Malassezia globosa and *restricta*: Breakthrough Understanding of the Etiology and Treatment of Dandruff and Seborrheic Dermatitis through Whole-Genome Analysis

Thomas L. Dawson Jr[1]

Dandruff and seborrheic dermatitis (D/SD) share an etiology dependent upon three factors: sebum, microbial metabolism (specifically, *Malassezia* yeasts), and individual susceptibility. Advances in microbiological and analytical techniques permit a more detailed understanding of these etiologic factors, especially the role of *Malassezia*. *Malassezia* are lipid-dependent and demonstrate adaptation allowing them to exploit a narrow niche on sebum-rich skin. Work in our and our collaborators' laboratories has focused on understanding these adaptations by detailed analysis of biochemistry and gene expression. We have shown that *Malassezia globosa* and *M. restricta* predominate on dandruff scalp, that oleic acid alone can initiate dandruff-like desquamation, that *M. globosa* is the most likely initiating organism by virtue of its high lipase activity, and that an *M. globosa* lipase is expressed on human scalp. Considering the importance of *M. globosa* in D/SD (and the overall importance of commensal fungi), we have sequenced the *M. globosa* and *M. restricta* genomes. Genomic analysis indicates key adaptations to the skin environment, several of which yield important clues to the role *Malassezia* play in human disease. This work offers the promise of defining new treatments to D/SD that are targeted at changing the level or activities of *Malassezia* genes.

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INTRODUCTION

Dandruff and seborrheic dermatitis (D/SD) are common abnormal skin conditions characterized by flaking and itch. In dandruff, the flakes are loosely adherent, oily, generally not associated with overt inflammation, and restricted to the scalp. In seborrheic dermatitis, the flakes are greasy and yellowish, and inflammation is observed. In SD, the most common affected sites are the scalp, nasolabial folds, ears, eyebrows, and chest. Although the conditions differ in some respects, they appear to represent a continuum of symptoms with a common etiology (Pierard Franchimont *et al.*, 2000; Gupta *et al.*, 2003). More than 50% of adults may be affected by these conditions and their socioeconomic impact is very high. For seborrheic dermatitis alone, the health care direct, indirect, and intangible costs exceeded $1.4 billon in the United States in 2004 (Bickers *et al.*, 2006). Despite the impact of these conditions, their etiology is poorly understood.

It is clear that D/SD are more than superficial disorders of the stratum corneum. Instead, the epidermis is substantially altered, with hyperproliferation, excess intercellular and intracellular lipids, interdigitation of the corneal envelope, and parakeratosis (McOsker and Hannon, 1967; Warner *et al.*, 2001). In previous work, we have shown that these abnormalities are seen throughout the scalp of affected individuals, not just in areas of flaking, and are improved by treatment with anti-fungal agents, including pyrithione zinc shampoo. Recent technical advances, including improved microbial and analytical techniques (Gemmer *et al.*, 2002; Batra *et al.*, 2005), have provided new insights into the underlying pathology. Based upon the most recent evidence, the etiology of D/SD appears to be dependent upon three factors: sebaceous gland secretions, microfloral metabolism, and individual susceptibility (DeAngelis *et al.*, 2005; Ro and Dawson, 2005). This paper will describe recent advances in the understanding of these factors, especially the role of the yeast *Malassezia.* These advances may provide new avenues to effective therapies.

DISCUSSION

Role of sebaceous gland activity

The role of sebaceous gland activity in D/SD etiology is suggested by the observation that common scalp flaking

[1]*The Procter & Gamble Company, Cincinnati, Ohio, USA*

Correspondence: Dr Thomas L. Dawson Jr, The Procter & Gamble Company, 11810 East Miami River Road, Cincinnati, Ohio 45252, USA. E-mail: dawson.tl@pg.com

Abbreviation: D/SD, dandruff and seborrheic dermatitis

conditions have a strong temporal correlation with sebaceous gland activity. This temporal correlation includes increased incidence during infancy (cradle cap), low incidence after infancy until puberty, increase in adolescence and young adulthood, and a decrease later in life (Ramasastry *et al*., 1970; Cotterill *et al*., 1972; Wheatley, 1986; Dawber, 1997). In addition, D/SD occur exclusively on skin in areas with high levels of sebum.

