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PRODUCTION OF XYLANASE BY *ASPERGILLUS* SPP. IN SOLID STATE FERMENTATION

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Abstract

Isolation and screening of fungal hetero-culture for the production of xylanase were dealt with. The fungal strains were isolated from different soil samples and subjected to primary and secondary screening for xylanase production. The hetero-culture LCWU-31consists of *Aspergillus niger* and *Aspergillus flavus* which showed highest xylanlolytic potential. Six different fermentation media were screened and M_2 medium containing wheat bran was designated as an optimized medium. Different physico-chemical parameters including rate of fermentation, incubation temperature, pH, moisture content, size of inoculum were studied. The optimum xylanase production was obtained at 96 hrs, 30°C, pH 6 and 40% moisture content with 2 ml of inoculum. The 2% glucose and 0.075% of $NaNO_3$ act as best carbon and nitrogen source, respectively. The maximal xylanase production was obtained in the presence of $CaCl_2$.The novelty of this study was the use of synergistic phenomena of fungi by the use of hetero-culture technique for the enhanced production of xylanase.

Introduction

Xylanases (EC 3.2.1.8) belonging to hydrolytic group are extracellular enzymes and can be produced by a number of microorganisms. It hydrolyses xylan and produces xylose which is a prime carbon source. Xylan is the most important and major portion of the hemicellulose in the plant cell wall. They are long hetero-polymers, in which β-D-xylopyranosyl units join together to form 1 - 4 glycosidic linkages. They mainly consist of xylose and arabinose. Xylanase breaks the β-1,4 bond present in xylan (Collins *et al.* 2005). Xylanase has enormous biotechnological applications. Most commonly in animal nutrition, bio bleaching, papermaking, textile industry, pharmaceutical industries, bread making and in clarification of fruit juices (Kulkarni *et al.* 1999, Guimaraes *et al*. 2013). Xylanase can be derived from various microorganisms like fungi and bacteria (Knob *et al.* 2014). However, xylanase produced by fungi is preferred over the bacterial xylanase because bacterial xylanase are not subjected to post translational modifications like glycosylation, which is an important process that protects protein proteolysis, affects the stability of protein confirmation, and improves protein stability (Polizeli *et al*. 2005). The biosynthesis of xylanase can be carried out either by solid state fermentation (SSF) or submerged fermentation (SmF). However, SSF is preferred over SmF because of its simple technique, more stable product, low investment, higher productivity and reduced energy requirements (Pandey *et al.* 1999). For the efficient production of xylanase it is very important to choose the ideal substrate because the outcome of fermentation highly varies for each substrate. Different agricultural products including wheat bran, sugarcane bagasse, cotton hull, rice husk, corn cob, corn meal, saw dust, oat straw, gram husk have been used. Among these wheat bran proved to be best and economical substrate for fungal xylanase production (Ahmad *et al.* 2009). The hetero culturing of microorganisms can

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improve production levels of biological metabolites such as enzymes. As compared to monocultures, hetero cultures of fungi may lead to improved productivity, better substrate utilization, increased adaptability to changing conditions and increased resistance to contamination. Nutritional limitations may be overcome in synergetic interactions between compatible partners (Ahmed *et al.* 2008).

Materials and Methods

The fungal strains were isolated from the different soil samples collected from the different areas of Punjab. Isolation of fungal strains was made using serial dilution method (Clark *et al*. 1958). Xylan agar plates were used for primary screening. The fungal colony showing bigger xylan hydrolysis zones were collected. Compatibility of all the collected strains was tested. The strains showing compatibility with each other were screened for xylanase production employing solid state fermentation. Conidial inoculum was prepared by adding 10 ml of sterilized saline water in each slant containing 3-4 days old fungal culture and mixed thoroughly for preparing uniform suspension with the help of inoculating loop. The 0.5 ml of inoculum from both slants was transferred in 250 ml Erlenmeyer flasks containing sterilized solid substrate moistened with mineral salt medium. All the flasks were incubated at 30°C for 72 hrs. After incubation, 100 ml of distilled water was added in the fermented bran and placed in an incubator shaker. After incubation the content of flasks was filtered. The filtrate was used for the estimation of xylanase. Different fermentation media were screened for xylanase production include g/l

M_1: 10 g of sugar cane bagasse and 15 ml of Mandel's Medium (g/l) containing Urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/l): $FeSO_4.7H_2O$, 5; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.7H_2O$, 1.4 and $CoCl_2.6H_2O$, 20.0 .

