Plant hormones are molecules essential for normal plant growth and development associated to myriad of processes in plant and microorganisms. Biofertilizers are products that contain living microorganisms that when in contact with plants enhance growth or produces other beneficial response. The two objectives of this research were to evaluate a solid phase extraction procedure for extraction of indole-3-acetic acid (IAA) from a liquid biofertilizer developed by Embrapa Hortalicas and to evaluate the changes in this plant hormone level at three different periods time (10, 20 and 30 days of fermentation) and the general group of microorganism associated. The method of preparation and analysis of IAA from biofertilizer was performed by centrifugation, followed by pH adjustment and solvent extraction and a further cleaning by cartridge containing mixed-mode anion-exchange and reverse-phase sorbents. A nucleosil C<sub>18</sub> column was used followed by fluorimetric detection ( $\lambda_{EX}$  = 280 /  $\lambda_{EM}$  = 350 nm) by Reversed Phase High Performance Liquid Chromatography (RP-HPLC) determination. The linear regression equation of calibration curve (P < 0.0001) had the  $r^2$  = 0.995 and the limit of detection was below 0.06 µg mL<sup>-1</sup>. The IAA amount at 10, 20 and 30 days was 0.57, 0.32 and 0.15 µg mL<sup>-1</sup> respectively Both total number of bacteria and actinomycetes were higher at day 10, coinciding with the higher IAA production. The use of biofertilizers with potential biostimulant activity (IAA activity) in agriculture may help enhance plant rooting and productivity as a result. This is particularly important for low input agriculture such as organic farming.