

New insights into the microevolution of two aquatic bacterial pathogens

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STRAIN TYPING:

Key tool for epidemiological outbreak investigations. Multi locus sequencing (MLST) and other established sequence-based typing methods have limited resolution. Typically determine SNP/indel variations in 6-10 genes.

WHOLE GENOME TOTAL SNP COMPARISONS:

Using high throughput sequencing technologies: now possible to identify inter-isolate variations in single nucleotide polymorphisms (SNP's) in thousands of genes at once (Harris, Feil et al. 2010. Science 327, 469).

PROJECT AIMS:

Whole genome sequence more than 90 *R. salmoninarum* and *V. vulnificus* isolates. Determine phylogenetic and evolutionary relationships.

RENIBACTERIUM SALMONINARUM

- Causative agent of Bacterial Kidney Disease in salmonids
- 3.4 Mb; single chromosome
- Highly clonal, intracellular pathogen
- Very difficult to type (limited genetic and phenotypic variation)
- Slow growing diplobacillus (>24h doubling time *in vitro*)
- Transmitted horizontally and vertically



Rainbow trout (*Oncorhynchus mykiss*) with clinical BKD

72 strains sequenced

- Isolated between 1960 and 2009
- From different hosts: farmed rainbow trout, Atlantic salmon (wild and farmed), brown trout, Pacific salmon spp.
- From different geographic regions: UK, Norway, W & E Coast Canada & US

VIBRIO VULNIFICUS

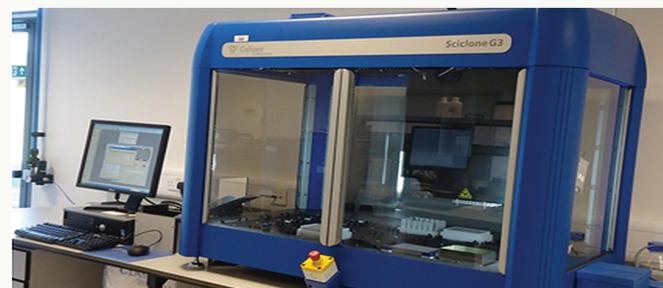
- Causes severe disease in both humans and fish
- Two chromosomes (circa 3.4, 1.8 MB)
- Component of estuarine/ euryhaline environments
- High intraspecific genetic and phenotypic variation. 3 biotypes (BT) and several serovars.
- Subset of strains (BT 2) can cause clinical disease in humans and fish (eels)



V. vulnificus wound infection
Ruppert et al. (2004)

24 strains sequenced

- Isolated between 1980 and 2005
- From different sources: diseased humans, diseased fish healthy fish, oysters, sediments, water.
- From different geographic regions: Spain, France, USA, Israel, South Korea, Australia, Taiwan and Denmark



Liquid handling robot used to construct all 96 isolate DNA libraries at a single time

METHOD OVERVIEW

DNA extracted separately from all 96 isolates. Sent to sequencing centre (TGAC, Norwich). DNA from each isolate sheared, tagged ('bar coded') and pooled prior to sequencing

96 isolates sequenced on equivalent of 1 x full run of Illumina HiSeq 2000 (12 isolates pooled for each lane of 8 lane flow cell).

Average coverage: > 50x per isolate (paired-end data, average read length 100nt)

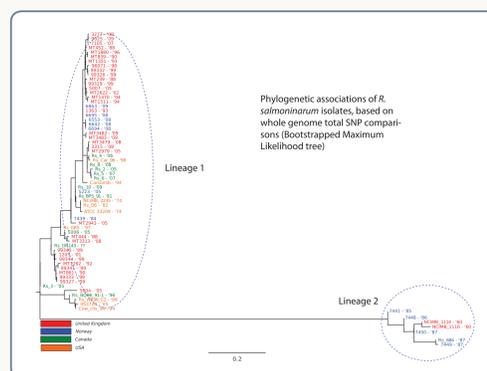
Compared reads to published genomes to identify SNPs in conserved genes across two datasets

Cost: approx £200 per isolate

Demonstrated possible to sequence > 150 isolates on one run of HiSeq 2000 in future projects

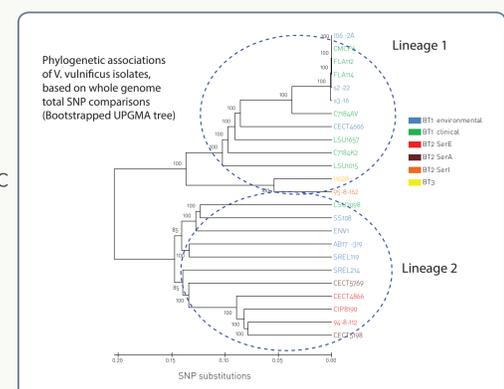
RESULTS: R. SALMONINARUM

- Only 3802 SNPs identified in *R. salmoninarum* core genome (all isolates > 99.8% similar). Further evidence that pathogen is highly clonal
- Evidence for two distinct subpopulations
 - Lineage 1: related to *R. salmoninarum* Type strain ATCC 33239 and other isolates from North American West Coast Pacific salmon (*Onchorhynchus*) spp.
 - Lineage 2: related to two isolates from wild Atlantic salmon from the River Dee, Scotland 1960 (Isolates NCIMB 1114 and NCIMB 1116)
- *R. salmoninarum* co-evolved separately with Pacific (*Onchorhynchus* genus) and W Atlantic (*Salmo* genus)?
- Movements of rainbow trout ova from US in 19th and 20th century may have transferred Lineage 1 into N. European salmonid (rainbow trout and Atlantic salmon) culture ?
- Early Norwegian farmed Atlantic salmon outbreaks caused by Lineage 2 isolates: possible infection from wild Atlantic salmon reservoirs?



RESULTS: V. VULNIFICUS

- Approx 400000 SNPs identified (in both chromosomes). Further evidence of high species heterogeneity
- SNP analysis divides the species in two populations
 - Lineage 1: variety of BT 1 isolates (independent of origin), BT 2 serovar I, and BT 3
 - Lineage 2: Variety of BT 1 isolates, BT 2 serovar A & E isolates.
- Chromosome analysis: also divides species in to two populations. Similar division to SNP analysis, except: all BT2 and 3 isolates grouped within the same group
- Further confirmation likely polyphyletic species origins
- Different biotype 2 serovars likely emerged from different BT 1 lineages
- BT3 isolate 11028 most similar to BT2 serovar I isolate 95-8-162
- BT2 serovar I: eel pathogens, endemic in Denmark; BT3: human pathogens, endemic in Israel. Do the two groups have a common evolutionary origin?



SUMMARY

- Whole genome SNP typing: very powerful technique. Provided unique insight into the evolutionary relationships of both species
- Likely to replace MLST and other typing methods: much higher resolution, allowing better determination of likely origins and spread of pathogens at microscale
- Time taken and ease of sample preparation/ data analysis: now major bottle necks to routine adoption, rather than cost

Acknowledgements

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