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REGULAR ARTICLE

A SIMPLE STAINING METHOD FOR INCREASING SENSITIVITY IN DETECTION OF ALKYL SULFATASE IN NATIVE POLYACRYLAMIDE GELS

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SUMMARY

To increase the sensitivity in detection of alkyl sulfatases in Native PAGE gels, we report here a simple staining method based on Coomassie brillent blue R 250. This method facilitates in detection of faint alkyl sulfatase bands which are otherwise difficult to visualize.

Key words: Alkyl sulfatase, Colloidal coomassie, Polyacrylamide gel (PAG)

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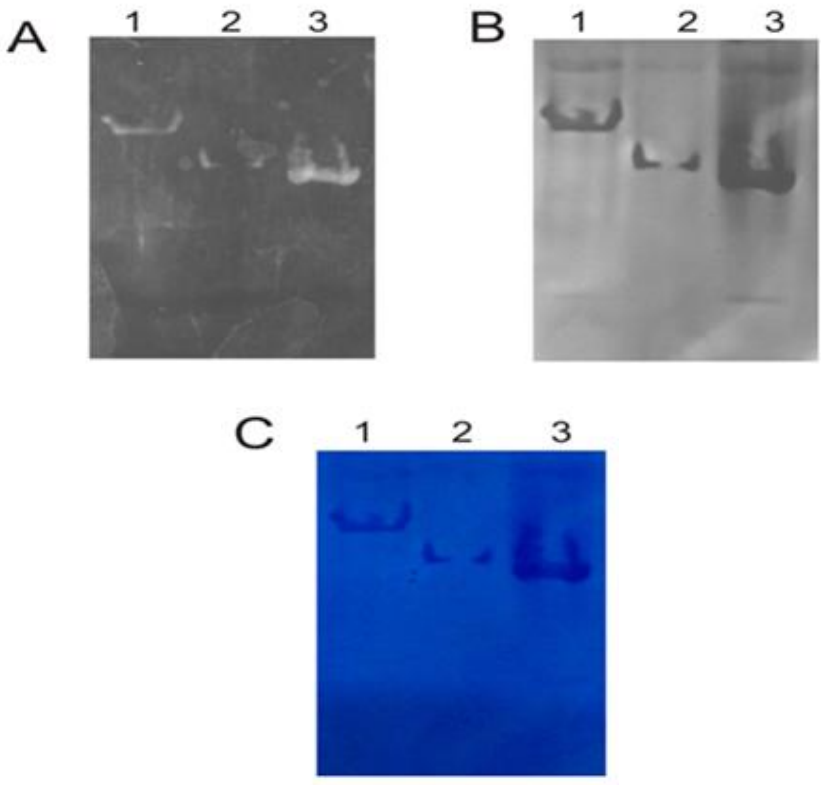
Alkyl sulfate esters are known to possess excellent cleansing properties and are widely used as detergents. Sodium dodecyl sulfate is a member of the group and is one of the widely used detergents in households and Industry. Apart from its use in household, SDS is also used in many other applications (Karsa, 1992). It is well documented that SDS is toxic to animals and microorganisms, especially to fishes, which come in direct contact with this detergent as mainly it is disposed in huge amounts in water bodies. So, it is considered as a pollutant in water systems (Rosety et. al., 2001, Bantseev et. al. 2003). Due to its toxicity, it was realized that bioremediation of this detergent is important. This initiated the screening and identification of bacteria, which could degrade and metabolize SDS. Members of *Pseudomonas* were found capable of degrading this detergent and until now, most of the work on biodegradation of SDS is done on this bacterium. Pathway of biodegradation of SDS is initiated by cleavage of sulfate group from SDS, resulting in the formation of 1-dodecanol (C12 alcohol). This step is catalysed by the enzyme alkyl sulfatase. 1-dodecanol is then oxidized to 1- dodecanoic acid by alcohol dehyrogenases. 1-dodecanoic acid is then metabolized via beta oxidation pathway and used as a carbon source (Thomas and White, 1989). In *Pseudomonas sp.*, it has been reported that multiple alkyl sulfatases of different molecular weights and substrate specificities exist. Until now, only two alkyl sulfatases have been fully characterized. So, in work involving biodegradation of SDS, it is essential to study the enzyme involved in biodegradation. Alkyl sulfatase can be easily detected by Native PAGE Zymography. Zymographic detection of alkyl sulfatase involves incubation of PAG in a solution containing 20mM SDS in 0.1M Tris-Cl for 30 mins to 1-2 h. Alkyl sulfatase cleaves SDS and 1- dodecanol is formed, which forms a white precipitate in PAG. This method is simple but it is difficult to visualize because many a times, these white bands are very faint and are unstable. They disappear quickly in 2-3 h due to diffusion of 1-dodecanol in to the medium (Ellis et. al., 2001). This limits in the characterization and identification of the enzyme. Since, for the identification of enzyme, involves aligning the bands on the zymogram gel with corresponding protein bands on an equivalent gel without substrate. Bands are typically excised from the equivalent gel for protein identification by mass spectrometric analysis. Also for molecular weight estimation, the zymogram gel is aligned with

