Microbial L-glutaminase screening protocol development for high-throughput screening

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ABSTRACT

L-glutaminase (E.C 3.5.1.2) is bio catalyst, able to hydrolyze L-glutamine, then release L-glutamic acid and ammonium as product and by-product respectively. The L-glutamic acid has outstanding characteristic of umami taste which enhance food flavor. With this, L-glutaminase is widely applied in food industry especially soy sauce industry. The ultimate purpose of this research is to develop adequate microbial L-glutaminase screening protocol for high-throughput screening (microplate). This screening protocol result in both efficiency and effectiveness which approach to food industry application. The protocol development was mainly emphasized on down scale of quantitative screening protocol (Nessler's reagent assay) by examined on the effect of various down scale on ammonium detection. The adequate Nessler's reagent protocol that compliable with microplate is 15 µL of reaction and stop solutions and 150 µL of Nessler's reagent which is 225 µL as final volume. There were 14 strain of microbial; *Streptomyces, Bacillus, Pseudomonas* and *Aspergillus* were cultivated for 7 day on agar plate and 5 day in broth media which supernatant was collected daily for Nessler's reagent assay. Result shown that *Streptomyces lydicus* (TBRC 1884) showed the highest ability to produce L-glutaminase in both qualitative and quantitative screening. Despite, the overall result was relied on ammonium, by-product which can be synthesized by various pathway of microbial and easily influence by nitrogen composition in medium used. Thus, detection of L-glutamic acid was necessary to affirm accurate result.

KEY WORD: L-glutaminase/Down scale/Nessler's reagent assay/Ammonium