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## A survey of bacterial diversity in ticks, lice and fleas from Australia

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**Abstract** We isolated bacteria from ticks, lice and fleas. Partial small subunit rRNA sequences were obtained for each isolate and the closest matches in the FastA database were determined. These bacteria were mostly Gram-positive (Firmicutes), although representatives from the Proteobacteria ( $\alpha$ ,  $\beta$ ,  $\gamma$  subdivisions) and CFB group were also isolated. Most of the isolates we found were from genera that were present in most of the ectoparasites studied, but a few genera were restricted to one species of ectoparasite. The most commonly isolated genera were *Stenotrophomonas*, *Staphylococcus*, *Pseudomonas*, *Acinetobacter* and *Bacillus*. Species of *Bacillus* and *Proteus*, which have biopesticide potential, were found in some of these ectoparasites. Overall, the communities of bacteria were similar to those found in other studies of parasitic arthropods.

### Introduction

Ticks, lice and fleas carry and may transmit bacteria to their vertebrate hosts. Many of these bacteria are important pathogens of people and domestic animals. For example, *Yersinia pestis*, which causes plague, is transmitted predominantly by the oriental rat flea, *Xenopsylla cheopis*, whereas *Rickettsia prowazekii*, which causes louse-borne epidemic typhus, is transmitted by the human body louse, *Pediculus humanus*

var. *humanus*. *Anaplasma marginale*, which causes tick fever in cattle, is transmitted by cattle ticks like *Boophilus microplus*. Consequently, most work on the bacteria of ectoparasites has been with the purpose of characterising pathogens of medical and veterinary importance. This sort of work has been particularly common for ticks (see Sparagano et al. 1999) but has also been done for other parasitic arthropods. Quan et al. (1986) surveyed mammals and their fleas for *Pasteurella multocida*, which causes pasteurellosis, the authors isolating bacteria from pools of rabbit fleas (*Cediopsylla inaequalis*), rodent fleas (*Diamanus montanus*, *Hoplopsyllus anomalus*) and carnivore fleas (*Pulex simulans*). Roux and Raoult (1999) surveyed body lice (*Pediculus humanus* var. *humanus*) for bacteria causing human disease.

Other work on ectoparasites has been to assess bacterial diversity in ticks like *Ixodes scapularis* (Martin and Schmidtman 1998) and in lice from goats (Nunez-Sevilla et al. 1987) and captive common buzzards (Perez et al. 1994). Another aspect of the study of bacteria from parasitic arthropods is the potential for some bacteria to be effective biopesticides against ectoparasites. For example, females of the tick, *B. microplus*, may be infected with *Cedecea lapagei*, which destroys the epithelium of the vaginal area (Brum et al. 1991); and adult *Dermacentor andersoni* ticks may be killed by *Proteus mirabilis* (Brown et al. 1970). A well known example of a bacterium that is used commercially as a biopesticide is *Bacillus thuringiensis*, which was first used against lepidopteran insects. Studies of other *B. thuringiensis* strains have resulted in commercial strains effective against a range of insect pests. Some authors have assessed the effectiveness of different strains of *B. thuringiensis* against ectoparasites, including ticks (Hassanain et al. 1997), fleas (Maciejewska et al. 1988) and lice (Lonc and Lachowicz 1987; Drummond et al. 1995). In the present study, our aim was to find, isolate and characterise bacteria from a sample of ticks, lice and fleas.

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## Materials and methods

### Ectoparasites

We screened five species of ticks: five individuals of *Ixodes holocyclus*, 11 *Boophilus decoloratus*, two *Amblyomma triguttatum*, two *Haemaphysalis longicornis* and one *Aponomma fimbriatum*. We also screened four species of flea: eight individuals of *Ctenacephalides felis*, two of an unidentified flea (ex *Isoodon macrourus*?) and one individual each of a flea (ex pig) and another flea (ex wombat). Finally, we screened three species of lice: four individuals of *Bovicola* sp. (ex goat), three individuals of *B. ovis* (ex sheep) and one individual of *Bovicola* sp. (ex alpaca).

### Surface sterilisation of ectoparasites

Parasites were submerged in a solution of household bleach (40 g available chlorine/l) diluted 1:100 with 0.7% Decon-90 (non-ionic detergent; Bloomfield and Arthur 1994). This disinfectant solution was prepared fresh each time that ectoparasites were sterilised. Ectoparasites were submerged in the solution for 3 min and then rinsed well with sterile H<sub>2</sub>O prior to crushing.

### Culturing of bacteria

Ectoparasites were crushed with a micropestle and micromortar in a 0.5% solution of tryptone. The volume varied, depending on the size of the ectoparasite. Half the volume was frozen and half was serially diluted. Dilutions were plated on nutrient agar (Oxoid), blood agar (Colombia blood agar PP2001; Oxoid) and ISP2 agar (yeast extract/malt extract agar; Pridham et al. 1957; Shirling and Gottlieb 1966) and then incubated at 25 °C. Nutrient agar was our general purpose medium, whereas ISP2 was the medium we selected to isolate streptomycetes. Blood agar was selected because blood is the sole source of nutrition for ticks and fleas. Some cultures were first grown on nutrient agar and then subcultured on nutrient agar, blood agar or ISP2 agar.

