SHORT COMMUNICATION

A Screening Method for the Estimation of Filter Paper Activity

By FRANZISKA WABNEGG,* KURT MESSNER AND MAX RÖHR

*Institut für Biochemische Technologie und Mikrobiologie, Technische Universität Wien,
Getreidemarkt 9, A-1060 Wien, Austria*

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A simple method which enables rapid screening of cellulase activity was devised. The results of this test correlated with those obtained using published methods for determining filter paper activity.

INTRODUCTION

Various methods for the detection of cellulolytic activity of micro-organisms have been described. These can be grouped into two categories. In the first, the end-products of cellulose degradation are measured as reducing sugars – either the degradation of non-crystalline substrates (e.g. carboxymethylcellulase activity) or of crystalline substrates (filter paper activity) is measured. In the other category, changes in the qualities (e.g. loss of tensile strength) or in the amounts of cellulose (weight loss) are measured. Measurements in the second category cannot be directly compared with the liberation of reducing sugars. For example, the depth of clearing test correlates with the weight loss of cotton fibres rather than with carboxymethylcellulase activity (Rautela & Cowling, 1966). No correlation with filter paper activity has been demonstrated either (Wabnegg *et al.*, 1978). Thus, the depth of clearing test, which is frequently used to detect cellulolytic fungi, does not give information on the amount of reducing sugars found by measurements of carboxymethylcellulase or filter paper activity. However, since the determination of reducing sugars is a useful measure of the commercial value of a fungus with respect to saccharification or cellulase production, the depth of clearing test is not very suitable for screening purposes. The method described in this paper offers the possibility of predicting fairly accurately the filter paper activity of a large number of organisms and requires little time and material.

METHODS

Organisms. These are listed in Table 1. Strains 1 to 34, 36, 37, 39, 40, 42 and 43 were from the collection of the Institut für Biochemische Technologie und Mikrobiologie an der Technischen Universität Wien, Austria; strains 38 and 46 were from the collection of the Commonwealth Mycological Institute, Kew, Surrey; and strains 35, 41, 44 and 45 were from the Culture Collection of Fungi, Mycological Services, Amherst, Mass., U.S.A.

Medium and test procedures. The test medium was that of Sternberg (1976) without Tween 80 and with the addition of 2% (w/v) agar for plate cultures. The concentration of cellulose was 1% (w/v) instead of 0.75%. Cultures were grown in 100 ml Erlenmeyer flasks containing 60 ml medium on a rotary shaker at 250 rev. min⁻¹. Mesophilic fungi were incubated for 9 d at 25 °C and thermophilic fungi for 5 d at 45 °C. After filtration, the filter paper activity of the cultures was determined by the procedure of Mandels *et al.* (1976).

Agar plate cultures were inoculated and then incubated at the same temperatures as above for 4 to 14 d until colony diameters reached 3 to 6 cm. A 1 cm diam. disc was then cut with a cork borer 0.5 cm from the colony edge. This disc was placed on the centre of a filter paper circle (Whatman no. 1, diam. 7 cm) which had previously been dipped into 0.05 M-citrate buffer (pH 4.8). The lid of a 6 cm diam. Petri dish was placed

Table 1. Filter paper activity and colour intensity in the screening test for various cellulolytic fungi

