Brazilian Journal of Microbiology (2001) 32:320-326
ISSN 1517-8382

# SCREENING AND GENETIC IMPROVEMENT OF PECTINOLYTIC FUNGI FOR DEGUMMING OF TEXTILE FIBERS

**Silvia M.G. Molina[1]*; Francisco A. Pelissari[1]; Cláudia B.M. Vitorello[1]**

[1]Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, SP, Brasil

Submitted: December 12, 1999; Returned to authors for corrections: July 07, 2000; Approved: October 11, 2001

## ABSTRACT

Aiming at contributing to technological improvements in plant fiber processing methods, this paper reports research work on the obtainment of more efficient pectinase-producing fungi strains. More specifically, this work reports the analysis of 18 strains of filamentous fungi, with the purpose of obtaining enzymes for textile fibers degumming. The strains were evaluated for production of pectinolytic enzymes under several growth conditions (culture medium and growth temperature). Production of pectinases was measured by an enzymatic index (EI) in solid pectin medium. Among the tested strains, *Penicillium chrysogenum* IFO 4626 (Q 176) showed the best performance. Genetic improvement of this strain was carried out to increase its pectinase production, while keeping cellulase activity down to a negligible level, since cellulases are known to decrease the resistance of the fiber. Variability was induced through several cycles of mutation and selection by exposing conidea to ultra-violet light (UV). We selected 39 out of 390 isolated colonies. Resulting mutants produced nine times more pectin lyase (PL) than the original strain in terms of PL specific activity, and five times more in terms of PL activity (i.e. mmoles liberated per minute of reaction per mL of medium). Periodically, mutant performance was evaluated in solid pectin medium. Genetic stability was maintained for four years after isolation.

**Key words:** *Penicillium chrysogenum,* pectin lyase, pectinolytic enzyme, degumming of textile fibers.

## INTRODUCTION

In developing countries large amounts of plant fibers are available as agricultural products (sisal, ramie, manila hemp and jute) or as agro-industrial waste (banana tree pseudostem fiber). However, technological modernization needs to be introduced in processing methods (8,15) to fulfill the economical potential of these fibers. The characteristics of banana tree fibers for textile purposes are present in all species of banana, mainly in Manila hemp (*Musa textilis*). Fibers from commercial species of banana trees have been used in Brazil, but only in craftsmanship, using rough processing methods. Pectinolytic enzymes have important applications in food processing, such as in the fruit juice and wine industries. Their comparative economic and technological advantages justify their application also in textile production (21).

Research has been conducted to develop and to evaluate new techniques for the enzymatic processing or maceration of textile fibers (23,24). Brumano *et al*. (10) found that for *Penicillium griseoroseum* the optimum level of activity for pectinolytic enzymes occurred at near-neutral pH levels and at high temperatures. These conditions are ideal for natural processing of material. High temperatures inhibit mesophylic contaminant growth and can act as a pasteurization process. The requirement of neutral pH values means that the enzymes can be used directly on the plant tissue, with no need for alteration. Authors also compare the performance of the commercial enzyme Flexzyme, with those obtained from *P. griseoroseum.* After treatment with the commercial enzyme, the resulting fiber is softer but less resistant, probably because the commercial enzyme is not free of cellulases.

* Corresponding author. Mailing address: Laboratório de Ecogenética de Resíduos Agroindustriais, Departamento de Genética, ESALQ/USP. Av. Pádua Dias, 11. 13418-900, Piracicaba, SP, Brasil. Tel.: (+5519) 429-4225, Fax: (+5519) 433-6016. E-mail: smgmolin@esalq.ciagri.usp.br

Other experiments also point to *Penicillium* as a good choice for fiber degumming. Bacarat *et al.* (5) evaluated species of *Aspergillus*, *Penicillium*, *Eupenicillium*, and *Humicola.* The best performance for total pectinase production was obtained by *P. versicolor* #1, and the highest production of poligalacturonase (PG) was obtained by *P. expansum*. *P. frequentans* has been reported as having good PL activity (22,25). Among isolates of 80 wild fungi selected for pectinolytic enzymes, Fawole and Odunfa (12) reported high levels of activity for *Aspergillus*, *Fusarium*, *Penicillium* and *Rhisopus.* Deshpande and Gurucharanan (11) reported their success with the degumming of ramie fibers by selected strains of *Aspergillus versicolor*. A direct relationship between inoculum concentration and fiber separation was verified. Enzymatic treatment of cellulose fibers of ramie xylanases produced by *Penicillium janthinillum* has been reported with good results in facilitating the pulp whitening process (17).