### Extraction of nucleic acids from bacteria

Two methods were used to extract DNA from bacteria: a guanidinium thiocyanate method and a chelex method. The guanidinium thiocyanate method was modified from Hubbard et al. (1995). A lysis buffer was made by dissolving 24 g of GuSCN (guanidinium thiocyanate) in 20 ml of 0.1 M Tris-HCl (pH 6.4) and 4.4 ml of 0.2 M EDTA (pH 8.0). After the addition of 52 g of Triton X-100, heating to 65 °C in a water bath dissolved the solution. The solution was then filter-sterilised. A suspension was made by adding 20 ml of dH<sub>2</sub>O and 200 µl of HCl (32% w/v) to 4 g of diatomaceous earth; and this suspension was then autoclaved. A scrape of bacteria was added to 900 µl of the lysis buffer and 50 µl of the diatomaceous earth suspension was added, vortexed and then spun at maximum speed in a microcentrifuge for 1 min. The supernatant was discarded and the pellet was cleaned with two washes of 70% ethanol (900 µl of 70% ethanol added each time, followed by 1 min of microcentrifugation and removal of the supernatant). Acetone (190 µl) was added to the pellet and the solution was then microcentrifuged at maximum speed for 1 min before the supernatant was discarded. Finally, when the remaining acetone had evaporated, 100 µl of Tris-EDTA buffer was added to the dried pellet and incubated at 37 °C for 15 min to elute the DNA. After a 5-min spin in a microcentrifuge at maximum speed, the supernatant was collected.

The chelex method involved adding 1 ml of chelex solution (5% chelex in Tris-EDTA, pH 8.0, with 0.25 µg RNase A/ml) to bacteria. The suspension was placed in a boiling water-bath for 20 min, with occasional inverting. After cooling for 10 min, the

solution was microcentrifuged for 5 min at top speed and then 5 µl of the top layer was used in PCR.

### Polymerase chain reaction

Extracted DNA (chelex or guanidinium thiocyanate methods) was added to PCR reactions. Sometimes, whole cells were added to the PCR mix, using toothpicks. The degenerate PCR primers used amplified approximately 880 bp of the small subunit rRNA: BACF (5'-CTGAHSVAGCVAHGYMGCCTG-3') and RCAB (5'-ACGTCRTCCHCDCCTTCCTC-3'), where H = A or C or T, S = G or C, V = G or C or A, Y = C or T, M = C or A, R = G or A and D = G or A or T. An annealing temperature of 50 °C was used for PCR and the recipe for a 50-µl reaction was 5 µl of 10× buffer, 3 µl of 25 mM MgCl<sub>2</sub>, 10 µl of 1 mM dNTPs, 5 µl of dimethyl sulfoxide, 0.4 µl of 2.5 units Redhot Taq polymerase/µl, 5 µl of a 1 µM solution of each primer, 14.6 µl of H<sub>2</sub>O and 2 µl of DNA (or, in the case of chelex-extracted DNA, 11.6 µl of H<sub>2</sub>O and 5 µl of DNA).

### Sequencing and analysis

The Applied Biosystems BigDye terminator cycle sequencing kit was used. The primers used for sequencing were the same as those used for PCR. Sequences were edited in Sequencher ver 3.1.1 (Gene Codes Corporation) and then subjected to FastA (ver 3.08, July 1997) searches (ktup: 6; gap-penalty: -16/-4; width: 16; optimised, DNA matrix) in ANGIS (Australian Genomic Information Centre) to determine closest matches. Only matches with *E* values for the *z*-score < 1e<sup>-04</sup> were considered and the closest matches according to the *z*-score are presented here. Similarity scores were calculated by dividing the number of perfectly matched nucleotides by the length of sequence from each isolate, excluding ambiguous nucleotides (Tables 1, 2, 3).

## Results

A total of 239 bacterial isolates were sequenced (53 from fleas, 33 from lice, 153 from ticks); and their closest matches, determined in FastA searches between September 1999 and October 2000, are shown in Tables 1, 2, 3. Note that small subunit rRNA sequences, especially if partial sequences like those collected here, do not necessarily distinguish different species of the same genus and are highly unlikely to distinguish different sub-species or strains of the same species. The taxonomic arrangement of the organisms isolated is shown in Fig. 1.

### Culture specificity

Bacteria from the genera *Curtobacterium* and *Rahnella* were isolated only on ISP2 agar. However, streptomycetes, which should be selectively isolated on ISP2 agar, were not detected among the bacteria from ticks, lice and fleas cultured on this medium. The only streptomycetes cultured and isolated were *Streptomyces* spp. from *Ctenacephalides felis* on nutrient agar. Bacteria from the genera *Klebsiella*, *Nocardioides* and *Arthrobacter* were isolated only on blood agar. Bacteria from the genera *Kocuria* and *Renibacterium* were isolated on

**Table 1** List of bacteria found in fleas from a bandicoot ( $n=2$ ), dog ( $n=7$ ), wombat ( $n=1$ ) and pig ( $n=1$ ), with their FastA nearest match ( $z$ -score). Numbers in brackets indicate the raw number of nucleotide differences. Media used: *N* nutrient agar, *I* ISP2 agar, *B*

blood agar. *N/I*, *N/B* and *N/N* indicate that bacteria were first cultured on nutrient agar and then subcultured on ISP2 agar, blood agar or nutrient agar, respectively. *No.* Specimen number for ectoparasite individual

