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Biotechnol. Agron. Soc. Environ. 2008 **12**(2), 111-118

Improvement of xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation

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Received on May 2, 2006, accepted on September 10, 2007.

Among hemicellulases, xylanases are catalysts of considerable interest so as fundamental than applied point of view. However, it is paradoxical to note that the high cost of their production limits their use on a large scale. The use of purified xylan as culture substrate increases the production cost of the enzyme. Consequently, for commercial applications, it is advisable to develop processes starting from inexpensive substrates. The purpose of this study is to optimise xylanases production in solid-state fermentation based on agricultural residues. The strain is *Penicillium canescens* 10-10c, selected in our laboratory for his ability to produce xylanase activity free of cellulase. Assays concern optimization of different culture parameters in order to develop in the future a solid-state fermentation reactor with soya oil cake. These parameters are: medium composition, temperature incubation, induction and repression mechanisms. Soya oil cake in pellets (size > 10 mm) gave a higher enzymatic activity. Great volume of culture medium reduced the enzymatic production. The presence of lactose, saccharose or starch of corn has a positive effect on the production of xylanase while the presence of xylose, mannose, galactose, arabinose, cellobiose and pectin or methylcellulose reduces the production of xylanase. The sources of phosphorus (di-potassic and di-sodic) enhance xylanase production. The enzymatic production obtained in Erlenmeyer flasks (250 ml) after 7 days incubation at 30°C is about 14000 $U \cdot g^{-1}$ of carbon source. The nature of inoculum affects the enzymatic productivity. Indeed, better productivity was obtained with inoculation by solid preculture (956 $U \cdot g^{-1}$ per day) than liquid preculture (473 $U \cdot g^{-1}$ per day) and spores suspension (383 $U \cdot g^{-1}$ per day). These observed enzymatic activity levels are higher than those related in the literature, which shows all the potentialities of this strain and this technique for the production of xylanase and allow to develop our solid-state fermentation bioreactor.
Keywords. *Penicillium canescens* 10-10c, hemicellulases, xylane, xylanase, solid-state fermentation, soya oil cake.

Optimisation de la production des xylanases par *Penicillium canescens* 10-10c en culture solide. L'intérêt que suscitent les hémicellulases, plus particulièrement les xylanases, est de plus en plus important, que ce soit d'un point de vue théorique ou d'un point de vue appliqué. Or, il est paradoxal de constater que le coût élevé de leur production limite leur utilisation à grande échelle. L'emploi de xylane purifié comme substrat de culture augmente le coût de production de l'enzyme. En conséquence, pour des applications commerciales, il convient de développer des procédés à partir de substrats peu coûteux. C'est dans ce cadre que se situe cette étude. Elle vise à optimiser la production de xylanases en milieu solide à partir de résidus agricoles. La souche envisagée est *Penicillium canescens* 10-10c, sélectionnée dans notre laboratoire pour son aptitude à produire une activité xylanase exempte d'activité cellulase. Les travaux concernent l'optimisation de différents paramètres de culture avec comme optique le développement ultérieur d'un réacteur semi-solide à base de tourteau de soja. Ces paramètres sont : la composition du milieu de culture, la température d'incubation, les phénomènes d'induction et de répression. Le tourteau de soja non broyé (taille > 10 mm) donne une bonne activité enzymatique. L'augmentation de la quantité de milieu de culture entraîne une baisse de la production enzymatique. La présence de lactose, de saccharose ou d'amidon de blé a un effet positif sur la production des xylanases tandis que la présence de xylose, de mannose, de galactose, d'arabinose, de cellobiose, de pectine ou de méthylcellulose réduit la production de xylanase. Les phosphates dipotassique ou disodique améliorent la production. La production enzymatique obtenue en fiole (250 ml) après une incubation de 7 jours à 30 °C est de l'ordre de 14000 $U \cdot g^{-1}$ de substrat carboné. La nature de l'inoculum affecte la productivité enzymatique. En effet, les productivités enzymatiques respectives suivantes sont obtenues : 965 $U \cdot g^{-1}$ par jour avec une pré-culture solide, 473 $U \cdot g^{-1}$ par jour avec une pré-culture liquide et 383 $U \cdot g^{-1}$ par jour avec des spores. Les niveaux d'activités enzymatiques obtenus avec cette technique