Many experiments have attempted to increase the performance of enzyme production by improving culture conditions. There are reports about the amount of pectin used in culture media (2); the stimulating effect of yeast extract (1); using onion peel as source of carbon (7); and the effect of glucose on the production of pectinases (18,26,27).

Concerning genetic improvement, Leuchtenberger and Mayer (16) reported two and threefold increases of PG and PL production in mutants of *Aspergillus niger*. Fernandes-Salomão *et al.* (13) obtained mutants of *Penicillium expansum* with a two-fold increase in PL and PG production, as compared with the original strain.

## MATERIALS AND METHODS

### Strains

This paper reports the selection and characterization of 18 strains of fungi: 14 of *A. niger*, one of *Humicola* sp. wild, two of *Penicillium frequentans* and one of *Penicillium chrysogenum*. Strains IFO 4626 (Q 176) of *Penicillium chrysogenum*, 10V10 of *Aspergillus niger*, and strains of *Humicola sp.* were granted by the Laboratory of Microbial Genetics, at the Escola Superior de Agricultura “Luiz de Queiroz” - University of São Paulo, Brazil. All other strains were generously granted by the Department of Agroindústria, Alimentos e Nutrição at ESALQ/USP. They are: IZ 36 (AATCC-10581); IZ 37 (AATCC-1027); IZ 45 (AATCC-1040); IZ 47 (AATCC-10582); IZ 52 (AATCC-1015); IZ 85 (AATCC-10575); IZ 100 (AATCC-6276); IZ 109 (AATCC-1044553); IZ 122 (AATCC-10578); IZ 126 (AATCC-8740); IZ 129 (AATCC-9142); IZ 132 (AATCC-10254); e IZ 135 (AATCC-7797) of *A. niger*, and IZ 86 (AATCC-10449) and IZ 1619 (JBR-19K5) of *P. frequentans*. An experimental growth test and morphological evaluations on complete media were carried out as a preliminary characterization of these strains (14, modified by 4).

### Enzymatic index

The enzymatic index (EI) was calculated as the measurement of the degradation halo formed on the pectin solid media of colonies growing at 30ºC for three days divided by the diameter of colonies. Three repetitions per treatment were made. The tested culture media were: Pectin 0.5% medium Type 1 (1 liter of medium = 6 g $NaNO_3$; 1.5 g $KH_2PO_4$; 0.5g KCl; 0.5g $MgSO_4.7H_2O$, $FeSO_4$, $ZnSO_4$ and $H_3BO_3$; 1 g of yeast extract; 5.0 g citric pectin; 15 g common agar; pH 7.0, $H_2O$ up to a liter); Pectin 0.75% medium Type 1.1 (the quantity of pectin was changed from the previous medium to 7.5); Pectin 0.5% medium Type 3 (1 liter of medium: 1 g $(NH_4)_2SO_4$; 2.0g $KH_2PO_4$; 0.9g $Na_2HPO_4$, trace of $MnSO_4$, 5.0g pectin, 1.0g yeast extract, 15g common agar, pH 7.0; $H_2O$ up to a liter); Pectin 0.5% medium Type 4 (add to previous trace of $CaCl_2$); Pectin 0.5% medium Type 5 (related to Type 3: no yeast extract, but add 0.5g of glucose. A 1% (w/v) hexadecyl-trimethyl-amonium bromide solution was added to the plates after three days of incubation, revealing the halo of pectin degradation. This solution precipitates pectin present in the medium, resulting in a translucent halo where pectin was degraded.

### Effect of temperature and media on enzymatic index

In order to characterize the effect of temperature on EI, the pectinolytic activity of strains IFO 4626 of *P. chrysogenum*; IZ 36, IZ 47, IZ 85, IZ 100 and 10V10 of *A. niger;* IZ 1619 of *P. frequentans;* and the wild strain of *Humicola* sp. was evaluated. Conditions: a) pH 7.0; b) temperature: 30ºC and 37ºC; c) types of solid culture media: 1, 3, 4 and 5; and d) length of time: 3 days; e) inoculum per plate: 4.

