

BRIEF REPORT

ENVIRONMENTAL MICROBIOLOGY



Identification of putative coral pathogens in endangered Caribbean staghorn coral using machine learning

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Abstract

Coral diseases contribute to the rapid decline in coral reefs worldwide, and yet coral bacterial pathogens have proved difficult to identify because 16S rRNA gene surveys typically identify tens to hundreds of disease-associated bacteria as putative pathogens. An example is white band disease (WBD), which has killed up to 95% of the now-endangered Caribbean *Acropora* corals since 1979, yet the pathogen is still unknown. The 16S rRNA gene surveys have identified hundreds of WBD-associated bacterial amplicon sequencing variants (ASVs) from at least nine bacterial families with little consensus across studies. We conducted a multi-year, multi-site 16S rRNA gene sequencing comparison of 269 healthy and 143 WBD-infected *Acropora cervicornis* and used machine learning modelling to accurately predict disease outcomes and identify the top ASVs contributing to disease. Our ensemble ML models accurately predicted disease with greater than 97% accuracy and identified 19 disease-associated ASVs and five healthy-associated ASVs that were consistently differentially abundant across sampling periods. Using a tank-based transmission experiment, we tested whether the 19 disease-associated ASVs met the assumption of a pathogen and identified two pathogenic candidate ASVs—ASV25 *Cysteiniphilum litorale* and ASV8 *Vibrio* sp. to target for future isolation, cultivation, and confirmation of Henle-Koch's postulate via transmission assays.

INTRODUCTION

The global rise in coral disease epizootics associated with human-induced climate change has caused unprecedented coral declines (Bruno et al., 2007; Burge et al., 2014; Harvell et al., 1999), especially in the greater Caribbean where white band disease (WBD) has killed up to 95% of the now-endangered *Acropora* corals since 1979 (Aronson & Precht, 2001; Gladfelter, 1982), and stony coral tissue loss disease (SCTLD) is currently causing die-offs in more than 21 common coral species (Alvarez-Filip et al., 2022; Precht et al., 2016). Despite the devastating impacts of coral diseases, specific coral pathogens have been identified in only five of the 20 or more described coral

diseases (Sutherland et al., 2004). Coral bacterial pathogens have proved difficult to identify because culture-independent, genetic analyses typically identify hundreds of disease-associated amplicon sequencing variants (ASVs)/operational taxonomic units (OTUs) as candidate pathogens (e.g., Gignoux-Wolfsohn & Vollmer, 2015) coupled with difficulties culturing these putative coral pathogens to fulfil Henle-Koch's postulate in controlled transmission experiments. Large numbers of disease-associated bacteria have led to the emerging view that many coral diseases are caused by a dysbiosis between the coral host, its symbiotic algae and its associated microbiome in stressed or compromised corals (Voolstra et al., 2024), even for host-specific coral diseases with clear transmission dynamics like

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