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Screening of Xylanase-Producing *Rhizopus* spp.

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A total of 67 strains of *Rhizopus* spp. were screened on their capability to produce xylanase under solid state and liquid culture. The highest xylanase activity of 516 unit/mL for liquid culture was exhibited by strain *Rhizopus peka* from the Philippines. For solid state culture, the highest activity of 7802 unit/mL was achieved by *Rhizopus* sp. MKU 32 which originated from Thailand. Results showed that liquid culture was more suitable for xylanase production using *Rhizopus* strains from the Philippines.

INTRODUCTION

Investigation on new and different xylan–digesting microorganisms has increased through the years because of the importance of bio–converting waste xylan, which is abundant in agriculture and the farm–industry. Wong *et al.* (1988) has reported the application of xylanase (EC3.2.1.8, 1,4 β–D–xylanohydrolase) for bio–conversion processes, such as bio–pulping and food processing. In these aspects, several types of microbial xylanases were reported like bacterial xylanase from *Bacillus* sp. (Horikoshi and Atsukawa, 1973, Honda *et al.*, 1985, Dey *et al.*, 1991, Park *et al.*, 1992), fungal xylanase from *Aspergillus niger* (John, *et al.*, 1978, Frederick *et al.*, 1984), yeast xylanases from *Cryptococcus albidus* (Biely *et al.*, 1980, Iefuji *et al.*, 1996) and *Trichosporon cutaneum* (Stuttgen and Sahm, 1982). Some of these xylanases were previously purified and characterized. Other xylanase producing microorganisms known to date are *Aeromonus* sp. (Ohkoshi, *et al.*, 1985), *Humicola lanuginisa* (Kitpreechavanich *et al.*, 1989), *Sporotrichum cellulophilum* (Lerrwerawat and Hinoshita, 1989), *Aureobasidium pullulans* (Li, *et al.*, 1993), *Trichoderma viride* (Hashimoto, *et al.*, 1971), *Talaromyces byssochlamydoides* (Yoshioka, *et al.*, 1981), *Clostridum* (Wong, *et al.*, 1988) and *Streptomyces* (Wong, *et al.*, 1988).

Since there has been no report on *Rhizopus* as a source of xylanase, this paper deals with the screening of xylanase–producing *Rhizopus* strains.

MATERIALS AND METHODS

Microorganisms

A total of 67 *Rhizopus* strains were used in the study. Forty six strains came from the laboratory stock cultures including TISTR type culture, MKU collection, UQM collection and new isolates. The other 21 strains came from the MNH–MCC, UPLB and IFST, UPLB culture collection in the Philippines. These strains were stocked on a PDA slant agar.

Liquid medium

The liquid medium composition (Morita and Fujio, 1997) was 10 g liquefied cassava starch, 4 g ammonium acetate, 1 g dipotassium hydrogen phosphate, 0.5 g magnesium sulfate heptahydrate, 0.01 g iron sulfate heptahydrate, 0.03 g zinc sulfate heptahydrate, 0.21 g calcium chloride, 3.3 g citric acid and 1.0 L deionized water. The pH was adjusted to 6.0 and 100 mL of the medium was distributed in a 500 mL shaking flask with a cotton plug. The shaking flask was autoclaved at 121 °C for 20 min.

Solid medium

The solid medium was composed of 20 g wheat bran, 2 g cassava starch and 20 mL tap water which was distributed in a 500 mL Erlenmeyer flask with a cotton plug. After mixing well, the medium was autoclaved at 121 °C for 20 min.

Inoculum preparation and inoculation

Rhizopus strain from stock culture was transferred in a fresh PDA slant and pre-cultured at 30 °C for one week. Spore and mycelium suspension was prepared by adding 13 mL sterile water to pre-cultured *Rhizopus* and scratching the agar surface using platinum loop. A 2 mL spore suspension per a flask was inoculated for both solid and liquid media.

Culture condition

Liquid cultivation was carried out at 30 °C for 2 days on a rotary shaker while solid medium was incubated at 30 °C for 7 days.

Preparation of enzyme solution

After incubation, the culture broth from liquid culture medium was filtered using filter paper (Toyo No. 7) and the filtrate was used as crude enzyme solution. For solid culture, 200 mL deionized water was added in the cultured flask and mixed well. The mixture was placed in a cold room at 4 °C for 20 h and then centrifuged at 7,012×at 4 °C for 20 min. The supernatant liquid was used as crude xylanase solution.

Determination of xylanase activity

Xylanase activity was determined using 1% xylan (from Oat-spelts, Nacalai Tesque, Kyoto, Japan) as a substrate suspended in a 0.1 M sodium acetate buffer (pH 4.5). A 0.5 mL xylanase solution was added in a 0.5 mL substrate suspension and incubated at 40 °C for 30 min. The amount of reducing sugar liberated was determined by the DNS method (Miller, 1959) and measured the absorbance at 547 nm using D-xylose as the standard. One unit of xylanase activity was defined as the amount of enzyme that produces 1.0 μmol of xylose per min under the given conditions.