M_2: Wheat bran, 10 g; 10 ml trace element solution contained (g/1); $FeSO_4$, 0.05; $ZnSO_4.7H_2O$, 0.014; $CoCl_2$, 0.02 and $MnSO_4$, 0.016.

M_3: Rice Bran, 5 g; Nutrient salt solution (15 ml) containing g/l 1.5 KH_2PO_4, 4.0 NH_4Cl, 0.5 $MgSO_4.7H_2O$, 0.5 KCl, 1.0 Yeast extract with 0.04 ml/l trace elements having µg/l 200 $FeSO_4.7H_2O$, 180 $ZnSO_4.7H_2O$ and 20 $MnSO_4$.

M_4: Corncob, 10.0 g; Mandel's medium, 15 ml containing Urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/l): $FeSO_4.7H_2O$, 5; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.7H_2O$, 1.4 and $CoCl_2.6H_2O$, 20.0.

M_5: 10 g of Wheat straw, Mandels and Stenburg's Basal Medium containing (g/l) 10.0 g; Urea, 0.3; peptone, 0.75; yeast extract, 0.25; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0 $CaCl_2$, 0.3; $MgSO_4.7H_2O$, 0.3 and trace elements (mg/l): $FeSO_4.7H_2O$, 5; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.7H_2O$,1.4; $CoCl_2.6H_2O$ and Tween 80, 0.1%.

M_6: Saw dust; 5.0 g and Mandels and Sternburg's basal (MS) medium adjusted to 1:5 (substrate: medium, w/v) containing peptone, 1 g/l; Tween 80, 0.1% (v/v); $(NH_4)2\ SO_4$, 1.4; KH_2PO_4, 2.0; Urea, 0.3; $CaCl_2$, 0.3; $MgSO_4\ 7H_2O$, 0.3; trace elements (mg/l): $FeSO_4\ 7H_2O$, 5.0; $MnSO_4\ H_2O$, 1.6; $ZnSO_4\ 7H_2O$, 1.4 and $CoCl_2$, 2.0.

The estimation of xylanase activity was carried out according to Ahmad *et al.* (2009). The enzyme activity was determined by taking 1mL of enzyme along with 1.0mL of xylan (0.6%).The enzyme substrate complex was incubated at 30^{o}C for 30 min. The reducing sugar was estimated at 546 nm by using the DNS method (Miller 1959). One unit of enzyme activity was defined as the "amount of enzyme that releases one mg of reducing sugar from 1% xylan equivalent to 1mg of xylose under standard assay conditions". Total protein was estimated according to the Bradford (1976). All the experimental data were subjected to statistical analysis. Post hoc multiple

comparison tests were applied under one way ANOVA. The significance has been presented at $p \leq 0.05$ by using SPSS (16.0).

Results and Discussion

The isolation and selection of appropriate fungal hetero-culture are paramount for successful and economic production of xylanase. In addition to this, optimization of effective parameters equally plays an important role for successful biosynthesis of xylanase. In the present study, different filamentous fungal strains were isolated from the soil samples collected from different localities of Pakistan by using serial dilution method (Clark *et al*. 1958). After testing the compatibility of each strain, 45 compatible fungal hetero-cultures were screened for xylanase production by employing solid state fermentation (data not shown). The hetero-culture LCWU-31 gave maximum xylanase production (139 U/ml). This hetero-culture identified according to Mazza *et al*. (1997) consists of *Aspergillus niger* and *Aspergillus flavus*. Results revealed that hetero-culturing of fungal strains is more effective as the fungal strains act synergistically and substrate shows colonization thus become accountable for improving the enzyme activity.

