Chemical Mutagenesis of *Penicillium italicum* for the Development of Catabolite Insensitive Mutants

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ABSTRACT

The objective of this study was to enhance mannanase production through chemical mutagenesis and to develop catabolite insensitive mutants. Mutants were generated by incubating spore suspension of *Penicillium italicum* with varying concentrations of Ethyl Methane Sulfonate (EMS). Wild type and mutants were screened for mannanase production in basal media supplemented with Locust Bean Gum (LBG) as an inducer. Mannanase activity was determined by dinitrosalicylic acid method, while protein content was determined by Lowry method. Approximately 46% of the mutants generated showed higher mannanase activity in comparison with the wild type, while repression of enzyme biosynthesis was observed in others. The isolated mutants were screened for catabolite activation studies in the presence of different mannose concentrations (0.1, 0.5 and 1% w/v) as a carbon source. The supplementation of 0.1% (w/v) mannose in the fermentation media caused enhancement of mannanase synthesis in approximately 54% of the mutants. The supplementation of 0.5 and 1% (w/v) mannose in the fermentation media caused improvement of mannanase biosynthesis in 100% and approximately 62% of the mutants, respectively. The results indicated that EMS might be an effective mutagenic agent for the development of catabolite insensitive mutants.

Key words: Chemical mutagenesis, mannanase, ethyl methane sulfonate, catabolite insensitive mutants, *Penicillium italicum*

INTRODUCTION

Mannans in soft and hardwoods occur in two forms, galactomannan and acetylated galactomannan. Galactomannan is made up of mannose residues and it is abundant in the seeds of leguminous plants while acetylated galactomannan is a principal component of hemicelluloses with heterogenous backbone mannose and glucose units. Mannanases otherwise known as 1,4-β-D-mannan mannanohydrolases occurs in a variety of microorganisms such as fungi, yeasts and bacteria as well as in leguminous seeds of terrestrial plants (Heck *et al.*, 2005; Lin *et al.*, 2007; Olaniyi *et al.*, 2014a).

Mannans are stored in the bulbs and endosperm of some plants as carbohydrate. Mannans either in form of galactoglucomannans or glucomannans in plants are both branched heteropolysaccharides that require a consortium of hydrolase enzymes for their complete decomposition (Youssef *et al.*, 2006). Mannanases is the enzyme that breaks down β-1,4-mannosidic
bonds in the main chain of mannans, galactomannans, glucomannans and galactoglucomannans into mannooligosacharides and small amounts of mannose, glucose and galactose (Olaniyi et al., 2014b). The mannanases have been classified into two major families, glycosyl hydrolase 5 (GH5) and glycosyl hydrolase 26 (GH26) (Lin et al., 2007). The protein folding, its mode of catalytic action and mechanism of glycosidic and mannosidic bond cleavage are conserved within mannosidic enzyme families (Abdel-Fattah et al., 2009).

The increased demand of mannanases for various industrial applications has necessitated expansion both in qualitative and quantitative enhancement. Quantitative demand for mannanases require strain improvement coupled with optimization of cultural parameters for enhanced productivity as the quantities elaborated by parent strains are usually unappreciable (Haq et al., 2010). The extensive application of mutation and selection of improved mutants have been the spectacular success of research input (Bapiraju et al., 2004). Strains’ improvement can be achieved by mutagenesis, which is a successful method. The improved mutants with higher yielding enzyme potential can be obtained through Ultraviolet (UV), gamma (γ) irradiations, ethyl methyl sulphonate (Femi-Ola, 2008; Haq et al., 2010; Olaniyi et al., 2014a) and N-methyl-N’-nitro-N-nitroso guanidine (Iftikhar et al., 2010). They had been reported as effective mutagenic tools for strain improvement of certain microorganisms.

Microbial enzyme biosynthesis is regulated by induction and repression mechanisms. Enzyme production is repressed in microorganisms when cultured in a medium supplemented with high concentration of readily metabolized carbon source (Olaniyi et al., 2014a). The rate of enzyme-mediated hydrolysis of cellulose has been reported to be inhibited by products of hydrolysis and fermentation products, particularly when hydrolysis and fermentation are carried out at the same time (Lynd et al., 2002). Increase in the cost of enzymes production has always been the consequence of repressing substance and end product associated with the fermentation. A numbers of research had been carried out on catabolite repression of cellulase biosynthesis. However, there is scanty information available on catabolite repression of mannanase synthesis in mannolytic microorganisms.