sont supérieurs aux niveaux présentés dans la littérature et démontrent donc toutes les potentialités de cette souche et de cette technique pour la production des xylanases.
Mots-clés. *Penicillium canescens* 10-10c, hémicellulases, xylane, xylanase, fermentation solide, tourteau de soja.

1. INTRODUCTION

Among hemicellulose, xylan is a heteregeneous polysaccharide in which β-1, 4-linked-D-xylopyranose residues are the main constituents. Depending on their origin, xylans may also contain variable amounts of arabinosyl and 4-O-methylglucuronic acid residues and acetyl groups. The most important enzyme in the xylan biodegradation is the endo-1, 4-β-xylanase (EC 3.2.1.8) that releases xylopyranose units. Xylanases offer considerable interest as catalysts in various biotechnological applications, such as bleaching of kraft pulps (Niku-Paavola et al., 1994; Vicuna et al., 1997; Madlala et al., 2001), clarifying juices and wines (Philippe, 1997), improving the nutritional value of animal feed (Buchert et al., 1994; Medel et al., 2002).

A variety of micro-organisms, bacteria and filamentous fungi have been reported to produce xylanolytic enzymes (San-Lang et al., 2003; Henning et al., 2005; Virupakshi et al., 2005). Among these, *Penicillium canescens* 10-10c is able to produce xylanase free of cellulase activities (Gaspar, 1999). In submerged culture, xylanase production by this filamentous strain is optimized by reducing hydrodynamic stress and by increasing oxygen transfer (Yasser, 2003). The best production was obtained in medium containing soya meal and wheat straw and reached 535 $U \cdot ml^{-1}$ after 120 h (Gaspar, 1999). The process of enzyme production in solid-state fermentation was previously investigated with this strain (Yasser, 2003). The maximum of activity produced after 12 days at 30°C has reached 9632 $units \cdot g^{-1}$ of carbon source.

The purpose of this study was to improve this enzyme production by testing different culture conditions in order to enhance the productivity (in terms of $units \cdot g^{-1}$ substrate per day) on agricultural substrate available in quantities and easy to manipulate.

2. MATERIAL AND METHODS

2.1. Strain

P. canescens 10-10c was supplied by G.I Kvesidatse, Institute of Plant Biochemistry, Academy of Sciences, Tbilisi, Georgia.

Enzyme production by using solid-state fermentation.

Erlenmeyer flasks (250 ml) containing 5 g of substrate plus 20 ml of distilled water supplemented by casein peptone (0.75% W/V) were autoclaved, inoculated with a suspension of spores to reach 10^6 $CFU \cdot g^{-1}$ of carbon source. The flasks were then incubated at 30°C.

2.2. Enzyme extraction

The enzyme from each flask was extracted with distilled water in a total volume of 100 ml by stirring during 1 h at room temperature. After filtration, the clear supernatant was used as the enzyme source.

2.3. Enzyme assay

Xylanase activity was measured according to Bailey et al. (1992) using 1% birchwood xylan as substrate. Reducing sugars were assayed by dinitrosaycylic acid method with xylose as standard. One unit of xylanase activity is defined as the amount of enzyme that released 1 µmol of reducing sugar equivalent to xylose per minute.

2.4. Protein assay

Protein was measured by bicinchoninic acid method (Sigma acid protein assay kit).

2.5. Cultivation in trays

Enamel metallic trays of sizes 17*11*5 cm^3 were used to cultivate the fungus strain after covering with aluminium foil and autoclaving. The wet *Penicillium* soya oil cakes were extracted from trays and assayed for xylanase.

