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Metabolic engineering of a high biomass C4 grass for commercial scale production of bioplastics

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Sugarcane is an important high biomass crop grown throughout the tropical and subtropical regions of the world. In Brazil, sugarcane has a central role in not only the production of sucrose but also in the production of biofuels and bio-based chemicals. In the future, these bio-based chemicals may be derived not only from biorefineries but also from genetically engineered sugarcane itself. We have been metabolically engineering sugarcane to function as a biofactory producing bioplastics and bioplastic precursors. Sugarcane was metabolically engineered to produce polyhydroxybutyrate (PHB), a member of the class of biopolymers known as polyhydroxyalkanoates (PHA). Most of our work has focused on PHB using a three enzyme pathway from a soil bacterium (*Ralstoniaeutropha*) [β-ketothiolase (PhaA), acetoacetyl-reductase (PhaB) and PHB synthase (PhaC)]. Transgenes were expressed in the nucleus of sugarcane and the enzymes were targeted to plastids. In plastids, using a maize polyubiquitin promoter, PHB granules were detected in all cell types in sugarcane stalks and leaves, however, the highest accumulation was in bundle sheath cells. A number of strategies were used to increase the levels of PHA accumulation in sugarcane leaf tissue. An early detection system was developed using Nile Blue A staining and fluorescent microscopy showing that PHA could be detected in leaf tissue from tissue culture plantlets regenerated from the transgenic callus. When a stronger promoter was used (maize chlorophyll A/B binding protein promoter) PHA accumulation was significantly higher in leaf tissue of sugarcane. Knocking out competition for acetyl-CoA in sugarcane resulted in a 50% increase in PHB levels in leaves. Replacing PhaA with a novel enzyme resulted in another 4 fold increase in PHA levels resulting in leaves producing commercial levels of PHA. In addition to plastid production, PHB and PHB/PHA copolymers were engineered into sugarcane peroxisomes by targeting the PhaA, PhaB, and either PhaC or the *Pseudomonas* aeruginosaPhaC1 to peroxisomes. We found both PHB and PHB/PHA copolymer granules localized in peroxisomes and vacuoles in sugarcane leaves. The biopolymers we are producing have very high molecular weight with the highest at close to two million daltons. The highest production level of PHA polymer recorded to date in sugarcane is 12% of the total leaf dry weight.

Biography

Stevens M Brumbley completed his PhD at the University of Georgia in 1992 and postdoctoral studies at the University of California Riverside in 1993. He worked in the Australian Sugarcane Industry from 1993 to 2006 and at the University of Queensland's Australian Institute for Bioengineering and Nanotechnology for 2006 to 2011. He is currently a tenured Associate Professor of Plant Metabolic Engineering at the University of North Texas. He has published more than 50 papers in high impact journals.

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