HVCfP PROOF OF CONCEPT FUNDED PROJECTS – ROUND TWO

A SYNTHETIC METABOLON FOR THE PRODUCTION OF HIGH VALUE CAROTENOID PIGMENTS

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Carotenoids are natural pigments used industrially as feed supplements, food colorants, over the counter medicines (OTC), and bioactives. Their industrial exploitation means they are chemicals of high value, with global markets over $1 billion per annum and, in some cases, are set to increase to $334 million by 2018 (BCC Research report FOD025C). Presently, chemical synthesis which uses petroleum derived precursors is the commercial method of choice for carotenoid production. Despite synthesis being expensive, difficult and yielding non-native stereo isomers no competitive plant, algal or microbial sources exist to date. Using a genetic intervention approach the applicants have created tomato varieties, which produce high levels of non-endogenous fruit carotenoids that are suitable for the industrial feed sector. Despite achieving high level production, under certain environmental conditions and upon scale-up, the accumulation of intermediates can occur. In order to increase efficient metabolite channelling an enzyme fusion ("metabolon") of the biosynthetic enzymes has been created. The function of these enzymes has been shown in bacteria. Our goal now is to assess its effectiveness *in planta*.

DEVELOPMENT OF NATURAL SWEETENERS FROM *STEVIA REBAUDIANA*

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Steviol glycosides (SGs) are natural, low- to zero-calorie sweeteners found exclusively in *Stevia rebaudiana*, a perennial herbaceous plant belonging to the Asteraceae family. Being native to Paraguay, the sweetening properties of the plant have been used by indigenous Indian tribes for centuries. In addition to their sweetness, SGs possess other beneficial characteristics such as heat-stability and high solubility in water that have allowed them to become quickly established in the sweetener market in recent years. Steviol glycoside-based sweeteners from *Stevia* are now available throughout the UK both as sugar substitutes in some beverages and as table-top sweeteners. About a dozen different steviol glycosides occur naturally in the *Stevia* plant. They differ in type and number of sugar moieties attached to the steviol backbone which also affect their sweetness and bitter aftertaste. Among the more abundant SGs in *Stevia* leaves, rebaudioside A possesses the highest sweetening potency and has the best palatable characteristics. The project aims to maximise the utility and value of SGs extracted from *Stevia* leaves by converting stevioside, another of the more abundant SG found in the leaves, to the sweeter and better tasting rebaudioside A using a biotransformation approach. Some SGs may be even sweeter and have even better palatable characteristics than rebaudioside A but their low abundance in *Stevia* leaves is currently preventing their commercial application. Therefore, the project also seeks to identify novel enzymatic activities capable of converting more abundant SGs to these desirable but low-abundance SGs, thus developing a pathway for their commercial application.