A three-stage cultivation technique was employed: spores suspension, liquid-state preculture, solid-state preculture. In the first, trays containing 50 g of soya oil cake plus 200 ml of distilled water containing 1.5 g of casein peptone and 2 g of $Na_2HPO_4.2H_2O$ were autoclaved, inoculated with a suspension of spores to reach 250.10^5 spores. In the second, 10 ml distilled water containing 300 mg of soya oil cake, 100 mg $Na_2HPO_4.2H_2O$, 75 mg casein peptone were autoclaved, inoculated with 250.10^5 spores. Tubes containing the mixture were incubated at 30°C for 1 day in an orbital shaker (150 rpm) for mycelium production. In the third stage, an inoculum of 250.10^5 spores was added to the enzyme production medium (EPM) in Erlenmeyer flasks (250 ml) after autoclaving and incubation at 30°C for 1 day. The composition of EPM was 5 g soya

oil cake (5 mm) plus 20 ml of distilled water containing casein peptone (0.75% W/V).

Then, the 1 day old preculture was used to inoculate the trays containing soya oil cake (49.70 g in the second and 45 g in the third stage). The carbon sources, supplemented by the mineral solution prior to heat sterilisation (121°C, 20 min) were prepared as follows:

– in the second stage: 190 ml of distilled water plus 1.425 g of casein peptone, and 1.9 g of $Na_2HPO_4.2H_2O$.
– in the third stage: 180 ml of distilled water plus 1.35 g of casein peptone and 1.8 g of $Na_2HPO_4.2H_2O$.

3. RESULTS AND DISCUSSION

3.1. Enzyme production on various carbonaceous substrates

Penicillium strain was grown on medium containing casein peptone (0.75% W/V), 20 ml of distilled water and 5 g of soya oil cake alone or soya oil cake supplemented with various substrate (soya meal, wheat bran, pulp beet) at two ratios (70 : 30, 50 : 50 respectively). **Table 1** shows that the soya oil cake at 100% yielded the highest xylanase production. The maximum activity after 7 days reached 12000 units·g^{-1} of soya oil cake. The combination of soya oil cake and another agricultural waste gradually decreased the levels of xylanase production. The variations of the production level observed with various lignocellulosic materials are probably related on differences in composition and the accessibility of the substrates (Yasser, 2003). The soya oil cake could constitute a significant source of nitrogen. Thus, it is also probable that the effect of these two sources of nitrogen, soya bean oil cake and yeast extract, on the xylanasic production, is related to the evolution of the neutral pH that they induce (Gaspar, 1999).

With wheat straw, xylanase production by *P. canescens* 10-10c attained 9632 units·g^{-1} of substrate after 12 days of culture (Yasser, 2003; Yasser et al., 2003).

Table 1. Combination effect of soya oil cake and other carbonaceous substrates on xylanase production by *Penicillium canescens* 10-10c — *Effet de la combinaison de tourteau de soja et d'autres substrats carbonés sur la production de xylanases par* Penicillium canescens *10-10c*.

Proportion of substrates (5 g)	**Xylanase activity** (U·g^{-1} of carbon source)
Soya oil cake 100%	12096
Soya oil cake 50% + soya meal 50%	10040
Soya oil cake 70% + soya meal 30%	9677
Soya oil cake 50% + wheat straw 50%	6290
Soya oil cake 70% + wheat straw 30%	8225
Soya oil cake 50% + wheat bran 50%	7983
Soya oil cake 70% + wheat bran 30%	8588
Soya oil cake 70% + complete wheat 30%	3145
Soya oil cake 50% + complete wheat 50%	6411
Soya oil cake 70% + maize raid 30%	7137
Soya oil cake 50% + maize raid 50%	9007
Soya oil cake 70% + pulp beet 30%	7257
Soya oil cake 50% + pulp beet 50%	8798

