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The mechanism behind the Heart Shaped Herb's purple glow

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Abstract
This paper will investigate the mechanism behind the purple glow produced by the heart shaped herb seen in the Black Panther movie. Auto-luminescent plants *Nicotiana tabacum* (tobacco plant) have been produced facilitated by the horizontal gene transfer of the lux operon gene from bacteria. There are many bioluminescent marine species which live deep in the sea; these include anglerfish and *Watasenia scintillans*, also known as a firefly squid. Their luminescent properties are due to bioluminescent bacteria and photophores. This paper will investigate whether these bacteria can be used to produce the glow in the heart shaped herb.

Keywords: *Film; Biology; Bioluminescence; Heart Shaped Herb; Marvel; Black Panther*

Introduction
In the Black Panther movie there is a heart shaped herb which contains vibranium [1]. This plant is only grown in Wakanda and is believed to be a gift from the Panther God [2]. To become Black Panther the juice from the heart shaped herb is extracted and applied to their body which can provide people with enhanced abilities due to the vibranium within the plant [2]. The heart shape herb has a purple glow, and this paper will be investigating the potential for bioluminescent plants, and whether luminescent bacteria seen in deep sea animals could be applied to plants to produce luminescent plants such as the heart shaped herb.

Auto-luminescent plants
Plants and bacteria share similar DNA and due to these similarities parts of the DNA can be exchanged [3]. Some bacteria contains genes in there DNA which allow the bacteria to glow [3]. In a 2010, study they produced two independent lines of *Nicotiana tabacum,* a tobacco plant, containing lux operon genes from the bacteria *photobacterium leiognathi* [4]. The Lux operon encodes genes to produce luminescent proteins such as the enzyme luciferase [4]. Luciferase is an enzyme that catalyses light emitting reactions, and their substrate is a chemical called luciferin [3]. When luciferase and luciferin are exposed to each other a chemical reaction occurs, releasing energy as visible light [3]. This allows *photobacterium leiognathi* to glow [4]. Through horizontal gene transfer the lux operon gene from bacteria was inserted into the DNA of the plant sex cells [3]. The vectors plasmid contained lux operon and a code for antibiotic resistance as a checkpoint [3]. The lux operon was inserted into the sex cell of the plant as they would develop into seeds for new plants to grow [3].

Of the two lines of tobacco plants generated the first line was able to integrate the lux operon gene into the rps12/TrnV locus of the chloroplast genome; in the second line, the gene was inserted into the TrnI/TrnA locus of the chloroplast [4]. They tested the glow ability of these plants once fully developed by measuring the number of photons released by the plants [3]. The plants that had successfully expressed the 6 genes of the lux operon in the chloroplast generated autonomous light emission [3]. When placed in the dark the plants visibly glowed releasing visible light [3]. This could potentially be applied to the heart shaped herb in Black Panther, and the lux operon gene could be transferred from *photobacterium leiognathi* into the plants genome and used as a mechanism to cause its purple glow.

Angler fish bioluminescence
Anglerfish are organisms that live in complete darkness over 1,000 metres below the ocean's surface [5]. As the anglerfish live in darkness and are unable to produce their own bioluminescence, they form a symbiotic relationship with bacteria [5]. There

is not a great amount of knowledge on this relationship due to the difficulty in acquiring anglerfish to study. Due to these symbiotic relationships, they do not live in darkness, as female anglerfish have an esca which is a glowing lure on the top of their forehead containing bioluminescent bacterium [5].

From current research, it is known that the bacteria have lost majority of the genes required to live in water freely due to this symbiotic relationship, where it supplies the anglerfish with light and, in return, the anglerfish provides nutrients and amino acids to the microbe [5]. As the genes lost by the bacteria are associated with breaking down nutrients and producing amino acids [5]. These bioluminescent bacteria in angler fish genome are 50% reduced compared to freely swimming bacteria [5]. There symbiotic relationship differs as the bacteria is not permanently stuck with the host as the bacteria moves between the fish and the anglerfish [5]. Their Additional each species of anglerfish has its own varying bacterial symbiont. For an organism to gain a bacterial symbiont, it is either by vertical transmission, which is via direct transmission from organism, or by taking in the bacteria after encountering the bacteria in its environment [6]. This luminescent bacterium could be potentially assimilated by the heart shape herb if they encounter each other in the bacteria environment however, due to the lack of knowledge on the bacteria itself this is less likely to be the mechanism behind the herbs glow.

Photophores

Photophores are light producing organs that allow bioluminescence in fish species including *Watasenia scintillans:* a bioluminescent firefly squid in the deep sea [7]. The name stems from its flashing lights that resemble a firefly. Its bioluminescent properties come from photophores, which cause them to emit a deep blue light all over there body [7]. In general, these bioluminescent properties can either be due to photocytes, which are specialised cells that emit light or from symbiotic fluorescent bacteria cultured in photophores [8]. The latter is used in the case of firefly squid, as small photophores are found all over the squids' body whereas larger photophores are found at the tips of the tentacles and the outlining of their eyes [7]. The basis of the visible light emitted by the squid is a series of luciferin-luciferase reactions as mentioned prior. Therefore, fluorescent bacteria produced by Photophores could potentially be used as a mechanism to facilitate the production of bioluminescent properties in the heart shaped herb. The pigment would have to be changed as heart shaped herb glows purple.

Evolutionary advantages of bioluminescent plants

There can be many direct and indirect advantages of the heart shaped herb having these luminescent properties such as aiding in attracting pollinators as it can advertise the plant to insects [9]. This is important for reproduction to sow seeds and breed, which is vital for the ecosystem, also for mimicry as plants can resemble insects which leads the insects to believe they are mating, rather they can spread the flowers pollen [9, 10]. Deep sea animals use their bioluminescence on the under part of their body as this prevents them from being spotted by predators [9]. They are uniform with the light from the sky, and this is a form of camouflage which could possibly be applied to bioluminescent plants to protect them from predators [9]. It can also be used to protect prey from predators as the luminescent leaves could distract the predators. This has indirect advantages for the plant as the continuous visitation of the prey and predators would lead to more defecation which helps in increasing soil fertilisation [9].

Conclusion

To conclude, bioluminescent bacteria and photophores can be used to produce bioluminescent properties in many marine species. A symbiotic relationship is formed between the marine species and the fluorescent bacteria this is seen in anglerfish and *Watasenia scintillans.* Photophores facilitate the symbiont relationship between luminescent bacteria and firefly. The luminescent bacteria produced by photophores could be used to produce the glow in the hear shaped herb although it produces a blue light whilst the heart shaped herb glows purple. How to facilitate this transfer is not fully understood. The production of luminescent tobacco plants by horizontal gene transfer of lux operon encoded gene from *photobacterium leiognathid,* is the most feasible method to produce the glow seen in the heart shaped herb. They successful produced two lines of luminescent plants they visibly glowed in the dark due to luciferin-luciferase reaction. In terms of a glowing a purple pigment, this is yet to be seen therefore the possibility of producing luminescent heart shaped herb is possibly, however it may not glow purple

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