Plants as bio-resource for PHB-producing bacteria

***Ilona Gasser*, Henry Müller, Gabriele Berg**

Institute of Environmental Biotechnology, Petersgasse 12, 8010 Graz, Austria

Introduction

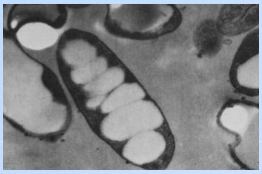


Fig.1: Accumulation of PHA granules in Rhodobacter sphaeroides (thanks to EPOBIO)

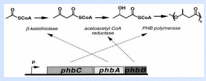
Biopolymers are an alternative to petroleum-based polymers with a wide range of environmental advantages. Bacteria are able to produce Polyhydroxybutyrate (PHB) as a storage substance, which has properties similar to those of Polypropylen (PP). Therefore it is important to find such PHB-producers which cope with industrial demands. Among terrestrial ecosystems, the soil layer influenced by plant roots, the so called rhizosphere, with its high microbial activity is expected to be a good habitat for PHB-producers. Indigenous microorganisms must be adapted to changing conditions of their environment and fluctuations in the concentration of nutrients exuded by plant roots. Inclusion of storage substances are a competitive advantage. Here we studied the occurrence of PHB-producing bacteria associated with different plants at the genetic and phenotypic level.

Methods

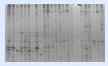
Cultivation-independent

Oilseed rape and olive

Total DNA extraction from the rhizo- and endosphere



Amplification of PHB synthesis gene (*phbC*) by polymerase chain reaction (PCR) from total community DNA



Seperation of amplified DNA fragments by Single Strand Conformation Polymorphisms (SSCP) analysis

Fig. 2: Cultivation-independent approach to analyse the presence of PHB synthesis genes in the rhizosphere of oilseed rape and olive

Cultivation-dependent

Sugarbeet, oilseed rape and wheat

Amplification of PHB synthesis gene (*phbC*) by polymerase chain reaction from single isolates

Isolation and cultivation of bacteria from rhizo- and endosphere of sugar beet, oilseed rape and wheat

Microscopic analysis

Fig. 3: Screening for PHB-producing bacteria by both molecular and microscopic approaches

Results

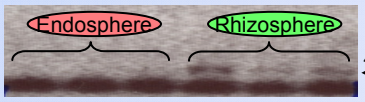


Fig.4: PCR amplification of PHB synthesis gene phbC from total DNA of the rhizo- and endosphere of olive

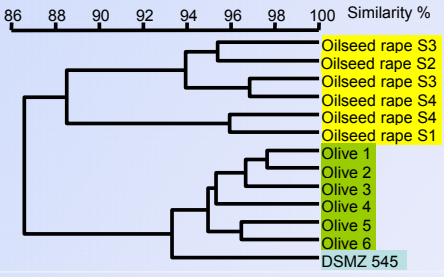


Fig. 5: Dendrogram *generated from SSCP patterns of PHB synthesis genes phbC in the rhizosphere of oilseed rape and olive after calculation of band-based DICE similarity coefficient. DSMZ 545 represent phbC amplicon from the reference strain Cupriavidus necator .*

The gene *phbC* could be amplified only from the DNA extract of the rhizosphere but not from the DNA of the endosphere, suggesting that bacteria which are able to synthesise PHBs predominantly colonise the rhizosphere (Fig. 4). SSCP profiles of PHB synthesis gene *phbC* of the bacterial community in the rhizosphere of oilseed rape and olive form distinct cluster which indicates that the investigated plants harbour a specific group of PHB producers.

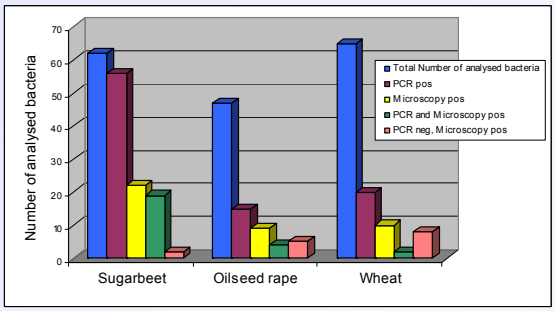


Fig. 6: Results from the screening of bacteria isolated from the rhizosphere of sugar beet, oilseed rape and wheat on their capacity to form PHBs by using PCR and microscopic methods.

Comparing PCR results, sugarbeet were found to be associated with a higher number (90%) of PHB producing bacteria than oilseed rape and wheat. Overall out of 174 analysed bacteria 41 isolates form PHBs in vitro. 25 isolates present a correlation between intracellular granules and a positive PCR signal. However, 66 isolates carry the *phbC* gene but show no visible PHB accumulation during cultivation, indicating a discrepance between results from cultivation and the genetic potential to synthesise PHBs.

Conclusions

By applying both cultivation-independent as well as cultivation-dependent methods the results clearly demonstrate that plants are colonised by numerous bacteria which are potentially able to accumulate polyhydroxybutyrates as energy and carbon source. Particularly the rhizosphere, which is in contrast to the endosphere characterised by temporal and spatial changes in nutrient availability, appears to be a good source for the isolation of PHB producers for biotechnological applications.
For the screening procedure two techniques were applied. On the one hand, the cultivation of the bacterial isolates under nutrient limitation resulted in the selection of few PHB positive strains. In contrast, using PCR analysis a higher number of investigated bacteria were shown to carry genes for enzymes involved in PHB biosynthesis. For a comprehensive study of the population of PHB producing bacteria in any habitat a combined strategy was approved.