3.2. Effect of carbon source size

The particles size of the substrate had an influence on the ratio surface/volume (S/V) and thus on the accessibility of the substrate for the microorganisms, on the possibilities of diffusion of the oxygen, heat, and exo-enzymes. The availability of oxygen is determined by diffusion, which in turn is determined by porosity, particle size and consistency of the substrate (Alvarez-Martinez, 1987; Mitchell et al., 1988). It is clear that particle size affected enzyme production. Smaller particle sizes of wheat straw < 0.5 mm having greater surface to volume ratio (Weiland, 1988) gave the higher xylanase production by *Thermoascus aurantiacus* in solid-state culture (Kalogeris et al., 1998). In our study, two sizes of soya oil cake were tested. Production of enzyme was carried out on pellets of soya oil cake (size >10 mm) and on pellets crushed in order to obtain after sieving particles of 1 mm. **Figure 1** shows that the activity produced by *P. canescens* 10-10c is more important with the soya oil cake remained in pellets. The enzymatic level of production is higher 26% (14485 U·g^{-1}) than with the crushed soya oil cake (11496 U·g^{-1}). Perhaps this negative effect is a result

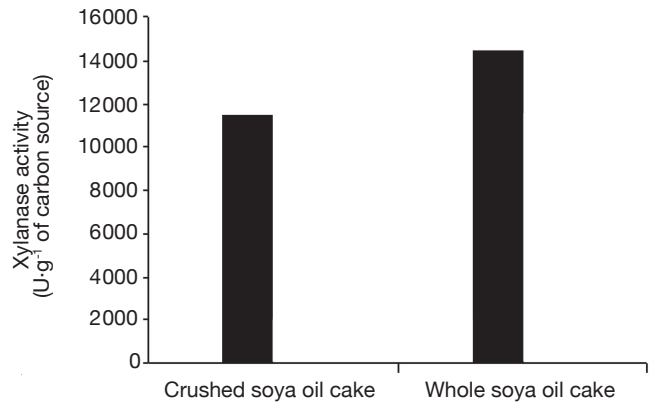

Figure 1. Production of xylanase by *Penicillium canescens* 10-10c on pellets of soya oil cake whole or crushed — *Production des xylanases par* Penicillium canescens *10-10c en présence de tourteau de soja en pellets ou broyés.*

of a reduction of aeration in the medium by settling. These findings confirm relevant data before reported (Gaspar, 1999) on xylanase production by *P. canescens* 10-10c with micronisation of soya meal.

3.3. Effect of quantity of medium

For the static cultures, an adequate thickness of medium is necessary to allow a good ventilation of the mass in order to maintain aerobic conditions favourable to the cell multiplication and the synthesis of the metabolites (Durand et al., 1989). Generally in solid fermentation, the optimal thicknesses of culture are low. Indeed, great volumes or thicknesses of culture release significant heat in the culture medium. That constitutes difficulties for the large-scale productions. In the tempe production, similar observations have been reported concerning metabolic heat accumulation when increasing the fermentor size (Aidoo et al., 1982). Consequently, the temperature within a bed or package of tempe may rise 10-16°C above that of environment. A steep temperature gradient of 3°C·cm^{-1} bed thickness during active growth has been reported in a fermentor employing a bed height of 6.5 cm (Rathbun et al., 1983). Production of enzyme was measured by loading the medium in flasks in order to multiplicate it by 2, 3, etc. and these media were respectively named 2X, 4X, etc. The contents of the flasks were extracted and assayed from each set. The highest xylanase production was observed when the bed height of the medium is the lower (**Figure 2**). The same result is observed during the production of penicillin in solid-state fermentation by *Penicillium chrysogenum* (Barrios-Gonzalez et al., 1988). The optimal production of penicillin is obtained with increasing the culture medium concentration only by two. Then increasing medium concentration had an adverse effect on penicillin production by *P. chrysogenum* (Barrios-Gonzalez et al., 1988). Increasing of medium concentration causes a bad aeration and a bad heat transfer in the mass, and then reduces enzymatic productivity. Thus, with a concentration of the medium of 10X (the height of bed is 30 cm), important putrefaction releases itself from the flasks at the end of the 7 days of incubation. This situation causes a weak development of the strain. The culture is almost impossible under these conditions.

