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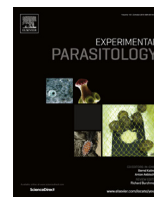

Full length article

Identification and disruption of bacteria associated with sheep scab mites-novel means of control?

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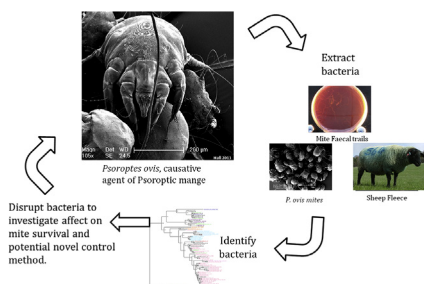
HIGHLIGHTS

- Characterisation of bacteria associated with *Psoroptes ovis* mites, faecal trails and sheep fleece.
- Identification of *Comamonas sp* in *P. ovis* mites, a potential endosymbiont.
- Disrupting bacteria within *P. ovis* using antibiotics reduces mean mite survival time compared to controls.

GRAPHICAL ABSTRACT



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ABSTRACT

Psoroptes ovis mites, which cause psoroptic mange (sheep scab), were investigated to identify potential bacterial targets for endosymbiont control of sheep scab. In addition, transmission of bacteria to the sheep skin was investigated through the characterisation of bacteria present in *P. ovis* faecal trails and on the fleece environment by internal transcribed spacer (ITS) sequencing. A diverse range of bacteria was identified in addition to a potential endosymbiont candidate, *Comamonas* sp, which was detected in *P. ovis* by both ITS PCR and endosymbiont-specific PCR. Disruption of these bacteria within *P. ovis*, through the use of antibiotics, was explored; with significant reduction in mean mite survival when administered antibiotic diets compared with controls ($LR_4 = 23.12$, $P < 0.001$). The antibiotic treatments also significantly affected the bacterial density (CFU/mite) within *P. ovis*, indicating that mite survival may be linked to the bacterial communities that they harbour. Although antibiotics are not suitable for practical application, these results suggest disrupting bacteria associated with *P. ovis* should be further investigated for novel control.

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1. Introduction

Sheep scab is an important disease of sheep which causes significant welfare concerns. It is caused by the obligate non-burrowing mite, *Psoroptes ovis* which lives its whole life on sheep. This disease is currently treated with chemicals, either by dipping or injections, but resistance has been reported to all classes except the macrocyclic lactones (Lewis, 1997). Because of the weaknesses in control of this disease, there is a need to investigate alternatives.

P. ovis mites cause extreme pruritis (itching) and development of lesions (Baker and York, 1999) which may be exacerbated by opportunistic bacteria (Kirkwood, 1986). These bacteria may be ingested by mites from the skin surface (Sinclair and Filan, 1989) and subsequently potential mite luminal gut bacteria are deposited

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on the sheep skin in guanine-rich faecal pellets (Bates, 1999b; Lewis, 1997; Mathieson, 1995) and opportunistically infect open wounds on the sheep skin as a result of irritation (Bates, 1999a, 2003; Hogg and Lehane, 2001; Mathieson and Lehane, 1996).

There are a number of hypotheses for the presence and function of bacteria associated with *P. ovis*, as several other arthropods have close relationships with internal bacteria where they serve as a food source (Zouache et al., 2009a), or as obligate endosymbionts that are necessary for physiology and successful life cycles (Brune, 2003). The negative effect on the host of removing these endosymbionts has been shown with a number of studies (Eutick et al., 1978; Fukatsu and Hosokawa, 2002; Hogg and Lehane, 1999). Endosymbionts have been detected in many arthropod species, including predatory mite (*Metaseiulus occidentalis*) (Hoy and Jeyaprakash, 2005) and poultry red mite (*Dermanyssus gallinae*) (De Luna et al., 2009). Douglas (1989) suggested the control of arthropod pests through disruption of their endosymbionts. Bacteria have previously been observed internally within *P. ovis* (Mathieson, 1995; Mathieson and Lehane, 1996) but their function is unclear.

In this study, bacteria excreted in *P. ovis* faecal trails were compared with bacteria found on healthy and scab-infected sheep fleece to elucidate transmission of these bacteria between environments. The microbial composition of sheep fleece has been carried out previously and a shift in microbial diversity/composition has been reported to occur with disease occurrence (Lyness et al., 1994; Merritt, 1980; Merritt and Watts, 1978; Tadayon et al., 1980).

Identification of bacteria from *P. ovis* mites and the sheep fleece environment was achieved through cloning and sequencing of the internal transcribed spacer (ITS) region of bacterial DNA using PCR, where products can be separated by sequence heterogeneity to provide phylogenetic differentiation (Garcia-Martinez et al., 1999) and bacterial identification (Cardinale et al., 2004; Kolbert and Persing, 1999). Individual bacterial species can then be identified from a complex community based on the ITS sequence.

