

## CHAPTER 2

### THE ANTIMICROBIAL ACTIVITY OF HONEY

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It is now widely accepted that honey has antimicrobial activity and that this is dependent upon a variety of different modes of action (Molan, 1992a). These are known to include osmolarity, acidity, limited availability of water molecules, peroxide generation and antimicrobial chemicals. This chapter outlines the evidence for these different actions and illustrates how, even after dilution (as occurs upon interaction with wound exudate), the antimicrobial activity remains (Molan, 1992a, b).

#### **Properties of honey that restrict microbial growth**

All micro-organisms require supplies of nutrients that provide sources of carbon, nitrogen, minerals and water. Any restriction in the supply or availability of each key nutrient will tend to compromise microbial metabolism.

Honey contains approximately 80% sugar by weight, which is comprised of four main sugar molecules (fructose, glucose, maltose and sucrose), but with many others in lower quantities. The acids present in honey also help to restrict microbial growth. Complex mixtures of acids, particularly gluconic acid, contribute to low acidity and low pH (between 3.4 and 6.1) (White, 1979). These characteristics alone make all honeys unsuitable to support the growth of micro-organisms and explain why honeys destined for human consumption rarely spoil during storage in the home.

Honey is a super-saturated solution of sugars, with low water content, and the binding of water molecules to sugars makes them unavailable for micro-organisms. The availability of free water is expressed as water activity ( $A_w$ ); pure water is  $A_w$  1.00 and blood 0.99. Most honeys possess an  $A_w$  of approximately 0.6, and many microbial species require  $A_w$  between 0.94 and 0.99 to grow. The most osmotolerant bacteria (ie. those which can survive in high sugar concentration) are staphylococci; the minimum concentration of sucrose required to prevent growth of these bacteria is 29% (v/v) (Molan 1992a), at an  $A_w$  of 0.86 (Chirife *et al*, 1982).

## Antimicrobial activity of honey

The first report of the antibacterial activity of honey has been attributed to a Dutch scientist, Van Ketel, in 1882 (Dustman, 1979). An indication that antimicrobial effects were not solely due to sugars came when Sackett (1919) showed that activity increased on dilution. Dold (1937) suggested the involvement of an antimicrobial substance termed 'inhibine', but the identity of the inhibine was not recognised until 1962, when Adcock demonstrated that catalase (an enzyme that degrades hydrogen peroxide) destroyed antibacterial activity. White *et al* (1963) deduced that inhibine was hydrogen peroxide, which was generated by the action of glucose oxidase (an enzyme transferred from the hypopharyngeal glands of bees during nectar collection and honey processing in the hive). Levels of hydrogen peroxide are at undetectable levels in undiluted honeys because the enzyme is not active, but on dilution the uninhibited enzyme converts glucose into gluconic acid in the presence of oxygen. The rate of hydrogen peroxide generation on dilution in different honeys is not constant, and maximum levels of hydrogen peroxide were generated in honeys that were diluted by a factor of 2 to 3 (Bang *et al*, 2003).

The potency of the antibacterial activity of honeys shows marked differences (James *et al*, 1972; Molan *et al*, 1988; Al-Jabri *et al*, 2003). In a survey of 345 New Zealand unpasturised honeys from largely single floral sources, antibacterial activity was investigated using a bioassay based on an agar well diffusion technique (Allen *et al*, 1991). The extent of inhibition of a test bacterium (*Staphylococcus aureus*) was compared to that achieved by dilutions of phenol. Phenol (as carbolic acid) was the first antiseptic used in aseptic surgery and is an accepted reference chemical in evaluating disinfectants and antiseptics in laboratory tests.

In the New Zealand honeys, potency ranged from the equivalent of <2% (w/v) phenol to 58%, with significant differences between floral sources. When the test was performed with catalase added to the honey solutions, antibacterial activity disappeared from many honey samples, but was retained in only two types of honey tested, Manuka (*Leptospermum scoparium*) and viper's bugloss. Therefore, honeys with detectable activity were distinguished by their ability to generate hydrogen peroxide in the test (peroxide honeys), or their ability to retain potency in the presence of catalase (non-peroxide honeys). Some Australian non-peroxide honeys are known, for example, jellybush honey (*Leptospermum polygalifolium*). Since honeys do not significantly vary in their total sugar content (White *et al*, 1962), the antibacterial activity demonstrated in the bioassay used by Allen *et al* (1991) was attributed to either hydrogen peroxide or factors other than hydrogen peroxide. An example of a bioassay is presented in Figure 2.1.

