Does a DNA barcoding gap exist in bioactive marine bacteria?

Evidence from analyses of 16S rRNA gene sequence data of pigmented pseudoalteromonads

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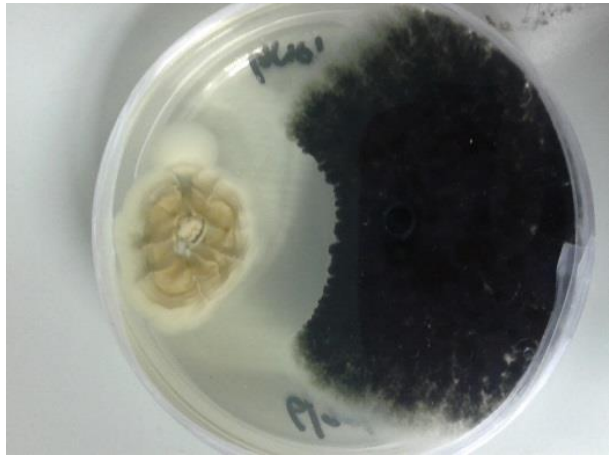
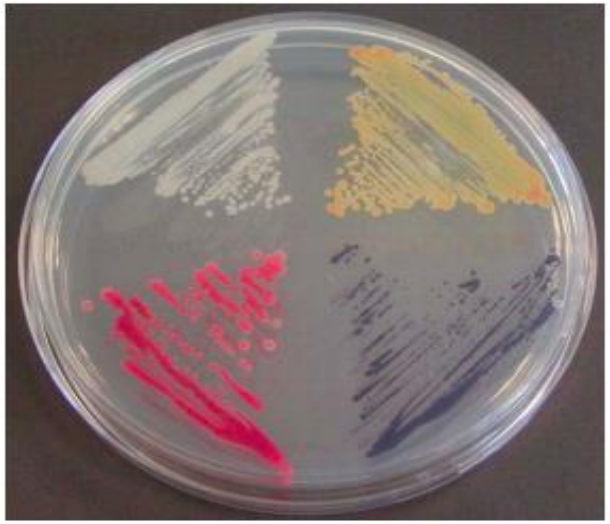
Background of the Study