It is very important to select a suitable fermentation medium that must be economical and have better capability for enzyme production. Therefore, six different media were tested (Fig. 1a). Among them M_2 gave maximal enzymatic activity. It might be due to the presence of minute quantities of trace elements in the medium that allow the fungal strains to grow and enhance the production in a natural environment and thus it is very economical too (Isil and Nilufer 2005). Various concentrations of selected substrate wheat bran (5 - 30 g) were tested. The 25 g of wheat bran produced maximum xylanase (Fig. 1b). Further increase or decrease from 25 g resulted decline in enzyme production. The reason might be that lower wheat bran concentrations had less availability of nutrients for the growth of fungal hetero-culture as well as enzyme production.

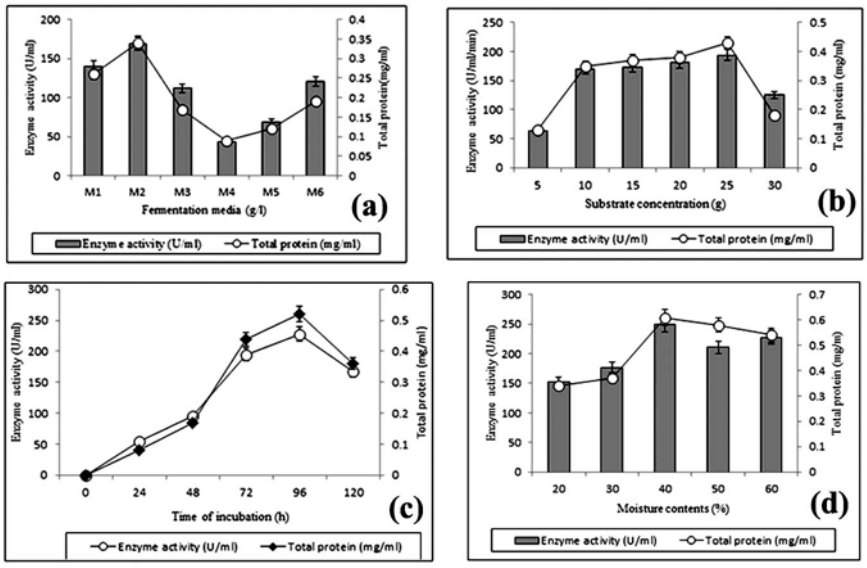

Fig 1. (a) Screening of fermentation media for the biosynthesis of xylanase by fungal hetero-culture, (b) Influence of different concentration of substrate on the biosynthesis of xylanase by fungal hetero-culture, (c) Effect of rate of fermentation on the biosynthesis of xylanase by fungal hetero-culture and (d) Effect of moisture content on the biosynthesis of xylanase by fungal hetero-culture.

While high concentrations of substrate (30 g) were not supportive because of poor availability of oxygen (Ahmed 2009). The time of incubation plays a key role for significant production of xylanase. In the current study xylanase productivity show increasing trend up to 96 hrs (Fig. 1c). A further increase in the incubation period resulted decline in output of enzyme. Numerous factors might be responsible for this reduction like nutrient deficiency, formation of by products that inhibits the growth of fungi and secretion of enzyme. Kavya and Padmavathi (2009) optimized the xylanase production in 144 hours of incubation. These results are in contrast of the present work. This shows that present work is more significant than previous reported work because it is more economical to reduce the time. In the present study influence of varying moisture contents (20 - 60%) on the biosynthesis of xylanase was observed (Fig. 1d). The 40% moisture content was observed to be optimum. Any rise or fall in moisture contents resulted decline in xylanase production. Perhaps it might be due to the more moisture content caused fall in the production of enzyme because of difficult growth of producer strain. It caused particles to stay together, thus interfering critically the oxygen diffusion in the substrate (Gendy and Bondkly 2014). Lesser moisture content resulted in decrease of nutrients solubility of the substrate, low level of swelling and high water tension (Moat *et al.* 2002). Different temperature 20 - 70°C were studied (Fig. 2a). The maximal xylanase production was recorded at 30°C. At higher temperature decrease in xylanase production occurred. The reason might be that higher temperature caused evaporation of moisture contents of substrate resulting inhibition or slow down the growth of fungi and in turn enzyme production (Sanghvi *et al*. 2010). In current research, influence of varying pH (3 - 8) was analyzed (Fig. 2b). The maximal enzyme production was recorded at pH 6. Any rise and fall in pH resulted

