Biosynthesis of Monoterpene Indole Alkaloids in vitro†

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Introduction

The medicinal value of monoterpene indole alkaloids continues to attract interest in their phytochemistry, chemical structure, biosynthesis, and chemotherapeutic activity. Structural complexity and non-profitability of total synthesis, perceived or real shortages in the supply of botanicals, and recent concerns for the environmental impact of wild cropping, made production of these alkaloids in vitro, i.e. by plant cells cultured in bioreactors, a viable prospect. The synthesis and accumulation of monoterpene indole alkaloids in cell cultures received great attention when commercial production of compounds like quinine (1), ajmalicine (2) and catharanthine (6), vinblastine (8) and vincristine (9) appeared attainable. Technologies aimed at increasing alkaloid accumulation by employment of production media, by precursor feeding, elicitation, semi-continuous culture, and by application of enhanced bioreactors were designed to achieve this goal. At present, however, efforts to further promote cell cultures as medicinals have softened somewhat due to regulatory barriers, but also due to biological barriers. Vinblastine and vincristine, the most desirable Catharanthus alkaloids, did not occur in concentrations which would warrant commercial exploitation of an in vitro process. Research will be required to overcome these barriers. Research into the biosynthesis of monoterpene indole alkaloids, when supplemented with molecular biological approaches, appears to be the most sensitive approach.

Plant cell culture

The response of tissues excised from stems and leaves of Catharanthus roseus (L.) G. Don to in vitro culture has been most favorable: crown gall tissue, habituated, and regular callus tissue have been grown in vitro since 1945. Culture in bioreactors and occurrence of various alkaloids have been demonstrated as early as 1969 [5]. DeLuca and Kurz [11] reviewed efforts to produce alkaloids in Catharanthus cell cultures by one- and two-phase culture systems and produced a list of 30 plus alkaloids found. Single cell clones obtained with protoplasts derived from leaves showed extreme variation in alkaloid spectrum [7]. Cryopreservation allowed stability of such clones, potentially over several years

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Plant regeneration from cells [8], of importance for genetic engineering with this plant, may have gained in efficiency recently through embryogenesis in callus derived from anthers of specific germplasm, i.e. cv Little Delicata, using seeds from Takii & Comp., Tokyo [21].

**Biosynthesis and production**

Since the discovery of the hypoglycemic effects of *Catharanthus* alkaloids, but in particular since the demonstration of the antileukemic effect of vincaleucoblastine (VBL) in 1958, the structure of *Catharanthus* alkaloids has been under

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Fig 1. Schematic representation of the biosynthesis of monoterpenoid indole and quinoline alkaloids (L.H. Stevens, 1994).

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