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Saccharopolyspora sp. NFXS83 in Marine Biotechnological Applications: From Microalgae Growth Promotion to the Production of Secondary Metabolites †

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Abstract: Marine bacteria are a significant source of bioactive compounds for various biotechnological applications. Among these, actinomycetes have been found to produce a wide range of secondary metabolites of interest. *Saccharopolyspora* is one of the genera of actinomycetes that has been recognized as a potential source of these compounds. This study reports the characterization and genomic analysis of *Saccharopolyspora* sp. NFXS83, a marine bacterium isolated from seawater from the Sado estuary in Portugal. The NFXS83 strain produced multiple functional and stable extracellular enzymes under high-salt conditions, showed the ability to synthesize auxins such as indole-3-acetic acid (IAA), and produced diffusible secondary metabolites capable of inhibiting the growth of *Staphylococcus aureus*. Furthermore, when *Phaeodactylum tricornutum* was co-cultivated with strain NFXS83 a significant increase in microalgae cell count, cell size, cell-fluorescence, and fucoxanthin content was observed. Detailed analysis revealed the presence of clusters involved in the production of various secondary metabolites, including extracellular enzymes, antimicrobial compounds, terpenes, and carotenoids in the genome of strain NFXS83. Ultimately, these findings indicate that *Saccharopolyspora* sp. NFXS83 has a significant potential for a wide range of marine biotechnological applications.

Keywords: *Saccharopolyspora*; marine; microalgae; secondary metabolites; biotechnology

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1. Introduction

Marine environments harbor diverse and rich bacterial populations, which play key roles in several aspects of marine ecology and global nutrient cycles [1]. Amongst marine microorganisms, actinobacteria are of special interest due to their relevant impacts in marine ecosystems and increased ability to synthesize a wide variety of secondary metabolites and bioactive compounds of biotechnological interest, including antibiotics, anti-tumoral agents, pigments, and enzymes [2–5]. These bacteria play a role in the cycling of nutrients in marine ecosystems and can interact with other marine organisms in a wide range of trophic interactions. For example, some actinobacteria have been shown to produce compounds that inhibit the growth of harmful algae, potentially affecting their abundance and distribution in the ocean [6]. On the other hand, some actinobacteria producing antimicrobial compounds may have a mutualistic relationship with marine organisms such as corals, protecting these organisms from pathogens and other predators [7].

The stressful nature of marine environments (e.g., high salinity, fluctuating temperatures and light intensity, low nutrient concentrations, competition) greatly impacts their associated microorganisms, including marine actinobacteria. This leads to strong adaptations (genetic and phenotypic) to stress conditions by these microorganisms, favoring the biosynthesis of unique bioactive compounds [8]. Recent studies have revealed the biotechnological properties of some marine actinobacteria [5], however, due to their increased genetic and phenotypic diversity much of their biotechnological potential is still untapped.

Saccharopolyspora are gram-positive, aerobic, non-motile actinobacteria and are largely distributed throughout the terrestrial and marine environments with half of their species described as halotolerant [9]. Moreover, *Saccharopolyspora* are one among the various genera of actinomycetes recognized as a potential source of novel bioactive compounds [9].

In this work, the marine actinomycete *Saccharopolyspora* sp. NFXS83, a bacterium isolated from the seawater surface in the Sado estuary, Portugal, is characterized in detail and its genome sequence is analyzed and discussed. The results obtained herein bring new insights into the role of *Saccharopolyspora* in marine environments and their potential for use in a wide range of biotechnological applications, including the ability to promote microalgae growth and accumulation of valuable compounds, and the production of several secondary metabolites of relevance.

2. Materials and Methods

2.1. Isolation and identification of the strain NFXS83

Strain NFXS83 was isolated from surface seawater of the Sado estuary, Portugal, in June 2021. Bacterial identification was made by 16S rRNA gene sequencing.

2.2. Characterization and biotechnological potential of Saccharopolyspora sp. NFXS83

2.2.1. Production of extracellular lytic enzymes

Strain NFXS83 was cultured in spots in marine basal solid media supplemented with different substrates (cellulose, chitin, starch, alginate, pectin, skimmed milk, tributyrin, olive oil). The enzymatic activities were assessed through the measurement of the degradation halos formed.

2.2.2. Synthesis of indolic compounds

Strain NFXS83's ability to produce the indolic compounds IAA (indole-3-acetic acid), IPA (indole-3-propionic acid) and IBA (indole-3-butyric acid) from tryptophan was analyzed through a colorimetric assay with Salkowski's reagent, as described by Glickman and Dessaux [10].

2.2.3. Screening for antimicrobial activity

For this screening, NFXS83 was placed in four distinct spots in Luria Agar. Three incubation days later, a culture of *Staphylococcus aureus* was streaked around the NFXS83 spots. Upon growth, the inhibition halo was measured.

