

types 1 and 2, HSV-1 and HSV-2) and Papovavirus (Simian Virus 40, SV-40).

CHO cells were grown in the dark in presence of 5-bromodeoxyuridine (1 $\mu\text{g}/\text{ml}$) for 2 cell cycles during logarithmic phase. One set of cultures was inoculated by adsorption method with 3 dex $\text{TCID}_{50}/\text{ml}$ of each of the following viruses: Vaccinia (671061), HSV-1 (753166), HSV-2 (753167) and SV-40 (776), and the cultures were incubated for about 10 hr thereafter. Mock-infected and uninfected cultures were used as controls. c-Metaphase chromosome preparations were made with a 2 hr colchicine (0.05 $\mu\text{g}/\text{ml}$) treatment. The slides were coded for temporarily masking their identity. Sister chromatid differential staining was performed following the 'fluorescence-plus-Giemsa' technique¹ with minor modifications. Thirty well-spread metaphase plates, each containing 21 chromosomes, were scored from each slide. The slides were then decoded and the results were analyzed.

The average SCE increase, obtained from 3 identical experiments, in Vaccinia-, HSV-1-, HSV-2- and SV-40-infected cultures was about 2.77, 3.0, 3.85 and 2.76 exchanges per cell over the control base levels, respectively (Student's *t*-test, $p < 0.01$). There was no significant difference between uninfected control and mock-infected control cultures. Other chromosome abnormalities were not observed in any of these cultures.

Brown and Crossen² reported elevation in the SCE frequencies but not chromosome aberrations in a mouse embryo cell line (JLS-V16) infected with Rauscher leukemia virus; on the other hand, Kato and Sandberg³ have shown that infection of human diploid fibroblasts with HSV-1 and HSV-2 viruses induces chromosome aberrations but not SCEs. The present observations therefore agree with the view that the mechanisms leading to SCEs and to chromosome aberrations are distinct³.

It is interesting to note that the induction of SCEs in CHO cultures was observed with all the viruses tested, irrespective of the differences among their nature, replication mechanisms and lytic/transformation cycles: Vaccinia virus multiplies in cytoplasm while HSV-1 and HSV-2 multiply in nucleus; CHO cells are non-permissive to SV-40 virus multiplication, but can be transformed by this virus. It is therefore apparent that some as yet unidentified virus-specific event(s), common to all these viruses, may play an important role in the induction of SCEs in CHO cells. Attempts to resolve this issue will be described elsewhere.

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XYLANOLYTIC ACTIVITY OF *ASPERGILLUS OCHRACEUS-42*

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MANY studies have been carried out concerning the industrial use of amylase and cellulase. However, only a few reports are available on xylanase which degrades xylan to xylose. Xylan is a polymer of xylose containing β -1, 4-xylosidic linkages and is widely distributed in plant cell walls and forms a primary part of the hemicellulose portion. In some higher plants and agricultural wastes, xylan is 20–40% of dry weight¹. Because of its natural abundance, xylan, like cellulose, is potentially a good fermentation substrate for production of feedstuff².

In recent years, interest has increased in the use of microbial xylanase for the economical production of xylose, a sweetening and antidiabetic agent, for clarification of fruit juices. It is also used as digestive aid in pharmaceutical industry. Various microorganisms such as *Aspergillus niger*^{3,4}, *Chaetomium trilaterale*⁵, *Streptomyces xylophagus*⁶, *Cryptococcus albidus*⁷, *Irpex lacteus*⁸, *Bacillus*. sp⁹, *Bacillus subtilis*¹⁰, have been reported to be sources of xylanases. *Aspergillus ochraceus-42* has outstanding ability to produce sufficient xylanolytic enzymes in xylan medium. But it has not been reported earlier. Therefore the present paper deals for the first time with the xylanolytic activity of *A. ochraceus-42*.

The strain was isolated during a screening programme for xylanase producing microorganisms from soils of W. Bengal. It possesses appreciable xylan decomposing activity. Colonies on CD-medium attain a diameter of 2–3 cm in 6 days at 28°C, usually plane or slightly furrowed, less zonate, characterized by a tough basal mycelium that is submerged. Conidial structures are crowded. It appears as brownish yellow. Conidial

heads are globose. Conidia measure 2.14μ to 4.28μ ; conidiophores 1 to 2 mm in length by $10-20 \mu$ in diameter; vesicles globose, 32.10μ to 40.66μ in diameter; sterigmata in two rows, 7 to 13.7μ in length.

The microorganisms were screened according to their ability to produce sufficient xylanase. On the agar plates containing 0.5% xylan as a sole source of carbon, a little drop of spore suspension was given and the plates were incubated at 28°C . After 5 days of incubation microorganisms, formed Clear-cut Zones around the colonies, were scored as a positive indication for extracellular production of xylanase. Quantitative assay for the enzyme supported *A. ochraceus* to be the best producer. The identification of the strain was confirmed from the Commonwealth Mycological Institute, Kew, Surrey, England. To give separate entity, the organism was named as *A. ochraceus*-42.

One ml of conidial suspension (6×10^6 spores/ml) of *A. ochraceus*-42 was inoculated with 20 ml CD medium, supplemented with 0.5% xylan in 100 ml Erlenmeyer flask and incubated for 12 days at 28°C under shaking condition. The pH of the medium was kept constant at 6.5 by adding 2N HCl or 2N NaOH as needed. The readings were taken on every alternate day of incubation. The culture filtrate was centrifuged at 10,000 r.p.m. for 10 min and the supernatant was used for enzyme assay.

Xylanase activity was determined by measuring the reducing sugar liberated from larch wood xylan (Sigma Chemicals Co., U.S.A.). The reaction mixture consisting of 1 ml of 1% xylan solution, 1 ml of 0.1 M citrate buffer (pH 6.0), and 1 ml diluted enzyme solution, was incubated at 50°C for 30 min. The reducing sugar liberated was measured according to Somogyi¹¹. One unit of xylanase was defined as the amount of enzyme liberating $1 \mu\text{mol}$ of reducing sugar as xylose in one minute.

Determination of protein of the culture filtrate was done following the method of Lowry *et al*¹² using bovine serum albumin as standard.

The results of the experiments are presented in table 1, from where it is clear that the enzyme production started from the 2nd day of incubation. The optimum production of xylanase was on the 6th day and then it decreased gradually while the extracellular protein production was in continuous increase. This experiment also indicated that xylanase activity did not increase with the gradual increase of extracellular protein production. There was a sharp fall in enzyme activity following the 8th day of incubation. This was due to mycelial autolysis interfering with xylanase

Table 1 Effect of incubation period on the production of extracellular protein and xylanase by *A. ochraceus*-42

Days of incubation	Extra-cellular protein ($\mu\text{g/ml}$)	Xylanase activity (μml)	Specific activity (units/mg protein)
2	72.40	0.02	0.277
4	106.40	1.12	10.56
6	122.00	2.24	18.36
8	155.00	2.07	13.55
10	250.00	1.92	7.68
12	283.32	1.77	6.25

production which was determined from the specific activity. This enzyme produced by *A. ochraceus*-42 also acts on the hemicellulosic parts of various agricultural wastes such as wheat straw, rice straw, etc. Thus the strain, reported in this investigation, can be successfully utilized for the economical production of xylose, ethanol and other chemicals. Further work to increase the enzyme production is in progress.

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