Session D: Improvement of organisms for bioreactors and photo bioreactors

DPL 1: Novel plant cell systems, vis-à-vis cultivation methodologies for the production of valuable phytochemicals

R.A. Gokare

Dr. C.D. Sagar Center for Life Sciences, Dayananda Sagar Institutions, Bengaluru 560078, India,
e-mail: rgokare@yahoo.co.in (corresponding author)

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Abstract

The bioreactor technologies are gaining importance as means of production of metabolites, food, pharmaceuticals, enzymes, bio-molecules and specialty chemicals. Though these technologies have been extensively adopted, the use of genetically modified cells/organisms and the problems related to their exploitation due to environmental concerns have laid emphasis on the contained-cultivation methods. Furthermore, utilization of solar energy and renewable energy systems as drivers of photo bioreactor systems is emerging as novel green technology. In order to enhance the efficiencies of the above mentioned systems, there is need for a two- pronged approach: firstly the engineering the organisms commensurate with the scale up technologies in bioreactors, and also the innovations of the scale up technologies, themselves.

With respect to the high value metabolites from medicinal plant systems, the aspects of pathway engineering to produce high quantities of the active principles is gaining attention. The production of desired metabolites using cell cultures, hairy root cultures, and genetically modified (GM) cells are of importance in controlled processes. To achieve this, they need to be integrated with bioreactors, immobilized cell culture systems and novel organisms (including cultivated transgenic plants as bioreactors), coupled to downstream processing.

The author's group at the Central Food Technological Research Institute at Mysore and presently at DSI, Bengaluru, has been engaged in the development of cell culture mediated production of secondary metabolites, design of novel bioreactor, immobilized cell cultures, and algal production in outdoor bioreactors and design of cells / organism through genetic engineering. The studies on cell culture / immobilized cell culture / hairy root culture mediated production of capsaicin, anthocyanin, betalains, *Withania* alkaloids , coupling them with elicitor mediated technologies for the enhancement of production capability; large scale production of metabolites of importance through algal technologies; pathway engineering of metabolites such as capsaicin, astaxanthin, gamma linolenic acid (GLA) were pursued.

We attempted novel methods of enhancing the metabolites by 2-3 folds, using microbial elicitors. The aqueous extracts from organisms viz., *Aspergillus niger*, *Rhizopus oligosporus*, were very effective in eliciting capsaicin in immobilized cell cultures of *Capsicum*. Similar results were obtained in the elicitation of several other secondary metabolites such as anthocyanin in the in vitro tissues of *Daucus carota* or annatto dye in *Bixa orellana*.

The elicitor technology has now been adopted in several industrial processes to enhance the metabolites of importance such as Shikonin in *Lithospermum erythrorhizon* and many more. The release of the metabolites to the exterior through permeabilization using agents appropriate to the system will be an added advantage for using the cell cultures in bioreactors in a continuous mode. In case of capsaicin, the molecule is naturally effluxed from cultured immobilized cells. These unit operations coupled to downstream processing technologies have been responsible for the enhancement of efficiency of the production process for the target compound(s).

Development of hairy root cultures of *Beta vulgaris* using *Agrobacterium rhizogenes*, as an alternative to the cell culture mediated process, resulted in enhanced productivity of betalains. Our studies have shown the advantage of using this system in terms of the enhanced production through nutritional stress, elicitors and also use of permeating agent for the release of betalains to the exterior in a continuous production mode.

The pathway engineering of carotenoids, especially astaxanthin, in algal systems was done in our laboratory in order to clone the genes for Betacarotene ketolase (BKT) and Betacarotene hydroxylase. The BKT cloning from the green algal form *Haematococcus pluvialis* was successfully done and introduced into *Dunaliella* sps. This opened up the possibility of production of astaxanthin in *Dunaliella* which otherwise is a known producer of Beta carotene only. Such examples will be of relevance in producing the genetically modified strains in bioreactors for high value compounds of importance as exemplified by our studies on pathway engineering of astaxanthin. Similarly we have successfully demonstrated cloning of cloning of gene for Delta-6 desaturase from the cyanobacterium *Spirulina* and transformed Soybean plants to produce a vegetable oil enriched with GLA.

Capsaicinoids are the pungency causing alkaloids synthesized in placental tissues of *Capsicum* fruits. Capsinoids are the non-pungent analogues also found to be synthesized in placental tissues of *Capsicum* fruits. Capsaicinoids, and Capsinoids are unique to *Capsicum* sp. pungency is regulated by involvement of either of two genes viz Pun1 (Acyl-transferase involved in condensation of C9-C11 Fatty acids with vanillylamine) & pAMT (Aminotransferase involved in vanillylamine synthesis). We have purified and characterized the pAMT protein and are now exploring the synthesis of various pAMT catalyzed pharmaceutically important compounds. For the first time we have functionally validated the involvement of pAMT in regulation of vanillylamine through Agrobacterium mediated genetic transformation experiments. We were able to show the synthesis of Vanillylamine in *Nicotiana* sp. using a binary vector sense construct, thereby proving the function of pAMT in alternate species. pAMT mutant species like CH-19 sweet pepper (*Capsicum* sps) produce vanillyl alcohol instead of vanillylamine. Capsinoids are fatty acid esters (C9-C11 fatty acids linked with vanillyl alcohol) in pAMT mutant plants.

It has now been possible to use this information to overproduce capsinoids. Capsinoids are recently explored compounds with a promising potential of anti-obesity properties. Capsinoids also exhibit chemopreventive and anticancer properties. They lack nociceptive responses and are most promising among vanilloid receptor agonists, hence safer for therapeutic usage.

The developments mentioned above will be presented to provide an overview of our attempts to produce novel compounds of importance through engineered cell systems to produce desired metabolites through scale up processes.