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Culture Characteristics of *Streptomyces* spp. on Improved Polyacrylamide Gel and Agar Media

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Application of polyacrylamide gel (PAG) instead of agar to solid cultures of *Streptomyces* spp. was studied. The improved media were prepared by 1) gelling 20 ml of 5% acrylamide in a glass petri dish at room temperature, 2) washing by running water for more than 8 hr to remove residual reaction reagents, 3) drying at 50°C for 12 hr to make a gel film, 4) autoclaving at 121°C for 15 min, and 5) swelling gel for about 4 hr by adding sterile liquid medium. In PAG media there were no differences from the observation of morphological characteristics showing during the cellular differentiation on agar media, whereas the ability to utilize carbohydrates differed somewhat from agar media. Agar media thus were little favorable for biochemical tests which the growth was determined depending on the formation of colony, but washed PAG was superior to serve as a solidifying agent.

Key words: *Streptomyces*, culture, polyacrylamide gel

We reported in 1992 that polyacrylamide (PA) gel could be used in cultures of *Streptomyces* and other microorganisms as a solidifying agent instead of agar(1). PA gel media were simply prepared by polymerizing constituents of culture media at 121°C for 5 min in autoclave. PA gel media prepared by this method has since been used in our laboratory but later disadvantages were found: the method was limited to some microorganisms, because after polymerization reagents remained and inhibited the growth of *Streptomyces* spp. and also it was not convenient to remove excess water in petri dish(1). So in order to improve gel medium, PA gel without components of medium was polymerized and remaining reagents was washed out by water, and then gel was dried at 50°C for 12 hr to make thin gel film and thereafter swollen by adding liquid medium and autoclaved. This method also was not successful in that gel was separated from the wall of petri dish and the loss was produced about 20% of total media(7). Recently it was found that to prevent gel separartion dried gel plates first should be autoclaved immediately before swelling gels with broth media.

Here we report improved method for solid cultures of *Streptomyces*. The following method (for 5% PAG plates) is recommended: 1) dissolve 4.75 g acrylamide, 0.25 g BIS (N, N-methylene-bisacrylamide) in 100 ml distilled water and add 0.2 g ammonium persulfate, 2) agitate the mixture for 15 min, 3) pour 20 ml mixture into glass petri dish, 4) stir uniformly with L-shape glass rod immediately after the addition of 20 μl of 10% TEMED (N,N,N',N'-tetramethylethylene diamine), 5) stand for 20 min at room temperature for gellation, 6) wash gel plates by running water for at least 8 hr to remove remaining reagents, 7) dry for 12 hr at 45~50°C to make thin gel film, 8) autoclave the thin gel film at 121°C for 15 min, 9) add 7~8 ml sterile medium by membrane filtration asceptically, 10) stand for 3-4 hr to swell gels for inoculation, and 11) incubate PA gel plates. Such a PAG medium was used for checking the cellular differentiation and biochemical properties of *Streptomyces* spp.

Table 1 shows an example of PAG solid cultures. Morphological characteristics of *S. cellulosae*, *S. coelicolor* and *S. diastitacus* were compared on PAG contained components of media which are recommended by ISP (International Streptomyces Project) with agar medium (5). The results about spore chain, spore surface ornamentation, color of spore mass, production of diffusible pigment were not distinctly different except for that *S. cellulosae* do not produce melanin on tyrosine agar (ISP-7) as in the description of Bergey's manual (3). It was

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Table 1. Morphological characteristics of *Streptomyces* spp. on polyacrylamide gel(PAG) and agar

Species / haracters	*S. cellulosae*	*S. coelicolor*	*S. diastaticus*
1. Spore chain morphorlogy	straight to flexuous	hook or spiral	stdraight to flexuous
2. Spore surface ornaentation	spiny, warty	smooth	smooth
3. Color of spore mass	white	violet	white
4. Pigmentation of Substrate mycelium	yellow	red-violet	yellow-brown
5. Diffusible pigment produced	-	+ (violet)	+ (yellow-brown)
6. Melanin on tyrosine agar	+	-	-

