained an activity of 0.08 U/ml after 155 to 165 h. In shaken flask cultures, supplemented with 1% mutan, mutanase activity would reach its maximum yield of 0.16 U/ml after 120 h of incubation. Meanwhile using the same fungal strain, Quivey and Kriger [12], reached after 4 d in shaken flask cultures, the specific mutanase activity of 0.37 U/mg protein. Also, basing on *Streptomyces chartreusis*, Inoue et al. [4], obtained, after 3 d in shaken flask cultures, the maximum mutanase activity of 0.005 U/ml. In case of bacterial mutanases, Matsuda et al. [8] achieved enzyme activities of 0.17 U/ml and 0.039 U/mg protein in a flask culture of *Bacillus circulans*, grown for 36 h on mutan composed mainly of $\alpha$-1,3 glucan, and Ebisu et al. [2] – after 24 h in a 10-l fermenter culture of *Flavobacterium* – an enzyme yield of 0.003 U/ml.


## *Mutanase properties*

The crude enzyme (not stabilized) was stable for about two weeks at 4 °C, and the diluted enzyme (1 : 19, 0.2 M acetate buffer, pH 5.5) was also stable at room temperature for 24–48 h. The enzyme in crude state was also found to be highly stable after two treatments–freezing and thawing. The effects of pH and temperature on the activity and stability of *T. harzianum* mutanase are illustrated in Fig. 3. The optimum pH for the enzyme activity was pH 5.5, and the enzyme was the most stable over a pH range of 4.5–6.0. The effect of temperature on mutanase activity was investigated over the range of 20–60 °C. The enzyme hydrolyzes mutan with the highest rate at 40 °C. Thermostability studies showed that after 1-h incubation the enzyme was
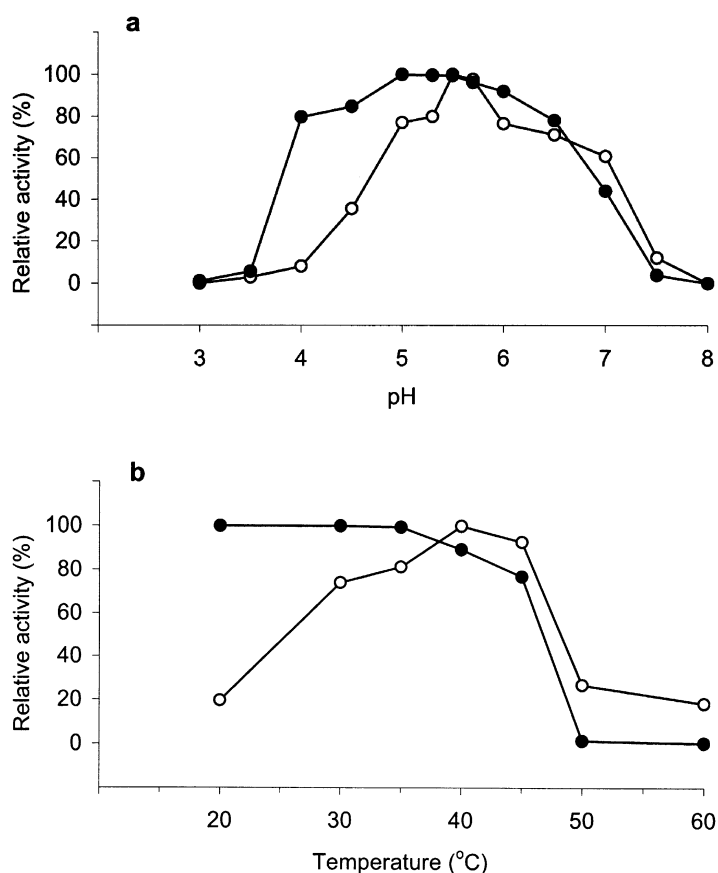
*Fig. 3.* Effects of pH (a) and temperature (b) on activity (●) and stability (○) of *T. harzianum* mutanase. (a) 0.2 M McIlvaine buffer of various pH values was used instead of acetate buffer in the standard assay. (b) The activity was assayed by the standard method except for the reaction temperature that changed as indicated. Other experimental details as described in Materials and Methods

stable at temperatures up to 35 °C. At 45 °C the enzyme lost 23% of its maximum mutanase activity, which rapidly decreased above 45 °C to get totally lost at 60 °C.

The pH optimum observed is not exactly in agreement with the one reported earlier at pH 6.0 for the *T. harzianum* OMZ 779 mutanase [3], yet comparable to that obtained for the *Microbispora rosea* mutanase [1]. Correspondingly, bacterial mutanases from *B. circulans* [8], *Flavobacterium* [2] and *Microbispora rosea* [1] have similar (5.5) or higher (6.3–6.9) pH optima than fungal mutanases, and similar temperature optima (40–50 °C). Although pH in the oral cavity is around pH 6–7, the slightly acidic pH profile of the fungal mutanase may be of importance in its application for plaque removal, as low pH values have been observed locally in the plaque [11].

In conclusion, the data presented show that the selected strain of *T. harzianum* F-470 represents a potentially new and highly effective producer of extracellular mutanase and hence justifies further investigations on application of this fungus to obtain an active mutanolytic preparation for biotechnological purposes.

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