The challenge associated with catabolite repression of mannanase production in microorganisms necessitated this study. A series of efforts have been made to generate mutants that are resistant to catabolite repression. The use of catabolite insensitive mutants might eliminate this challenge and allow high yields of enzymes in direct microbial fermentation. The aims of the present investigation were to generate chemical mutants from P. italicum capable of producing large quantities of mannanase and screen the mutants for the isolation of catabolite insensitive mutants.

MATERIALS AND METHODS
Fungal strain: Penicillium italicum was sourced from the culture collection of Research Laboratory, Federal University of Technology, Akure, Nigeria. The strain whose origin was yam peels, screened and confirmed to be positive for mannanase activity by plate assay technique was used in this study (Arotupin and Olaniyi, 2013). This strain was selected based on its performance in terms of quantitative mannanase production both in solid and submerged state fermentation. The identity of this strain was ascertained to ensure its purity and viability through cultural characters and microscopic structure. The culture was maintained on Locust Bean Gum (LBG) containing agar slant at 4°C throughout the study.
Inoculum preparation: The inoculum of *P. italicum* grown for 96 h at 30°C on LBG agar medium slants was prepared by adding 10 mL of sterile distilled water, which contained 0.1% (v/v) Tween 80 to the agar slant and shaking vigorously. The spore suspension was adjusted to the spore concentration of $10^6$ spores mL$^{-1}$ (as the initial inoculum size) (Ibrahim *et al*., 2012).

Mutation by ethyl methane sulfonate: Mutant strains were generated with EMS using eight test tubes with 2 mL of cell suspension ($10^6$). One of them was kept aside as control and the rest were incubated with EMS concentrations varying from 20-100 μL for 30 min at room temperature (32±2°C) as described by Radha *et al.* (2012). Each test tube was made up to 5 mL with distilled water. After required period of treatment, the cells were centrifuged at 3000 rpm for 15 min and washed with sterilized phosphate buffer (pH 7.0) twice. A volume of 0.1 mL of EMS-treated fungal spore suspension was poured into sterilized petri plates containing malt extract agar medium. The spores of mutants generated were kept for subsequent use at 4°C (Radha *et al*., 2012).

Mannanase production by wild and mutant strains: The mutants and the wild type of *P. italicum* were screened for mannanase production under submerged state fermentation. Enzyme production was performed in 250 mL Erlenmeyer flask containing 50 mL of enzyme Production Medium (PM). This medium contained (g L$^{-1}$) LBG 10, NaNO$₃$ 2, KH$_₂$PO$_₄$ 1, MgSO$₄·₇H₂O$ 0.5, KCl 0.5, FeSO$_₄·₇H₂O$ traces, pH 6.8. The medium was sterilized at 121°C for 15 min. After sterilization, each flask was inoculated with 2 discs of 8 mm diameter of both wild type and mutant strains of *P. italicum* (Olaniyi *et al*., 2014b). The cultures were harvested after 5 days of incubation by centrifugation at 6000 rpm for 15 min at 4°C using refrigerated centrifuge. The supernatants were used as the crude extracellular enzyme source. Each treatment was carried out in triplicate and the results obtained throughout the work were the arithmetic mean of 3 experiments.

Mannanase assay: Mannanase activity was assayed in the reaction mixture composing of 0.5 mL of 1% LBG prepared in 50 mM potassium phosphate buffer pH 6.8 and 0.5 mL of supernatant at 45°C for 60 min (Youssef *et al*., 2006). The control tube contained the same amount of substrate and 0.5 mL of the enzyme solution heated at 100°C for 15 min. Both experiment and control tubes were incubated at 45°C for 60 min. At the end of the incubation period, tubes were removed from water bath (Lamfield Medical England Model DK-600) and the reaction was terminated by the addition of 2 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube. The tubes were incubated for 5 min in a boiling water bath for colour development and were cooled rapidly. The activity of reaction mixture was measured against a reagent blank at 540 nm. Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) (Miller, 1959). One unit of mannanase activity was defined as amount of enzyme producing 1 μmole of mannose per minute under standard assay conditions.