2.2.4. Microalgae (*Phaeodactylum tricornutum* CCAP 1055/1) growth promotion assay

The microalga *Phaeodactylum tricornutum* CCAP 1055/1 was co-cultured with NFXS83 in 6-well plates for 10 days and analyzed by flow cytometry and microscopy. Fucoxanthin content was quantified by HPLC.

2.3. Saccharopolyspora sp. NFXS83 genome sequencing and analysis

Bacterial DNA sequencing was performed using Illumina Novaseq. Assembly was performed using Spades v.3.15.2 [11]. The NCBI Prokaryotic Genome Annotation Pipeline [12] was used for strain NFXS83 genome annotation.

The functional genome annotation was conducted using BlastKOALA [13] and BLASTp [14] searches against the UNIPROT database [15] performed in the Geneious Prime software [16]. Genes encoding carbohydrate active enzymes were predicted using the dbCAN2 webserver [17]. GH and other lytic protein domains were predicted using the InterProScan tool [18] also in the Geneious Prime software. Proteolytic enzymes were predicted using BLASTp searches against the MEROPS Peptidase Database [19] in the Geneious Prime software. Secondary metabolite production genes/clusters were predicted using antiSMASH bacterial version v.6.0 [20].

3. Results and Discussion

3.1. Characterization in vitro of Saccharopolyspora sp. NFXS83

3.1.1. Production of extracellular lytic enzymes

The strain NFXS83 was able to degrade the eight different substrates tested (**Figure 1**), indicating that the lytic enzymes produced by this bacterium are extracellular, functional, and stable under high salt conditions. These characteristics are of great interest for biotechnological applications. For example, Chakraborty and colleagues [21] showed that the marine *Saccharopolyspora* sp. A9 produced an extracellular α-amylase that was stable in the presence of wide range of NaCl concentrations and of laboratory surfactants, detergents, and oxidants, thus presenting novel properties that could lead to applications in detergent, food and other industrial processes involving high salt concentrations.

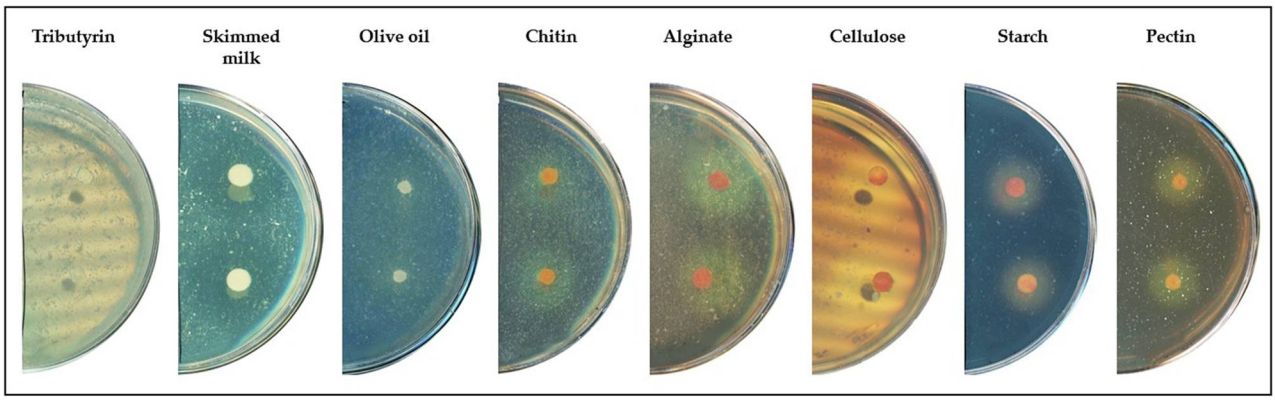

Figure 1. Lytic enzymatic activities observed for *Saccharopolyspora* sp. NFXS83 when cultivated in marine basal medium supplemented with different substrates.

3.1.2. Biosynthesis of indole-3-acetic acid (IAA) and other indolic compounds

Saccharopolyspora sp. NFXS83 presented the ability to synthesize IAA (6.25 ± 0.21 μg/mL), IBA (62.12 ± 6.35 μg/mL) and IPA (40.95 ± 3.89 μg/mL) from tryptophan, suggesting that this strain may influence auxin levels in marine environments/organisms.

3.1.3. Antimicrobial activity of *Saccharopolyspora* sp. NFXS83

The antimicrobial activity of the strain NFXS83 against *S. aureus* was confirmed by the visualization of an inhibition zone surrounding the NFXS83 colony spots (**Figure 2**).

These results indicate that strain NFXS83 produces diffusible secondary metabolites, such as antibiotics, capable of inhibiting the growth of *S. aureus*.

Figure 2. Antimicrobial activity of *Saccharopolyspora* sp. NFXS83 against *Staphylococcus aureus* ATCC 6538 in solid media.