Flea species	No.	Medium	Length	Closest match in GenBank, according to FastA search	% Similarity	
Ex bandicoot (host code F1)	1	N/I	320 bp	<i>Pseudomonas tolaasii</i>	99.7% (1)	
		N/I	239 bp	<i>Leminorella grimontii</i> , <i>Enterobacter cloacae</i> , <i>Pantoea agglomerans</i>	99.6% (1)	
	2	N/B	439 bp	<i>Acinetobacter johnsonii</i> , <i>A. junii</i> , <i>A. lwoffii</i>	98.6% (6)	
		N/B	374 bp	<i>A. calcoaceticus</i> , <i>A. haemolyticus</i>	100%	
		N/B	524 bp	<i>Stenotrophomonas maltophilia</i>	99.8% (1)	
		N/B	371 bp	<i>Staphylococcus sciuri</i> , <i>S. lugdunensis</i> , <i>S. warneri</i>	100%	
		N/N	479 bp	<i>S. saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%	
		N/N	512 bp	<i>S. sciuri</i>	100%	
	Ctenocephalides felis ex dog (F2)	1	N/I	424 bp	<i>Bacillus thuringiensis</i> , <i>B. cereus</i>	99.8% (1)
			N/I	542 bp	<i>Acinetobacter radioresistans</i>	99.8% (1)
		2	N	425 bp	<i>Bacillus psychrophilus</i>	98.2% (7)
			N	444 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
			N/I	480 bp	<i>S. succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		3	N/B	526 bp	<i>S. succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
N/N			372 bp	<i>Flavobacterium spiritivorum</i>	96.7% (12)	
4		N/N	330 bp	<i>Bordatella bronchiseptica</i> , <i>B. holmesii</i> , <i>B. pertussis</i> , <i>B. parapertussis</i> , <i>Alcaligenes xylosoxidans</i> , <i>Alcaligenes</i> sp.	99.3% (2)	
		5	N/I	382 bp	<i>Staphylococcus sciuri</i> , <i>S. lugdunensis</i> , <i>S. auricularis</i> , <i>S. warneri</i>	100%
			N/B	543 bp	<i>Proteus mirabilis</i>	100%
	N/B		576 bp	<i>Serratia marcescens</i>	99.3% (4)	
	6	N/I	447 bp	<i>Serratia entomophila</i> , <i>S. rubidaea</i> , <i>S. ficaria</i> , <i>Leminorella grimontii</i>	99.3% (3)	
		N/I	430 bp	<i>Enterobacter amnigenus</i> , <i>Pantoea ananas</i>	98.8% (5)	
	7	N/I	507 bp	<i>Stenotrophomonas maltophilia</i>	99.8% (1)	
		N/I	498 bp	<i>Staphylococcus kloosii</i> , <i>S. gallinarum</i>	99.8% (1)	
		N/I	477 bp	<i>Enterococcus malodoratus</i> , <i>E. mundtii</i> , <i>E. hirae</i> , <i>E. durans</i> , <i>E. avium</i> , <i>E. raffinosus</i> , <i>E. pseudoavium</i> , <i>E. dispar</i>	100%	
		N/N	460 bp	<i>Streptomyces lividans</i>	100%	
N/N		524 bp	<i>Streptomyces</i> sp.	99.6% (2)		
N/N		544 bp	<i>Nocardiosis tropica</i>	99% (5)		
N/N		531 bp	Human oral bacterium	99.6% (2)		
N/N		496 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i> , <i>Bacillus</i> sp.	100%		
N/N		388 bp	Unidentified bacterium	98.8% (4)		
N/I		560 bp	<i>Brevibacterium iodinum</i>	100%		
N/I		591 bp	<i>Staphylococcus equorum</i>	100%		
8		N/I	530 bp	<i>Paenibacillus lautus</i>	97.7% (12)	
		N/B	585 bp	<i>Bacillus megaterium</i>	100%	
		N/B	649 bp	<i>Staphylococcus schleiferi</i>	99.7% (2)	
	N/N	420 bp	<i>Streptomyces sampsonii</i>	100%		
	N/N	446 bp	Unidentified bacterium clone CAR1	98.2% (8)		
	N/I	489 bp	<i>Bacillus pumilus</i>	99.8% (1)		
	Ex wombat (F3)	1	N/B	373 bp	<i>Serratia marcescens</i>	100%
			N/B	321 bp	<i>Pseudomonas putida</i> , unidentified eubacterium	100%
2		N/N	352 bp	<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	100%	
		N/I	401 bp	<i>Acinetobacter</i> sp.	100%	
Ex pig (F4)	1	N/B	594 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%	
		N/B	374 bp	<i>Pseudomonas putida</i> , unidentified eubacterium	99.7% (1)	
		N/B	645 bp	<i>Enterobacter aerogenes</i>	100%	
		N/N	520 bp	<i>Acinetobacter junii</i> , <i>Acinetobacter</i> sp.	100%	
		N/N	567 bp	<i>Stenotrophomonas maltophilia</i>	99.5% (3)	
		N/I	340 bp	<i>Enterobacter aerogenes</i> , <i>E. cancerogenus</i> , <i>Citrobacter freundii</i>	100%	
		N/I	398 bp	<i>Acinetobacter calcoaceticus</i>	100%	
		N/I	455 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%	
		N/I	396 bp	<i>Acinetobacter calcoaceticus</i>	100%	
		N/I	320 bp	<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	99.7% (1)	
		N/I	512 bp	<i>Acinetobacter</i> sp.	99.6% (2)	

both ISP2 and blood agar, but not on nutrient agar. *Corynebacterium* spp were isolated on nutrient and blood agar, but not on ISP2. Genera that were only isolated on nutrient agar were *Rhodococcus*, *Micrococcus*, *Stomatococcus*, *Paracraurococcus*, *Terrebaacter*,

