DIRECT QUANTIFICATION OF CELLULASE ACTIVITY BY CLEARING ZONES ON PETRI DISHES ASSAY

María Teresa Torres-Mancera, Arturo Figueroa-Montero, Sevastianos Roussos, Ernesto Favela-Torres, Gerardo Saucedo-Castañeda. Universidad Autónoma Metropolitana, Departamento de Biotecnología. Av. San Rafael Atlixco 186, CP 09340, México D.F. te1982re@gmail.com

Key words: Cellulase, Petri Dishes, Agar Diffusion

Introduction. The Petri dishes assay is a method that can be used for selection of microorganisms with special characteristics. Antier *et al*[1] determined pectinase activity by the appearance of clearing zones and Montville[2] determined protease activity by a similar way. The diameter of the clearing zones was linearly proportional with the $\log_{10}$ of the enzyme activity. The aim of this work was to demonstrate that the agar assay can be used for direct quantifying of cellulase activity measuring the clearing zones in Petri dishes.

Material and methods. Assays were realized in 55 mm diameter Petri dishes using 10 ml a medium containing (g/L): 15, agar and 2, CM-cellulose (Sigma C3896) as substrate and incubated at 30ºC and 40ºC. The culture medium was sterilized at 15 lb, and 121ºC for 15 min. Enzymes activities were tested from aqueous preparations (U/dishes) from commercial cellulose from *Trichoderma reesei* (Sigma C2730). A standard curve (clearing zone area vs enzyme concentration) was prepared. One international unit (U) of activity enzyme was defined as the amount of enzyme needed to produce 1 µmol of product (glucose) per min.

Results. Figure 1 shows the cellulase activity in Petri dishes.

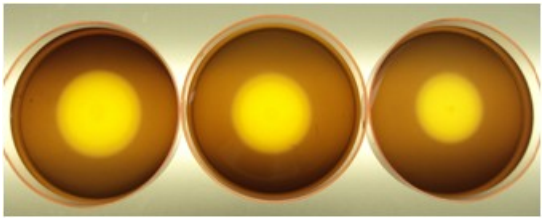

Fig.1 Clearing zones for PecA and XylA

Table 1 shows the clearing zone area measured with different enzyme concentrations, temperature and incubation time.

Table 2 shows linear regression results for clearing zone area vs enzyme concentration at different, temperatures and incubation times for commercial cellulase.

Table 1. Clearing zone measured

Time (h)	24	48	72
Concentration (U/caja)	Clear zone area (cm^2)		
0.41	4.16	6.72	8.04
0.33	3.93	6.32	7.61
0.25	3.55	5.89	6.95
0.17	3.18	5.31	6.32
0.08	2.95	4.86	5.78
0.02	2.58	4.25	4.96

Table 2. Linear regression

T (ºC)	t (h)	Equation	R^2
30	24	$A = 4.01C + 2.55$	0.96
	48	$A = 6.19C + 4.26$	0.96
	72	$A = 7.69C + 4.99$	0.97
40	24	$A = 4.22C + 2.73$	0.96
	48	$A = 6.82C + 5.30$	0.97
	72	$A = 8.36C + 5.87$	0.97

$\alpha < 0.001$

A: Clearing area (cm^2); C: Concentration (U/dishes)

From the 24 h to 48 h at 30°C and 40°C the slope increased 1.5 and 1.6 times, respectively. From the 48 h to 72 h at 30°C and 40°C the slope increased 1.2 times for both incubation temperatures. This can be explaining because diffusivity of cellulose activity is affected by temperature[3]. The slope increased 10% for all incubation times from the 30°C to 40°C. The hydrolysis (clearing zone) area was linearly proportional to the concentration enzyme.

Conclusions. This assay allows the qualitative selection of microorganisms by the appearance of clear zone and quantitatively to enzyme activity by a standard curve. The area of the clear zones area was linearly proportional to the enzyme concentration.

Acknowledgements. CONACyT 204412

References.

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