3.4. Effect of temperature

Variations of a few degrees around the optimal temperature can notably modify the growth and the metabolism of the microorganism. Indeed, the solid substrates, because of their heterogeneity, their nature and their low moisture, involve a bad evacuation of calories (Saucedo-Castaneda et al., 1990; Prior et al., 1992). Great quantities of calories can accumulate in the medium, generating micro gradients, which progressively form more significant gradients. It is thus significant to be able to optimize precisely the temperature of culture. The influence of temperature incubation on the enzymatic production, cellular viability, level of extracellular proteins and lost of fresh weight (initial weight / final weight) were studied by following kinetic evolution of these parameters during fermentation process respectively at 23°C, 30°C and 37°C. **Figures 3** and **4** indicate respectively a fall of the enzymatic production, level of extracellular proteins and cellular viability at 23°C and at 37°C. At 37°C, **figure 5**

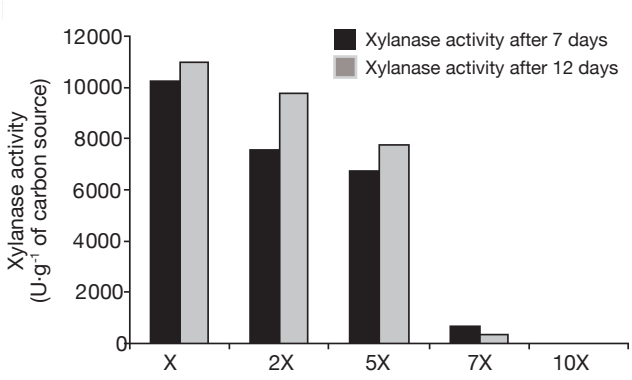

Figure 2. Effect of medium quantity on xylanase production by *Penicillium canescens* 10-10c — *Influence de la quantité de milieu de culture sur la production des xylanases par* Penicillium canescens *10-10c*.
X represents 5 g of whole soya oil cake supplemented with 20 ml of distilled water, yeast extract at 0.75% W/V and 10^6 spores·g^{-1} of soya oil cake — *X représente 5 g de tourteaux de soja additionnés de 20 ml d'eau distillée, d'extrait de levure (0,75 % W/V) et 10^6 spores·g^{-1} de tourteaux de soja.*

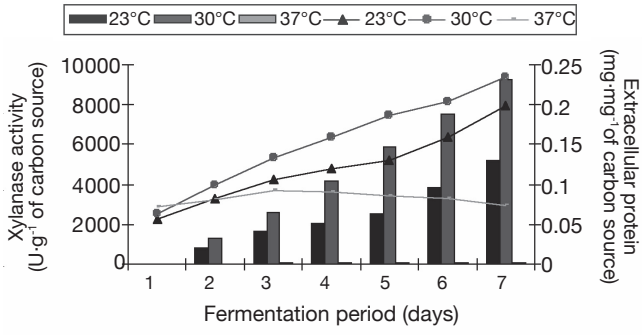

Figure 3. Times profiles of xylanase production by *Penicillium canescens* 10-10c and extracellular proteins according to the culture temperature — *Évolution de la production de xylanases et du taux de protéines extracellulaires en fonction de la température d'incubation par* Penicillium canescens *10-10c*.
Histograms represent time profile of xylanase production and curves represent time profile of extracellular proteins — *Les histogrammes représentent l'évolution de la production de xylanases et les courbes représentent l'évolution des protéines extracellulaires.*