### Mycelium growth

Mycelium growth was evaluated by cultivating the strains in 50 mL of Type 3 liquid medium, at 30ºC, which was subjected to a 160 rpm shaking for 48 hours. Initial inoculum was $10^7$ spores.

### Survival curve to U.V. light and evaluation of mutants

The survival curve of strain IFO 4626 to U.V. light was plotted (4). The mutants were evaluated in solid media with pectin 0.5% (w/v) (media Type 1 and 5); pH 7.0, 30ºC for 4 days; it was inoculated 4 colonies per Petri plate. This choice of media was made because in Type 5 medium the best IFO 4626 strain performance took place with 0.5% (w/v) pectin, followed by Type 1, which was also good for the rest of the strains under study. It is worth mentioning that the effect of the Type 1 medium is no different from that of Type 1.1, with 0.75% (w/v) pectin (Table 1, ahead). Essays with mutants in solid medium (EI evaluation of enzymatic activity), showed that Type 5 medium, with glucose and no yeast extract, was the best inductor, when compared to Type 1, with pectin as the only carbon source.

### Enzyme extraction

For the purpose of enzyme extraction, the IFO 4626 strain and derived mutants were cultured at first in a solid sporulation medium (20): for 6 days at 30ºC. Inoculation in the liquid pectinolytic medium was done with $10^7$ spores/mL, in 50mL of Type3 pectin medium (the best inductor in previous assays in liquid media - data not showed), with 3 replicates per strain. Inoculated flasks (250 mL Erlenmeyers) were incubated in orbital shaker (160 rpm) at 30ºC during 5 days. The dried weight of the mycelial mass were determined. The filtered material was used to determinate pectin lyase activity. One unit of pectin lyase activity was defined as the amount of enzyme that produces 1 mMol of product (glucuronic acid) per minute of reaction. Three assay tubes were prepared with 0.6mL of the filtered material (enzyme) per evaluated strain. To each tube was added 0.4 mL of pectin substratum 2% (100mM $KH_2PO4$, 100mM $Na_2PO_4$, 2% Sigma pectin, pH 6.8-7.0, corrected with KOH, freezer conservation). This preparation was incubated in water bath at 40ºC for 30 minutes. After this period the reaction was stopped by the addition of 9mL of HCl 0.01N. Blanks were prepared by immediately adding 9mL of HCl 0.01N in the tube with 0.6mL of the filtered material plus 0.4mL of the pectin 2% substrate. The OD measure was done at 235 nm.

### Re-evaluation of strains

A re-evaluation of strain IFO 4626 and its derived mutants for cellulose activity was also made in solid culture medium (see description above). This work also evaluated the performance of the IFO 4626 mutants of *Penicillium chrysogenum*, under the same conditions of cultivation, within a 4 year storage period.

### Benomyl resistant mutants

In order to obtain benomyl resistant mutants (9), a spore suspension (with $10^7$ conidia) was seeded in solid sporulation medium and benomyl addition (stock solution of 5mg/mL of distilled water, sterilized in auto-clave, at 1 atmosphere, during 30 minutes), in final concentration of 0.5 µg/mL. The colonies, which grew in this culture medium, were isolated in sporulation medium with benomyl. Mono-spore colonies were isolated and selected at random. 50 Petri plates were seeded, with sporulation medium, with $10^6$ conidia in each one.

### New mutation-selection cycle

A new mutation-selection cycle was carried on with the isolated M350 (resistant to benomyl). Mutagenic EMS was used under the same described conditions and 468 colonies were isolated. New mutants evaluation experiments were carried over with three repetitions, with statistical analyses for each group of 26 colonies, plus M350 and IFO 4626.

## RESULTS AND DISCUSSION

### Strains

Strain IFO 4626 of *Penicillium chrysogenum* was found to be the most adequate and promising for genetic improvement research according to results (see below).