an equivalent gel containing the molecular weight markers. Since, in the present case, the alkyl sulfatase bands are very unstable and they disappear in 2-3 h due to diffusion of 1-dodecanol in the medium, so it is very difficult to identify the proteins with this method. So, we wanted to develop a method to prevent diffusion of 1- dodecanol into the medium. So that, the alkyl sulfatase bands arising from activity staining can be aligned with an equivalent gel which is stained with CBB staining. The corresponding band can be cut and analyzed by maldi tof analysis.

In the present study, we report here a simple staining procedure for increasing the sensitivity and thereby, detection of the alkyl sulfatase bands by staining the bands with Coomassie brillent blue R 250. In this process, crude cell extracts of SDS grown cells belonging to *P. aeruginosa* strain SDS3 (EF197939) (Lane 1), *P. stutzeri* strain K2 (GQ328718) (Lane 2) and *P. alcaligenes* strain JN2 (GQ328720) (Lane 3) were prepared according Ellis et. al. (2001) and were run on native PAG containing 5% Acrylamide in stacking and 6% in resolving gel. The electrophoresis was conducted at 4^0C and 15 mA current, for minimizing the heat inactivation of enzymes. After electrophoresis, the PAG gels were incubated in solution containing 20 mM SDS in 0.1M Tris-Cl at 37^0 C. Until, the bands were observed (Figure 1A). These PAG were transferred to Colloidal coomassie staining solution for 4-5h. The fixing step in 40% ethanol and 10% Acetic acid was omitted because it was thought that it would facilitate diffusion of 1- dodecanol in the medium. After incubation it was observed that, the white bands of 1- dodecanol were stained dark blue and the gel was light blue in color (Figure1 B and C).

Figure1.Zymographic detection of alkyl sulfatases in native PAG. (A) Detection of alkyl sulfatases by incubation in 20mM SDS in 0.1M Tris-Cl, (B)and(C) Colloidal coomassie stained bands, (B) Image acquired by gel documentation Unit, (C) Image acquired by Canon digital camera A530. Lane 1, 2 and 3 represent *P. aeruginosa* strain SDS3 (EF197939) (Lane 1), *P. stutzeri* strain K2 (GQ328718) (Lane 2) and *P. alcaligenes* strain JN2 (GQ328720) (Lane 3) respectively.



After staining the gel was taken out from the solution and washed 2 times with double distilled water and photograph was taken. It was easily evident that faint bands corresponding to alkyl sulfatase which were faint (Lane 2 A) and not clearly visible were easy to identify. The staining of bands by Coomassie brillent blue R 250 can be attributed to its high solubility in alcohols rather than water. Since product of SDS cleavage is 1-dodecanol (C12 alcohol). So, it is obvious that the dye has higher affinity towards alcohols as a result bands were stained blue. Also, staining of bands with the dye blocks the diffusion of 1–dodecanol in the medium. Previously it was observed that when PAG were incubated in solution of SDS in Tris-Cl, the bands were visible after 1 h of incubation and they disappeared after 2-3 h due to diffusion of 1-dodecanol into the medium but here after staining with the dye for 4-5h, the bands are still present. It can be concluded that staining of bands with Coomassie brillent blue R 250 increases the sensitivity for the detection of alkyl sulfatases in native PAG. This method is simple and efficient for detection of Alkyl sulfatase by native PAGE Zymography.

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