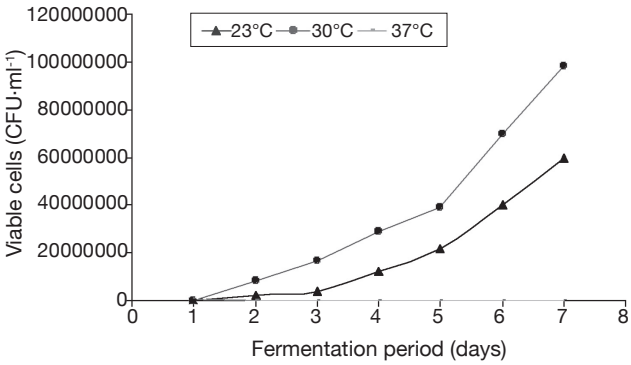

Figure 4. Time profile of *Penicillium canescens* 10-10c cellular viability according to the culture temperature. — *Évolution de la viabilité cellulaire de* Penicillium canescens *10-10c en fonction de la température d'incubation.*

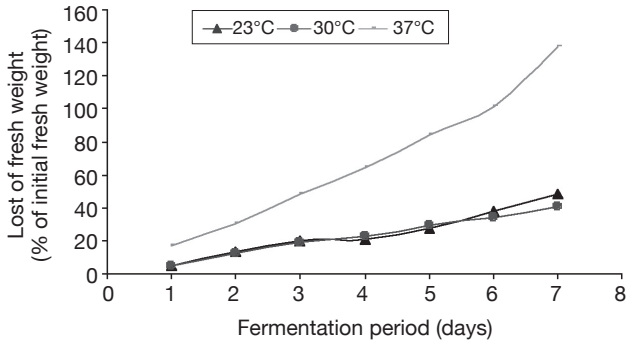

Figure 5. Time profile of fresh weight lost (initial weight / final weight) according to the culture temperature — *Évolution de la perte de poids frais (poids initial / poids final) en fonction de la température d'incubation.*

indicates also great losses of fresh weight in flasks. The losses would be then mainly constituted by water evaporation. The optimum temperature cultivation for xylanase production by *P. canescens* 10-10c in solid-state fermentation is 30°C. That temperature allows obtaining a good cellular viability, best enzymatic production and extracellular protein synthesis, low loss of fresh weight. An adequate temperature control was an important factor to reach these productivities in reactor scale.

3.5. Effect of different salts

Mineral salts are usually used in enzymatic production (San-Lang et al., 2003; Yasser, 2003; Yasser et al., 2003; Henning et al., 2005; Virupakshi et al., 2005). Experience was made with incorporating in the 20 ml of water used to moisten the substrate respectively:

– at a concentration of 0.75% (W/V), different salts (NaCl, KCl, $MgSO_4$, $FeSO_4$, $MnCl_2$, $ZnSO_4$, $CaCl_2$, and $CuSO_4$).
– at a concentration of 1% (W/V), different sources of inorganics phosphorus (K_2HPO_4, KH_2PO_4, $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$).

Among them added at 0.75% (W/V) concentration, NaCl and KCl did not have effect on xylanase production (**Table 2**) whereas the other salts are negative for enzyme excretion: $MgSO_4$, $FeSO_4$, $MnCl_2$, $ZnSO_4$, $CaCl_2$, and $CuSO_4$. Phosphorus (mono-potassic and mono-sodic) induces a fall of enzyme production whereas sources of phosphorus di-potassic and di-sodic enhance xylanase production. This would be due partly to the modification of the initial pH induced by these ions. Sources of phosphorus mono-potassic and mono-sodic involve a fall of initial pH and sources of phosphorus di-potassic and di-sodic at 1% (W/V) increase initial pH towards neutrality. Indeed, our studies have shown us that in solid-state fermentation, initial neutral pH is very favourable to the xylanase synthesis by *P. canescens* 10-10c. An initial pH different of neutrality involves a fall of the enzymatic production. These results confirm relevant data (Gaspar, 1999; Yasser, 2003).