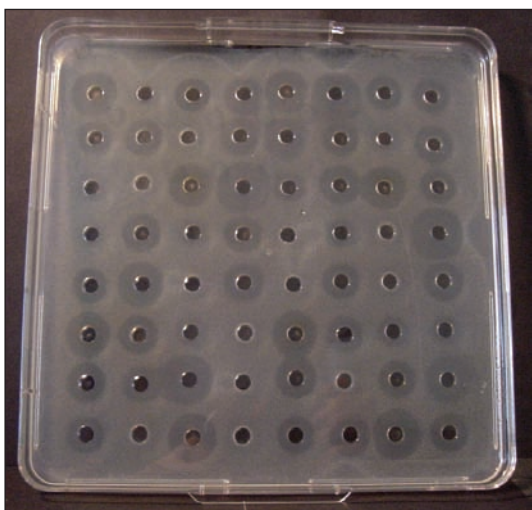


Figure 2.1: Bioassay to evaluate the antibacterial potency of honey. The clear zones show where bacteria that had been seeded into the agar have failed to grow. Wells contained diluted honey solutions or solutions of a range of concentrations of phenol

## The nature of the non-peroxide antimicrobial activity of honeys

Despite numerous investigations into the nature of the non-peroxide components that confer antimicrobial activity to honey, definitive

identifications and characterisations have not yet been published. The 'inhibine' mentioned earlier has been described as both a hydrogen peroxide activity and the effect of natural flavonoids and phenolic acids of plant origin (caffeic and ferulic acids) by Wahdan (1998).

The two readily identifiable sources of such components in honey are the bee and the floral source. A comparison of the antimicrobial activity of honey produced by a stingless bee and a honey bee in Costa Rica has suggested that plants, rather than bee species, influence potency (DeMera and Angert, 2004). However, Bogdanov (1997) suggested that both bee and plant influence activity. Phytochemicals, including the flavonoids, are secondary metabolites that confer colour, flavour and defence against infection to plants. Many possess antimicrobial potential and can be divided into several major groups (Cowan, 1999). The phenolic compounds and flavonoids have received the greatest attention, and these include some of the antioxidants known to be present in honeys. Separation of honey into four fractions showed Manuka to be exceptional in that most antibacterial activity was associated with the acidic fraction, whereas for other types of honey antibacterial activity was detected in all fractions (Bogdanov, 1997). Analysis of flavonoids in Swiss honeys indicated the presence of pinocembrin, which is an antimicrobial component characteristically found in another bee product, propolis or bee glue (Bogdanov, 1989). The phenolic components of ten Brazilian honeys have been extracted by HPLC and antibacterial activity evaluated. Compounds typical of Brazilian propolis were present in honeys, and geographical location was shown to influence chemical composition (Miorin *et al*, 2003). However, phenolic fractions of nineteen Manuka honeys neither varied with geographical location nor in their antibacterial potency (Weston *et al*, 2000). The antibacterial activity of high potency Manuka honey has been suggested to be associated with the carbohydrate fraction and not confined to the phenolic components (Weston *et al*, 1999).

## **The inhibition by honey of microbial species implicated in wound infections**

More than a century after Van Ketel's observation, a comprehensive review of the inhibitory properties of honey was provided by Molan (1992a, b). By collating most of the previously published studies on the microbial species

inhibited by honey, it could be seen that seventy-seven different species were inhibited by *in vitro* tests, but agreement about the susceptibilities of each species was absent. Comparisons between studies were impossible because varying techniques had been utilised, and descriptions of methods were incomplete or ambiguous; often neither the floral origin of the honey under investigation, nor detail of its processing was specified. Both heat and light can be shown to affect activity (Molan, 1992b), but these effects may not have been recognised in earlier studies.

Modern studies into the inhibition of wound pathogens by honey *in vitro* have confirmed its broad spectrum of activity, irrespective of whether honey was tested by being incorporated into broths (Willix *et al*, 1992; Karayil *et al*, 1998; Wahdan, 1998; Miorin *et al*, 2003), or agar plates (Nzeako and Hamdi, 2000; Subrahmanyam *et al*, 2001; Cooper *et al*, 2000; Cooper *et al*, 2002a, b; Blair, 2003). Using specified honeys of known potency, and comparing their antimicrobial activity to sugar solutions of similar osmolarity, some of these studies have confirmed that the activity of natural honeys is not solely attributable to their sugar content (Wahdan, 1998; Cooper *et al*, 2000; Cooper *et al*, 2002a, b; Blair, 2003). In many of these studies, *Leptospermum* honey, diluted by factors between 10 and 30, has inhibited test bacteria. It would not be expected that such low concentrations of honey would be used in wound care products, but the magnitude of the activity assures that dilution by wound exudates should not easily remove activity.