The function of human sebum has been controversial, but recent advances in analytical technology have made some progress possible. Sebum is involved in epidermal development and barrier maintenance (Pilgram *et al*., 2001), transporting antioxidants (Theile *et al*., 1999), protection, body odor, and generation of pheromones (Kligman, 1963). It has also recently become understood that sebum is directly involved in hormonal signaling, epidermal differentiation, and protection from UV (Thiboutot *et al*., 2003; Zouboulis, 2003).

Human sebum is a complex mixture of triglycerides, fatty acids, wax esters, sterol esters, cholesterol, cholesterol esters, and squalene (Figure 1) (Stewart *et al*., 1978; Strauss *et al*., 1983; Wertz and Michmiak, 2000; Ro and Dawson, 2005). When secreted, sebum consists of triglycerides and esters, which are broken down by microbes into diglycerides, monoglycerides, and free fatty acids. The free fatty acids play a key role in initiation of the irritant response at the base of D/SD. The role of sebaceous secretion also underlies the impact of stress and hormones on D/SD. It is well known that these are affecters of human sebum secretion and therefore impact D/SD (Cotterill *et al*., 1973; Downing *et al*., 1986; Saint-Léger, 2003).

Role of Malassezia. Although they are members of the normal skin flora, yeasts of the genus *Malassezia* have been known for many years to play a role in human skin diseases, including dandruff, seborrheic dermatitis, pityriasis versicolor, and *Malassezia* folliculitis, and they may play a role in exacerbation of atopic dermatitis and psoriasis (Gupta *et al*., 2004a; Batra *et al*., 2005). The importance of fungal species in development of D/SD is supported by the fact that effective treatments include a wide variety of agents whose only common property is their anti-fungal activity. Further, the improvement in flaking following treatment is highly correlated with the reduction in the level of scalp *Malassezia* (Schwartz *et al*., 2004). The study of this genus has been complicated by their fastidious culture requirements and a complex series of changes in nomenclature (Batra *et al*., 2005).

Although the genus has also been called *Pityrosporum*, that name is no longer preferred. At one time, the members of *Malassezia* were classified into two species: a lipid-dependent species *Malassezia furfur*, and a non-lipid-dependent species, *M. pachydermatis*. More recently, it has been recognized that there are multiple different lipid-dependent species (including *M. globosa*, *M. restricta*, *M. furfur*, *M. obtusa*, *M. slooffiae*, *M. sympodialis*, *M. japonica*, *M. nana*, *M. dermatis*, and *M. yamatoensis*), in addition to the non-lipid-dependent, primarily zoophilic species, *M. pachydermatis* (Batra *et al*., 2005). Use of molecular markers is generally required to correctly differentiate between the various lipid-dependent species (Guého *et al*., 1996; Ashbee and Evans, 2002; Sugita *et al*., 2003, 2005; Gupta *et al*., 2004b). Using an advanced molecular technique, terminal fragment length polymorphism, we previously identified *M. globosa* and *M. restricta* as the predominant species present on the scalp of D/SD sufferers (Gemmer *et al*., 2002). The *Malassezia* yeasts are most common on sebum-rich areas of the body and degrade sebum. Specifically, the organisms contain lipases that hydrolyze triglycerides, freeing specific saturated fatty acids that the yeast requires to proliferate. To demonstrate that *Malassezia* generated free fatty acids can induce dandruff-like flaking in humans, we applied a