3.6. Effect of carbon sources as additive

Soluble carbonaceous substrates have already been tested in order to evaluate their effects on xylanases production by other strains (Leathers et al., 1986; Priem et al., 1991; Smith et al., 1991; Haltrich et al., 1996; Abdel-Sater et al., 2001; Souza et al., 2001). Xylose, mannose, galactose, arabinose, lactose, cellobiose, saccharose, wheat starch, methylcellulose and pectine were used at a concentration of 5% (W/W) to supplement soya oil cake in order to evaluate their

Table 2. Effect of minerals additives on xylanase production by *Penicillium canescens* 10-10c — *Influence des sels minéraux sur la production de xylanases par* Penicillium canescens *10-10c.*

Additives	**Concentration W/V** (%)	**Relative xylanase activity** (%)
Control	0	100
KCl	0.75	107
$MgSO_4.7H_2O$	0.75	41
$FeSO_4.7H_2O$	0.75	25
$MnCl_2.4H_2O$	0.75	24
$ZnSO_4.H_2O$	0.75	11
$CaCl_2.H_2O$	0.75	17
$CuSO_4.5H_2O$	0.75	17
NaCl	0.75	108
KH_2PO_4	1	66
K_2HPO_4	1	112
$NaH_2PO_4.2H_2O$	1	67
$Na_2HPO_4.2H_2O$	1	136

effect on xylanase production by *P. canescens* 10-10c in solid-state fermentation. The enzyme was assayed after 7 days incubation at 30°C. Among carbon sources assayed, mono- (xylose, mannose, galactose, arabinose), di- (cellobiose) and polysaccharides (methylcellulose and pectin) reduce xylanase production (**Table 3**). That can be explained by a catabolic repression of various substrates. On the other hand, lactose, saccharose and wheat starch increased enzyme production by *P. canescens* 10-10c.

3.7. Effect of addition of dextrose and xylane on xylanase production in solid-state fermentation

Several works highlighted the respective inducer or repressive character of xylan and dextrose on the production of xylanases (Espinar et al., 1992; Espinar et al., 1994; Singh et al., 1995; Yasser, 2003; Yasser et al., 2003).

However, this observation depends in certain cases on culture conditions (Archana et al., 1997; Souza et al., 2001). To evaluate these effects, different levels of xylane (5, 10, 15, and 20%), dextrose (5, 10, 15, and 20%) and xylane with dextrose (5, 10, 15, and 20% for each) were incorporated into soya oil cake.

Our results (**Figure 6**) confirm these observations. Indeed, we observed that the addition of xylan to soya oil cake incresead slighthy xylanase production. Previously, Yasser et al. (Yasser, 2003; Yasser et al., 2003) have showed similar observations for the same strain cultivated on wheat bran. On the other hand, xylanase production was repressed by dextrose. Addition of dextrose to the medium containing xylan produced also catabolic repression. Negative effect in this case is more important. Enzyme production was reduced of 50%. Perhaps, is it possible that accumulated xylose issued from the hydrolyse of xylan leaded to this important repression (Senior et al., 1989). But no complete repression was observed even at 20% dextrose.

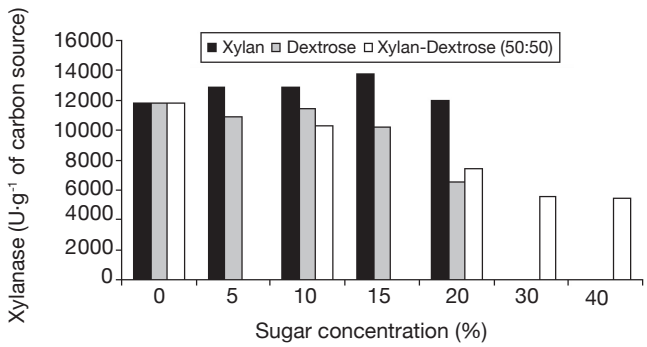

Figure 6. Induction and repression of xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation medium supplemented with dextrose and xylan — *Induction et répression de la production de xylanases de* Penicillium canescens *10-10c en présence de glucose et de xylane.*