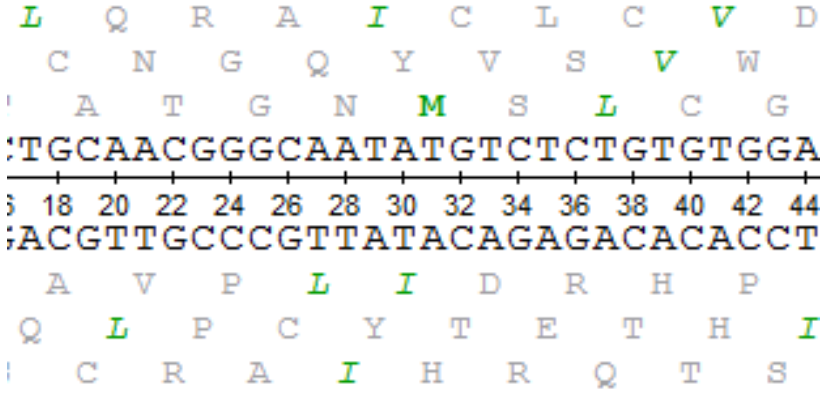




***Pseudoalteromonas* spp.**

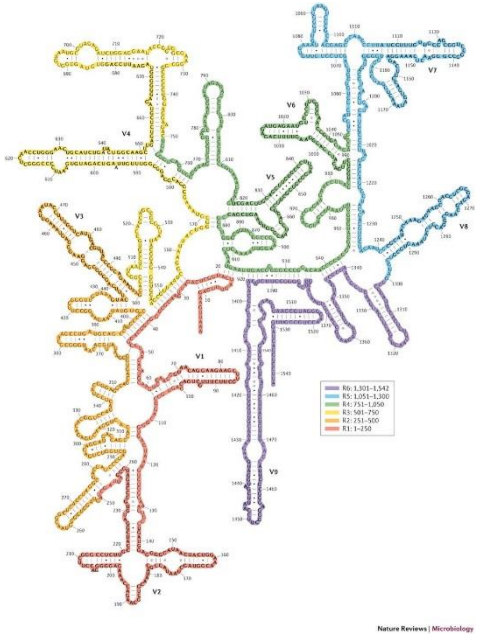
- Marine, Gram -, Rod-shaped
- Non-pigmented & Pigmented
  - ✓ Anti-biofilm activity
  - ✓ Bactericidal activity
  - ✓ Quorum sensing inhibition

Background of the Study



16s rRNA gene sequencing

- OTU determination
- Taxonomic assignment
- Phylogeny reconstruction
  - ✓ 3% sequence dissimilarity
    - Limited evidence of suitability to pigmented pseudoalteromonads



Research Objectives

1. Compare sequence divergence of pigmented *Pseudoalteromonas* species included in this *in silico* analysis;

2. Construct 16S rRNA gene trees to confirm and support the clustering of species that have been previously reported; and

3. Determine the presence of a DNA barcoding gap in pseudoalteromonads' 16S rRNA gene sequences.

Methodology

Data sources	***Data treatment and analysis***	***Gene tree construction***	***Gap determination***
• GenBank® • 195 partial sequences • 1000-1500 bp • 2013-2017	• Sequence alignment • Optimal model selection (*AIC) • Test for data set saturation (*Xia test et al., 2003)	• Maximum Likelihood • Maximum Parsimony • 1000 replicates as bootstrap support	• Sequence divergence • Frequency distribution of distances • Barcoding gap determination

Results

1. Alignment of 16S rRNA gene sequences, test for saturation, and optimal DNA substitution model

2. 16S rRNA gene sequence divergences among pigmented pseudoalteromonads

3. Gene trees of pigmented pseudoalteromonads

4. Absence of DNA barcoding gap in bioactive pseudoalteromonads

Results 1

- **195** strains and isolates of pigmented pseudoalteromonads belonging to **13** species were included in the analyses.
  - The length of the aligned sequences was **1342** nucleotides.
  - Index of substitution saturation for 32 OTUs was significantly smaller than those for completely symmetrical and asymmetrical trees **(0.041).**
  - Optimal DNA substitution model chosen for the dataset is **TPM2uf+G.**