*Streptomyces*, *Nocardiosis*, *Flavobacterium*, *Bordatella/Alcaligenes* and *Leucobacter*. Many genera grew in all three culture media, for example, *Bacillus*, *Staphylococcus*, *Enterococcus*, *Brevibacterium*, *Pseudomonas*, *Acinetobacter*, *Serratia*, *Enterobacter*, *Pantoea*, *Steno-*

**Table 2** List of bacteria found in lice, with their FastA nearest match (*z*-score). Numbers in brackets indicate the raw number of nucleotide differences. Media used: N=nutrient agar; I=ISP2 agar; B=blood agar; N/I, N/B and N/N indicates that bacteria

were first cultured on nutrient agar then subcultured on ISP2 agar, blood agar or nutrient agar. No. specimen number for ectoparasite individual

Louse	No.	Medium	Length	Closest match in GenBank according to FastA search	% Similarity
<i>Bovicola ovis</i> ex sheep (L1)	1	N/B	527 bp	<i>Microbacterium esteraromaticum</i> , <i>M. arabinogalactanolyticum</i>	99.8% (1)
		N/N	459 bp	<i>Corynebacterium coyleiae</i>	98.6% (6)
		N/N	452 bp	<i>Staphylococcus condimentii</i> , <i>S. piscifermentens</i> , <i>S. carnosus</i> , <i>S. auricularis</i>	99.6% (2)
	2	N/I	518 bp	<i>Microbacterium</i> sp.	99.6% (2)
		N/B	563 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		N/B	477 bp	<i>S. condimentii</i> , <i>S. piscifermentens</i> , <i>S. carnosus</i> , <i>S. auricularis</i>	99.6% (2)
	3	N/I	303 bp	<i>S. pasteurii</i>	100%
		I	352 bp	<i>Bacillus licheniformis</i>	100%
		I	474 bp	<i>Citrobacter braakii</i> , <i>C. freundii</i> , <i>C. werkmanii</i> , <i>Enterobacter cancerogenus</i> , <i>E. cloacae</i> , <i>E. aerogenes</i> , <i>Pantoea agglomerans</i>	100%
		I	314 bp	<i>Proteus mirabilis</i>	100%
<i>Bovicola</i> sp. ex alpaca (L2)	1	I	334 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i> , <i>S. equorum</i>	100%
		B	451 bp	<i>Bacillus simplex</i>	99.5% (2)
	2	B	521 bp	<i>Enterococcus faecalis</i>	100%
		B	370 bp	<i>Bacillus megaterium</i>	98.4% (6)
		B	397 bp	<i>Staphylococcus saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%
		B	450 bp	<i>Bacillus pumilus</i>	99.8% (1)
		B	331 bp	<i>Kocuria rhizophila</i>	100%
		B	379 bp	<i>Paenibacillus lautus</i>	100%
		I	403 bp	<i>Bacillus spaericus</i>	99% (4)
		I	461 bp	<i>Staphylococcus saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%
<i>Bovicola</i> sp. ex goat (L3)	1	N/N	461 bp	<i>Staphylococcus saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%
	2	N/B	480 bp	<i>Corynebacterium</i> sp.	98.9% (5)
	3	N/I	358 bp	<i>Bacillus licheniformis</i>	100%
	4	B	517 bp	<i>Bacillus thuringiensis</i>	99.2% (4)
		N	453 bp	<i>Stomatococcus mucilaginosus</i>	97.8% (10)
		N	363 bp	<i>Paracraurococcus ruber</i>	95.2% (17)
		N	396 bp	<i>Staphylococcus saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%
		N	263 bp	<i>Enterococcus faecalis</i>	99.6% (1)
		N/N	529 bp	<i>Terrebacter</i> sp.	99.8% (1)
		N/N	377 bp	<i>Enterococcus faecalis</i>	100%
N/N		385 bp	<i>Paracraurococcus ruber</i>	95.2% (18)	
N/N	489 bp	<i>Enterococcus faecalis</i>	100%		
N/N	423 bp	<i>Stomatococcus mucilaginosus</i>	97.1% (12)		
N/N	502 bp	<i>Micrococcus luteus</i> -like	97.7% (10)		

*trophomonas*, *Proteus*, *Microbacterium* and *Paenibacillus*.

#### Commonly occurring genera

Some genera of bacteria were isolated from almost all of the ectoparasites sampled.

*Stenotrophomonas* spp were detected in the tick species *Ixodes holocyclus*, *Aponomma fimbriatum* and *Boophilus microplus* and in all the flea species studied. However, none of the 38 bacterial cultures from lice had matches with the genus *Stenotrophomonas*. *Pseudomonas* and *Acinetobacter* spp were also restricted to ticks and fleas: these bacteria were not found in lice. *Staphylococcus* spp were detected in three ticks, *I. holocyclus*, *B. microplus* and *Haemaphysalis longicornis*, in all fleas except those from the wombat and in all lice studied. *Bacillus* spp were detected in: (1) three ticks, *I. holocyclus*, *Amblyomma triguttatum* and *B. microplus*, (2) the bandicoot flea and *Ctenacephalides felis* and (3) in all lice.

