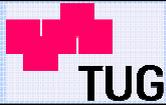


Molecular and microscopic analyses of PHB-producing bacteria associated with plants



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Introduction

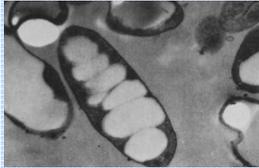
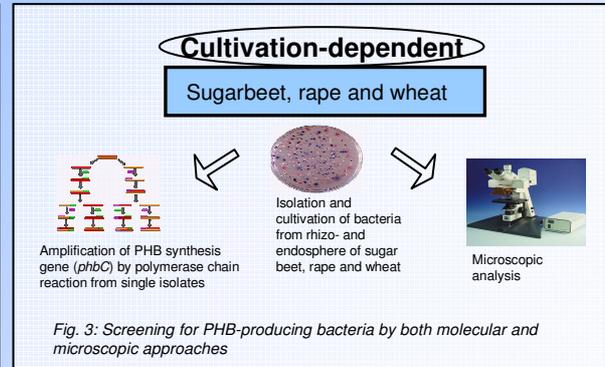
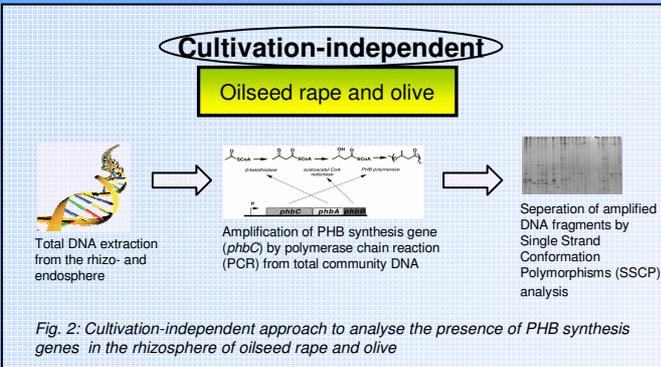


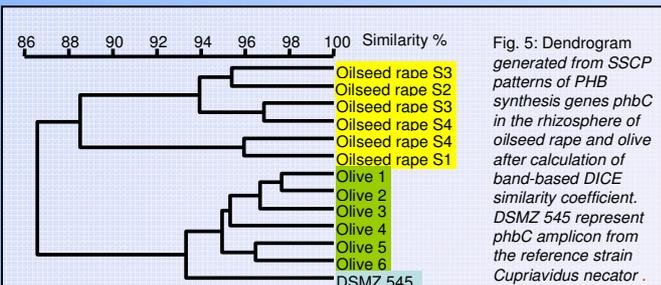
Fig. 1: Accumulation of PHA granules in *Rhodospirillum rubrum* (thanks to EPOBIO)

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



Results



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.

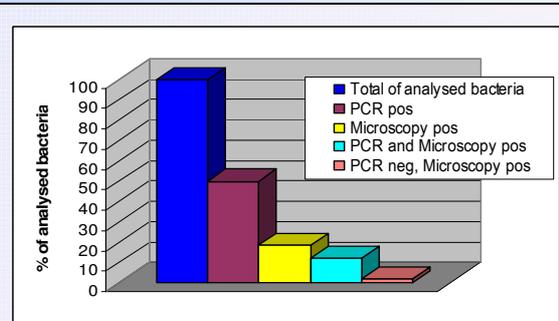


Fig. 6: Results from the screening of bacteria isolated from the rhizo- and endosphere of sugar beet, rape and wheat on their capacity to form PHBs by using PCR and microscopic methods.

Out of 308 analysed bacteria 57 isolates were shown to form PHBs in vitro. For 37 isolates a correlation between intracellular granules and a positive PCR signal was demonstrated. However, 154 isolates carry the *phbC* gene but show no visible PHB accumulation during cultivation, indicating a discrepancy 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.