Assessment of the *in-vitro* inhibitory activity of HiveAlive™ against *Paenibacillus larvae*, the causative agent of American Foulbrood infection in honeybees

Aleksandra Pavlenkova[1], James Comer[1], Linda Connor[2] ✉ & Dara Scott[2]

[1] *Department of Life & Physical Science, Galway-Mayo Institute of Technology, Galway, Ireland*
[2] *Research & Development Department, Advance Science Ltd., Galway, Ireland.*
✉ *info@advancescience.com*

Abstract

American Foulbrood (AFB) is caused by a Gram-positive, rod shaped, spore-forming bacterium called *Paenibacillus larvae*. Oxytetracycline is used to treat infections but strains of oxytetracycline resistant *P. larvae* have been identified in some countries. Hives and hive equipment are usually incinerated to prevent spread of the disease which is quite costly to the beekeeper. HiveAlive is a feed supplement for honeybees that demonstrates antibacterial activity. Here, different concentrations of HiveAlive were evaluated for their inhibitory potential using agar-dilution assays and 96-well microdilution assays. A concentration of 0.25% HiveAlive in agar was sufficient at inhibiting growth on solid media. In liquid media, growth appeared to be inhibited at 0.625%. Further tests are required to define the minimum inhibitory concentration and to assess if this inhibitory action can be replicated in larvae.

Introduction

AFB is caused by a Gram-positive, rod shaped, spore-forming bacterium called *Paenibacillus larvae*. Spores of this bacteria can be found all over a contaminated hive but they only germinate within honeybee larvae and demonstrate tolerance to heat and chemical stress. The matchstick test is used to assess presence in a hive whereby a matchstick is inserted into a suspected brood cell and drawn back out. Infected larvae will present as thready material as shown in Figure 1. Treatments available are limited, one of which is an antibiotic (oxytetracycline) to which resistance has been reported. In order to eradicate the infection and prevent spread, beekeepers must incinerate colonies and equipment used. Novel strategies of control of this microorganism are required.

Figure 1: Matchstick test for AFB. Image taken from Coffey 2016

Materials & Methods

Paenibacillus larvae (LMG 9820) was cultured on Brain Heart Infusion agar supplemented with thiamine hydrochloride at 28°C to obtain single colonies. A single colony was then added to liquid BHI media also supplemented with thiamine hydrochloride and incubated at 28°C in a rotary shaker at 195 rpm. This overnight culture was then used in a 96 well plate microdilution assay where varying concentrations of HiveAlive were added to media and growth monitored spectrophotometrically over a period of 72 hours. Assays were carried out in triplicate. Overnight culture was also inoculated onto BHI agar supplemented with varying concentrations of HiveAlive and incubated. Growth was assessed visually after 4 days of incubation.

Results & Discussion

A concentration of 0.625% of HiveAlive was shown to inhibit growth of *P. larvae* in 96 well plate assays (figure 2). At the end of the experiment, 100µl of culture in HiveAlive media was taken from wells and spread on to BHI agar plates (figure 3). No growth was evident at concentrations of 1.25% and above but was present at 0.625% and below. In the agar dilution assays, 0.25% of HiveAlive in BHI agar and above inhibited the growth of *P. larvae*. Growth was present on plates with a lower concentration of HiveAlive.

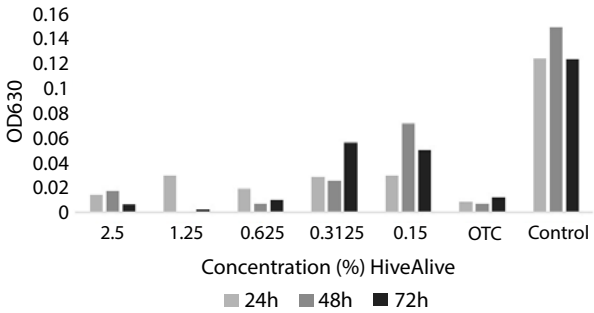

Figure 2: Growth of *P. larvae* in media supplemented with HiveAlive over 72 hours represented by absorbance at 630nm. *P. larvae* growth was also monitored in the presence of oxytetracycline (OTC) and control media (no HiveAlive added).

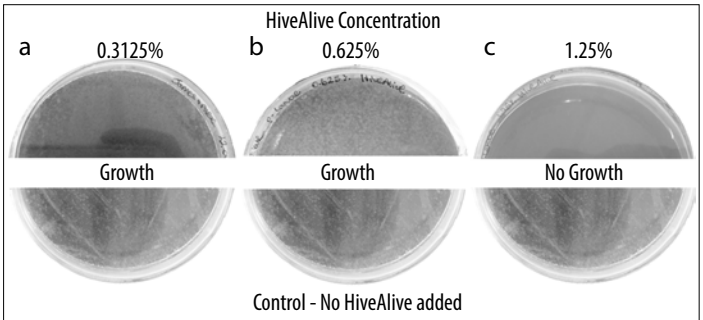

Figure 3: Culture from 96 well plate assays at the end of the assay. 100µl was spread on agar plates and incubated. Growth is present on *P. larvae* in 0.3125% HiveAlive (a) and *P. larvae* in 0.625% HiveAlive (b) but no growth was present on plates from wells containing *P. larvae* in 1.25% HiveAlive (c) and above. Each plate in images a, b and c (top) is compared to control *P. larvae* plates with no HiveAlive added (bottom).

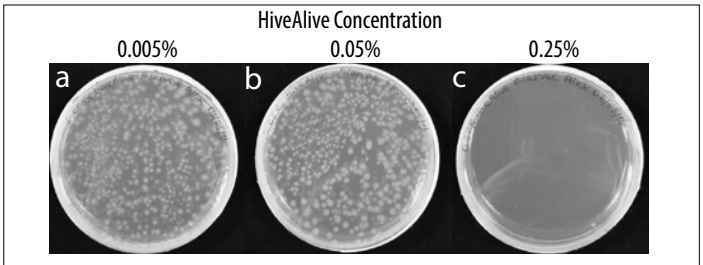

Figure 4: Effect of addition of HiveAlive to agar medium on *P. larvae*. Growth of *P. larvae* was not inhibited at 0.005% (a) and 0.05% (b) HiveAlive but no growth was present on plates that had 0.25% (c) HiveAlive added.

References: Coffey, M. (2016). FIBKA Policy on American Foulbrood. http://www.irishbeekeeping.ie/index.php/about-us/education/bee-health/fibka-policy-on-afb