The mode of bacterial inhibition by honey has been seen to be bactericidal (killing), rather than bacteriostatic or growth-inhibiting (Wahdan, 1998; Cooper *et al* 2002a; Blair, 2003).

Antibiotic-resistant bacterial strains have been shown to be as susceptible to honeys as sensitive strains (Karayil *et al*, 1998; Cooper *et al*, 2000; Cooper *et al*, 2002a, b; Blair, 2003). Recent studies have, significantly, added methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci and *Burkholderia cepacia* (a pseudomonad-like bacterium associated with lung infection in cystic fibrosis with known resistance patterns) to the list of susceptible microbial species. Yeasts and fungi have also been reported to be susceptible to honey (Brady *et al*, 1996; Wahdan, 1998; Blair, personal communication).

At present, the mode of action of honey on bacterial cells has not yet been elucidated. An investigation into molecular effects using microarrays has indicated that the stress response is up-regulated in *Escherichia coli*, while some of the genes involved in protein synthesis are down-regulated (Blair, 2003). Morphological changes in bacteria exposed to Manuka honey (Cooper and Henriques, unpublished data), suggest that Gram

positive and Gram negative bacteria do not respond identically. Further studies into cellular and molecular events are on-going.

## **The relevance of antimicrobial activity of honey to wounds**

Polymicrobial communities are associated with wounds, but varying species may be found in different kinds of wounds (Bowler *et al*, 2001). The presence of micro-organisms in a wound may not necessarily be a cause for concern, since many do not give rise to infection. Routine application of antimicrobial agents to wounds can never be justified, because wounds do not have to be rendered sterile to heal successfully. There are complex interactions between the microbial inhabitants of wounds and human hosts that can result in differing outcomes, depending on microbial virulence and host immunocompetency. Infection, colonisation and contamination are three possible outcomes. Wound infection always interrupts the healing process and appropriate antimicrobial intervention is indicated. Guidelines for the selection of antibiotics in severe infections, and algorithms to manage hospital admissions are available (Eron *et al*, 2003). Nevertheless, rapid clearance of infection from honey-treated wounds that failed to respond to conventional therapies has been reported. The effectiveness of honey in limiting infection in burns patients was compared by Subrahmanyam (1998) to silver sulfadiazine. In this study, lower infection rates and faster healing was observed in those patients treated with honey.

Situations where topical agents are appropriate are not always immediately obvious; the reasons why wounds fail to heal are diverse and the involvement of micro-organisms is not yet entirely understood. The eradication from wounds of either  $\beta$ -haemolytic streptococci (Schraibman, 1990), or staphylococci and pseudomonads (Gilliland *et al*, 1988) before attempting skin grafting is accepted. The cases studies presented later in this book illustrate the clinical benefits of this activity.

In chronic wounds, the reduction of certain microbial species, such as anaerobic bacteria, has been advocated to limit undesirable odour (Bowler *et al*, 2001). Examples of clinical success where reduction of malodour was achieved have been reported (Dunford *et al*, 2000; Kingsley, 2001; and *Chapters 8 and 9* of this book).

MRSA-colonised wounds act as a potential reservoir of cross-infection,

as well as having the potential to develop into an infection. Successful eradication of MRSA using honey has been achieved in reports of two patients to date (Dunford *et al*, 2000; Natarajan *et al*, 2001).

## Conclusions

Already there is extensive laboratory evidence to demonstrate the antibacterial activity of honey, but to what degree this is relevant to the clinical situation can only be judged with its use. Certainly, reports of clinical studies tend to support the use of honey in reducing wound bioburden. Its broad spectrum of antimicrobial activity promises to make it an effective barrier to infection when applied to wounds, controlling ingress of environmental organisms, and, egress of wound pathogens. Many healthcare practitioners have been sceptical about the benefits of honey in managing wounds. It is encouraging to note the recent availability of CE marked products containing honey within the UK. More widespread use will ultimately provide the clinical evidence to prove or disprove previous claims.

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