We can't go a year without....

The attempt to gain information about the host associated spirochete, *Cristispira pectinis*.

This time by:

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Introduction:

Cristispira pectinis was first identified in 1882 as a host-associated spirochete which lives in the style organ of bivalves. Since then, studies on its morphology and attempts to culture it have been made in almost every Microbial Diversity course at MBL.

Through numerous attempts, a media was developed that allows for *Cristispira* survival in vitro for up to six days. Numerous cultures have been attempted, however, no actual cultures have survived in lab.

Suggestions have been made as to what *Cristispira* needs from the style (Mayasich and Smucker 1987). It is thought that cellobiose and N-acetyl-D-glucosamine are the main attractants that bring *Cristispira* to the style. It is this hypothesis that I am interested in testing by using chemotaxis assays to cellobiose and GlcNAc.

Also interesting is the transmission of *Cristispira* (CS) between oysters. It is not yet known if CS travels through the environment or through larval stages. Since oysters can be found with styles without CS, is this a lack of vertical transmission in the population, or a lack of horizontal transmission from the environment? I am interested in exploring these questions using DNA methods to analyze community structure and also looking at the relationship of oyster to oyster and also oyster to mussel.

Materials and Methods:

Oysters and mussels can be obtained from the MRC (Marine Resources Center at Marine Biological Laboratory). Oysters, *Crassostrea virginica*, should be shucked as soon as possible to avoid style degradation. To shuck an oyster, it is best to talk with Roxanna Smolowitz D.V.M. on the second floor of the MRC in order to learn oyster anatomy. Then, Falmouth Hardware (on main street by the army-navy store) sells oyster shucking knives and filleting gloves. Oyster shucking knives are small knives with a slightly curved tip for prying the oyster open. A filleting glove is a necessity to avoid long hospital trips.

To open an oyster, insert the curved end of the knife into the dorsal ligament (pointy end of the oyster). Push down on the knife until the oyster pops open. Then slide the knife into the oyster and proceed to separate from the shell the muscle that holds the shells together. Once this muscle is broken, the oyster will open. To find the gut, hold the oyster with the dorsal ligament pointing at you. The gut will be on the right, it is usually seen as a dark object under the surface. Under Dr. Smolowitz's direction, I cut into the gut and then probed with tweezers until the style emerged. Previous attempts were made to just "pop" the style out (Lazer 1995), however, sometimes the style is buried under the gut and tweezers are needed for extraction. Styles, once removed, were placed into 1mL of 2% methylcellulose pH 5.7 with MES (Klappenbach 1998).

Styles were dissolved in the methylcellulose solution and 10ul was observed at 10X magnification under darkfield. CS cells can be seen by their obvious large size and morphology.

Chemotaxis assays were performed with a mini U-tube system. U-tubes were made by bending 1.5 mm capillary tubes. With this smaller system, capillary tube assays were able to be performed using only 40ul of inoculum. Capillary tubes were made with

cellobiose and GlcNAc at concentrations of 1M, 100mM, 10mM, 1mM in methylcellulose and seawater. Assays were observed under the Zeiss "Superscope" at 40X magnification.

DNA was extracted from styles in the methylcellulose solution using the MoBio Soil DNA extraction kit. 16S rDNA was amplified using PCR under standard class conditions. The forward primer used was 8F (universal bacterial). The reverse primer was Spir1400R, made by Dan Buckley to specifically hit spirochetes:

Spir1400R: 5'-ACTCRGRTGGTGTGACGGGC-3'

An annealing temperature of 65°C was needed to use this primer set as spirochete specific, as determined by gradient PCR with *E.coli* DNA as a bacterial DNA. PCR products were sent for sequencing directly. PCR products were also used for ARDRA analysis using the enzyme Rsa I.

Results:

Style Collection:

The first oyster collection yielded 15 styles, 10 of which had CS out of 20 oysters. From 20 mussels, 2 styles were collected, neither of which had CS apparent by microscopy.

The second oyster collection yielded 15 styles from 15 oysters, 12 of which had CS.

These statistics are much higher than past years. The new style extraction technique may be responsible.

Survival in Lab:

Only one style out of the 22 examined had CS that were motile after 6 days. The vast majority of style samples stopped having motile CS within 1 day. The styles with the most dead CS always seemed to contain the highest number of other bacteria in culture, mostly long Cytophaga-like rods and cocci.

Chemotaxis Assays:

The chemotaxis assay was exceedingly difficult to determine definitive results from. In order to use a capillary U-tube assay, a thick layer of cells was needed. However, in the methylcellulose media a thick layer means much obstruction of view. Movies could not be taken of assays since the scope could not catch the motility of CS among the gunk of the methylcellulose.

What was observed by watching cultures when attractant was added is suspect, but follows:

CS seemed to direct swimming towards cellobiose at 100mM and 10mM.

