Statistical Optimization of Poly-β-Hydroxybutyrate Biosynthesis Using the Spent Mushroom Substrate by *Bacillus tequilensis* PSR-2

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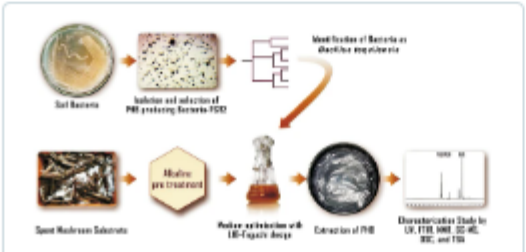
Kanagavel Suvithra, Kannan Badri Narayanan ✉, Uthandakalaipandian Ramesh, Chelliah Edward Raja, Gandhi Premkumar ✉, Govindaraju Vaseeharaju, Arunachalam Vijayakumar, Marikani Kannan & Kaniappan Rajarathinam

Abstract

Poly-β-hydroxybutyrate (PHB) belonging to the polyhydroxyalkanoates family is a natural polyester used as a biodegradable plastic for various commercial applications. In this study, soil samples from the vegetable oil processing industry were used to screen for PHB-producing bacteria using Sudan black B staining. Among the isolated bacteria, PHB-positive PSR-2 isolate was chosen as a potent PHB producer. The phylogenetic tree revealed that the PSR-2 isolate has a high 16S rRNA gene sequence similarity of 99.9% with *Bacillus tequilensis*. The PHB content of 2.8 ± 0.09 g/L was produced by PSR-2 isolate in 48 h in a nutrient broth medium containing 1% glucose compared to the PHB production of 1.6 ± 0.08% by the reference strain, *Bacillus circulans*. Taguchi method was used to optimize PHB production using the alkali-pretreated spent mushroom substrate of sugarcane bagasse (SMS-SB) as an additional carbon substrate along with other energy sources. The optimized factors in the contribution of PHB production from the highest- to the lowest-ranking are as follows: alkali-pretreated SMS-SB, glucose, glycerol, peptone, ammonium chloride, and potassium dihydrogen phosphate at 30 °C, pH 7.0, which resulted in the production of 12.4 ± 0.95 g/L. PHB was higher than the predicted value of 11.59 g/L. The synthesized PHB was characterized using Ultraviolet–visible spectrophotometry, Fourier transform infrared spectrometry, differential scanning calorimetry, thermogravimetric analysis, nuclear magnetic resonance spectroscopy, and gas chromatography–mass spectrometry. The results revealed the presence of hydroxyl (–OH), methyl ($-CH_3$), methine (=CH–), methylene ($-CH_2-$) and ester carbonyl (>C=O) groups, which confirmed the PHB structure. Thus, alkali-pretreated SMS-SB plays a significant role as an energy substrate for the production of PHB. This gives the knowledge to utilize cost-effective lignocellulosic agro-waste materials as a feedstock for the sustainable production of biodegradable PHB for many biomedical applications.

Graphic Abstract



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