

## PREPARATION OF A SUBSTITUTE FOR NUTRIENT BROTH TO ACTIVATE *BACILLUS LICHENIFORMIS* 6346

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Fish (200 g) suspended in saline (1000 ml) was left at 30°C for 48 h. The extract (1000 ml) was mixed with pepsin (270x10<sup>5</sup> Units in 250 ml 0.5N HCl) and incubated at 30°C for 24 h (Fish extract hydrolysate.) The residue (150g) was pressure cooked with 6N HCl (400 ml) for 2 h and the extract was collected (Fish hydrolysate). Three activation media for *Bacillus licheniformis* 6346 were prepared by taking either the mixture of fish hydrolysate and fish extract hydrolysate (2.0 : 5.0, volume ratio, 20 ml) or fish extract hydrolysate (20 ml) or nutrient broth (20 ml, 25g l<sup>-1</sup>), 3.0 g l<sup>-1</sup> soluble starch and 3.0 g l<sup>-1</sup>, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>. The activation media (20 ml) were inoculated with 2 loopsful of the bacteria and incubated at 42°C for 18 h. The activated bacteria (5.0 ml) was inoculated with 20 ml of the respective activation media and incubated for 5 h at 42°C to prepare the inocula. The different inocula (20 ml) were mixed to solid medium (80 g) (containing (g kg<sup>-1</sup>) paddy husk, 300; rice flour, 10; soya flour, 32 and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 6.4 and (ml kg<sup>-1</sup>) gingili oil, 9.0; coconut oil, 3.0 and tap water, 440) and incubated at 42°C. At 4<sup>th</sup> day α -amylase activity obtained in the solid medium inoculated with the *B.licheniformis* containing inocula prepared by taking either the mixture of fish hydrolysate and fish extract or fish extract hydrolysate or nutrient broth in terms of glycine were 25.5, 0.37 and 0.88 mg ml<sup>-1</sup> respectively. Then to a fixed amount of fish extract hydrolysate (13.6 mg amino acid) varying amount of fish hydrolysate (from 6.6 to 33.3 mg amino acid) was added to prepare the activation medium. Highest α -amylase activity (1218 m mole g DBM<sup>-1</sup>min<sup>-1</sup>) was obtained in the solid medium which was inoculated with the activation medium containing the fish extract hydrolysate: fish hydrolysate in the volume ratio of 4.0 : 10.0 (amino acid ratio of 1.0 : 2.5). Hence this media could be used as a substitute for nutrient broth.

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