RESULTS AND DISCUSSION

Xylanase production

Table 1 shows the results of the screening of 64 laboratory stock of *Rhizopus* strains on xylanase production under liquid and solid culture. For liquid culture, a maximum

activity of 310 units was achieved by *Rhizopus* sp. MKU 10. Sixteen strains did not produce any xylanase under this condition. In the case of solid culture, maximum activity was achieved by *Rhizopus* sp. MKU 32 units and only one strain, MKU 24, did not exhibit xylanase production.

Higher xylanase production was also noted in solid culture. Thirty two strains resulted in more than 100 units, compared to 18 strains for liquid culture. From the results, it can be said that solid culture is more suitable for higher xylanase production.

Table 2 shows the result for 21 *Rhizopus* strains originating from the Philippines. Maximum xylanase activity was exhibited by *Rhizopus peka* (P8) at 516 units and *Rhizopus stolonifer* (P4) at 436 units for liquid and solid culture, respectively. All *Rhizopus* strains tested, showed capability to produce xylanase under liquid medium. Poor xylanase production, between 0–100 units, was observed in 13 strains under solid medium. In contrast to laboratory stock cultures (Table 1), liquid culture is said to be more suitable for xylanase production for *Rhizopus* strains from the Philippines. Except for *Rhizopus stolonifer* (P4), all strains have higher xylanase activity under liquid culture. Some of the strains from Japan showing high xylanase activity were further tested and are still under study for purification and characterization of the enzyme.

Table 1. Xylanase production by liquid culture (LC) and solid culture (SC) of *Rhizopus* sp. and some type culture from laboratory stock culture.

	Xylanase activity (unit)				
Code No.	LC	SC	Code No.	LC	SC
MKU 4	248	312	IFO 4697	88	312
MKU 7	210	148	IFO 5441	38	100
MKU 8	300	522	IFO 5442	86	260
MKU 10	310	110	UQM 186F	60	200
MKU 11	120	452	F60	272	200
MKU 12	236	394	F61	0	330
MKU 17	210	8	F62	100	236
MKU 18	248	8	F64	0	110
MKU 21	100	110	F67	0	8
MKU 24	200	0	F68	148	336
MKU 32	184	702	F89	60	468
MKU 38	20	300	F94	0	20
MKU 40	126	60	F98	122	260
MKU 42	10	312	G6	60	110
T3001	50	136	G7	184	88
T3052	0	386	G82	100	134
T3079	0	70	LKN	50	86
T3155	0	50	A11	48	574
T3189	36	236	UM	0	74
T3165	0	386	WJ	0	312
T3211	0	8	ON	0	136
T3241	0	74	Rh3	0	110
T3247	0	248			
T3324	0	36			

MKU, T (TISTR) IFO and QUM were from culture collection. Others were isolates.

Table 2. Xylanase production by liquid culture (LC) and solid culture (SC) of *Rhizopus* from the Philippines (MNH–MCC).

	Xylanase activity (unit)				
Rhizopus strain	LC	SC	*Rhizopus* strain	LC	SC
Rh. oryzae, P1	372	50	*Rh. oligosporus*, P12	316	232
Rh. oligosporus, P2	432	128	*Rh. oligosporus*, P13	308	32
Rh. oryzae, P3	508	96	*Rh. oligosporus*, P14	386	206
Rh. stolonifer, P4	134	436	*Rh. sp.*, P15	250	68
Rh. cohnii, P5	448	144	*Rh. javanicus*, P16	364	12
Rh. tamarii, P6	436	138	*Rh. oryzae*, P17	306	0
Rh. arrhizus, P7	440	364	*Rh. oligosporus*, P18	202	0
Rh. peka, P8	516	0	*Rh. arrhizus*, P20	280	0
Rh. oligosporus, P9	268	0	*Rh. oligosporus*, P21	252	0
Rh. oligosporus, P10	220	188	*Rh.* sp., P22	372	0
Rh. oligosporus, P11	188	50			

Rh. (Rhizopus).

Xylanase production and other hydrolases

In previous studies by Elegado and Fujio (1993) on the production of soluble starch digestive glucoamylase (GA) and raw starch digestive glucoamylase (GARS), it was reported that *Rhizopus* strains Rh3, IFO 5441 (from Table 1) are good GA and GARS producers. Similarly, these strains produced xylanase activity but were reported to produce high GA. Other strains like F60, F61, F62, F67, F68, F89, F98, IFO 5442 and G8 were reported to produce less than 100 units of GA, although most of these strains have more than 100 units of xylanase activity.

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