3.2. Saccharopolyspora sp. NFXS83 promoted the growth of microalgae

The co-cultivation assay showed that the inoculation of *P. tricornutum* CCAP 1055/1 with *Saccharopolyspora* sp. NFXS83 led to an increase of the microalgae cell count, red fluorescence, size and fucoxanthin accumulation when compared to the microalgae cultivated under axenic conditions (**Figure 3**). These results suggest that this strain produces substances that benefit microalgae development, such as the previously detected IAA.

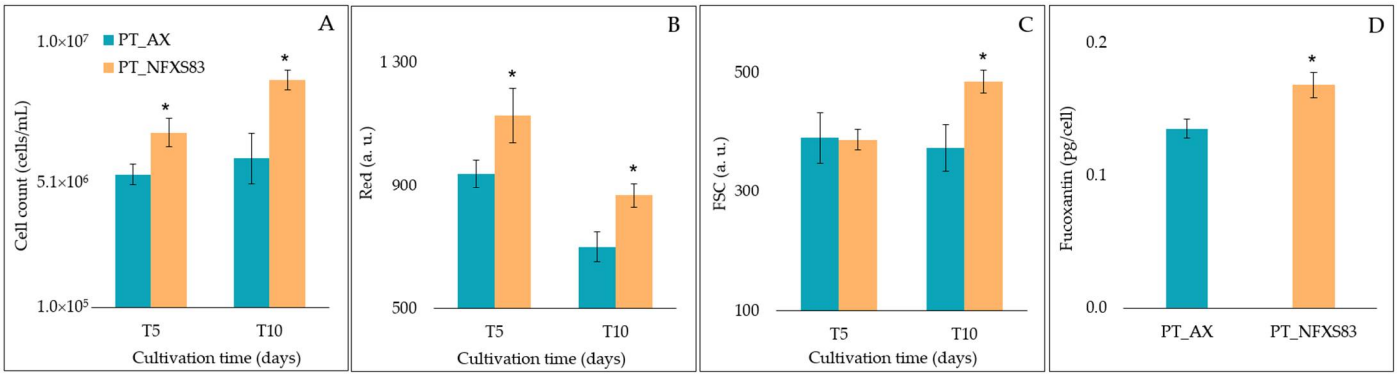

Figure 3. Culture dynamics of *Phaeodactylum tricornutum* cultivated under axenic conditions (PT_AX) and in co-cultivation with *Saccharopolyspora* sp. NFXS83 (PT_ NFXS83). (A) *P. tricornutum* cell count (cells/mL); (B) auto-fluorescence (Red); (C) front scatter (FSC); (D) fucoxanthin content (pg per cell) for T10. *Statistically significant ($p < 0.05$).

3.3. Characterization in silico of Saccharopolyspora sp. NFXS83

3.3.1. Genes encoding extracellular lytic enzymes

Genome mining revealed the presence of genes encoding enzymes capable of breaking down chitin, starch, cellulose, alginate, and pectin. Additionally, the genome of NFXS83 also contained three genes encoding lysozymes and several lipases, esterases, proteases and peptidases containing signal-peptide sequences.

3.3.2. Gene clusters involved in the production of secondary metabolites

Multiple biosynthetic gene clusters (BGCs) involved in secondary metabolites production were discovered in the genome sequence of *Saccharopolyspora* sp. NFXS83,

including several antimicrobial compounds, such as erythromycin, kiamycin and ε-poly-L-lysine, as well as ectoine, terpenes and carotenoids.

3.3.3. Genes involved in the production of microalgae-growth promotion compounds

Genomic analysis revealed the presence of multiple genes that could be involved in the beneficial interactions between *Saccharopolyspora* sp. NFXS83 and microalgae, such as genes involved in the production of IAA and of several vitamins and co-factors, including pantothenate (vitamin B5), biotin (vitamin B7), tetrahydrofolate (vitamin B9) and cobalamin (vitamin B12).

4. Conclusions

This study provides an in-depth analysis of *Saccharopolyspora* sp. NFXS83 and its biotechnological potential. The strain produced extracellular lytic enzymes functional and stable under high salt conditions, presented antimicrobial activity and promoted the growth and pigment accumulation of *Phaeodactylum tricornutum*. The genomic analysis of the strain revealed the presence of several unique genes involved in lytic enzymes production, various gene clusters involved in the production of secondary metabolites (e.g., antimicrobial compounds, terpenes, and carotenoids) of great interest, as wells as genes involved in the biosynthesis of microalgae growth promoting compounds, further reinforcing the marine biotechnological potential of this strain. Overall, this study lays the foundation for future studies exploring *Saccharopolyspora* sp. NFXS83 applications in various marine biotechnological processes.

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Data Availability Statement: The genome of strain NFXS83 is available in the NCBI database under the accession number JAPFGB000000000.1.

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Conflicts of Interest: The authors declare no conflict of interest.

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