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Pigments and Pathogenesis

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Abstract

In recent years pigments have been identified in human nutrition to have a positive effect on human health and reduction to oxidative stress exposure. In the media it has become common wisdom that colourful food is naturally better to consume for humans and animals. Now recently it has been shown that pigments aid microbial species as well, and conversely these microbial pigments may result in more morbidity and mortality for the human host infected by these colourful microbes. Similar pigments that are available for consumption in food are also present in many bacterial species. Presumably these pigments aid the bacteria in their survival in the environment and within a human or animal host. Importantly, interference with the production of certain microbial pigments results in some bacterial strains that are more susceptible to environmental stressors and the host immune system. These studies seem to indicate a role of pigments for in vivo survival by microbial species.

Keywords: Bacterial pathogenesis, Carotenoid, Mycobacteria, Pigment

Pigments have evolved in nature to serve a variety of purposes. In higher animals colour can signal sexual fitness as brightly coloured birds attest to [1]. But pigments can serve a more fundamental purpose that includes protection from oxidative stress, absorption of light in plants and the retina in animals, and protection from harmful UV light [1]. The appearance to have colour may be incidental to the chemistry of these pigments which contain varying amounts of conjugated double bonds [1]. These double bonds serve to absorb light which leads to the phenomenon of colour. In addition, conjugated double bonds are efficient antioxidants, reducing oxidative stress inside cells that can result from UV light exposure or other stresses [2]. For bacteria where there are no visualization systems, pigments functioning in other capacities is only logical [1].

Carotenoids may have evolved to reinforce bacterial membranes. Conjugated double bonds provide rigidity, that when inserted into a membrane serve to stabilize it. Carotenoids seem to have evolved within archaebacteria serving mechanical functions [1]. In addition carotenoids present within the ecto-skeleton of crustaceans and similar animals serve to protect and reinforce the various proteins present within these structures. Carotenoids complexed with proteins in certain ova can also prevent protein digestion from native proteases [1].

In pathogenic bacteria pigments have been implicated in bacterial survival and virulence. The gram positive pathogen *Staphylococcus aureus* is named such because it constitutively produces a golden yellow pigment [3]. When genes encoding synthetic enzymes responsible for the production of staphyloxanthin are deleted, the bacterium appears colourless. More significantly these mutant bacteria are more susceptible to killing via host immune cells, presumably due to less resistance to reactive oxygen species produced within the phagosomes of these cells [4]. In addition these mutant strains are much less virulent in a mouse model of skin abscess or during disseminated infections. In a study where the genes responsible for carotenoid production were transferred to a group *A streptococcus*, this new recombinant streptococcal strain was both pigmented and produced enlarged skin lesions in a mouse model of infection [5]. A cholesterol synthesis inhibitor was revealed to inhibit carotenoid synthesis in *S. aureus* when placed in the growth media of the microorganism [6]. The bacteria grown in the presence of the cholesterol synthesis inhibitor also had increased sensitivity to immune cell killing and reactive oxygen species [6].

While some species of bacteria produce pigment constitutively, others produce pigment in response to environmental stresses. Synechococcus species accumulate carotenoids in response to low iron stress [7]. Methanol exposure can induce pigment production in *Acinetobacter wofii* [8]. Oxidative stress may possibly be increased due to methanol exposure and carotenoids may decrease the effects of this stress. Vibrio cholera makes melanin under hyperosmotic stress as well as acidity [9,10]. This response of increased melanin may aid survival in aquatic environments as well as within the upper gastrointestinal tracts of infected humans. In addition, pigments have been shown to combat mutagenesis and act as scavengers of reactive oxygen species thus protecting DNA [11]. Norbixin is a carotenoid from seeds of a tropical shrub from Brazil. This pigment supplied to *Escherichia coli* protects these microbes from damage due to UV light exposure as well as reactive oxygen species [2].

The genus *Mycobacterium* includes many bacteria that produce pigments [12-14]. The Runyon classification system groups mycobacteria based on growth rate and pigment production [15]. Carotenoid pigment production in mycobacteria was previously thought to only be produced constitutively or in response to UV light exposure [16]. Interestingly, recently it has been shown that mycobacteria also produce pigment in response to acidic stress at pHs 5.0-6.0 [17]. The pigment has a similar absorption profile to other carotenoids and *Mycobacterium smegmatis* carotenoid production genes were upregulated by acidic stress at pH 5.5 and 6.0 [17]. This

pigment production may protect mycobacteria from acidic stress or concurrent oxidative stress found in the environment or in the host.

Some species of mycobacteria produce pigment constitutively and are designated as scotochromogens [18]. Other mycobacteria such as *M. smegmatis* and *Mycobacterium goodii* produce pigment only after extended growth on agar plates of 7-10 days [15,19]. These species are considered to be late pigment producers. Recently both these species have been shown to produce pigment at 3 days growth on agar plates at pH 5.5 and 6.0 but not 7.0 (Figure 1). Perhaps the late formation of the pigment is actually in response to an increase in acidity in the agar media due to extended incubation and metabolism by the mycobacterial cells [17]. As the mycobacteria metabolize sugars present within the media, they also produce excess protons which cause the media to acidify. Other rapid growing mycobacteria were also shown to produce pigment in response to acidic pH at 5.5-6.0, including *Mycobacterium abscessus*, *Mycobacterium fortuitum* and *Mycobacterium chelonae* [17]. *M. chelonae* and *M. abscessus* were also exposed to pH 5.0, however only *M. abscessus* continued to produce the pigment and *M. chelonae* ceased production of the pigment possibly due to the toxic effects of the more extreme pH on pigment production [17]. Several slow growing species such as *Mycobacterium avium* intracellulare and *Mycobacterium avium* also produce pigment in response to acidic stress at pH 5.5-6.0 [17,20, 21]. As these environmental mycobacterial species may encounter acidity in stagnant water in bogs and swamps, rivers, streams, brooks, and certain soils, pigment production may protect mycobacteria against environmental stress. Pigment production may aid in the resistance to acidity and help these mycobacteria survive.