Genera restricted to one type of ectoparasite

Many genera of bacteria were isolated only from one species of ectoparasite (Fig. 1). For example, the genera *Curtobacterium*, *Clavibacter*, *Renibacterium*, *Arthrobacter*, *Leucobacter*, *Sphingobacterium*, *Citrobacter*, *Salmonella* and *Rahnella* were only identified from isolates from the tick, *Boophilus microplus*, whereas the genera *Nocardioopsis*, *Streptomyces* and *Flavobacterium* were only identified from isolates from the flea, *C. felis*.

#### Discussion

The greatest diversity of bacteria was obtained with nutrient agar. Indeed, many of the isolates obtained from blood agar and ISP2 agar were initially also cultured on nutrient agar. The two speciality agars, ISP2 and blood agar, allowed us to isolate bacteria that were not isolated with nutrient agar, e.g. bacteria from the genera *Klebsiella*, *Nocardioides* and

**Table 3** List of bacteria found in ticks, with their FastA nearest match (z-score). Numbers in brackets indicate the raw number of nucleotide differences. Media used: N=nutrient agar; I=ISP2 agar; B=blood agar; N/I, N/B and N/N indicates that bacteria were first cultured on nutrient agar then subcultured on ISP2 agar, blood agar or nutrient agar. No. specimen number for individual ectoparasite

Tick	No.	Medium	Length	Closest match in GenBank according to FastA search	% Similarity
<i>Ixodes holocyclus</i> (T1)	1	N	504 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i> , <i>Pasteuria penetrans</i>	100%
		N	372 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i> , <i>S. equorum</i>	100%
		N	375 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
	2	N	449 bp	<i>Brevibacterium iodinum</i>	100%
		B	278 bp	<i>Pseudomonas azotoformans</i>	100%
		B	483 bp	<i>Pantoea stewartii</i>	99% (5)
		B	417 bp	<i>Pantoea stewartii</i>	
		B	628 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		B	529 bp	<i>Brevibacterium iodinum</i>	98.3% (9)
		N	598 bp	<i>Pseudomonas tolaasii</i>	99.5% (3)
		N	479 bp	<i>Pantoea stewartii</i>	98.9% (5)
		N	590 bp	<i>Bacillus mycoides</i>	100%
		I	455 bp	<i>Pantoea stewartii</i>	98.9% (5)
		I	461 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
	3	N	566 bp	<i>Sphingomonas paucimobilis</i>	99% (5)
		N	494 bp	<i>Burkholderia</i> sp. isolate N3P2	100%
		I	432 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
		I	485 bp	<i>Staphylococcus pasteurii</i>	100%
		I	458 bp	<i>Burkholderia cepacia</i>	100%
		I	530 bp	<i>Sphingomonas asaccharolytica</i> , <i>S. pruni</i>	98.3% (9)
	4	I	549 bp	Star-like micro-colonies	99.8% (1)
		N	471 bp	<i>Paenibacillus lautus</i>	97.1% (13)
		N	456 bp	<i>Paenibacillus</i> sp.	97.9% (9)
		N	445 bp	<i>Paenibacillus</i> sp.	97.9% (9)
		5	N/N	522 bp	<i>Bacillus cereus</i> , <i>Bacillus</i> sp., <i>B. thuringiensis</i>
	N/N		586 bp	<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	100%
	N/N		633 bp	<i>Acinetobacter lwoffii</i>	100%
N/N	588 bp		<i>Pseudomonas putida</i>	99.5% (3)	
N/N	587 bp		<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	100%	
N/N	586 bp		<i>Pseudomonas</i> sp. <i>P. aureofaciens</i> , <i>P. putida</i>	99.8% (1)	
<i>Amblyomma triguttatum</i> (T2)	1	B	483 bp	<i>Bacillus</i> sp.	98.1% (9)
		B	517 bp	<i>Bacillus licheniformis</i>	97.1% (15)
		B	481 bp	<i>Bacillus megaterium</i>	100%
		B	445 bp	<i>Bacillus cohnii</i>	97.5% (11)
		B	437 bp	<i>Bacillus cohnii</i>	97.2% (12)
		N	502 bp	<i>Bacillus licheniformis</i>	97% (15)
		N	539 bp	<i>Bacillus</i> sp.	97.5% (13)
		I	406 bp	<i>Bacillus pumilus</i>	100%
		I	465 bp	<i>Bacillus pumilus</i>	100%
		I	457 bp	<i>Bacillus pumilus</i>	99.8% (1)
	2	I	424 bp	<i>Kocuria kristinae</i>	100%
		B	334 bp	<i>Nocardioides thermophilacinus</i>	97.1% (9)
		I	448 bp	<i>Bacillus psychrophilus</i>	98.1% (8)
		I	417 bp	<i>Bacillus cohnii</i>	97.5% (10)
<i>Aponomma fimbriatum</i> (T3)	1	I	491 bp	<i>Bacillus pumilus</i>	99.8% (1)
		I	317 bp	<i>Bacillus pumilus</i>	100%
		N/B	231 bp	<i>Paracoccus solventivorans</i>	97% (6)
		N/B	306 bp	<i>Pseudomonas putida</i> , <i>Pseudomonas</i> sp.	100%
		N/N	532 bp	<i>Stenotrophomonas maltophilia</i>	99.8% (1)
		N/N	335 bp	<i>Pseudomonas putida</i> , <i>Pseudomonas</i> sp.	100%
		N/N	489 bp	<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	99.8% (1)
		N/N	365 bp	<i>Stenotrophomonas maltophilia</i> , <i>Stenotrophomonas</i> sp.	100%
<i>Boophilus microplus</i> (T4)	1	N/N	422 bp	<i>Stenotrophomonas maltophilia</i>	100%
		N/I	589 bp	<i>Stenotrophomonas maltophilia</i>	99.5% (3)
		B	547 bp	<i>Paenibacillus thiaminolyticus</i>	97.2% (15)
		N	488 bp	<i>Staphylococcus auricularis</i>	99.6% (2)
		I	534 bp	<i>Staphylococcus chromogenes</i>	100%
		I	337 bp	<i>Bacillus megaterium</i>	100%
		I	497 bp	<i>Bacillus halmapalus</i>	97.9% (10)
2	B	465 bp	<i>Renibacterium salmoninarum</i>	98.7% (6)	