### Enzymatic index

The strains differ significantly among themselves (prob. >F=0.0001) according to analysis of variance (Table 1). A Tukey test shows the superiority of IFO 4626 strain over all the others ($a$ = 0.05 and $a$ = 0.01): the halo of IZ 85, the largest among all other strains, attained an EI of only 1.162, and these values decreased down to strain IZ 52 which produces no halo at all beyond the growth limits of the colony. Effects of different culture media on the value of EI (prob. > F = 0.01177) were also found. In Table 1A it is shown that the 0.75% (w/v) pectin medium (Type 1) was more efficient than all others at $a$ = 0.05; but at $a$ = 0.01 there was no significant difference among them. The effect of different media on strain IFO 4626 reveals that Type 5 media (with glucose, no yeast extract) stands above all other media, which do not differ among themselves ($a$ = 0.05). At a 1% probability level, however, Type 1.1, with 0.75% (w/v) pectin, was also significantly superior to all others, but did not differ from type 5 (Table 1B, ahead). These results suggest that the quantity of carbon source was a limiting factor to strain development, as well as evidence that glucose, for IFO 4626 strain, contributes mostly as inductor to enzymatic activity, rather than as inhibitor.

### Effect of temperature and media on enzymatic index

It was found a combination of both factors medium and temperature effects (Prob. >F = 0.00103), and of strains (prob.> F = 0.00076) was found. Enzymatic indexes at 37º C were lower than those at 30ºC. Higher performances were reached in the Type 1 and 5, both at 30ºC; and lower than Types 3 and 4 at 37ºC ($a$ = 0.05). In level of $a$ = 0.01, it can be considered higher only in Type

**Table 1.** Enzymatic index means in different culture media.

| A. All Strains (18) | | | | B. Ifo 4626 Strain | | | |
|---|---|---|---|---|---|---|---|
| Media | Enzymatic Index (means)* | 5% | 1% | Media | Enzymatic Index (means)* | 5% | 1% |
| Type 1.1 | 1.744 | a | A | Type 5 | 2.180 | a | A |
| Type 1 | 1.162 | ab | A | Type 1.1 | 1.800 | b | AB |
| Type 5 | 1.160 | ab | A | Type 1 | 1.687 | b | B |
| Type 4 | 1.134 | b | A | Type 4 | 1.609 | b | B |
| Type 3 | 1.109 | b | A | Type 3 | 1.442 | b | B |

* Means followed by distinct letters differ among themselves at indicated significance level.

**Table 2.** Enzymatic index means in different culture media and temperatures.

| A. | All Strains (18) | | | | B. | Ifo 4626 strain | |
|---|---|---|---|---|---|---|---|
| Temp (ºC) | Culture Media | Enzymatic Iindex (means)* | 5% | 1% | Temp (ºC) | Culture Media | Enzymatic Index (means)* |
| | Type 1 | 0.937 | a | A | | Type 5 | 2.250 |
| 30 | Type 5 | 0.756 | a | AB | 30 | Type 3 | 2.050 |
| | Type 3 | 0.586 | ab | AB | | Type 4 | 1.950 |
| | Type 4 | 0.450 | ab | AB | | Type 1 | 1.950 |
| | Type 1 | 0.374 | ab | AB | | Type 1 | 0.000 |
| 37 | Type 5 | 0.245 | ab | AB | 37 | Type 3 | 0.000 |
| | Type 3 | 0.000 | b | B | | Type 4 | 0.000 |
| | Type 4 | 0.000 | b | B | | Type 5 | 0.000 |

* Means followed by distinct letters differ among themselves at indicated significance level.

1 medium (Table 2A - the values presented are arithmetic means and include parcels, in which there was no growth, because of low pectinolytic activity). In terms of strains performance, again IFO 4626 showed the highest levels of enzymatic activity. However, this strain had no growth at 37ºC. This fact produced too strong data variations, within the experiment. Statistical analysis showed no significant difference between media and temperature. For analytical purposes, data were transformed according to the following expression: $(x+2)^{1/2}$.

### Mycelium growth

IZ 36 strain presented the highest mycelium mass (38mg); followed by IZ 85 (33mg), IZ 37 and IZ 132 (both with 30 mg) and IFO 4626 (28mg); remaining strains are: IZ 47; IZ 86, and IZ 1619, respectively with 23 mg, 22 mg, and 18 mg. These experiments showed that there is no association between mycelium growth and pectinolytic enzyme production, as evaluated by EI, for those strains subjected to evaluations, in Type 3 medium, at 30ºC (IZ 36; IZ 47; IZ 85; IZ 1619, and IFO 4626). For example: IZ 36 strain, which produced the biggest mycelium mass (38 mg), presented EI equal to 1.00, while IFO 4626, with the higher EI (2.05) developed mycelium mass of 28 mg. (Last placed strains: IZ 47, 23 mg, no growth in solid medium; IZ 85, 33 mg, EI=1.00; IZ 1619, 18 mg, EI=1.00).

