

TITLE: Homoscleromorpha-derived *Bacillus* spp. as potential sources of biotechnologically-relevant hydrolases and biosurfactants.

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Abstract

Marine sponges are sessile and benthic organisms that have developed efficient ecological interactions with microorganisms. Homoscleromorpha is the most recently described class of the phylum Porifera and light has been shed just lately about its microbiology. Despite hydrolytic exoenzymes and biosurfactants having been gradually reported from the poriferan microbiome, biotechnological exploitation of microorganisms inhabiting Homoscleromorpha sponges remains scarce. Thereby, this study aimed to identify and characterize proteases, cellulases, lipases and biosurfactants produced by bacteria isolated from three shallow-water homoscleromorph species, *Oscarella* sp., *Plakina cyanorosea*, and *Plakina cabofriense* collected at the coast of Cabo Frio, RJ - Brazil. A total of 99 of 107 sponge-associated bacterial isolates exhibited activity for at least one of the analyzed hydrolases. Following fermentation in Luria-Bertani (LB) and Tryptic Soy Broth (TSB), two isolates, 80BH11 and 80B1:1010b, showed higher lipase and peptidase activities. Both of them belonged to the *Bacillus* genus and were isolated from *Oscarella* sp.. Central composite design leveraged up the peptidase activity in 280% by *Bacillus* sp. 80BH11 in the TSB medium for 48 h at 30 °C. The optimized model also revealed that pH 6.5 and 45 °C were the best conditions for peptidase reaction. In addition, the production of biosurfactants and/or bioemulsifiers was evaluated by six Homoscleromorpha-derived strains in both LB and TSB fermentation media. *Bacillus* sp. 80BH11 was able to release highly emulsifying and remarkable stable surfactants in the LB medium. Moreover, this bacterium was able to reduce water surface tension from 72 mN/m to 32.51 mN/m in TSB medium and 30.7 mN/m in LB medium. Surfactin was finally unveiled as the biosurfactant released by this *Oscarella*-derived *Bacillus*. Conclusively, we hope to have set the scenery for further prospecting of industrial enzymes and biosurfactants in Homoscleromorpha microbiomes.

Keywords: lipase, *Oscarella*, peptidase, sponge-associated bacteria, surfactin.

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