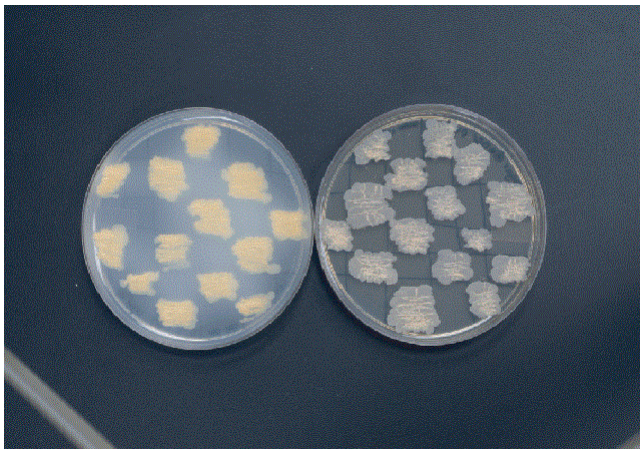

Figure 1: *M. smegmatis* were patched onto 7H10 agar media containing ADC (albumen dextrose, and sodium chloride) at pH 5.5 (left) or pH 7.0 (right) and were incubated for 72 hours at 37°C. The bacteria on the left plate appear to be contain a bold o yellow colour

The promoter driving expression of carotenoid synthesis genes has regions that respond transcriptionally to exposure to light in *Mycobacterium marinum* [22,23]. In *M. smegmatis*, exposure to acidity transcriptionally upregulates the homologue of this promoter region [17]. In addition, at least for *M. smegmatis*, pigment production is thought to be controlled by the sigma factor SigF [24,25]. In *M. smegmatis* a deletion in the sigF gene resulted in a bacterium which not only cannot produce pigments, but a mutant mycobacterium sensitive to oxidative stress as well [24,26]. Likely the presence or absence of carotenoid compounds dictates resistance to oxidative stress. In addition, lack of sigF increases transformation efficiency indicating the cell envelope has become potentially more porous [24]. Possibly carotenoid compounds lead to stabilization of the cell envelope, as well as making this structure less traversable. With the lack of stabilizing carotenoids, DNA may be able to travel through the cell wall/membrane more easily. SigF may also regulate other cell wall/membrane components that are altered within the mutant mycobacterium, and it remains to be determined what contribution carotenoid compounds make to cell wall/membrane stability. In *Mycobacterium tuberculosis* sigF is thought to control genes that aid in persistence and survival in late stage disease and a deletion in the sigF gene results in decreased mycobacterial burden *in vivo* [27].

Many mycobacteria including *M. tuberculosis* inhabit the phagosomes of macrophages [28-30]. When rapid and slow growing environmental mycobacteria enter the human body they are phagocytosed by macrophages where the mycobacteria often reside within the phagosomes. Resistant individuals may quickly eliminate these mycobacteria via many antibacterial mechanisms which include an increase in acidity to pH 5.0 and possibly rebounding to pH 6.0-6.5 [30]. In addition reactive oxygen intermediates are produced that also can damage these mycobacteria. In susceptible individuals these antibacterial mechanisms may be weakened and resistance mechanisms possessed by the environmental mycobacteria may allow for the successful replication of the pathogens in vivo. Pre-priming via environmental acidity to induce carotenoid compound induction may increase resistance to acidity in vivo, as well as resistance to oxidative stress. In fact, recently when carotenoid production was inhibited in *M. smegmatis* by a sigF disruption, it did increase sensitivity of these mycobacteria to oxidative stress [24,26]. Carotenoids are well known to detoxify oxidative stress due to their double bonds [1]. In addition carotenoids may stabilize the cellular membrane/cell wall of mycobacteria to decrease penetration of all types of environmental stresses including acidity and reactive oxygen intermediates [1].

Previously *Mycobacterium tuberculosis* was found to produce a pigment in response to para-aminobenzoic acid (PABA), however this was thought to be a metabolite of PABA [31]. Another pigment was also observed in response to para-aminosalicylic acid exposure [32]. In addition, *M. tuberculosis* seems to produce a non-carotenoid pigment that is cell wall associated, and this is produced due to long term growth in anaerobic culture [33]. These pigments may increase survivability under stressful conditions of low oxygen by serving to stabilize the outer membrane and the cell wall. It remains to be seen if *M. tuberculosis* also produces carotenoid pigments in response to acidic stress.

Pigment production in bacterial pathogens may increase their virulence. These pigments have been shown to increase resistance to oxidative stress, killing by immune cells, and mutagenesis. Pigments seem to increase virulence of pathogens by increasing invasiveness, survival in immune cells, and size of local abscesses. In fact in *M. marinum* pigmentation is linked to a locus important in intracellular survival [22]. Thus the finding that pigments in environmental mycobacteria are produced under conditions of acidity which can be found in the phagosomes of macrophages or the centers of caseating granulomas indicate that pigments may be important for pathogenesis [17]. However it is well known that mycobacteria are inherently resistant to a variety of environmental insults and stressors due a thick and waxy cell wall and possibly other genetic factors. Pigments may

augment this resistance in certain circumstances possibly to increase survivability in the face of microbe-external insults. In the future we may be able to inhibit environmental mycobacterial growth in vivo by inhibiting pigment production such as has been shown for *S. aureus* [6].

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