### Survival curve to U.V. light and evaluation of mutants

About 5% of the individuals survived, with 3-4 minutes of irradiation. We isolated 390 colonies of *P. chrysogenum*, derived from IFO 4626 strain.

### Enzyme extraction

Results (Table 3A and B, ahead) indicated that some of the mutants derivative of IFO 4626 exhibited superior performance in enzyme activity when compared to the original strain.

### Re-evaluation of strains

Strain IFO 4626 and derived mutants: 234, 272, 283, 309, 325, and 364 presented residual growth at 30ºC, but not at 37ºC. Cellulolytic FC-28 of *Trichoderma sp*. was the reference strain. Under the same conditions previously explained the reference strain presented normal growth, filling up the whole Petri plate with its mycelium. This work also evaluated the performance of the IFO 4626 mutants of *Penicillium chrysogenum*, under the same conditions of cultivation, after different storage periods. Enzymatic activity indexes were the same 4 years after isolation. These materials in stocks have been conserved in silica-gel. Fig. 1 presents the performance of the improved strains. As shown by a Tukey test, they are statistically superior to IFO 4626 strain, in an assay carried out in solid medium (14), after 4 years in stock, 3 days of cultivation at 30ºC, with the same enzymatic index production technique, as previously described.

### Benomyl resistant mutants

Industrial application success of improved strains depends on their genetics and physiological characterization, with a system that allows quick diagnosis. There are many available techniques for this purpose. There are reports using iso-enzymes associated with DNA molecular marker. However, resistant types of mutations can also be used for this purpose. This technique allows quick selection of improved strains, which become able to grow under new conditions (in this case, presence of benomyl (9)), what can not be done by common strains. Two spontaneous resistant mutants were isolated. An experiment was designed with the mutagenic agent, in order to increase the efficiency of mutants development. A survival curve of the IFO 4626 strains to EMS was obtained. A concentration of 100mg/mL of EMS, under 25 minutes of exposition, that determine 5% survival of the treated spore, was selected. Mutants resistant to benomyl

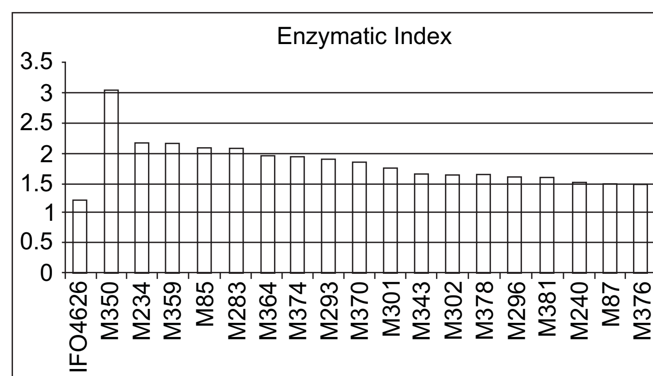


**Figure 1.** Enzymatic indez means of the improved strains higher than IFO 4626 in pectin media (14).

**Table 3.** IFO 4626 strain mutants pectin lyase activity means - selection first cycle.

A Essay 1

| Strains | Micelial Mass (mg/ml) | PL Activity | | Specific Activity | |
|---|---|---|---|---|---|
| | | (mu**) | Percentual performance | (Pl activity /Micelial Mass = mU Ml.mg$^{-1}$) | Percentual performance |
| M364 | 2.0 | 111.66 a* | 660 | 55.83 a* | 728 |
| M309 | 2.2 | 102.05 a | 606 | 46.39 a | 605 |
| M272 | 2.2 | 36.74 b | 218 | 16.70 b | 218 |
| IFO 4626 | 2.2 | 16.92 bc | 100 | 7.67 bc | 100 |
| M325 | 2.6 | 11.11 c | 66 | 4.27 c | 56 |
| M234 | 2.4 | 10.13 c | 60 | 4.22 c | 55 |