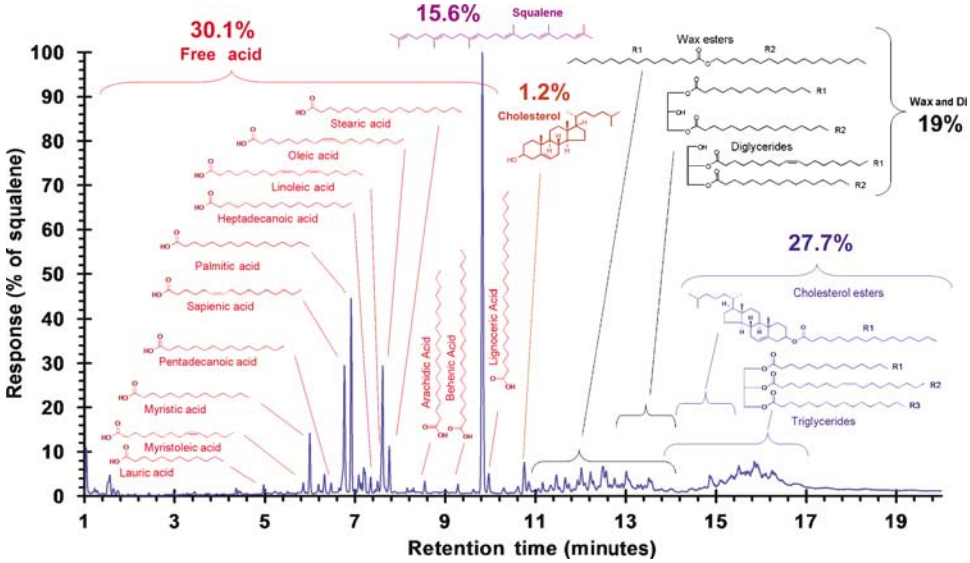

Figure 1. Relative composition of human sebum (reprinted from Ro and Dawson, 2005).

marker fatty acid, oleic, to human scalp. Even when *Malassezia* had been removed from the scalp oleic acid was able to elicit a flaking response in dandruff susceptible individuals (Ro and Dawson, 2005).

Role of individual susceptibility

We have shown that a fatty acid metabolite of *Malassezia*, oleic acid, induces flaking in dandruff-susceptible patients, but not in non-susceptible patients (Ro and Dawson, 2005). This finding provides evidence of role of these fatty acid metabolites in dandruff development and suggests an underlying difference between individuals that predisposes some to the development of dandruff or seborrheic dermatitis. Additionally, immunodeficiency such as acquired immune deficiency syndrome allows excess *Malassezia* proliferation, resulting in severe D/SD. Physical factors, nutritional disorders, drugs, and neurotransmitter abnormalities are additional aggravating factors. The difference between dandruff-susceptible and non-susceptible individuals remains unclear. Multiple possibilities exist, including innate differences in stratum corneum barrier function, skin permeability, and immune response to free fatty acids or proteins and polysaccharides from *Malassezia*. Further work will be necessary to fully understand the susceptibility response.

Initial forays into understanding lipase activity. Lipases play a key role in the lifestyle of *Malassezia* species on skin (Figure 2). In order to better understand this role, we isolated a lipase from *M. globosa* (DeAngelis *et al.*, in press). This protein was sequenced and the corresponding lipase gene (*LIP1*) was cloned and sequenced. This work was a first step toward a molecular description of lipid metabolism on the scalp and a more complete understanding of the role of microbial metabolism in the etiology of D/SD. Based on the limited activity of LIP1, we thought it likely that additional lipases were present in *Malassezia*, and that further work would be necessary to delineate the complete metabolic pathway.

