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棉花黄萎病生防芽孢杆菌Z-5菌株发酵培养基的优化

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Optimization of Fermentation Conditions of a Biocontrol Bacterial Isolate *Bacillus* Z-5 against Cotton Verticillium Wilt

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摘要 在农用微生物菌剂中芽孢杆菌菌剂产品多采用芽孢作为有效成分，因此产芽孢条件的优化对棉花黄萎病生防细菌的工业生产有着重要意义。本文通过单因素试验分析了8种碳源、8种氮源和6种无机盐对*Bacillus malacitensis* Z-5菌株芽孢产量的影响，采用Plackett-Burman试验设计确定了影响芽孢产量的主要因素，用最陡爬坡路径逼近最大响应区域，利用Box-Behnken试验设计及响应面分析法确定主要影响因素的最佳条件。结果表明，单因素试验筛选出3种碳源（玉米粉、麸皮、葡萄糖）、3种氮源（豆饼粉、花生饼粉、酵母粉）和2种无机盐（$MnSO_4 \cdot H_2O$、$CaCl_2$）；Plackett-Burman试验确定Z-5菌株生产芽孢最适碳源、无机盐和氮源分别为玉米粉、$MnSO_4 \cdot H_2O$和豆饼粉，最陡爬坡路径法获得此3种因子的最适浓度范围为：玉米粉1.0%~2.0%、MnSO4 · H2O 0.05%~0.09%、豆饼粉为1.0%~2.0%。响应面法得到芽孢产量与玉米粉、MnSO4 · H2O和豆饼粉含量的回归方程。最终确定优化培养基组合为玉米粉1.66%、豆饼粉1.30%、$MnSO_4 \cdot H_2O$ 0.07%、$NaH_2PO_4 \cdot 2H_2O$ 0.2%、$Na_2HPO_4 \cdot 2H_2O$ 0.4%，优化后芽孢产量达到$1.9^7 \times 10^9$ mL^{-1}，与理论值基本相符。

关键词： 响应面法 棉花黄萎病 生物防治 芽孢杆菌 发酵

Abstract： The spore was usually used as the active ingredient for most *Bacillus* products in agricultural microorganism preparation, so optimization of spores producing conditions of *Bacillus malacitensis* Z-5, a biocontrol bacterial isolate against cotton verticillium wilt, was of great significance in industrial production. The effects of eight kinds of carbon sources, eight kinds of nitrogen sources and six inorganic salts on the yield of spores produced by *Bacillus malacitensis* Z-5 were studied by single factor analysis in order to gain the optimum carbon, nitrogen and inorganic salt. Plackett-Burman(PB) experimental design was used to determine the main factors affecting spore production. The pass of steepest ascent was undertaken to approach the optimal response region of the three significant factors. Box-Behnken design and response surface analysis were adopted to further investigate the mutual interaction between the variables and identify optimal values that bring maximum spore yield. The result showed that corn flour, bran and glucose were the three most suitable carbon sources according to single factorial experiments, while soybean meal, peanut meal and yeast were the three best nitrogen sources, and $MnSO_4 \cdot H_2O$ and $CaCl_2$ were the best inorganic salt. It ' s also showed in PB design that corn flour, $MnSO_4 \cdot H_2O$ and soybean meal were the optimum carbon sources, inorganic salt and nitrogen sources, respectively. The path of steepest ascent experiment was employed to approach the optimal region of the medium concentration of corn flour 1.0%-2.0%, $MnSO_4 \cdot H_2O$ 0.05%-0.09% and soybean meal 1.0%-2.0%. The regression equation between the spore production of *Bacillus malacitensis* Z-5 and the variables from carbon source, inorganic salt and nitrogen sources content by the response surface method were computed. The final optimal culture medium was as followed: corn flour 1.66%, soybean meal 1.30%, $MnSO_4 \cdot H_2O$ 0.07%, $NaH_2PO_4 \cdot 2H_2O$ 0.2%, $Na_2HPO_4 \cdot 2H_2O$ 0.4%. The spore yield reached $1.9^7 \times 109$ mL^{-1} after fermentation experiment under optimal condition and basically consistent with the theoretical value.

Keywords： response surface method cotton Verticillium wilt biocontrol *Bacillus* spp. fermentation

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