EVALUATION OF POLY-β-HYDROXYBUTYRIC ACID CONTENTS OF SOME RHIZOBIAL STRAINS AND THEIR PERFORMANCE UNDER DIFFERENT STRESS CONDITIONS

By

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B.Sc. (Agric. Microbiology), Fac. Agric., Ain Shams University,2014

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ABSTRACT

Wafaa Hamdy Ali Radwan "Evaluation of Poly-β-Hydroxybutyric acid contents of some rhizobial strains and their performance under different stress conditions". Unpublished M.Sc. Thesis, Department of Agric. Microbiology, Faculty of Agriculture, Ain Shams University, 2020.

One hundred and eight *Rhizobium* isolates were isolated from the root nodules of *Vicia faba* and *Phaseolus vulgaris* grown in different locations belonging to nine governorates in Egypt. All isolates were confirmed as rhizobia after the authentication test in growth medium and pot experiment under controlled environmental conditions. *Rhizobium fabae* and *Rhizobium phaseoli* were identified based on morphological characteristics, Gram staining, and further confirmation by sequencing the 16SrRNA gene. A statistical approach of Response surface methodology (RSM) response was used for PHB production optimization by the selected isolates. Eleven variables including culture conditions (pH, incubation period, inoculum size, temperature and agitation speed) nutritional factors (media components), as mannitol, sucrose, yeast extract; glycine, K_2HPO_4, and $MgSO_4$ were analyzed by Plackett-Burman design for their effects, on PHB production. For *Rhizobium fabae,* the main effects which significantly affected PHB production were sucrose, yeast extract, incubation time, and agitation rate. For *Rhizobium phaseoli,* the main factors affected PHB production were sucrose, yeast extract, Glycine and $MgSO_4$. A central composite design (CCD) was carried out for selecting the optimum levels for all significant factors obtained from Plackett-Burman Design (PBD). For *Rhizobium fabae* strain, The maximum production (78.51%) was achieved at run number 19 in the presence of 25 g/l sucrose, 0.5 g/l yeast extract, 150 rpm incubated for two days. On the other hand, *Rhizobium phaseoli* reached the maximum production (77.70%) at run 36 in the presence of 25 g/l sucrose, 0.0 g/l yeast extract, 0.87 g/l glycine, 0.3 g/l $MgSO_4$ and 5% incubation size. The effect of different stress conditions (salinity, temperature, pH and drought) on these strains was studied at the lab scale. 5% NaCl showed 50% loss on the growth of both *Rhizobium fabae* and *Rhizobium phaseoli* when grown on base medium compared with the modified one. Thermal stress conditions illustrated that both of *Rhizobium fabae* and *Rhizobium phaseoli* can withstand high temperature (45^{o}C) in the modified medium when compared with the growth on the base medium to reach a growth loss 63.3and 56.4% for the *Rhizobium fabae* and *Rhizobium phaseoli*, respectively, on the base one. Acidity stress conditions showed that both of *Rhizobium fabae* and *Rhizobium phaseoli* can withstand alkalinity conditions (pH 10) in the modified medium when compared with the growth on the base medium to reach a growth loss 75 and 30% for the *Rhizobium fabae* and *Rhizobium phaseoli,* respectively, on the base one. Drought stress conditions showed that both of *Rhizobium fabae* and *Rhizobium phaseoli* can withstand drought conditions (by using PEG 3.5%) in the modified medium when compared with the growth on the base medium to reach a growth loss 90 and 65% for the *Rhizobium fabae* and *Rhizobium phaseoli,* respectively, on the base one.

Keywords: *Rhizobium fabae*; *Rhizobium phaseoli;* Poly-β-Hydroxybutyrate (PHB); Plackett–Burman design; central composite design; salinity; temperature; pH ; drought and affected soil .

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