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Improved detection of chitinase activity after SDS-polyacrylamide gel electrophoresis and coomassie blue staining

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ABSTRACT

An improved method in detecting chitinase activity using SDS-PAGE is described. The chitinolytic band and its molecular weight can be determined on the same SDS-gel without using an UV illuminator.

The detection of chitinolytic activities of chitinase enzymes in both native and denaturing polyacrylamide gels has been described by Trudel and Asselin[1,2]. After electrophoresis, the separation gel was overlaid with a gel containing 0.01% glycol chitin, which is a soluble modified form of chitin and substrate of chitinase. Lytic zones were then observed using an UV illuminator after staining for 5 minutes with 0.01% fluorescent brightener, Calcofluor white M2R. However, there are some disadvantages in this procedure, namely (a) it requires an extra overlay gel containing 0.01% Calcofluor white M2R; (b) it requires an UV illuminator which is biohazard; and (c) the molecular weights of the chitinolytic enzymes can not be estimated directly from the same gels. A modified method will be reported here.

Recently it has been shown that *Bacillus thuringiensis* (*Bt*) HD-1 produces chitinase[3,4] *Bt* culture media were first tested for the presence of chitinase activities using chitin-azure and bacterial culture agar plates containing 0.01% powder chitin[5,6]. For positive isolates, *Bt* was cultured in tryptone soy broth at 30^{0}C overnight, and then centrifuged. For chitinase induction the bacterial pellets were then washed once in sterile PBS (pH7.6), and finally re-suspended in 0.01M Tris buffer (pH 7.6) containing 0.1% colloidal chitin and cultured at 30^{0}C. After 4 days, the supernatant of the culture was collected by centrifugation. Protein was precipitated by 80% ammonium sulfate. The re-dissolved protein was finally dialyzed against distilled water and then PBS.

All the chemicals were obtained from Sigma (St. Louis, USA). Samples were separated by 12% SDS-polyacrylamide gel containing 0.05% glycol chitin in a Mini-gel electrophoresis apparatus (Bio-Rad, Richmond, USA). After separation, the two identical gels were then incubated in 0.1M sodium acetate (pH5.0) containing 1% Triton X-100 for 1 hr at room temperature to re-nature the enzymes. Then the gels were briefly washed with 0.01M sodium bicarbonate (pH 8.9), and were incubated in a moist box at room temperature. After 2 hr incubation, one gel was stained with Coomassie Blue R250 while the other gel was stained with 0.01% Calcofluor white M2R for 15 minutes, and then washed in distilled water for 1hr. The gel was then observed using an UV illuminator (Fotodyne, WI, USA). After photographed, the gel was rinsed in 0.01% acetic acid for half an hour. The gel was again visualized with an UV illuminator and re-photographed. The gel was finally stained with Coomassie Blue R250.

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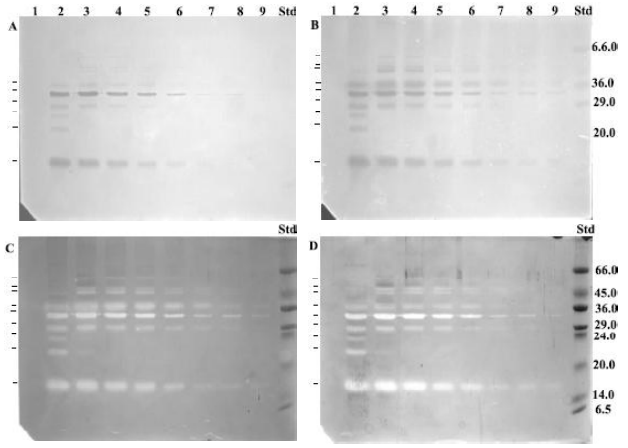

