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Synthesis of Black Soldier Fly Larvae Peptone as a Sustainable Nitrogen Source for Biofertilizer Microbial Consortia

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Abstract

The production of microbial inocula for biofertilizers is currently limited by the high cost of commercial peptone, which serves as a critical organic nitrogen source. Within a circular bioeconomy framework, black soldier fly larvae (BSFL, *Hermetia illucens*) offer a sustainable alternative due to their high protein content (39.59±0.29%) and ability to convert organic waste into nutrient-rich biomass. This study developed a multi-stage process to produce high-quality peptone from BSFL and evaluated its performance in both single-culture and multi-species microbial consortia representative of industrial biofertilizers. The production process involved initial defatting with n-hexane, increasing protein content to 49.95±0.77%, followed by alkaline extraction with a 33.91±0.30% protein recovery. Enzymatic hydrolysis using 3% bromelain at pH 8 and 50°C achieved a degree of hydrolysis (DH) of 53.76±0.66%. The final freeze-dried peptone yielded a product with 80.98±0.50% protein content and an amino nitrogen to total nitrogen (AN/TN) ratio of 0.21, meeting commercial specifications. SDS-PAGE analysis confirmed the presence of predominantly short-chain peptides (<17 kDa). Performance was validated against commercial bacteriological peptone. In single-culture tests using *Bacillus subtilis*, BSFL peptone supported growth kinetics comparable to commercial standards, with a maximum specific growth rate (μmax) of 0.50 h^{-1} and a doubling time of 1.39 h. Crucially, when applied to a biofertilizer consortium consisting of *Bacillus* sp., *Pseudomonas* sp., and *Saccharomyces* sp., BSFL peptone demonstrated superior performance. At a concentration of 15 g/L, BSFL peptone supported significantly higher cell density (1.15±0.17 × 10^9 CFU/mL) compared to commercial peptone (6.05±4.32 × 10^8 CFU/mL), effectively doubling the growth despite using half the nitrogen concentration of other complex sources like soybean or fish meal. The results indicate that BSFL peptone's unique amino acid profile, rich in glutamate, aspartate, and branched-chain amino acids, created favourable ecological conditions for consortium stability and growth. These findings confirm that BSFL-derived peptone is a technically viable, high-performance, and sustainable alternative to animal-derived nitrogen sources. By converting insect biomass into high-value biochemical ingredients, this approach aligns with circular economy principles and offers a scalable solution for reducing the environmental footprint of biofertilizer production.

Keywords: Hermetia illucens, peptone, microbial consortium, circular bioeconomy, biofertilizer