Results 2

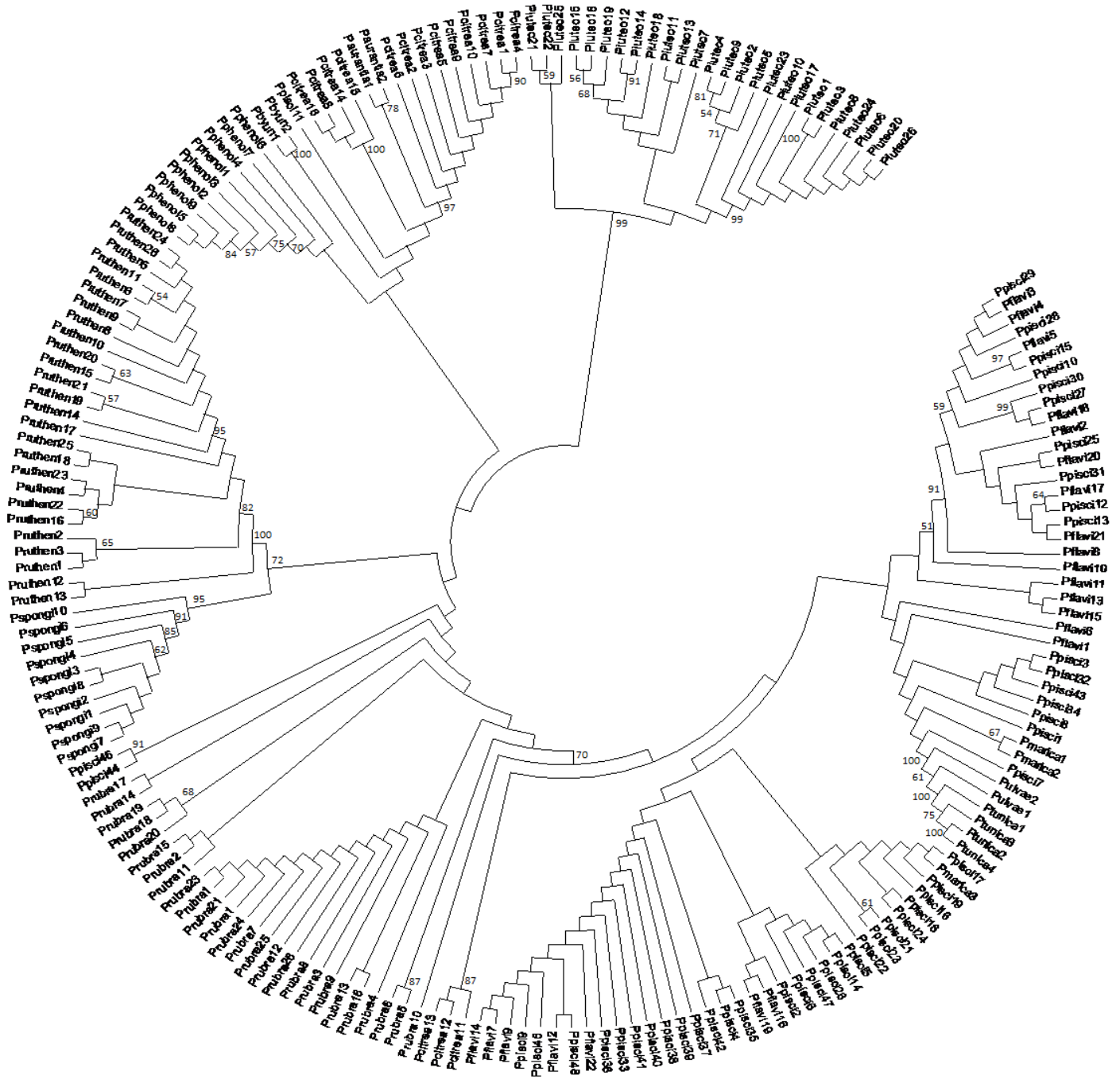
- Sequence divergences using average uncorrected distances of individuals within the same species were found to be less than **1% (0.98%)** compared to genetic distances of individuals from different species, which were at **3.54%.**
- Intraspecific genetic distances ranged from **0 to 0.140** while interspecific genetic distances ranged from **0 to 0.168.**