Screening of mutants for catabolite insensitive mutants: The mutants and wild type of *P. italicum* were subjected to catabolite activation studies in basal medium containing varying concentrations of mannose as an energy source (0.1, 0.5 and 1% w/v). The cultures were incubated at 30±2°C in static condition for 5 days (as described above under enzyme production). Mannanase synthesis by each mutant was induced by using 1% (w/v) LBG. Mutant which exhibited appreciable enzyme activity at screening stage were referred to as catabolite activation mutant (repression resistant mutants) (Olaniyi *et al*., 2014b).
**Statistical analyses:** The statistical analysis was performed using the general linear model function of Statistical Package for Social Science (SPSS), version 16.0. All data generated was subjected to one-way ANOVA while statistical differences of treatment were determined using Duncan’s Multiple Range (DMR).

**RESULTS**

**Production of mannanase by the wild type and mutant strains of *P. italicum:*** Table 1 shows mannanase activity, protein content, specific mannanase activity and percentage increase in mannanase activity of wild type and mutants of *P. italicum.* A total of thirteen mutants were developed from five different concentrations of EMS after 30 min of exposure. Mannanase was synthesized by the mutants at varying quantities. Approximately 46% of the mutant strains of *P. italicum* (EMSA32, EMSA41(b), EMSA412, EMSA41(a) and EMSA52 (b)) generated from 60, 80 and 100 μL EMS treatment showed higher mannanase activity when compared with parent strain, while repression of enzyme biosynthesis was observed in others. However, of all the mutants generated, mutant EMSA41(b) had the highest percentage enzyme activity of 169.14% with approximately 0.7 fold higher than the parent strain. Approximately 54% of the mutants generated exhibited 0.00 mg mL⁻¹ protein, while others had more than 0.49 mg mL⁻¹.

**Production of mannanase by the wild type and mutant strains of *P. italicum* supplemented with 0.1% (w/v) mannose:** Table 2 shows mannanase activity, protein content, specific mannanase activity and percentage increase in enzyme activity of mutants of *P. italicum.* The supplementation of 0.1% mannose in enzyme production media caused enhancement of enzyme biosynthesis in 53.85% of the mutants (EMSA11, EMSA12, EMSA22 (b), EMSA32, EMSA41 (b), EMSA412 and EMSA52 (a)) when compared with the wild type. However, of all the mutants generated, mutant EMSA32 had the highest percentage enzyme activity of 1512% with approximately 15 fold higher than the parent strain. The amount of protein generated in the fermentation medium inoculated with wild type was significantly (p<0.05) higher than the protein generated by any of the mutants.

| Table 1: Production of mannanase by the wild type and mutant strains of *Penicillium italicum* | EMS conc. | Mannanase activity | Protein content | Specific activity | Increase in activity (%) |
|-----------------------------------------------|-----------|--------------------|----------------|-----------------|------------------------|
| Mutants (μL/2 mL spore)                       | (U mL⁻¹)  | (mg mL⁻¹)          | (U mg⁻¹)       |                 |                        |
| Wild strains/control                         | Nil       | 70.56±0.00i        | 0.00±0.00a      | 1411.00±0.00n   | 100.00                 |
| Chemical mutagenesis                         |           |                    |                |                 |                        |
| EMSA11                                        | 20        | 21.63±0.03a        | 0.02±0.00b      | 1140.20±0.01d   | 72.44                  |
| EMSA12                                        | 20        | 119.02±0.02m       | 0.00±0.01a      | 13222±0.01n     | 40.16                  |
| EMSA22 (b)                                    | 40        | 60.91±0.02h        | 0.00±0.00i      | 12178±0.00i     | 30.71                  |
| EMSA21 (B)                                    | 40        | 44.32±0.01f        | 0.00±0.00a      | 8867±0.00a      | 51.34                  |
| EMSA31                                        | 60        | 36.11±0.02c        | 0.00±0.00a      | 7222±0.00a      | 51.18                  |
| EMSA32                                        | 60        | 102.33±0.01l       | 0.05±0.01c      | 1827±0.02g      | 131.66                 |
| EMSA41 (b)                                    | 80        | 36.22±0.01d        | 0.05±0.00c      | 710.21±0.01e    | 169.14                 |
| EMSA412                                       | 80        | 119.34±0.01k       | 0.00±0.00i      | 119.33±0.00b    | 130.08                 |
| EMSA41 (a)                                    | 80        | 51.12±0.01f        | 0.02±0.00c      | 2222.20±0.01l   | 168.66                 |
| EMSA52 (a)                                    | 100       | 28.34±0.01l        | 0.00±0.00i      | 5687±0.00a      | 77.95                  |
| EMSA51 (a)                                    | 100       | 92.90±0.01i        | 0.00±0.00i      | 92.90±0.02a     | 86.30                  |
| EMSA52 (b)                                    | 100       | 55.00±0.00f        | 0.02±0.00b      | 2381.20±0.16f   | 145.04                 |
| EMSA51 (b)                                    | 100       | 91.77±0.01l        | 0.02±0.00b      | 4830.50±0.01b   | 62.84                  |