Strain no.	Fungus	Filter paper activity (units ml ⁻¹)	Colour intensity
Basidiomycetes:			
1	<i>Bjerkandera adusta</i> W4	0.005	1.0
2	<i>B. adusta</i> W17	0.041	0.5
3	<i>Fomes fomentarius</i> W25	0.05	1.5
4	<i>Ganoderma</i> sp. D10	0.09	2.0
5	<i>Geopetalum</i> sp. W19	0.026	1.0
6	<i>Hypholoma capnoides</i> W15	0.01	0.5
7	<i>H. fasciculare</i> W33	0.02	1.0
8	<i>Hypochnicium</i> sp. H1	0.06	1.5
9	<i>Inonotus</i> sp. W5	0.05	1.0
10	<i>Ischnoderma resinosum</i> W12	0.05	1.5
11	<i>Lycoperdon</i> sp. W16	0.02	0.5
12	<i>Lyophyllum connatum</i> W35	0	0.5
13	<i>Panus strigosus</i> W14	0.06	2.0
14	<i>Peniophora</i> sp. H2	0	0.5
15	<i>Phellinus</i> sp. W13	0.04	1.0
16	<i>Phlebia rufa</i> D22	0.128	3.0
17	<i>Pleurotus ostreatus</i> W11	0.03	1.5
18	<i>Radulum orbiculare</i> W24	0.054	2.0
19	<i>Stereum</i> sp. W20	0.08	1.5
20	<i>Stereum</i> sp. W8	0.02	0.5
21	<i>Stereum</i> sp. D5	0.176	2.5
22	<i>Stereum</i> sp. H3	0.08	1.0
23	<i>Trametes confragosa</i> D3	0.173	3.0
24	<i>T. confragosa</i> D40	0.16	3.0
25	<i>T. gibbosa</i> D15	0.013	1.5
26	<i>T. gibbosa</i> D21	0	1.0
27	<i>T. hirsuta</i> D37	0.163	2.5
28	<i>T. hirsuta</i> D47	0.217	2.0
29	<i>T. versicolor</i> D1	0.093	5.0
Fungi Imperfecti (thermophilic):			
30	<i>Aspergillus fumigatus</i> X7	0.094	1.5
31	<i>Acremonium alabamensis</i> (<i>Thielavia terrestris</i>) X2	0.037	1.0
32	<i>Mucor pusillus</i> X5	0	0.5
33	<i>Penicillium emersonii</i> X9	0.01	1.5
34	<i>Sporotrichum pulverulentum</i> X28	0.04	2.0
35	<i>S. pulverulentum</i> 9145	0.08	3.0
36	<i>Thermoascus aurantiacus</i> X1	0.057	2.0
Fungi Imperfecti (mesophilic):			
37	<i>Botrytis cinerea</i> P1	0.11	2.0
38	<i>Fusarium solani</i> IMI 107511	0.22	4.5
39	<i>Graphium</i> sp. H4	0	0
40	<i>Mucor mucedo</i> F5	0	0
41	<i>Pestalotiopsis westerdijkii</i> 381	0.224	3.5
42	<i>Trichoderma viride</i> P10	0.25	4.0
43	<i>T. longibrachiatum</i> F6	0.21	4.0
44	<i>Trichoderma</i> sp. 6a	0.49	5.5
45	<i>Trichoderma</i> sp. 9414	0.59	5.0
46	<i>Trichoderma koningii</i> IMI 13022	0.46	5.5

upside down in an 8 cm diam. Petri dish. The filter paper circle was placed on this lid and the Petri dish was covered and incubated at 50 °C for 1 h. Subsequently, the paper was dried at 110 °C until the agar disc became detached. The filter paper was then dipped in aniline phthalate reagent (Peschke, 1965; 0.93 g aniline and 1.66 g *o*-phthalic acid dissolved in 100 ml water-saturated 1-butanol), put upside down on the lid of the Petri dish and developed at 110 °C for 30 min. Reddish green spots (3 cm diam.) appeared whose colour intensity varied depending on the cellulase activity of the fungus. To estimate the cellulase activity, a glucose calibration series was prepared. Glucose solutions (5 µl; 0.5 to 8.0 mg ml⁻¹) were applied 1 cm from the edge of a filter paper disc and about 2 cm from each other. After development, a concentric disc with its edge passing through the centres of the spots was cut from the calibration filter, thus enabling a ready comparison with the test spots. The glucose concentrations in the calibration series were graded so that the colour of spots of two adjacent concentrations could just be distinguished. In this way, individual colours on the test plates were clearly assigned to the corresponding glucose concentration, and the subjective error in measurement was no greater than the gradation of the concentrations in the calibration series.

RESULTS AND DISCUSSION

The test described above was carried out with 43 cellulolytic strains (29 Basidiomycetes and 14 Fungi Imperfecti) and 3 cellulase hyper-producing strains (*Trichoderma* sp. 6a, *Trichoderma* sp. 9414 and *Trichoderma koningii* 13022). The normal cellulolytic strains showed a correlation between filter paper activity and colour intensity values (Table 1). The correlation coefficient was 0.8, significant at the 0.1 % level. It should be noted, however, that filter paper activity is based on objective measurement, whereas the measurement of colour intensities is based on subjective comparison with standards.

The values for hyper-producing strains were not within the correlated population. The deviation of these strains from the correlation is probably explained by differences in the diffusion rates of enzymes. Nevertheless, the test does distinguish these hyper-producing strains, even though it does not indicate their actual activity.

The test described should be useful for rapid estimation of cellulose degrading activity of a variety of fungi and is suitable for screening experiments because it takes little time and enables the handling of a large number of samples.

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