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Bacteria associated with *Haemonchus contortus*

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Abstract

Internal parasitism, a major cause of production losses in sheep, is routinely controlled by anthelmintic drenches, however, alternative control strategies are needed to combat the increasing resistance to these chemicals. A possible novel method of controlling abomasal nematodes, such as *Haemonchus contortus*, is manipulation of their essential resident bacteria, as is currently used to control filarial nematodes. For the first time, bacteria have been identified in the reproductive tract, as well as in the gut, of *H. contortus*, using genetic fingerprinting, light and electron microscopy and fluorescence *in situ* hybridisation (FISH).

PCR-DGGE analysis showed that adult worms had less complex bacterial profiles than did abomasal contents. L3, eggs and adult worms had similar bacterial profiles; 16S rRNA sequences obtained from seven major common DGGE bands were dominated by lactic acid bacterial and Proteobacterial sequences. PCR-DGGE short sequences and clone libraries of nearly full length sequences from all three life-cycle stages contained sequences belonging to *Weissella, Lactococcus, Leuconostoc* and *Streptococcus*. Clone library sequences were used to design group-, class- and species-specific FISH probes to locate bacteria in the parasites.

The gut lumen of adult worms contained a mixed population of Gram-positive and Gram-negative bacteria, which appeared to be multiple morphotypes in TEM images. The FISH probe (EUB338), which targets most bacteria, hybridised with the gut bacteria, but only some of these were targeted by Strc493, which targets most *Streptococcus* sp. and some *Lactococcus* sp. Neither the lactic acid bacterial group- nor the *Weissella* species-specific probes targeted any bacteria in the gut.

A single morphotype of Gram-positive bacteria was seen in large numbers in the distal uterus of female *H. contortus* in the TEM. They were close relatives of either *Lactococcus* sp. or *Streptococcus* sp., as they were targeted by the FISH probe Strc493. These bacteria seemed to be non-pathogenic to the nematodes, as

adult female worms appeared to be healthy (normal in size and active) and carry normal eggs within them. Their roles in worm biology are unknown.

A smaller number of bacteria were seen in the TEM in eggs within female worms. They were closely related to *Weissella confusa,* as all were targeted by lactic acid bacterial group- and *Weissella* species-specific probes, as well as by EUB338. These bacteria were dispersed throughout the eggs, as they could be seen at different focal panels in confocal microscopy. DNA fingerprinting and visualisation of these bacteria in eggs strongly suggest they are maternally transmitted endosymbionts.

As this study was carried out on a parasite strain which has been maintained in the laboratory, practical applications of this research would depend on these bacteria being present in field strains of *H. contortus*.

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A	adenosine
A. aegypti	*Aedes aegypti*
A. albopictus	*Aedes albopictus*
A. cantonensis	*Angiostrongylus cantonensis*
A. stephensi	*Anophleles stephensi*
A. suum	*Ascaris suum*
ATCC	American type culture collection
BLAST	basic local alignment search tool
B. malayi	*Brugia malayi*
B. mucronatus	*Bursaphelenchus mucronatus*
bp	base pair
B. xylophilus	*Bursaphelenchus xylophilus*
C	cytosine
C. elegans	*Caenorhabditis elegans*
CTAB	cetyltrimethylammonium bromide
C. onchophora	*Cooperia onchophora*
Cy3 and Cy5	cyanine
DGGE	denaturing gradient gel electrophoresis
D. immitis	*Dirofilaria immitis*
D. melanogaster	*Drosophila melanogaster*
DNA	deoxyribonucleic acid

dNTP	deoxyribonucleotide triphosphate
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EDTA	ethylenediaminetetraacetic
EPN	entomopathogenic nematodes
FISH	fluorescence *in situ* hybridisation
FITC	fluorescein isothiocyanate
g	gram
g	gravitational force
G	guanidine
G. rostochiensis	*Globodera rostochiensis*
h	hour
H. contortus	*Haemonchus contortus*
H & E	hematoxylin and eosin
H. glycines	*Heterodera glycines*
H. goettingiana	*Heterodera goettingiana*
H. polygyrus	*Heligmosomoides polygyrus*
H. pylori	*Helicobacter pylori*
IJ	infective juvenile
Inc	Incorporated
J2	second-stage juvenile
Kg	kilogram
kV	kilovolt
LAB	lactic acid bacteria
LB	Luria Bertani

L1	first stage larva
L2	second stage larva
L3	third stage larva
L4	fourth stage larva
LM	light microscopy
Ltd	limited
M	molar
mg	milligram
MEGA	molecular evolutionary genetics analysis
min	minute
ml	millilitre
ML	maximum likelihood
mm	millimeter
mM	millimolar
MMIC	Manawatu Microscopy and Imaging Centre
M. punctatissima	*Megacopta punctatissima*
MQ	milli Q
N. brasiliensis	*Nippostrongylus brasiliensis*
NCBI	National Center for Biotechnology Information
N. dubius	*Nematospiroides dubius*
ng	nanogram
NJ	neighbour joining
nm	nanometre
O. ostertagi	*Ostertagia ostertagi*
OTUs	operational taxonomic units

O. volvulus	*Onchocerca volvulus*
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde
RDP	ribosomal database project
RNA	ribonucleic acid
RO	reverse osmosis
r.p.m	revolutions per minute
R. similis	*Radopholus similis*
SDS	sodium dodecyl sulphate
sec	second
sp	species
T	thymidine
TAE	tris-acetate EDTA
Taq	*Thermus aquaticus*
T. circumcincta	*Teladorsagia circumcincta*
T. colubriformis	*Trichostrongylus colubriformis*
TE	tris EDTA
TEM	transmission electron microscopy
TEMED	tetramethylenediamine
TGGE	temperature gradient gel electrophoresis
T. muris	*Trichuris muris*
T. spiralis	*Trichinella spiralis*

U	unit
UV	ultraviolet
[v/v]	volume per volume
[v/v/v]	volume per volume per volume
[w/v]	weight per volume
X. americanum	*Xiphinema americanum*
X. brevicollum	*Xiphinema brevicollum*
X. rivesi	*Xiphinema rivesi*
µg	microgram
µl	microlitre
µM	micromolar
16S rRNA	small subunit ribosomal RNA