Results 3

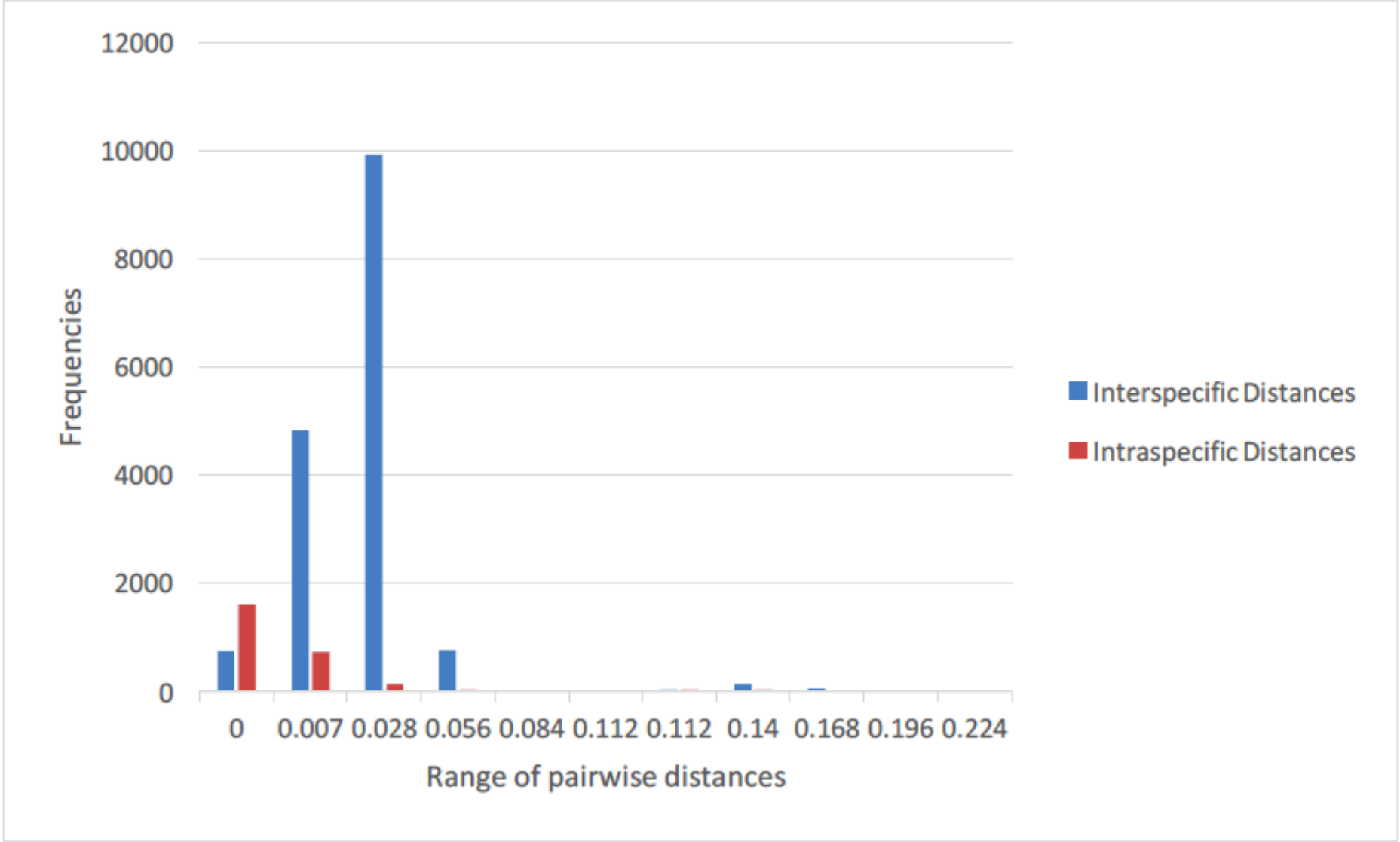
Maximum Parsimony gene tree of pigmented pseudoalteromonads based on the 16s rRNA gene

Results 3

Maximum Likelihood gene tree of pigmented pseudoalteromonads based on the 16s rRNA gene



Results 4


12000
10000
8000
6000
4000
2000
0
Frequencies
0
0.007
0.028
0.056
0.084
0.112
0.112
0.14
0.168
0.196
0.224
Range of pairwise distances
Interspecific Distances
Intraspecific Distances

Conclusions and Recommendation

- Use of 16S rRNA gene sequence data alone in identifying potentially bioactive marine prokaryotes is not sufficient
  - Intra- and interspecific sequence divergences are small
  - Individuals from different species clustered in MP and ML trees (n=8/13)
  - No DNA barcoding gap in the16S rRNA gene was detected
- Recommend use of multiple genes in taxonomic identification and phylogeny reconstruction of potentially bioactive marine prokaryotes such as pseudoalteromonads