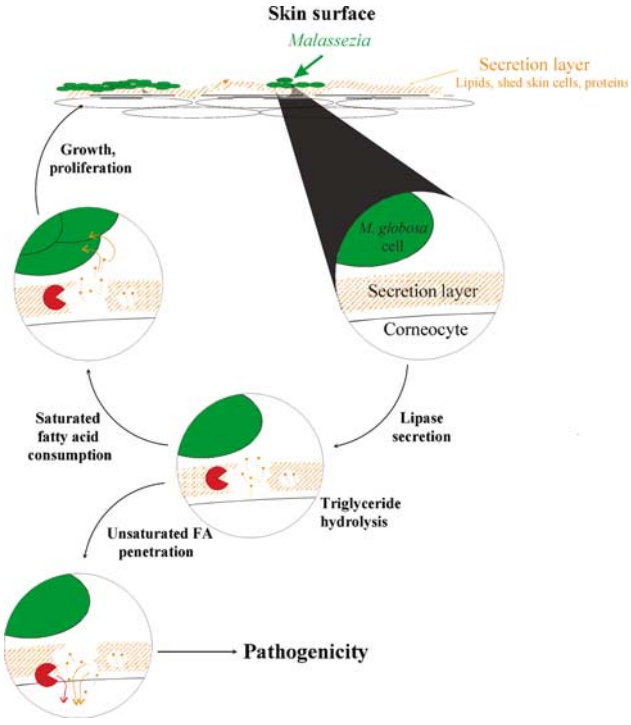

Figure 2. **A model of the role of *Malassezia* lipase-mediated hydrolysis of scalp lipids in the etiology of dandruff and seborrheic dermatitis.** Some fatty acids are consumed by the fungal cells, whereas other fatty acids cause scalp irritation (reprinted from DeAngelis *et al.*, 2007).

Sequencing of the Malassezia genomes. Increased understanding of the role of each of the three factors (sebaceous gland activity, microbial flora, and individual susceptibility) in D/SD offers the promise of new approaches to treatment. With this aim, we have been cooperating with an international team to further investigate the biochemistry of *Malassezia* species implicated in D/SD, including elucidation of the genomes of these organisms. Detailed understanding of the yeast's biochemical adaptations to its unique niche on sebum-rich skin may allow design of treatments specifically directed at altering the levels or action of *Malassezia* on affected skin.

The M. globosa genome. To understand *Malassezia* biology and elucidate the mechanism of their peculiar lipid dependence, we performed whole-genome sequencing of *M. globosa* and *M. restricta*. The *M. globosa* genome is 9 Mb, the smallest of any known free-living fungi (Dietrich *et al.*, 2004; Hermida *et al.*, 2005). To properly identify genes, the prediction of protein coding frames was improved by sequencing mRNA transcripts, allowing prediction of 4,289 protein-coding genes. Despite the small number of genes, the genome encodes the metabolic components for glycolysis, the tricarboxylic acid cycle, synthesis of all 20 canonical amino acids and the five nucleic acid bases, among others. The key deficiency is the absence of a fatty acid synthase, likely revealing why most *Malassezia* species are dependent on fatty acids for growth. In contrast to *M. globosa*, the available genomes of all other free-living fungi contain fatty acid synthases. The need for *Malassezia* to assimilate external fatty acids is also reflected in the number of multiple secreted lipases (13) and phospholipases (9). Reverse transcription-PCR and proteomics experiments confirmed the expression of multiple lipase and phospholipase genes on human scalp.

Of course, the enzymes would need to be extracellular to interact with host sources. We therefore performed proteomics experiments to identify over 50 secreted proteins. Some of the most abundant secreted proteins were, as hypothesized, lipases. In addition, many other secreted proteins were identified, including aspartyl proteases, members of the phospholipase C family, glucose-methanol-choline oxidoreductases, known *Malassezia* allergens (Chen and Hill, 2005), cell wall modifying enzymes, and unknown proteins. Because these proteins are secreted, they would be the most likely to interact with skin and would therefore mediate *Malassezia* pathogenicity and be relevant therapeutic targets.

Areas of future research. It will be necessary to conduct significantly more research into *Malassezia* biology and their interaction with human skin to understand the fundamentals of the interactions. The sequencing of these genomes, in conjunction with the already sequenced human genome, will allow detailed investigation of the metabolic interactions between human skin and *Malassezia*. As new pathways are elucidated, new intervention targets will arise. This new, groundbreaking research will enable development of new technologies to interrupt D/SD, which are not dependent on and can compliment existing anti-fungal treatments.

Summary. Work on the *Malassezia* genome and biochemistry provides insights into the mechanisms by which fungi adapt to the mammalian skin environment. These genomes will also provide new opportunities to dissect the specific interactions between ubiquitous mammalian commensal fungi and the skin. Deeper understanding of these interactions may well lead to new treatment paradigms and innovative ways to modify the effects of *Malassezia* species on human and animal health. Currently, anti-fungal treatments are the only effective means to control D/SD. Hopefully, new, more fundamental understanding of the interactions between *Malassezia* and human skin will enable development of new tools, which manage both the number and the activity of these unique fungi.

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