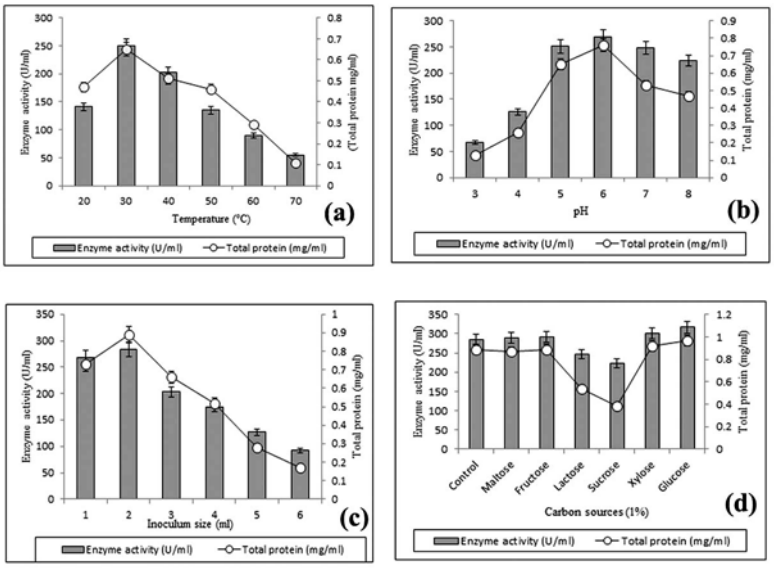

Fig 2. (a) Effect of temperature on the biosynthesis of xylanase by fungal hetero-culture, (b) impact of pH on the biosynthesis of xylanase by fungal hetero-culture, (c) effect of different size of inoculum on the biosynthesis of xylanase by fungal hetero-culture and (d) Influence of different carbon sources on the biosynthesis of xylanase by fungal hetero-culture.

reduction in xylanase production. Probably this might be due to the reason that fungi require acidic pH for growth, also enzymes are very sensitive to pH and any alteration in H^+ ions concentration extremely affects the enzyme production (Chidi 2008). Size of inoculum is one of

dynamic factor among physical factors. In the present study influence of various inoculum sizes (1 - 6 ml) was studied. The 2 ml of inoculum size was optimum for enzyme production (Fig. 2c). Increase in inoculum size caused fall in xylanase production. The reason might be that greater inoculum size provides greater amount of water content which eventually dropped the growth of fungi and also production of xylanase (Sharma *et al.* 1996). Below 2 ml a decline in enzyme production was also noted. It might be due to reason that lesser amount of inoculum formed slower growth of fungi which took a greater period to grow in an optimum quantity in consuming substrate and produced the desirable product (Kashyap *et al*. 2002).