Table 3 (Contd.)

Tick	No.	Medium	Length	Closest match in GenBank according to FastA search	% Similarity
		B	496 bp	<i>Microbacterium esteraromaticum</i> , <i>M. arabinogalactanolyticum</i>	100%
		B	388 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	99.7% (1)
		B	477 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		B	428 bp	<i>Microbacterium keratanolyticum</i>	100%
		B	507 bp	<i>Arthrobacter protophormiae</i>	100%
		B	449 bp	<i>Bacillus silvestris</i>	100%
		B	602 bp	<i>Microbacterium testaceum</i>	97.8% (13)
		B	378 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
		N	508 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
		N	479 bp	<i>Microbacterium esteraromaticum</i> , <i>M. arabinogalactanolyticum</i>	100%
		N	518 bp	<i>Microbacterium esteraromaticum</i>	100%
		N	422 bp	<i>Microbacterium testaceum</i>	100%
		N	440 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i> , <i>Pasteuria penetrans</i>	100%
		N	482 bp	<i>Microbacterium testaceum</i>	100%
		N	451 bp	<i>Microbacterium testaceum</i>	100%
		N	466 bp	<i>Microbacterium aborescens</i>	99.8% (1)
		N	463 bp	<i>Leucobacter komagatare</i>	95.9% (19)
		N/B	389 bp	<i>Microbacterium esteraromaticum</i> , <i>M. arabinogalactanolyticum</i>	100%
		N	564 bp	<i>Microbacterium lacticum</i>	100%
		N	484 bp	<i>Microbacterium aborescens</i>	100%
		N	404 bp	<i>Microbacterium lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. keratanolyticum</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	99.8% (1)
		N	469 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. oxydans</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	99.6% (2)
		N	350 bp	<i>Microbacterium testaceum</i>	100%
		N	547 bp	<i>Rhodococcus equi</i>	100%
		N/N	526 bp	<i>Microbacterium esteraromaticum</i>	100%
		N/N	502 bp	<i>Microbacterium esteraromaticum</i> , <i>M. arabinogalactanolyticum</i>	100%
		I	345 bp	<i>Microbacterium aborescens</i>	100%
		I	483 bp	<i>Bacillus silvestris</i>	100%
		I	365 bp	<i>Clavibacter xyli</i>	98.2% (6)
		I	457 bp	<i>Microbacterium testaceum</i>	97.8% (10)
		I	466 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		I	455 bp	<i>Bacillus silvestris</i>	99.8% (1)
		I	433 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		I	487 bp	<i>Renibacterium salmoninarum</i>	98.9% (5)
		I	445 bp	<i>Bacillus psychrophilus</i>	99.1% (4)
		I	376 bp	<i>Curtobacterium luteum</i>	100%
		I	507 bp	<i>Microbacterium keratanolyticum</i> , <i>M. lacticum</i> , <i>M. saperdae</i> , <i>M. schleiferi</i> , <i>M. aurum</i>	100%
3		B	367 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
		N	544 bp	<i>Bacillus longisporus</i>	98.2% (10)
		N	429 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i>	100%
4		B	436 bp	<i>Serratia proteamaculans</i> , <i>S. grimesii</i> , <i>Aranicola proteolyticus</i>	99.8% (1)
		B	371 bp	<i>Citrobacter braakii</i> , <i>C. freundii</i> , <i>C. werkmanii</i> , <i>Enterobacter cancerogenus</i> , <i>E. intermedius</i> , <i>E. aerogenes</i>	100%
		I	331 bp	<i>Rahnella aquatilis</i>	99.7% (1)
5		B	322 bp	<i>Pseudomonas mucidolens</i> , <i>P. putida</i> , <i>P. synxantha</i>	100%
		B	390 bp	<i>Pseudomonas hibiscicola</i>	100%
		B	428 bp	<i>Pseudomonas mucidolens</i> , <i>P. putida</i> , <i>P. synxantha</i>	100%
		B	471 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		B	274 bp	<i>Bacillus pumilus</i>	100%
		N	439 bp	<i>Pseudomonas mucidolens</i> , <i>P. putida</i> , <i>P. synxantha</i>	100%
		N	449 bp	<i>Pseudomonas hibiscicola</i> , <i>Stenotrophomonas maltophila</i> , <i>S. africana</i>	99.8% (1)
		N	469 bp	<i>Sphingobacterium multivorum</i>	99.1% (4)
		N	311 bp	<i>Acinetobacter lwoffii</i> , <i>A. johnsonii</i> , <i>A. junii</i>	100%
		I	495 bp	<i>Pseudomonas chlororaphis</i> , <i>P. coronafaciens</i> , <i>P. syringae</i>	99.4% (3)
		I	436 bp	<i>Pseudomonas hibiscicola</i> , <i>Stenotrophomonas maltophila</i> , <i>S. africana</i>	99.8% (1)
		I	317 bp	<i>Stenotrophomonas maltophila</i>	99.7% (1)
		I	452 bp	<i>Sphingobacterium multivorum</i>	99.3% (3)
6		B	413 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		B	328 bp	<i>Enterobacter asburiae</i> , <i>Pantoea stewartii</i>	99% (3)
		B	332 bp	<i>Enterobacter cloacae</i> , <i>Pantoea agglomerans</i>	100%
		B	404 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%