B. Essay 2

| Strains | Micelial Mass (mg/ml) | PL Activity | | Specific Activity | |
|---|---|---|---|---|---|
| | | (mu**) | Percentual performance | (Pl activity /Micelial Mass = mU Ml.mg$^{-1}$) | Percentual performance |
| M301 | 0.8 | 101.50 a | 507 | 126.88 a | 1901 |
| M304 | 1.0 | 99.40 a | 497 | 99.40 b | 1489 |
| M279 | 1.8 | 85.19 a | 426 | 47.33 c | 709 |
| M302 | 1.2 | 42.24 b | 211 | 35.20 cd | 529 |
| M359 | 1.0 | 31.48 bc | 157 | 31.48 d | 472 |
| M296 | 1.6 | 23.02 cd | 115 | 15.14 e | 227 |
| IFO 4626 | 3.0 | 20.02 cd | 100 | 14.39 e | 216 |
| M287 | 1.6 | 19.87 cd | 99 | 12.42 e | 186 |
| M239 | 2.8 | 18.32 cde | 92 | 11.62 e | 174 |
| M211 | 1.2 | 18.17 cde | 91 | 9.26 e | 139 |
| M178 | 1.4 | 16.26 cde | 81 | 8.44 e | 126 |
| M240 | 2.0 | 14.82 cde | 74 | 7.40 e | 111 |
| M281 | 1.6 | 13.51 cde | 67 | 6.73 e | 101 |
| M299 | 1.2 | 11.11 de | 55 | 6.67 e | 100 |
| M226 | 1.6 | 10.76 de | 54 | 6.54 e | 98 |
| M223 | 1.6 | 8.96 de | 45 | 5.60 e | 84 |
| M85 | 1.6 | 8.18 de | 41 | 5.11 e | 77 |
| M283 | 2.8 | 5.95 de | 30 | 2.13 e | 32 |
| M86 | 1.8 | 0.85 e | 4 | 0.47 e | 7 |

* Means followed by different letters are statistically different among themselves at the level of 5%. according to Tukey test; ** mU = nMoles of non saturated product liberated per minute of reaction per mL of enzyme solution.

were analysed in terms of pectinolytic enzymes, in order to verify whether they maintain equivalent production of pectinases. The results showed that all resistant mutants presented pectinolytic enzymes production similar to the non resistant mutant. For these evaluations, experimental designs completely randomized with 3 repetitions were used (do not shown).

**New mutation-selection cycle**

There is no evidence that exists isolated with better enzyme production than the original strains. In future experiments, we intend to change the selection criteria by isolating catabolic resistant mutants, related to pectin degradation. Fig. 2 presents a scheme of the mutation-selection cycle discussed in this paper.

**Additional remarks**

Starting with a group of pectinolytic strains, IFO 4626 of *Penicillium chrysogenum* was characterized. Improved strains were developed for textile fiber degumming. The oat medium was found to be the best sporulation media and the stock of original strain and their mutants in silica-gel showed good results. Additional work is being carried out on the pectinases production in terms of culture optimization, selection of new improved mutants, catabolic resistant mutantes, and kinetics of the enzyme.