Table 2. Utilization of carbohydrates and hydrolysis of macromolecules by *Streptomyces* spp. on polyacrylamide gel media (PAG)

species / Characters	*S. cellulosae*	*S. coelicolor*	*S. diastaticus*
carbon-free control on			
Agar	+	+	+
PAG	-	-	-
Utilization of			
1. Arabinose	-(+)	+(+)	+(+)
2. Xylose	-(+)	+(+)	+(+)
3. Cellobiose	+	+	+
4. Fructose	+(+)	+(+)	+
5. Galactose	-	+(+)	+
6. Glucose	+(+)	+(+)	+(+)
7. Lactose	-	+	+
8. Mannose	-	-	+
9. Rhamnose	-(d)	-(-)	+(+)
10. Sucrose	-(d)	-(+)	-(d)
11. Melezitose	-	+	+(d)
12. Melibiose	-(+)	-	+(d)
13. Raffinose	-(-)	-(-)	-(d)
14. Adonitol	-	-	-(d)
15. Inositol	+(+)	+(-)	-(d)
16. Mannitol	-(+)	+(+)	+(+)
17. Xylitol	-	-	+(d)
18. Salicin	-	-	+
Hydrolysis of			
19. Casein	+	+	+
20. Gelatin	+	-	-
21. Inulin	-	-	-
22. Starch	+	+	+

Note: positive or negative results in parentheses were cited from Bergey' manual of Systematic Bacteriology. vol 4. (1989) and Bergey's manual of Determinative Bacteriology, 8th ed. (1974)

characteristic that the size of colonies was smaller on PAG media than on agar media, and colonial growth was not overlapped in PAG media.

The utilization tests were carried out in ISP-9 media containing 1% of each carbohydrates. As shown in Table 2, in the utilization of carbohydrates there were many discrepancies between PAG and agar media unlike the results of morphological differentiation (3, 6). *Streptomyces* could form colonies on agar medium as carbon-free control, but not form any colony on PAG media. This means that agar contains to some extent carbon sources or sugars and other nutrients for their growth. This fact has already been known that agar supplies nutrients to allow scanty growth of streptomycetes(2). Therefore in case of *Streptomyces* it becomes obscure to evaluate on which carbon sources are utilized in agar media. In this respect, PAG media have some advantages, because PA gel have high purity and it can not be degraded and utilized by the test microorganisms.

For examples given in Table 2, according to Bergey's manual *S. cellulosae* can utilize arabinose, xylose, melibiose and mannitol in agar or broth media, but this organism could not use the same carbohydrates in PAG plates. *S. coelicolor* and *S. diastaticus* showed a little differences in the utilization of pentoses, hexoses, triose and sugar alcohols. Here still remains a problem as to which results will be chosen. However, we believe that it is necessary to reexamine the data which have so far been obtained from agar media.

The hydrolysis was carried out to know whether even macromolecules such as casein, gelatin, inulin and starch can diffuse into polyacrylamide gel when broth media were poured into polyacrylamide gel plates. As basal medium was used Bennett's medium as supplemented with 10 g of glycerol per litre (4). Macromolocules diffused well within 4 hr and also clearing zone was normally observed as in agar medium, which is formed by the diffusion of enzymes produced by tested strains.

In summary, the successful usage of PAG media depends on complete wash-out of reagents after polymerization and sterilization of dried gel plates by autoclaving. Once dried gel plates is prepared even if its preparation takes longer time in PAG than in agar, the plates can be stored for a considerable time till before use. (We expect forward that the time of preparation will be far lessened than that of agar if the manufacture of PAG plates is industrialized.) PAG media can be well employed in cultures of microorganisms not only which can degrade agar, but also which are acidophilic or al-

kalophilic because PAG is gellated under strong acidic or alkaline conditions, while agar is not solidified under such conditions.

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