Mean with the same supercript letters in the same column are not significantly different (p<0.05), EMSA: Ethyl methane sulphonate mutants of *P. italicum*
Production of mannanase by the wild type and mutant strains of *Penicillium italicum* supplemented with 0.1% (w/v) mannose

| Mutants          | EMS conc. (μL/2 mL spore) | Mannanase activity (U mL⁻¹) | Protein content (mg mL⁻¹) | Specific activity (U mg⁻¹) | Increase in activity (%) |
|------------------|---------------------------|----------------------------|---------------------------|---------------------------|--------------------------|
| Wild strain/control | Nil                       | 0.00±0.00                  | 1.07±0.01                 | 0.00±0.00                 | 100.00                   |
| **Chemical mutagenesis** |                           |                            |                           |                           |                          |
| EMSA11           | 20                        | 1.12±0.01                  | 0.56±0.01                 | 2.02±0.02                 | 112.00                   |
| EMSA12           | 20                        | 7.33±0.00                  | 0.82±0.01                 | 9.00±0.00                 | 733.00                   |
| EMSA21 (b)       | 40                        | 5.35±0.01                  | 0.92±0.01                 | 5.75±0.01                 | 535.00                   |
| EMSA21 (B)       | 40                        | 0.00±0.00                  | 0.60±0.01                 | 0.00±0.00                 | 0.00                     |
| EMSA31           | 60                        | 15.12±0.01                 | 0.90±0.01                 | 17.02±0.02                | 1512.00                  |
| EMSA32           | 60                        | 9.27±0.05                  | 0.45±0.01                 | 19.91±0.01                | 927.00                   |
| EMSA41 (b)       | 80                        | 2.35±0.01                  | 0.60±0.01                 | 3.94±0.03                 | 235.00                   |
| EMSA41 (a)       | 80                        | 0.00±0.00                  | 0.92±0.01                 | 0.00±0.00                 | 0.00                     |
| EMSA52 (a)       | 100                       | 4.66±0.02                  | 0.09±0.00                 | 50.15±0.01                | 466.00                   |
| EMSA51 (a)       | 100                       | 0.00±0.00                  | 0.21±0.01                 | 0.00±0.00                 | 0.00                     |
| EMSA52 (b)       | 100                       | 0.00±0.00                  | 0.00±0.00                 | 0.00±0.00                 | 0.00                     |
| EMSA51 (b)       | 100                       | 0.35±0.01                  | 0.39±0.02                 | 0.86±0.02                 | 35.00                    |

Mean with the same subscipt letters in the same column are not significantly different (p<0.05), EMSA: Ethyl methane sulphonate mutants of *P. italicum*

Production of mannanase by the wild type and mutant strains of *Penicillium italicum* supplemented with 0.5% (w/v) mannose