3.8. Culture on trays

Alternative ways of inoculation such as spore suspension, mycelium suspension obtained by liquid or solid precultures of spores were compared. The comparison (**Figure 7**) showed the interest of spores pregermination in solid medium, where phase of latency is reduced and where we quickly obtain optimum production of xylanase. Optimum productivity obtained are 965 $U \cdot g^{-1}$ per day with inoculation by solid preculture, 473 $U \cdot g^{-1}$ per day with inoculation

Table 3. Effect of different sugars on xylanase production by *Penicillium canescens* 10-10c — *Influence de saccharides sur la production de xylanases par* Penicillium canescens *10-10c.*

Additives (5%, W/W)	**Xylanase activity** ($U \cdot g^{-1}$ of carbon source)	**Relative xylanase activity** (%)
Control	11488	100
Control + xylose	3206	28
Control + mannose	6448	56
Control + galactose	2030	18
Control + arabinose	4808	42
Control + maltose	12502	109
Control + lactose	13518	118
Control + cellobiose	3900	34
Control + saccharose	12448	108
Control + wheat starch	13624	119
Control + methylcellulose	7266	63
Control + pectin	6180	54

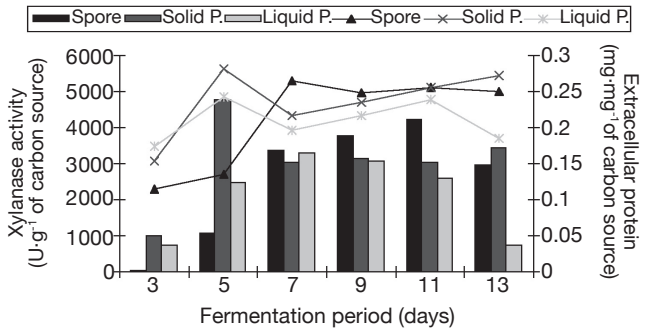

Figure 7. Effect of inoculum type on xylanase production by *Penicillium canescens* 10-10c in solid-state fermentation — *Effet de la nature de l'inoculum de* Penicillium canescens *10-10c sur la production de xylanases.*

Histograms represent time profile of xylanase production and curves represent time profile of extracellular protein — *Les histogrammes représentent l'évolution de la production de xylanases et les courbes représentent l'évolution des protéines extracellulaires.*

by liquid preculture and 383 $U \cdot g^{-1}$ per day with spores inoculation. In xylanase production by solid-state fermentation with *Thermoascus aurantiacus* (Kalogeris et al., 1998), better results were obtained with liquid preculture (4942 $U \cdot g^{-1}$) than spores suspension (4714 $U \cdot g^{-1}$) and solid state culture (4000 $U \cdot g^{-1}$) of wheat straw. However, in penicillin production by *P. chrysogenum* NRRL 1951, medium inoculation by spores gave better results than inoculation with mycelium obtained by preculture of spores (Barrios-Gonzalez et al., 1988).

4. CONCLUSION

The hyperproductor character of xylanase by *P. canescens* 10-10c was already proven by other work (Gaspar, 1999; Yasser, 2003; Yasser et al., 2003). The present study confirms this observation. Indeed, the maximal enzymatic production obtained after 7 days incubation is 14485 $U \cdot g^{-1}$ of substrate in Erlenmeyer. This level is higher than levels presented in the literature and shows all the potentialities of this strain and this technique for the production of xylanases. For commercial applications, the use of agricultural by-products for the production of these enzymes to replace purified xylan makes it possible to reduce the cost of the enzyme considerably. In addition, cultivation conditions (composition of the medium, cultivation temperature, induction and repression mechanisms, nature of inoculum) are essential to get good results.

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