ENTEROCOCCUS IN REPTILE FECAL MATERIAL

Karla N. Martinez Valeriano

Microbiology section 2k11

Professor Richard McLaughlin

Enterococcus bacteria are naturally found in the intestinal tract of humans and animals. These bacteria are associated with different types of healthcare associated infections. For example *Enterococcus faecalis* can cause endocarditis, bacteremia, urinary tract infection and meningitis which are all life threatening. The purpose of this experiment was to determine the presence of *Enterococcus* bacteria in the fecal material of 25 reptiles, 1 amphibian and 5 wood turtles as seen in Table 1. Each fecal sample was streaked on CHROMagar™ Orientation agar that differentiates between different bacteria, including *Enterococcus*, via colony color. In additional, a known *Enterococcus* species was streaked as a control. If *Enterococcus* was present in the fecal sample, the colony would be turquoise in color. The results are recorded +/- as shown in Tables 2 and 3. The colonies that most resembled the control plate of *Enterococcus* were streaked on Trypticase Soy Agar (TSA) plates to isolate an individual bacterium for 16S rRNA gene sequencing at Genewiz Inc. 16S rRNA gene sequencing is the gold standard used to identify bacteria. Once the 16S rRNA sequences were obtained, they were submitted to the BLAST database where the 16S rRNA sequences were matched with sequences of other known bacterial species. The results can be seen in Tables 2 and 3. There was no match for *Enterococcus* among the samples sent for sequencing. However, there was a very close relative to the *Enterococcus* and that is the *Aerococcus* which was detected. The BLAST results gave a 99% positive match for either *Aerococcus viridans* or *Aerocossus urinaeequi* (Table 2 #7). Originally, it was planned to sequence the *rho*B gene to discern between the various species of *Enterococcus* isolated. However, since no *Enterococcus* was isolated a different approach was used. Instead to determine which of the two was the correct match, various biochemical tests were performed including; phenol red lactose, that tested for the capacity of the bacteria to use lactose as a carbon and energy source, urea broth that tested for the enzyme urease, Simmon's

Citrate Agar that tested for the enzyme citrate permease, and SIM that tested for motility and hydrogen sulfide production. Two TSA plates were also inoculated with our sample. One plate was grown aerobically in the presence of O_2 and one plate was grown anaerobically in the absence of O_2. The bacteria grew on both TSA plates meaning it is a facultative anaerobe. This is a bacterium which is capable of growing in the presence or the absence of oxygen. All the biochemical test results were negative, as seen in Table 4. The fact that our sample is a facultative anaerobe meant that our sample is the *Aerococcus urinaeequi*. *Aerococcus viridans* is microaerophilic which means it cannot grow in an anaerobic atmosphere and it is also able to use lactose as a carbon and energy source to which our results were negative. What follows is that the *A. urinaeequi* sample and findings were sent to Dr. Michael Gilmore's lab at Harvard University for further studies.

Reptile Samples TABLE 1

	Common Name	Scientific Name	Material
A	Bearded Dragon	*Pogona vitticeps*	Fecal material
B	African Spur Thigh Tortoise	*Geochelone sulcata*	Fecal material
C	Red Footed Tortoise	*Chelonoidis carbonaria*	Fecal material
D	Box Turtle	*Terrapene carolina*	Fecal material
E	Elongated Tortoise	*Indotestudo elongata*	Fecal material
F	Green Basilisk	*Basiliscus plumifrons*	Fecal material
G	Russian Tortoise	*Testudo horsfieldii*	Fecal material
H	Plated Lizard	*Gerrhosaurus major*	Fecal material
I	Greek Tortoise	*Testudo graeca*	Fecal material
J	Standing's Day Gecko	*Phelsuma standingi*	Fecal material
K	Madagascar Giant Day Gecko	*Phelsuma madagascariensis grandis*	Fecal material
L	Knob Tail Gecko	*Nephrurus wheeleri*	Fecal material
M	White Tree Frog*	*Litoria caerulea*	Fecal material
N	Rhino Iguana	*Cyclura cornuta*	Fecal material
O	Asian Water Monitor	*Varanus salvato*	Fecal material
P	Tree viper	*Trimeresurus albolabris*	Shed skin
Q	Snake-neck Turtle	*Chelodina siebenrocki*	Fecal material

