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Issue 1, September 2000

www.proteinspotlight.org

Pretty pigments

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Had you ever wondered why strawberry ice creams are sometimes so pink? Food colouring is the answer. Natural pigments are widely used in the food industry and have been studied extensively. Long before the art of food colouring, however, there existed Nature's own art of plant colouring. And its palette is rich. Take betalains for example. Betalains are natural pigments synthesized exclusively in plants of the order *Caryophyllales* – where you find spinach and beetroot but also purslane, cacti and bougainvillea – and, surprisingly, in toadstools of the order *Amanita*.

Large-flowered purslane (*Portulaca grandiflora*) is one popular betalain-containing plant. The American botanist W.J.Hooker was the first to describe *Portulaca grandiflora* in 1829 after an excursion to the Rio Desaguardero in South America. He admired "the rich purple hue, here and there marked with spots of an orange colour, from the orange-coloured variety which grew intermixed with the other." His main interest however seemed to be the addition of the variety to his greenhouse….

DOPA-dioxygenase is the key enzyme involved in betalain biosynthesis. It converts 3,4-dihydroxyphenylalanine to betalamic acid, a yellow chromophore. Betalamic acid has then the choice of two fates. It can condense with amino acids or amines to form yellow betaxanthins (λmax 480nm), or condense with cyclo-DOPA derivatives to form violet betacyanins (λmax 536nm). Betaxanthins and betacyanins are the two subclasses of betalain pigment. They are synthesized in the cytoplasm and then filtered out and stocked in vacuoles. Their synthesis is highly regulated and depends on the plant's development and tissue.

There are a number of varieties of purslane which each express a specific amount of betacyanin and betaxanthin. Colours range from white (no pigmentation) to different shades of yellow, orange, red and violet. The palette is striking. And it was this fabulous range of colours that urged Japanese scientists – in the 1920s – to study the genetics of pigmentation with little knowledge of its chemical nature. Indeed, colours have been at the heart of many important discoveries. Modern genetics, for one, are based on Gregor Mendel's (1822-1884) understanding of the distribution of fruit and flower pigmentation!

Amanita muscaria, Beverly Hackett

Courtesy of the artist

Amanita is a betalain-containing fungus, which has met with much enthusiasm on behalf of researchers, let alone storytellers, artists and traditional medicine. *Amanita* is the familiar toadstool with a red and white-spotted cap, so frequently illustrated in children's books. There is a tradition on New Year's Eve in Germany, where 'Glückspilze' are offered. These are reproductions of *Amanita muscaria* in chocolate and marzipan, which are given as good luck presents. Much folklore is also associated with this toadstool and there are many reports of its use both in endemic medicine and religious ceremonies by nomadic tribes in Asia. Small quantities of *Amanita muscaria* are said to cause hallucinations, such as the distortion of size. Those who are acquainted with Lewis Carroll's 'Alice's adventures in Wonderland' will remember the caterpillar perched on a toadstool, smoking a hookah. Alice is no taller than the mushroom and is told that one side of it will make her taller, the other smaller… Besides these hallucinogenic sensations, *A.muscaria* has also been regarded as a fertility symbol probably because of its striking appearance when the fruiting bodies sprout from the Earth and unfold.

From a more biochemical point of view, *A.muscaria* synthesizes a second pigment besides betalain: muscaflavin which is a yellow pigment. Dr Ursula Hinz and her team at the Laboratory of cellular phytogenetics at the University of Lausanne (Switzerland) cloned DOPA dioxygenase from *Amanita muscaria* and expressed it in *Portulaca grandiflora*. They discovered that DOPA dioxygenase synthesized both betalain and muscaflavin. This led the researchers to the conclusion that the fungal enzyme is obviously not very specific. Indeed, the synthesis of betalain demands a 4,5-cleavage of DOPA whereas that of muscaflavin involves a 2,3-extradiol cleavage of DOPA. And in *Amanita*, DOPA dioxygenase performs both! What is more, this particular enzyme is a complete orphan: so far it resembles no other protein!

What are these pigments for? 'That is a difficult question', answers Ursula Hinz. 'Colours may be there simply to attract insects for pollination. However, in the case of spinach and beetroot, the flowers are green, not very visible and pollination is done by the wind.' What they are used for is easier to answer. Besides food colouring, the enzyme itself – DOPA dioxygenase – can be used as a marker gene in the transformation of betalain-containing plants. So far though, the use of betalain pigments has really only been commercial. In the past, betalains were added to wine to lend it a richer hue, and there remains little doubt that they will be used in the near future to create novel ornamental plants such as yellow geraniums or purple daffodils.

Cross-references to Swiss-Prot

DOPA 4,5-dioxygenase, *Amanita muscaris* (Fly agaric) : P87064

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