Table 3 (Contd.)

Tick	No.	Medium	Length	Closest match in GenBank according to FastA search	% Similarity
		B	485 bp	<i>Pseudomonas aureofaciens</i> , <i>P. putida</i>	100%
		N	355 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		N	481 bp	<i>Enterobacter asburiae</i> , <i>Pantoea</i> sp.	99.6% (2)
		N	424 bp	<i>Bacillus megaterium</i>	100%
		N	490 bp	<i>Enterobacter cloacae</i> , <i>Pantoea agglomerans</i>	100%
		N	527 bp	<i>Bacillus megaterium</i>	100%
		N	561 bp	<i>Pantoea ananas</i>	99.1% (5)
		N	400 bp	<i>Tatumella ptyseos</i> , <i>Pantoea</i> sp., <i>Enterobacter asburiae</i>	99.5% (2)
		I	270 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		I	388 bp	<i>Enterobacter cloacae</i> , <i>Pantoea agglomerans</i>	99.7% (1)
		I	456 bp	<i>Enterobacter cloacae</i> , <i>E. asburiae</i> , <i>Pantoea agglomerans</i> , <i>Pantoea</i> sp.	100%
		I	334 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		I	506 bp	<i>Enterobacter asburiae</i> , <i>Pantoea</i> sp.	99.6% (2)
		I	440 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
		I	470 bp	<i>Staphylococcus lugdunensis</i> , <i>S. warneri</i>	100%
	7	B	301 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i> , <i>Pasteuria penetrans</i>	100%
		B	475 bp	<i>Acinetobacter radioresistans</i>	100%
		B	546 bp	<i>Acinetobacter radioresistans</i>	100%
		B	401 bp	<i>Acinetobacter radioresistans</i>	100%
		B	507 bp	<i>Salmonella bongori</i>	99.4% (3)
		B	480 bp	<i>Acinetobacter radioresistans</i>	100%
		N	470 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	99.8% (1)
	8	B	230 bp	<i>Staphylococcus succinus</i> , <i>S. saprophyticus</i> , <i>S. xylosus</i> , <i>S. equorum</i>	99.6% (1)
	9	N	505 bp	<i>Bacillus cereus</i> , <i>B. thuringiensis</i>	100%
	10	N	472 bp	<i>Bacillus psychrophilus</i>	98.7% (6)
	11	N	358 bp	<i>Bacillus pumilus</i>	99.7% (1)
Haema-physalis longicornis (T5)	1	B	401 bp	<i>Serratia entomophila</i> , <i>S. rubidaea</i> , <i>S. ficaria</i>	100%
		N	430 bp	<i>Serratia entomophila</i> , <i>S. rubidaea</i> , <i>S. ficaria</i>	100%
	2	B	409 bp	<i>Klebsiella planticola</i> , <i>K. terrigena</i> , <i>K. ornithinolytica</i> , <i>K. oxytoca</i>	100%
		B	406 bp	<i>Staphylococcus saccharolyticus</i> , <i>S. capitus</i> , <i>S. caprae</i> , <i>S. epidermidis</i>	100%
		N	442 bp	<i>Pseudomonas pleoglossicida</i> , <i>P. putida</i> , <i>P. monteilii</i>	100%
		N	448 bp	<i>Pseudomonas marginalis</i> , <i>P. borealis</i> , <i>P. corrugata</i> , <i>P. aureofaciens</i> , <i>P. putida</i>	100%
		I	439 bp	<i>Pseudomonas pleoglossicida</i> , <i>P. putida</i>	100%
	I	422 bp	<i>Pseudomonas oleovorans</i>	100%	

*Arthrobacter* were only cultured on blood agar and bacteria from *Curtobacterium* and *Rahnella* were only cultured on ISP2 agar. In contrast, many genera were cultured only on nutrient agar, indicating this medium was effective for culturing a diverse range of bacteria.