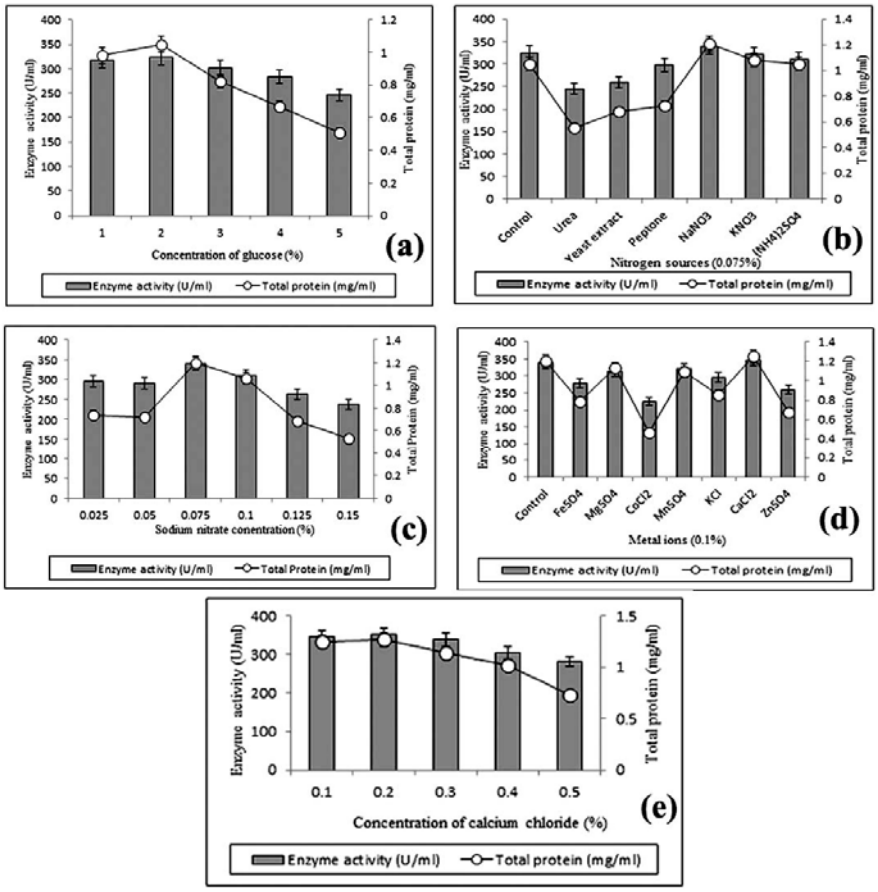

Fig. 3. (a) Effect of different concentration of glucose on the biosynthesis of xylanase by fungal hetero-culture, (b) impact of different nitrogen sources on the biosynthesis of xylanase by fungal hetero-culture, (c) influence of different concentrations of $NaNO_3$ on the biosynthesis of xylanase by fungal hetero-culture, (d) effect of different metal ions on the biosynthesis of xylanase by fungal hetero-culture and (e) impact of different concentration of $CaCl_2$ on the biosynthesis of xylanase by fungal hetero-culture

In the present study, various carbon sources and their effect were examined. The carbon sources as a supplement used were sucrose, maltose, fructose, glucose, xylose and lactose. Glucose provided the best supplementation and gave the maximum production (Figs 2d and 3a) at 2%. Addition of glucose enhances the level of xylanase production. Glucose can be easily

metabolized and thus may be used as a sole carbon source. Addition of 2% glucose was also supported by Bakri *et al.* (2003). Different nitrogen sources including urea, yeast extract, peptone, sodium nitrate, potassium nitrate and ammonium nitrate were analyzed for xylanase production. Sodium nitrate at the concentration of 0.075% proved to be the best nitrogen source (Figs 3b, c). The fungi were isolated from soil where the inorganic nitrogen sources are easily available as compared to organic nitrogen sources. Therefore $NaNO_3$ gave the more enzyme activity. Above 0.075% of $NaNO_3$ decline in enzyme production was noted, probably it was due to the reason that higher concentrations of free nitrogen sources produce toxicity. The present results are in accordance with the results of Kheng and Omar (2005) who had reported the same concentration of $NaNO_3$. metal ions perform a key role in the production of xylanase. A number of metal ions like $CoCl_2$, $ZnSO_4$, $FeSO_4$, $CaCl_2$, $MgSO_4$, $MnSO_4$ and KCl were screened for the enzyme production with the concentration of (0.1%). Out of all, $CaCl_2$ was found to be best for maximal xylanase production (Figs 3d, e). The reason might be that Ca^{+2} acts as an activator for xylanase production (Murugan 2011).

References

Ahamed A and Vermette P 2008. Enhanced enzyme production from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA grown as fed batch in a stirred tank bioreactor. Biochem. Eng. J.**42**: 41-46.

Ahmad Z, Butt MS, Anjum FM and Asgher M 2009. Effect of wheat bran concentration on xylanase biosynthesis by *Aspergillus niger***.** Int. J. Agric and Biol. **11**: 571-576.