Figure 1 : Chitinase activity after electrophoresis in a 12% (W/V) SDS-PAGE gel impregnated with 0.01% glycol chitin at pH8.9. *Bacillus thuringiensis (Bt)* HD-1 was cultured in Tris buffer pH7.6 containing 0.01% colloidal chitin for four days at 30^{0}C. Culture supernatant was collected by centrifugation, and precipitated with 80% ammonium sulfate. The precipitated protein was dissolved in 1/10 of its original volume and dialyzed against distilled water and then PBS. The final solution contains chitinase activity as tested by chitin azure. Wells 2-9 are two fold dilution of the final *Bt* chitinase preparation Equal volumes of sample and sample buffer containing mercaptoethanol were heated at 80^{0}C for two minutes before applying to the wells. After electrophoresis, the two identical gels were washed with 1% Triton X-100 in 0.1M sodium acetate (pH5.0), and then incubated at room temperature for 2 hr. Figure 1A : Gel was stained with 0.01% Calcofluor for 15 minutes and washed with water for 1 hr. Gel was viewed with an UV illuminator. Figure 1B: the same gel from Figure 1A was further incubated with 0.01% acetic acid for 30 min, and the gel was also viewed with an UV illuminator. Figure 1C: the same gel from Figure 1B was then stained with Coomassie Blue R-250. Figure 1D: An identical gel was stained with Coomassie Blue R250 immediately after incubation at room temperature for 2 hr. The dark bands in Figures 1A and B are chitinolytic bands. The chitinolytic bands in Figures 1C and D are clear, while the dark bands are proteins. (- indicates the approximate position of the chitinolytic band). Standards are in kDa.

Calcofluor white M2R has high affinity for chitin, but not digested glycol chitin. Therefore, the lytic zones appear as nonfluorescent dark bands in contrast to the fluorescent intact glycol chitin when the gels are viewed by UV illumination. Figure 1A showed the chitinolytic activity in the samples appeared as dark bands under UV illumination. There are at least 6 chitinolytic bands. After treating with 0.01% acetic acid for half an hour, more and darker chitinolytic bands appeared on the gel (Figure 1B), and the standard protein bands also appeared (Figure 1B, Std lane). When this gel was stained with Coomassie Blue R250, the chitinolytic bands appeared as clear bands and well defined, and there are at least 9 chitinolytic bands (Fig.1C). However, the chitinase protein bands did not appear within the clear chitinolytic bands. It suggests that either the chitinase proteins embedded in digested glycol chitin were stained very poorly by Coomassie Blue R250 or the concentration of the chitinase in the sample was very low. When the well was overloaded with sample, faintly dark protein bands appeared within the clear chitinolytic bands as shown in Figure 2 (lane 1). This suggests that the protein band within the clear chitinolytic band corresponds to the chitinase activity. When the other identical gel was stained with Coomassie Blue R250 immediately after two hr incubation at room temperature without Calcofluor white M2R staining, the gel (Figure 1D) also gave the same resolution as in Figure 1C but is better than Figure 1A. This suggests that direct staining with Coomassie Blue R250 without Calcofluor staining is more sensitive than using Calcofluor staining followed by UV illumination.

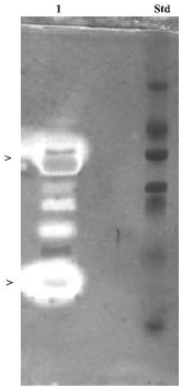

Figure 2 : Chitinolytic acitivity of the *Bt* culture medium sample after SDS-PAGE. Well 1 was overloaded with sample and chitinase activities were detected as described in Figure 1. The gel was stained with Coomassie Blue R250 immediately after incubating for 2hr at room temperature. Clear band indicates the chitinolytic acitivity, and the faint dark bands (as indicated by >) inside the clear bands indicate the protein staining of the enzymes.

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Apparently the presence or absence of mercaptoethanol in the sample buffer does not affect the positions of the chitinolytic bands (results no shown). Boiling the sample for 1/2 minute at 100^0C also does not appear to affect the *Bt* chitinase activities in the sample after re-naturing in sodium acetate-Triton X-100 buffer (results not shown).

The present method in detecting chitinolytic activities in samples has several advantages over that reported by Trudel and Asselin (1989). (1) The detection does not require overlaying another layer of gel containing glycol chitin on top of the SDS-gel. (2) It does not require an UV illuminator, which is biohazard, and detection can be achieved by direct staining with Coomassie blue R-250. (3) This method is more sensitive than the Calcofluor-UV method (Figure 1A and 1D). (4) Since the molecular weight standards can be observed from the same gel, the molecular weights of the chitinase can be determined more accurately. In conclusion, this is an improved and sensitive SDS-polyacrylamide gel electrophoresis in detecting chitinase activity and determining its molecular weight on the same gel.

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