R	European Legless Lizard	*Pseudopus apodus*	Fecal material
S	Crested Gecko	*Correlophus ciliatus*	Fecal material
T	Murray River Turtle	*Emydura macquarii*	Fecal material
U	Desert Crocodile	*Crocodylus suchus*	Fecal material
V	Veiled Chameleon	*Chamaeleo calyptratus*	Fecal material
W	Spotted Python	*Antaresia maculosa*	Fecal material
X	Ocelot gecko	*Paroedura pictus*	Fecal material
Y	Graceful Chameleon	*Chamaeleo gracilis*	Fecal material
Z	Eastern Corn Snake	*Elaphe guttata*	Fecal material

*amphibian

Identifying Bacteria TABLE 2

		Common Name	Scientific Name	*Enterococcus* (+/-) CHROMagar agar	BLAST results
1	A	Bearded Dragon	*Pogona vitticeps*	-	
2	B	African Spur Thigh Tortoise	*Geochelone sulcata*	+	*Bacillus aerophilus* (100%)
3	C	Red Footed Tortoise	*Chelonoidis carbonaria*	+	*Bacillus aquimaris* (100%)
4	D	Box Turtle	*Terrapene carolina*	-	
5	E	Elongated Tortoise	*Indotestudo elongata*	-	
6	F	Green Basilisk	*Basiliscus plumifrons*	+	*Lactococcus lactis* (100%)
7	**G**	**Russian Tortoise**	***Testudo horsfieldii***	**+**	***Aerococcus viridans*/*Aerococcus urinaeequi* (99%)**
8	H	Plated Lizard	*Gerrhosaurus major*	-	
9	I	Greek Tortoise	*Testudo graeca*	Nd	
10	J	Standing's Day Gecko	*Phelsuma standingi*	+	*Bacillus cereus* (100%)
11	K	Madagascar Giant Day Gecko	*Phelsuma madagascariensis grandis*	-	
12	L	Knob Tail Gecko	*Nephrurus wheeleri*	+	*Staphylococcus sciuri* (99%)
13	M	White Tree Frog	*Litoria caerulea*	-	
14	N	Rhino Iguana	*Cyclura cornuta*	-	
15	O	Asian Water Monitor	*Varanus salvato*	+	*Citrobacter freundii* (100%)
16	P	Tree viper	*Trimeresurus albolabris*	+	*Staphylococcus*

					saprophyticus (99%)
17	Q	Snake-neck Turtle	*Chelodina siebenrocki*	+	*Citrobacter freundii* (99%)
18	R	European Legless Lizard	*Pseudopus apodus*	+	*Citrobacter amalonaticus* (99%)
19	S	Crested Gecko	*Correlophus ciliatus*	+	*Citrobacter amalonaticus* (100%)
20	T	Murray River Turtle	*Emydura macquarii*	+	sequence not good
21	U	Desert Crocodile	*Crocodylus suchus*	-	
22	V	Veiled Chameleon	*Chamaeleo calyptratus*	+	*Bacillus* sp. (100%)
23	W	Spotted Python	*Antaresia maculosa*	-	
24	X	Ocelot gecko	*Paroedura pictus*	+	*Acinetobacter calcoaceticus* (99%)
25	Y	Graceful Chameleon	*Chamaeleo gracilis*	+	*Staphylococcus* sp. (100%)
26	Z	Eastern Corn Snake	*Elaphe guttata*	-	

Wood Turtle Fecal Samples Collected From the Wild TABLE 3

		Common Name	Scientific Name	*Enterococcus* (+/-) CHROMagar agar	BLAST results
27	A	Wood Turtle	*Glyptemys insculpta*	-	
28	B	Wood Turtle	*Glyptemys insculpta*	-	
29	C	Wood Turtle	*Glyptemys insculpta*	+	*Serratia proteamaculans* (99%)
30	D	Wood Turtle	*Glyptemys insculpta*	-	
31	E	Wood Turtle	*Glyptemys insculpta*	+	*Buttiauxella* sp.

Biochemical Tests TABLE 4

Biochemical test	Sample#7	*Aerococcus viridans*	*Aerococcus urinaeequi*
SIM tube (motility)	-	-	-
Hydrogen sulfide	-	-	-
Simmon's Citrate Agar slant	-	-	-
Urea broth	-	-	-
Phenol Red Lactose tube	-	+	-
Aerobe conditions	+	-	+
Anaerobe conditions	+	-	+