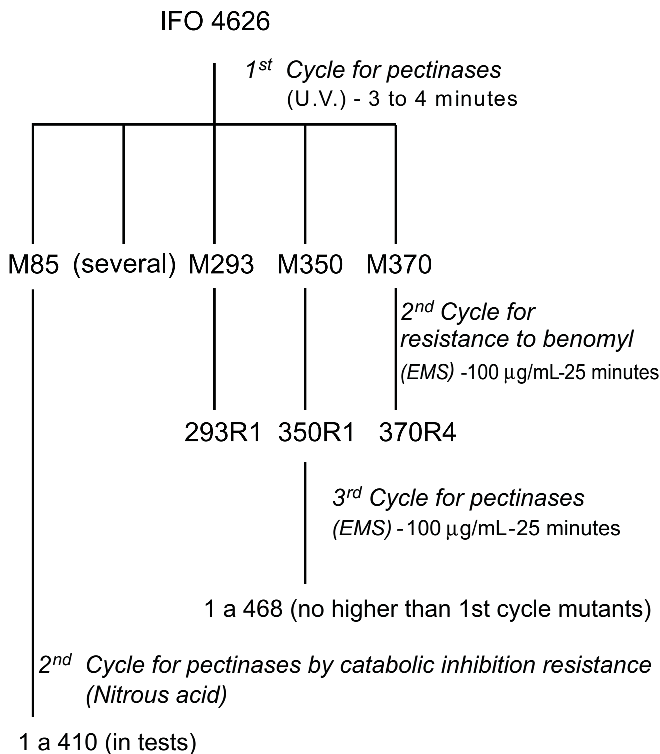


**Figure 2.** Mutation-selection cycle of *Penicillium chrysogenum.*


## ACKNOWLEDGEMENT

Authors thank Secretaria da Ciência, Tecnologia e Desenvolvimento Econômico do Estado de São Paulo, Brasil (Science, Technology and Economic Development Secretary of São Paulo State, Brazil; SCTDE-SP), for the grant that made the present work possible. The work is a part of the Project: "Aproveitamento de Resíduos da Agroindústria da Banana no Vale do Ribeira, SP" (Utilization of Banana Agroindustrial Wastes in Ribeira Valley, State of São Paulo, Brazil).


## RESUMO

### Triagem e melhoramento genético de fungos pectinoliticos para degomagem de fibras têxteis


Com a intenção de melhorar tecnologicamente os métodos de processamento de fibras vegetais, o presente trabalho comunica pesquisas feitas para obter linhagens mais eficientes de fungos produtores de pectinases. Mais especificamente com o objetivo de obter enzimas para degomagem de fibras têxteis caracterizou-se 18 linhagens de fungos filamentosos quanto a algumas condições de cultivo (meio de cultura e temperatura de crescimento) e quanto ao índice enzimático (IE) em meio de pectina sólido. Constatou-se superioridade da linhagem IFO 4626 (Q 176) de *Penicillium chrysogenum* e conduziu-se melhoramento genético da mesma com o objetivo de elevar sua produção de pectinases, mantendo em níveis insignificantes a atividade de celulases. Induziu-se mutações por meio de luz ultra-violeta e selecionou-se as 39 melhores linhagens entre 390 isolados. Obteve-se mutantes com produção de pectina liase aproximadamente nove vezes superior à da linhagem original no que se refere à atividade específica de PL e cinco vezes superior em termos de atividade de PL (liberação de µMoles por minuto de reação e mL de meio). Periodicamente, o desempenho dos mutantes foi avaliado em meio de pectina sólido. A estabilidade genética manteve-se por 4 anos após o isolamento dos mesmos.


**Palavras-chave:** *Penicillium chrysogenum*, pectina liase, enzimas pectinolíticas, degomagem de fibras têxteis.

## REFERENCES


1. Aguillar, G.; Huitrón, C. Constitutive exo-pectinase produced by *Aspergillus* sp. CH-Y-1043 on different carbon surce. *Biotechnol. Lett.*, 12(9): 655-660, 1990.
2. Alaña, A.; Gabilondo, A.; Hernando, F.; Moragues, M.D.; Dominguez, J.B.; Llama, M.J.; Serra, J.L. Pectin Lyase Production by a *Penicillium italicum* Strain**.** *Appl. Environ. Microbiol.*, 55(6): 1612-1616, 1989.
3. Amaral, D. *Experimentos de Microbiologia Geral.* MEC/Universidade Federal do Paraná, Curitiba, 1967, 107p.
4. Azevedo, J.L.; Costa, S.P. *Exercícios de Genética.* Ed. Nacional, São Paulo, 1973. 288p.
5. Baracat, M.C.; Valentim, C.; Muchovej, J.J.; Silva, D.O. Selection of Pectinolytic Fungi for Degumming of Natural Fibers. *Biotechnol. Lett.*, 11(12): 899-902, 1989.
6. Baracat-Pereira, M.C.; Venetti, M.C.D.; Araujo, E.F.; Silva, D.O. Partial characterization *of Aspergillus fumigatus* polygalacturonases for the degumming of natural fibers. *J. Ind. Microbiol.*, 11: 139-142, 1993.
7. Behere, A.; Satyanarayan, V.; Padwal-Desai, S.R. Separation and limited characterization of three polygalacturonases of *Aspergillus niger*. *Enzyme Microb. Technol.*, 15: 158-161, 1993.
8. Benatti Jr., R. *Rami: planta têxtil e forrageira.* Fundação Cargil, Campinas, 1985, 97p.
9. Bork, K.; Braymer, H.D. The genetic analysis of resistence to benomyl in *Neurospora crassa. J. Gen. Microbiol.*, 85: 51-56, 1974.
10. Brumano, M.H.N.; Coelho, J.L.C.; Araujo, E.F.; Silva, D.O. Pectin lyase activity of *Penicillium griseoroseum* related to degumming of ramie. *Rev. Microbiol.*, 24: 175-178, 1993.
11. Deshpande, K.S.; Guracharan, K. Degumming of ramie fibres: role of cell wall degrading enzymes of *Aspergillus versicolor. Ind. J. Bot.*, 8: 79-81, 1985.
12. Fawole, O.B.; Odunfa, S.A. Pectolytic moulds in Nigéria. *Lett. Appl. Microbiol.*, 15: 266-268, 1992.
13. Fernandes-Salomão, T.M.; Amorim, A.C.R.; Chaves-Alves, V.M.; Coelho, J.L.C.; Silva, D.O.; Araujo, E.F. Isolation of pectinase hiperproducing mutants of *Penicillium expansum*. *Rev. Microbiol.*, 27(1): 15-18, 1996.
14. Hanckin, L.; Anagnostakis, S.L. The use of Solid Media for Detection of Enzimic Production by Fungi. *Mycologia*, 67: 597-607, 1975.
15. Jarman, C.G.; Mykoluk, S.; Kenedy, L.; Canning, A.J. Banana fiber: a review of its properties and small-scale extraction and processing. *Tropical Science*, 19(4): 173-185, 1977.
16. Leuchtenberger, A.; Mayer, G. Changed pectinase synthesis by aggregated mycelium of some *Aspergillus niger* mutantes. Central