| Mutants          | EMS conc. (μL/2 mL spore) | Mannanase activity (U mL⁻¹) | Protein content (mg mL⁻¹) | Specific activity (U mg⁻¹) | Increase in activity (%) |
|------------------|---------------------------|----------------------------|---------------------------|---------------------------|--------------------------|
| Wild strain/control | Nil                       | 100.00±0.00                | 0.00±0.00                 | 100.00±0.00               | 100.00                   |
| **Chemical mutagenesis** |                           |                            |                           |                           |                          |
| EMSA11           | 20                        | 575.00±0.00                | 1.07±0.02                 | 526.55±0.01               | 575.00                   |
| EMSA12           | 20                        | 344.46±0.04                | 1.23±0.01                 | 281.42±0.02               | 344.43                   |
| EMSA21 (b)       | 40                        | 463.07±0.00                | 0.56±0.00                 | 832.86±0.02               | 463.07                   |
| EMSA21 (B)       | 40                        | 326.59±0.02                | 1.18±0.01                 | 279.61±0.02               | 326.57                   |
| EMSA31           | 60                        | 379.01±0.01                | 1.05±0.01                 | 358.91±0.02               | 379.00                   |
| EMSA32           | 60                        | 371.54±0.60                | 1.56±0.00                 | 239.22±0.01               | 372.21                   |
| EMSA41 (b)       | 80                        | 336.51±0.01                | 0.90±0.01                 | 379.92±0.02               | 336.50                   |
| EMSA412          | 80                        | 291.30±0.01                | 1.72±0.01                 | 436.05±0.01               | 291.29                   |
| EMSA41 (a)       | 80                        | 338.06±0.01                | 0.67±0.01                 | 198.41±0.02               | 338.07                   |
| EMSA52 (a)       | 100                       | 379.35±0.01                | 0.00±0.00                 | 378.34±0.02               | 379.36                   |
| EMSA51 (a)       | 100                       | 472.62±0.02                | 0.72±0.01                 | 652.83±0.01               | 472.64                   |
| EMSA52 (b)       | 100                       | 267.88±0.02                | 0.21±0.00                 | 1313.00±0.01              | 267.86                   |
| EMSA51 (b)       | 100                       | 288.05±0.03                | 0.62±0.01                 | 461.64±0.01               | 288.07                   |

Mean with the same subscipt letters in the same column are not significantly different (p<0.05), EMSA: Ethyl methane sulphonate mutants of *P. italicum*
Table 4: Production of mannanase by the wild type and mutant strains of *Penicillium italicum* supplemented with 1% (w/v) mannose

| Mutants               | EMS conc. (μL/2 mL spore) | Mannanase activity (U mL⁻¹) | Protein content (mg mL⁻¹) | Specific activity (U mg⁻¹) | Increase in activity (%) |
|-----------------------|---------------------------|----------------------------|---------------------------|---------------------------|--------------------------|
| Wild strain/control   | Nil                        | 100.00±0.00f                | 0.00±0.00a                 | 100.00±0.00c               | 100.00                   |
| Chemical mutagenesis  |                            |                            |                           |                           |                          |
| EMSA11                | 20                         | 104.11±0.07x                | 1.02±0.01f                 | 102.11±0.02e               | 104.03                   |
| EMSA12                | 20                         | 64.88±0.09b                 | 0.71±0.01f                 | 88.81±0.16b               | 64.97                    |
| EMSA21 (B)            | 40                         | 50.36±0.08g                 | 0.30±0.03d                 | 170.38±0.01h               | 50.44                    |
| EMSA22 (b)            | 40                         | 116.58±0.05i                | 0.12±0.01l                 | 1050.70±0.05m              | 116.64                   |
| EMSA31                | 60                         | 130.53±0.06j                | 1.07±0.02e                 | 119.36±0.01f              | 130.48                   |
| EMSA32                | 60                         | 324.33±0.02n                | 0.11±0.00o                 | 2922.00±0.01m              | 324.34                   |
| EMSA41 (b)            | 80                         | 116.29±0.05j                | 0.87±0.01i                 | 133.64±0.03g              | 116.29                   |
| EMSA412               | 80                         | 203.62±0.06m                | 0.54±0.00f                 | 379.25±0.03a              | 203.67                   |
| EMSA41 (a)            | 80                         | 87.73±0.01d                 | 0.29±0.01d                 | 315.58±0.03e              | 87.74                    |
| EMSA41 (a)            | 100                        | 66.04±0.01c                 | 0.93±0.01b                 | 69.93±0.01c               | 66.03                    |
| EMSA42 (a)            | 100                        | 171.93±0.02f                | 0.55±0.02e                 | 308.32±0.01i              | 171.98                   |
| EMSA42 (b)            | 100                        | 98.29±0.03g                 | 0.92±0.01i                 | 108.33±0.00h              | 98.25                    |
| EMSA51 (b)            | 100                        | 169.90±0.03h                | 0.04±0.01k                 | 4591.30±0.01m             | 169.88                   |

Mean with the same supercript letters in the same column are not significantly different (p<0.05), EMSA: Ethyl methane sulphonate mutants of *P. italicum*

production media caused varying degrees of mannanase enhancement in approximately 62% of the mutants (EMSA11, EMSA22 (b), EMSA31, EMSA32, EMSA41 (b), EMSA412, EMSA52 (a) and EMSA51 (b)), while others had their mannanase biosynthesis repressed (Table 4). The highest percentage increase in enzyme activity of 324.34% was obtained in EMSA32 which was approximately 3 fold higher than the value obtained for the parent strain. All the mutants had higher protein contents than the parent strain.