CS swimming did not change towards any concentration of GlcNAc.

Hard data is not available to support these statements, but perhaps better methods would achieve this.

ARDRA results:

As of the last day of class, I am still waiting on sequence results. However ARDRA patterns reveal at least two different ribotypes of CS (see figure 1 and 2). This might be substantiated upon receipt of the 16S sequences.

Discussion:

Cristispira tends to be a magical microbe that draws many a researcher to watch its swimming under the scope. It has remained sort of an enigma, out of our ability to culture. However, with further observation and the inclusion of molecular techniques to observations, we will one day find out what makes this large bug tick.

In this study, chemotaxis assays seemed very promising, but due to the limited time and inoculum available, no hard data was gained. Although it may be considered a failure, at least the determination has been made that chemotaxis studies on *Cristispira* should be done using different methods.

Study of the 16S region of *Cristispira* has only been done in one paper (Paster et al 1996). Further study of 16S variations in spirochetes of bivalve styles may yield some clue as to why the organism lives there and how it is transmitted.

Evidence from this study shows that there are at least two separate ARDRA patterns of *Cristispira* in Woods Hole oysters. When the sequence data arrives, it will hopefully support this observation.

If there are two separate 16S sequences, there is a possibility that the organism we have been viewing since 1882 and judging based on morphology is actually a collection of organisms. It will be interesting to see the outcome, especially if there is a geographic or host specific correlation.