There are a number of methods previously used to disrupt endosymbionts within arthropods, including heat treatment (van Opinjen and Breeuwer, 1999), lysozyme, which destroys symbiont membranes (Nogge, 1981) and antibiotics. Removal or disruption of arthropod internal bacterial communities by antibiotics has been shown to reduce survival (Koga et al., 2007), fecundity (Son et al., 2008; Zhong et al., 2007) and growth (Bandi et al., 1999; Hardie and Leckstein, 2007) but without inhibiting feeding (Ben-Yosef et al., 2008).

In this study, bacteria isolated from *P. ovis* faecal trails were used to determine effective antibiotics and concentrations through antimicrobial effect in solid and liquid cultures. These antibiotics (gentamicin and tetracycline) were then administered to *P. ovis* mites in specially constructed *in vitro* chambers to measure survival and bacterial density (Colony Forming Unit/mite). The antibiotics chosen have different bacterial targets and modes of action. Gentamicin is bactericidal, targeting Gram negative bacteria by its aminoglycosides. Tetracycline, however is bacteriostatic of both Gram positive and Gram negative bacteria by inhibiting protein synthesis within the bacteria (Hahn and Sarre, 1969). This present study aims to investigate bacteria associated with *P. ovis* mites and the effect of disrupting them on the survival of mites, for the potential novel application of parasitic control.

2. Materials

2.1. *P. ovis* samples

Mite samples (male and female) were received from SRUC Disease Surveillance Centres throughout Scotland (natural infections) and Moredun Research Institute, Edinburgh (*in vivo* cultures). Mites were used immediately for faecal trails or frozen at −80 °C for bacterial identification. For ITS-PCR three *in vivo* (M1, M2, M3) and three natural (S193, S21, S22) mite samples were selected following DNA extraction for PCR clean up, transformation and sequencing. For endosymbiont-specific PCR DNA was extracted from 14 different mite samples (eight *in vivo*, six natural).

2.2. Sheep fleece samples

Fleeces from healthy sheep were received from ewes housed at SRUC Easter Bush Estate, Edinburgh and fleeces with naturally occurring sheep scab infections were received from SRUC Disease Surveillance Centres throughout Scotland, after positive diagnosis of sheep scab infection. On receipt, samples were kept at 4 °C until use as previously suggested (Lyness et al., 1994). Twenty seven fleece samples (six healthy, 21 scab-infected) were used to extract DNA, from which three samples of each (healthy H24, H91, H109; scab-infected S9, S14, S23) were used for PCR clean-up, transformation and DNA sequencing.

3. Methods

3.1. *P. ovis* faecal trails

P. ovis mites received from *in vivo* culture were used to isolate bacteria from faecal trails, following the method of Mathieson (1995). Unique colonies were picked, purified and identified by ITS-PCR and sequencing as below.

3.2. DNA extraction

Mites were surface sterilised as described in Mathieson (1995) before DNA extraction. DNA was extracted from ten mites or approximately 20 mg of fleece using phenol/chloroform extraction (Fraaije et al., 1999) with an initial homogenisation with 440 μl of 2× TENs extraction buffer added (pH 8.0; 0.8 mM Tris-base, 0.5 mM NaCl, 0.3 mM EDTA, 1 mg/ml phenanthroline, 1 μl/ml mercaptoethanol, 0.02 g/ml PVP) with sterile Ballotini beads (Thistle Scientific). DNA quantity and purity was measured using an ND-1000 spectrophotometer (Nanodrop).

3.3. ITS PCR

Extracted DNA was amplified using forward primer ITSF (5′-GTC GTA ACA AGG TAG CCG TA -3′) and reverse primer ITSReub (5′-GCC AAG GCA TCC ACC-3′) (Cardinale et al., 2004) which targets the bacterial 16S–23S internal transcribed spacer (ITS). PCR was performed in a 25 μl reaction using 10 μl sterile water, 12.5 μl master mix (Promega; 1.5 mM MgCl2, 200 μM dNTPs, 1U colourless GoTaq), 0.5 μM primers and 2 μl DNA using a GeneAmp Thermal cycler (Biometra). Cycling conditions consisted of 2.5 min at 94 °C, 30 cycles of 45 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C, followed by a final extension of 7 min at 72 °C. 20 ng/μl of PCR product was run on a 2% agarose gel containing GelRed (Biotium) in TBE buffer (Eurogentec) with 100 bp or 1 Kb^+ ladder for size calibration (Invitrogen). The remaining PCR product was used for cloning and sequencing.

3.4. Endosymbiotic bacteria-specific primers

DNA extracted from whole *P. ovis* mites was screened for the presence of four known endosymbiont bacteria (*Wolbachia*, *Comamonas*, *Cardinium*, *Rickettsia*) using specific primer sets (Table 1). Optimised primer concentrations were 0.2 μM

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