**DISCUSSION**

The purpose of any mutagenesis is to generate and select mutants with improved properties. The wild type of *P. italicum* was subjected to chemical mutagenesis with a view to obtain mutants which could produce mannanase in large amounts and with high catalytic efficiency. It was well documented that EMS was one of the strongest and multi-potential carcinogens that has been frequently reported (Zhu et al., 2000; Zia et al., 2010; Olaniyi et al., 2014b). *Cellulomonas* sp. was treated with EMS as a mutagen to obtain a hype rxylanolytic mutant which was 2.5-fold higher than parent strain in terms of xylanase production (Zia et al., 2010). Chemical mutagenesis of *A. niger* was carried out with EMS and appreciable glucose oxidase activity was obtained (52.8 U mL⁻¹) (Khattab and Bazaraa, 2005). In a study conducted by Olaniyi et al. (2014b), the entire EMS mutants developed from *Klebsiella edwardsii* showed higher specific enzyme activities than the parent strain.

Efforts have been made to screen a large array of microorganisms with ability to produce enzymes intended for industrial application (Sae-Lee, 2007; Mabrouk and El Ahwany, 2008; Olaniyi et al., 2014b). Many research had been conducted with the aim of improving microbial strains for enhanced enzyme production; however there is no or scanty information on improved microbial strains for enhanced mannanase production. Improved strains could be developed through physical and chemical mutagenesis in which the use of EMS takes a lead (Rani and Prasad, 2012). Improved EMS mutants from *P. italicum* produced promising amounts of mannanase and can therefore be utilized at bioreactor level for industrial production. The improvement in the mutants for mannanase production could be due to ease of reconstitution of damaged genes by these strains with improved properties. The variation in mannanase production between mutant strains might be due to some factors like damaged DNA and differences in their ability to repair damaged genes (Rani and Prasad, 2012).
The supplementation of mannose in enzyme production caused varying degrees of repression in certain EMS mutants. Enzyme production in microorganisms is regulated by induction and repression mechanisms (Bakare et al., 2005; Femi-Ola, 2008). Low enzyme production is expected because the organisms (wild type and mutants) already have the simple sugar in their media, hence do not need to secrete hydrolytic enzymes (proteins) in which mannanase is one. It has been reported by Brock and Madigan (1991) that cyclic AMP plays a crucial role in catabolite repression mechanisms of exoenzyme production. This molecule exerts its influence through an allosteric protein known as catabolite gene activation protein (CAP) or cyclic AMP receptor protein (CRP). The cyclic AMP-CRP complex facilitates binding of RNA polymerase to the promoter site of catabolite sensitive operon which alleviates the repression state. As long as the sugar is available as an energy source, the cyclic AMP concentration is low, thereby preventing the synthesis of enzymes capable of utilizing other energy sources through CRP (Bakare et al., 2005; Brock and Madigan, 1991). This observation may also be applicable to mannanase production in mutants generated, because mannanase activity was reduced in some mutants in the presence of different sugar concentrations. Regulation of cellulase production had been well researched and documented (Moreno et al., 2001; Kotchoni et al., 2003); however, scanty information has been reported on regulation of mannanase production in microorganisms to date. The improvement in some of the mutants evaluated in media supplemented with mannose suggested that their regulatory genes might have lost their regulatory functions, thereby allowing the catabolite insensitive mutants to produce mannanase without inhibition by sugars.

CONCLUSION

These trials suggest that EMS mutants from P. italicum were developed and some of them had potential for increased production of mannanase. The development of catabolite insensitive mutants might eliminate the repression of mannanase biosynthesis and allow its continuous high yields in direct microbial fermentation. The mechanism involved in the generation of catabolite insensitive mutants is yet to be unraveled. Therefore, further molecular studies on catabolite insensitive mutants will be necessary to diagnose the mutation.

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