Institute of Nutrition, Bergholz-Rehbrücke, Germany. *Enzyme Microb. Technol.*, 14: 18-22, 1992.
17. Milagres, A.M.F.; Prade, R.A. Production of xylanases from *Penicillium janthinellum* and its use in the recovery of cellulosic textile fibers. *Enzyme Microb. Technol.*, 16: 627-632, 1994.
18. Phaff, H.J. The production of Exocellular Pectic Enzymes by *Penicillium chrysogenum*. I. On the formation and adaptative Nature of Polygalacturonase and Pectinesterase. *Arch. Biochem.*, 13: 67-81, 1947.
19. Pontecorvo, G.; Roper, J.A.; Memmons, L.M.; McDonald, K.D.; Bugton, A.W.J. The genetics of *Aspergillus nidulans*. *Advan. Gen.*, 5: 141-238, 1953.
20. Prescott, S.C.; Dumm, C.G.C.G. *Industrial Microbiology*. Mc Graw Hill, 1959, 945p.
21. Rosemberg, J.A. Microbiologia têxtil. *In*: Aquarone, E.; Borzani, W.; Lima, U.A. Tópicos de Microbiologia Industrial. Edgar Blücher, São Paulo, 1975. vol.2, p.97-112.
22. Said, S.; Fonseca, M.J.V.; Siéssere, V. Pectinase production by *Penicillium frequentans*. *World J. Microbiol. Biotechnol.*, 7: 607-608, 1991.
23. Sharma, H.S.S. Screening of polysaccharide-degrading enzyme for retting flax stem. *Int. Biodeter.*, 23: 1981-1986, 1987.
24. Sharma, H.S.S. Use of Chelating Compounds as Treatment for Removing Residual Non-Cellulosic Polysaccharides Present on Flax Fibre. *Cellulose Chem. Technol.*, 23(5): 531-534, 1989.
25. Siéssere, V.; Said, S. Pectic Enzymes Production in Solid-State Fermentation Using Citrus Pulp Pellets by *Talaromyces flavus*, *Tubercularia vulgaris* and *Penicillium charlessi*. *Biotechnol. Lett.*, 11(5): 343-344, 1989.
26. Solís, S.; Flores, M.E.; Huitrón, C. Isolation of Endopologygalacturonase Hiperproducing Mutants of *Aspergillus* sp. ch-Y-1043. *Biotechnol. Lett.*, 12(10): 751-756, 1990.
27. Spalding, D.H.; Abdul-Baki, A.A. *In vitro* and *in vivo* Production of Pectin Lyase by *Penicillium expansum*. *Phytopathol.*, 63: 231-235, 1973.