1st OYSTER COLLECTION

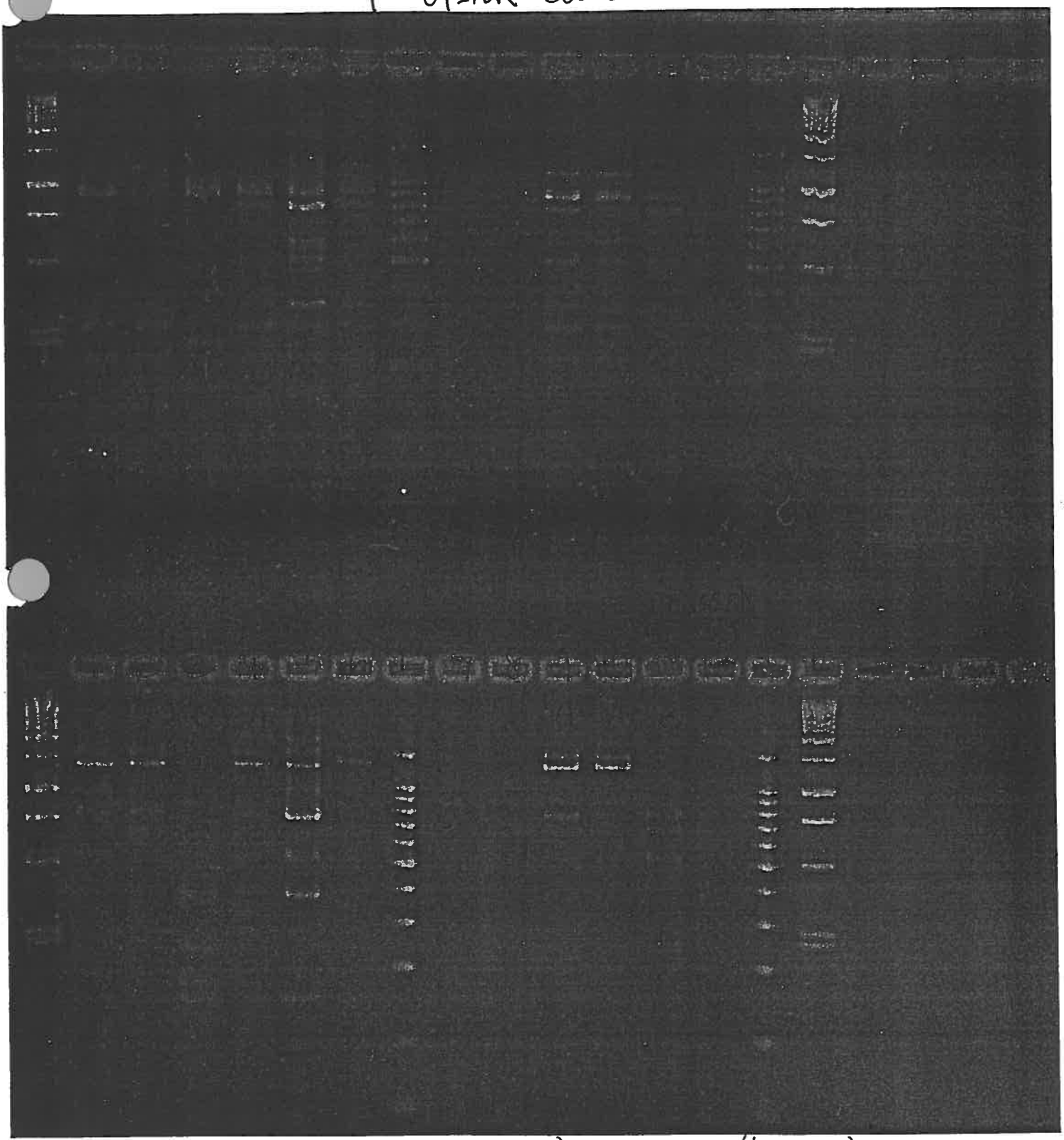

Fig 1: ARDRA with HpaI (top) and RsaI (bottom) lanes 11+12 are mussel styles.

RsaI 2nd OYSTER COLLECTION:

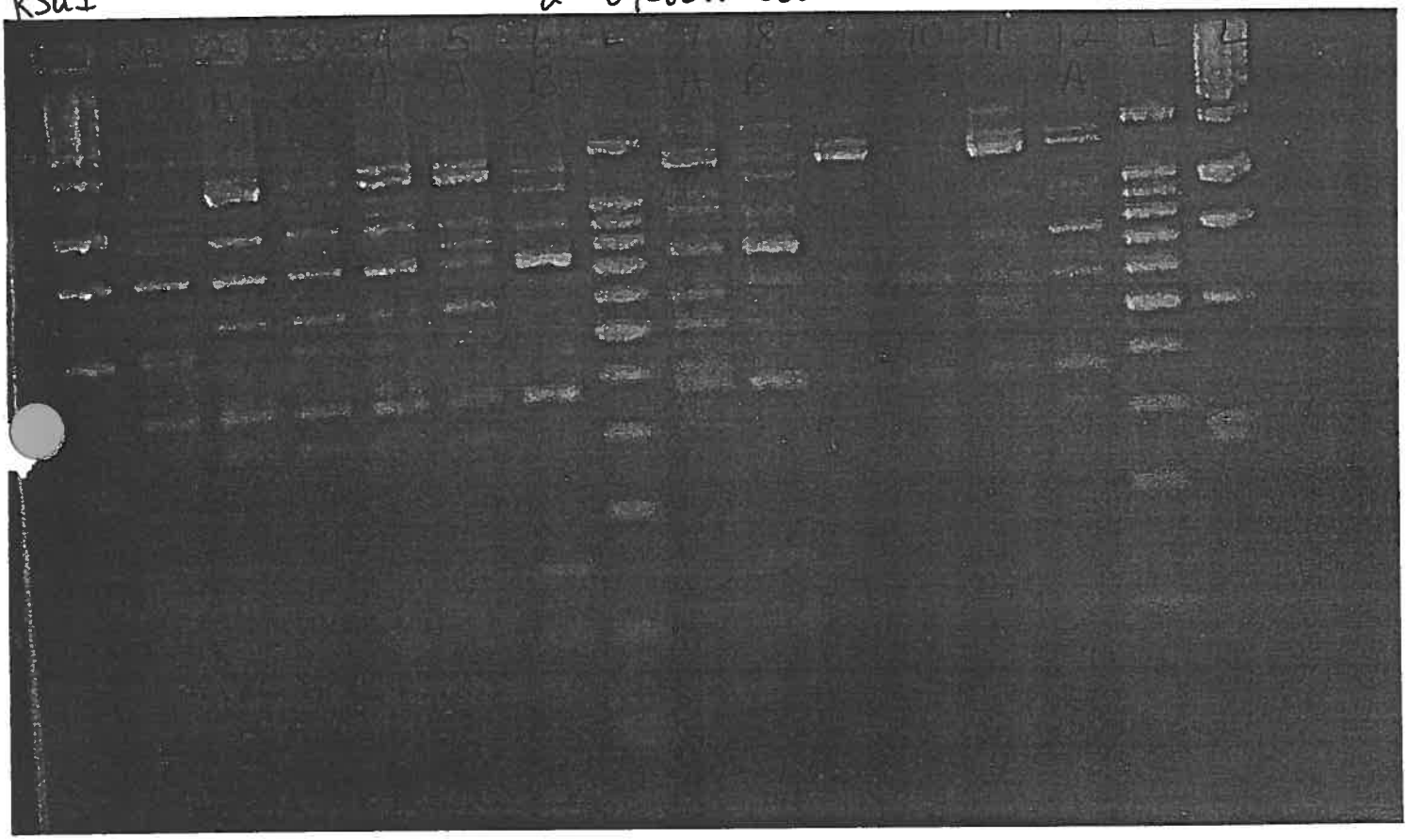

Fig 2: ARDRA with RsaI on 2nd oyster collection.

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