

Principelle Research Report

Antibacterial activity of Principelle PPIF15



C.J. Beukelman, PhD
PhytoGeniX B.V.
December 2012

Introduction:

Principelle is a specialist Company offering advanced wound care products and services to Distributors and Clinicians. Principelle is permanently focused on innovation and different approaches to achieve clinical results in a more effective or efficient way.

Honey is a natural product with a long history of medical application, mostly applied for wound healing.

PhytoGeniX is a research company focused on the improvement and development of herbal medicinal products. A major field of PhytoGeniX' expertise includes series of bioassays concerning immunomodulatory activity. PhytoGeniX is active in the area of wound healing for more than 20 years. PhytoGeniX has tested several series of honey from different sources in their bio-assays.

Principelle has asked PhytoGeniX to test PPIF15, a honey-based ionogen formulation, for antibacterial activity against a series of clinical important isolates. Since PhytoGeniX does not culture bacteria on a daily basis and does not possess standard wound care-relevant bacteria cultures, it was decided to subcontract specialists from the Eijkman Winkler Institute from the Academic Hospital in Utrecht to culture the bacteria and to determine antibacterial capacity. In this report the results of these experiments are presented.

Materials & methods:

Principelle PPIF15

PPIF15 (12D02 – 04.2015) was provided by Principelle.

Micro-organisms

Micro-organisms (most common clinical isolates of wound infections) were provided by the Eijkman Winkler Institute:

- *S.aureus*
- ESBL CTXM
- *S.aureus* MRSA
- *Enterobacter cloacea*
- *S.epidermidis*
- *B.cereus*
- *Pseudomonas aeruginosa*
- *Enterococcus faecalis*
- *Enterococcus faecalis* Vanco Res
- *E.coli* Ciprofloxacin Res

Physical characteristics

In a small pilot experiment PPIF15 was diluted in Mueller Hinton Bouillon combined with Mueller Hinton Agar. Subsequently agar thickness, pouring temperature, size of bacterial suspension droplets, and bacterial growth were determined and optimized (Data not shown).

Antibacterial activity of PPIF15

PPIF15 was added to double-strength nutrient agar at 50°C to produce final honey concentrations of 20%, 10%, 5%, 1% and 0 (control). The agar-PPIF15 mixture was immediately poured into Petri dishes. Overnight broth cultures of the micro-organisms were adjusted to the 0.5 McFarland standard, diluted 1:10 and 10 µl were applied to the surface of the plates to yield ~104 CFU per spot. Plates were incubated at 35°C for 24 hours and growth was compared to the control plate. Growth inhibition is independently scored by three persons from 0 – 3. Plates were incubated for another 24 hr period and scored again.

Results and discussion:

In fig 1 for each strain the growth scores are presented as determined after 24 and 48 hours by three independent co-workers. For ESBL it was also observed that spreading (as is characteristic for ESBL) was inhibited completely by PPIF15 from 5% on.

As expected, no differences were observed for honey resistance of ordinary *Staphylococcus aureus* versus Methycillin resistant *Staphylococcus aureus* (MRSA) as well as for *Enterococcus faecalis* versus Vancomycin resistant *Enterococcus faecalis*.

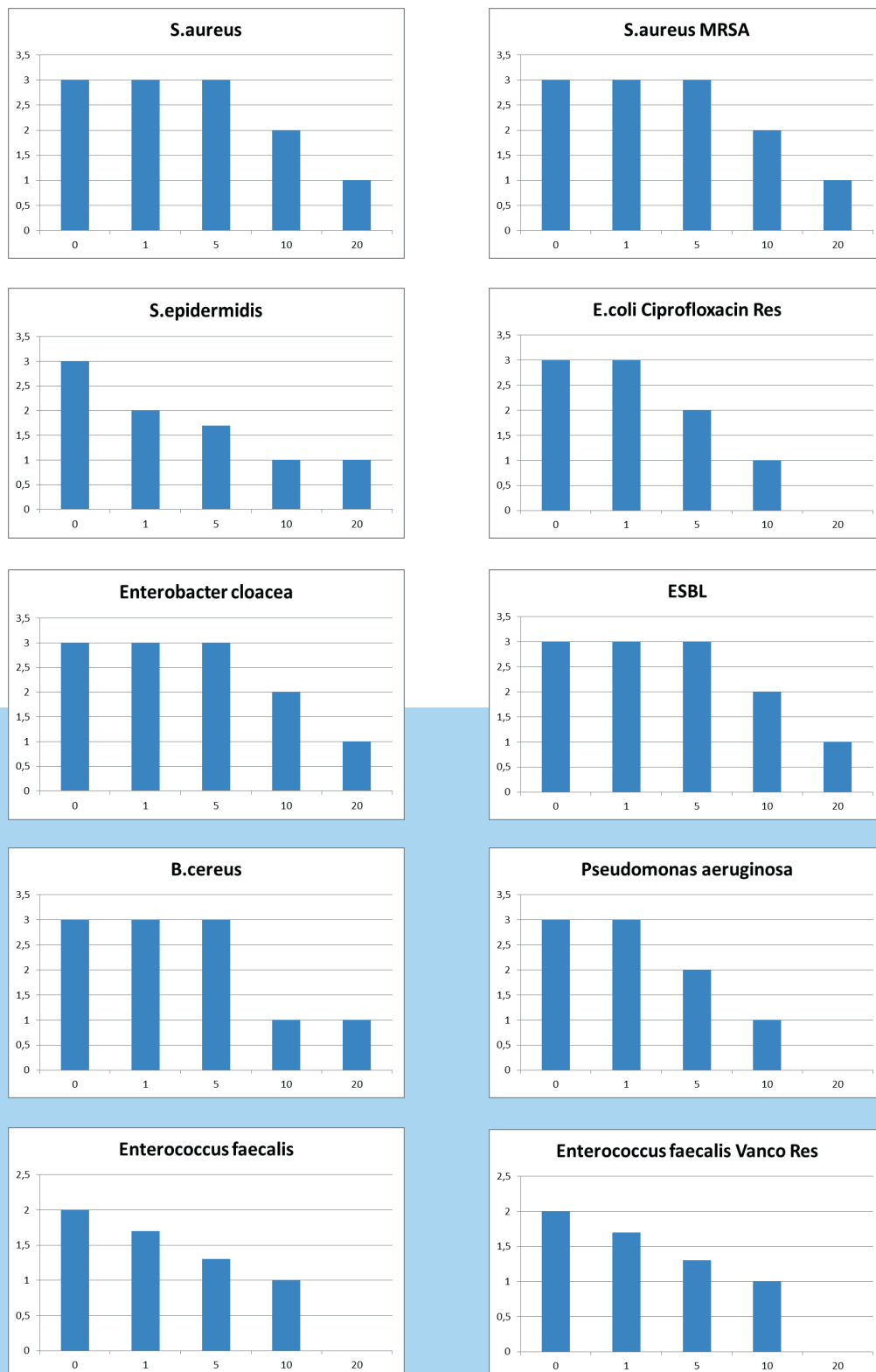


Fig1. Antibacterial activity of PPIF15 against 10 different bacterial strains.
 X-axis: percentage of PPIF15 mixed with agar.
 Y-axis: growth score.

Conclusions:

Concerning the antibacterial activity of PPIF15 it can be concluded that all bacterial strains were strongly inhibited at a concentration of 20% PPIF15 in agar. Four out of 10 strains were completely inhibited, six strains were strongly inhibited. Both methycillin and vacomycin resistant strains were strongly inhibited by honey-based ointment.