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SCREENING OF ISOLATED MICROBES FOR CELLULASE PRODUCTION

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Different microbes were propagated on substrates such as bagasse pith, wheat straw, rice straw and cotton seed hulls for cellulase production. Enzyme produced by these organisms varies from organisms to organism and from substrate to substrate.

INTRODUCTION

Enzymatic hydrolysis of cellulose is being increasingly investigated, as a means of converting this inexpensive raw material into glucose and other valuable products. Studies are commonly being made on conversion of waste products into food and energy sources as well as for pollution abatement. Cellulosic wastes can only be economically converted into sugar, single cell protein and ethanol, if cellulase is available.

Cellulase is an adaptive as well as induced [1-2] extracellular enzyme [3-5] which is generally produced by submerged fermentation. Locally isolated strains of molds and bacteria were propagated on bagasse pith, wheat straw, rice straw and cotton seed hulls for the production of enzyme cellulase.

Most of the studies for the production of cellulase have been carried out on *Trichoderma viride* [6-7]. The main objective of the present study was to determine the maximum amount of enzyme produced by cellulolytic microbes utilizing different agricultural wastes as carbon source.

MATERIAL AND METHOD

Preparation of Sample. Bagasse pith, wheat straw, rice straw and cotton seed hulls procured from the local market and ground to 20 mesh in a grinder.

Submerged Fermentation: Reese medium (500 ml) containing 0.5% of cellulosic waste was transferred into Erylenmayer flasks of one litre capacity and sterlized at 6.8 kg/2.5 sq. cm pressure for fifteen minutes. The flasks after incubation were shaken on a rotary shaker at 250 r.p.m. at 30° ± 2° for three days in case of bacteria and five days for molds.

One flask without inoculum was taken as blank.

Cellulase Assay: The enzymic activity was estimated by the method of Sumner and Somers [8] using 3 ml. of dinitrosalicylic acid (DNS), 1 ml. of enzymic solution, 2.5 ml. of 1% carboxymethyl cellulose (CMC) and 1 ml. of phosphate buffer (pH 4.6) was incubated at 37 ± 2° for one hour.

RESULTS AND DISCUSSION

The main reason for employing microorganisms as potential sources of enzyme is the case with which enzyme level may be increased by environmental and genetic manipulations. The maximum yield of enzyme was 72 and 144 units/g (Table 1) when bacteria, *Bacillus brevis* and mold Penicillium were propagated respectively on bagasse pith. The minimum yield was 42 units/g with *Bacillus laterosporus.* Similarly Trichoderma produced maximum cellulase i.e., 102 units/g when propagated in wheat straw, but it was 80, 40 and 38 units/g in rice straw, bagasse pith and cotton seed hulls respectively. Similarly the maximum enzyme production was 144 units/g in bagasse pith with Penicillium. The minimum yield was 80, 58 and 42 units/g with rice straw, cotton seed hulls and wheat straw respectively. Streptomyces produced 120 units/g in rice straw where as 64, 60 and 42 units/g were observed in wheat straw, bagasse pith and cotton seed hulls respectively.

Production of cellulase by *Bacillus brevis, laterosporus, pumilus* and *subtilus* was maximum i.e. 96, 88, 56 and 50 units/g when propagated on rice straw. While *B. polymyxa* and sphareicus showed maximum enzyme yield i.e. 56 and 48 units/g on bagasse pith where as no appreciable amount of enzyme production was observed by any other *Bacillus* spp. when wheat straw and cotton seed hulls were used as the test substrates.

It is evident from the above results that it is difficult

Table 1. Cellulase production from various agricultrural wastes by cellulolytic microorganisms units/g substrate

Microorganisms	Substrates Bagasse pith u/g substrate	Wheat straw u/g substrate	Rice straw u/g substrate	Cotton seed hulls u/g substrate
Bacillus laterosporus	42	50	88	56
Bacillus polymyxa	56	42	44	32
Bacillus pumilus	42	42	50	48
Bacillus sphericus	48	30	42	30
Bacillus subtilus	42	38	56	32
Bacillus brevis	72	70	96	38
Penicillium	144	42	80	58
Streptomyces	60	64	120	42
Chaetomium	78	88	100	64
Trichoderma	42	102	80	38

to find a direct corelation between the rate of growth of an organism and the amount of enzyme production, because the external enzyme secretion by bacteria is quite different from that of molds. In bacteria most of the enzyme synthesised is retained by the membrane and much less amount is secreted in the medium. In the case of molds however, all of the enzyme synthesised is secreted out.

These results are in accordance with the findings of Reese [9] who reported that *Chaetomium globosum* rapidly consumed cellulase during growth, but little or no extracellular enzyme was detectable.

Pestaliotropais westerdytic was reported to consume cellulose but only one member of the cellulase complex is found in the culture filtrate.

Trichoderma consumed cellulose slowly than other fungi, but the yield of the enzyme was more. Thus it was concluded that enzyme production varies from organism to organism and from substrate to substrate which are also the findings of the present research. It is concluded from the results that the best source of extracellular cellulase production are the molds and best medium for cellulase production is that developed by Mandels and Reese [1] with 0.5% substrate concentration.

Substrate specificity of the microbes for the production of the enzyme was also evident.

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