The majority of bacterial genera were isolated from most of the ectoparasites we studied but some genera were specific to particular types (e.g. ticks or lice) or even to a species. Some of these genera isolated may have potential for use as biopesticides; particularly those confined to one species of ectoparasite. For instance, many different *Bacilli* were isolated from all the ectoparasites studied. Martin and Schmidtman (1998) isolated mostly *Bacillus* spp from the tick, *Ixodes scapularis*, just as we isolated many *Bacillus* spp from the ticks, *Amblyomma triguttatum*, *Aponomma fimbriatum*, *Boophilus microplus* and *Ixodes holocyclus*. Perez et al. (1994) found *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptococcus*, *Corynebacterium*, *Pasteurella* and *Eikenella* spp inside the lice, *Degeeriella fulva*, *Craspedorrhynchus platystomus*, *Laemobothrion*

*maximum* and *Colpocephalum nanum* (formerly *C. meridionale*; see Price et al. 1997). We also isolated *Staphylococcus*, *Micrococcus*, *Corynebacterium* and *Bacillus* from lice. Nunez-Sevilla et al. (1987) isolated bacteria from biting lice of goats (*Bovicola* sp.) similar to those we isolated from *B. ovis*, *Bovicola* sp. from goats and *Bovicola* sp. from alpacas.

We cultured a wide range of bacteria from ticks, lice and fleas, some of which may have biopesticide potential. Although many of the genera we isolated were similar to those isolated from ticks and lice from other studies, we also isolated additional genera. The three culture methods used resulted in some redundancy but also enabled the isolation of some bacteria that were only isolated on one type of culture; and a better representation of bacterial diversity was thus obtained.

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Firmicutes (Gram-positive bacteria)	<i>Flavobacterium</i> F2
<i>Bacillus/Clostridium</i> group	Sphingobacteria
Bacillales	Sphingobacteriaceae
Alicyclobacillaceae	<i>Sphingobacterium</i> T4
<i>Pasteuria</i> (or <i>Bacillus</i> ) T1, T4	Proteobacteria
Bacillaceae	α-Proteobacteria
<i>Bacillus</i> T1, T2, T4, F1, F2, L1, L2, L3	Acetobacteriaceae
<i>Bacillus</i> (or <i>Pasteuria</i> ) T1, T4	<i>Paracraurococcus</i> L3
Paenibacillaceae	<i>Rhodobacter</i> group
<i>Paenibacillus</i> T1, T4, F2, L2	<i>Paracoccus</i> T2
Staphylococcaceae	Sphingomonadaceae
<i>Staphylococcus</i> T1, T4, T5, F1, F2, F4, L1, L2, L3	<i>Sphingomonas</i> T1
Lactobacillales	β-Proteobacteria
Enterococcaceae	Alcaligenaceae
<i>Enterococcus</i> F2, L2, L3	<i>Bordetella/Alcaligenes</i> F2
Actinobacteria	<i>Burkholderia/Oxalobacter/Ralstonia</i> group
Actinomycetales	<i>Burkholderia</i> T1
Corynebacterineae	γ-Proteobacteria
Nocardiaceae	Enterobacteriaceae
<i>Rhodococcus</i> T4	<i>Enterobacter</i> F4
Corynebacteriaceae	<i>Pantoea</i> T1, T4
<i>Corynebacterium</i> L1, L3	<i>Enterobacter/Pantoea</i> T4, F2
Micrococccineae	<i>Enterobacter/Citrobacter</i> F4
Microbacteriaceae	<i>Proteus</i> F2, L1
<i>Clavibacter</i> T4	<i>Serratia</i> T5, F2, F3
<i>Curtobacterium</i> T4	<i>Serratia/Aranicola</i> T4
<i>Leucobacter</i> T4	<i>Serratia/Leminorella</i> F2
<i>Microbacterium</i> T1, T4, L1	<i>Klebsiella</i> T5
Micrococcaceae	<i>Citrobacter</i> T4
<i>Arthrobacter</i> T4	<i>Salmonella</i> T4
<i>Kocuria</i> T2, L2	<i>Rahnella</i> T4
<i>Micrococcus</i> L3	<i>Tatumella/Pantoea/Enterobacter</i> T4
<i>Renibacterium</i> T4	<i>Leminorella/Enterobacter/Pantoea</i> F1
<i>Stomatococcus=Rothia</i> L3	<i>Enterobacter/Citrobacter/Pantoea</i> L1
Brevibacteriaceae	Pseudomonadaceae/Moraxellaceae group
<i>Brevibacterium</i> T1, F2	Pseudomonadaceae
Intrasporangiaceae	<i>Pseudomonas</i> T1, T3, T4, T5, F1, F3, F4
<i>Terreбактер</i> L3	Moraxellaceae
Propionibacterineae	<i>Acinetobacter</i> T1, T4, F3, F4
Nocardioidaceae	Xanthomonadales
<i>Nocardioides</i> T2	<i>Xanthomonas</i> group
Streptomycineae	<i>Stenotrophomonas</i> T1, T3, T4, F1, F2, F3, F4
Streptomycetaceae	
<i>Streptomyces</i> F2	
Streptosporangineae	
Nocardiopsaceae	
<i>Nocardiopsis</i> F2	
CFB group	
Flavobacteria	
Flavobacteriaceae	

**Fig. 1** List of bacterial genera isolated from ticks, lice and fleas. Codes indicate the hosts listed in Tables 1, 2, 3: F flea, L louse, T tick, T1 *Ixodes holocyclus*, T2 *Amblyomma triguttatum*, T3 *Aponomma fimbriatum*, T4 *Boophilus microplus*, T5 *Haemaphysalis longicornis*, L1 *Bovicola ovis*, L2 *Bovicola* sp. (ex alpaca), L3 *Bovicola* sp. (ex goat), F1 flea ex bandicoot, F2 *Ctenocephalides felis*, F3 flea ex wombat, F4 flea ex pig

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