Bakri Y, Jacques P and Thonart P 2003.Xylanase production by *Penicillium canescens* 10–10c in solid state fermentation. Appl. Biochem. Biotech. **108** :737-748.

Bradford MM 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem.**72**: 248-254.

Chidi SB, Godana B, Ncube I, Rensburg VEJ, Cronshaw A and Abotsi EK 2008. Production, purification and characterization of celullase free xylanase from *Aspergillus terreus* UL 4209. Afri. J. Biotechnol. **7**:3939-3948.

Clark H, Geldrich E, Kabler P and Huff C 1958. Applied Microbiology**.**International Book Companypp. 53-55. New York.

Collins T, Gerday C and Feller G 2005. Xylanases, xylanase families and extremophilicxylanases. FEMS Microbiol Rev. **29**: 3-23.

Gendy EMM and Bondkly EAM 2014. Optimization of solid state fermentation and leaching process parameters for improvement xylanase production by endophytic *Streptomyces* sp**.** ESRAA-301097. J. Microb. Biochem.Technol. **6**: 154-166.

Guimaraes DANC, Sorgatto M, Nogueira DCPS, Betini JHA, Zanoelo FF, Marques MR, Moraes DMLT and Giannesi GC 2013. Bioprocess and biotechnology: effect of xylanase from *Aspergillus niger* and *Aspergillus flavus* on pulp bio-bleaching and enzyme production using agro industrial residues as substrate. Springer Plus, **2**: 380-387.

Isil S and Nilufer A 2005. Investigation of factors affecting xylanase activity from Trichoderma harzianum 1073 D3. Braz. Arch. Biol. Technol.**48** (2): 187-193.

Kashyap P, Sabu A, Pandey A, Szakes G and Soccol C 2002. Extracellular L glutaminase production by *Zygosaccharoycerouxii* under solid state fermentation. Process of Biochem. **38**: 307-312.

Kavya V and Padmavathi T 2009. Optimization of growth conditions for xylanase production by *Aspergillusniger* in solid state fermentation. Pol. J. Microbiol. **58**: 125-130.

Kheng PP and Omar IC 2005.Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. Songklanakarin J. Sci. Technol**. 27**: 325-336.

Knob A, Fortkamp D, Prolo T, IzidoroSC and Almeida, JM 2014. Agro-residues as alternative for xylanase production by filamentous fungi. Bio. Resources **9**: 5738-5773.

Kulkarni N, Shendye A and Rao M 1999. Molecular and biotechnological aspects of xylanases.FEMS Microbiol Rev. **23**: 411-456.

Maza DL M, Pezzlo MT and Baron EJ .1997. *Color Atlas of Diagnostic Microbiology*, 3rd ed. pp. 336-338. International Book Company, New York.

Miller GL 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. **31**: 426-428.

Moat AG, Foster JW and Spector MP 2002. *Microbiology and Physiology*, 4th ed. pp.1-28 Wiley-Less, Inc. New York.

Murugan S, Arnold D, Pongiya UD and Narayanan P 2011. Production of Xylanase from *Arthrobacter* sp. MTCC 6915 using saw dust as substrate under solid state fermentation. Enzyme Res. **2**: 2-7.

Pandey A, Selvakumar P, Soccol CR and Nigam P 1999. Solid state fermentation for the production of industrial enzymes. Curr. Sci.**77**: 149-162.

Polizeli M, Rizzatti A, Monti R, Terenzi H, Jorge J and Amorim D. 2005. Xylanases from fungi: properties and industrial applications. Appl. Microbiol. Biotechnol.**67**: 577-591.

Sanghvi GV, Koyani RD and Rajput KS 2010. Thermostable xylanase production and partial purification by solid state fermentation using agricultural waste wheat straw. Mycology **1**: 106-112.

Sharma DK, Tiwari M and Behere, BK 1996. Solid state fermentation of new substrates for production of cellulase and other biopolymer hydrolyzing enzymes. Appl. Biochem. Biotechnol. **15**: 495- 500.

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