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Uncovering the Antibiofilm Potential of Moroccan Lichen-Associated *Actinomycetota* Against *Streptococcus Pneumoniae:* Isolation and Characterization Insights

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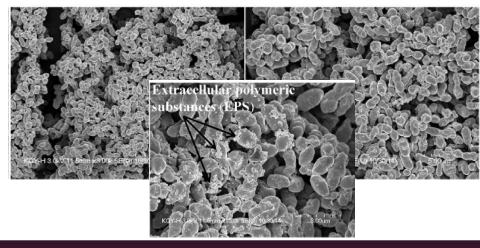
Introduction

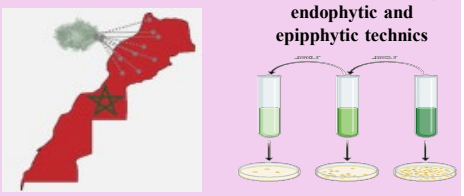
Streptococcus pneumoniae is a common commensal as well as a serious worldwide pathogenic bacterium in children. Its ability to form biofilms enhances its resistance to many first-line antibiotic treatments underscoring the urgent need for new effective metabolites. Lichen-associaetd *Actinomycetota*, known for producing potential secondary metabolites, has been shown to possess effective antibiofilm formation activity. This study investigated the antibiofilm activity of lichens associated *Actinomycetota* isolates. These isolates were screened against three *S. pneumoniae* strains obtained from the nasopharynx of healthy children (≤5years), with distinct serotypes and resistance profiles.



Methods

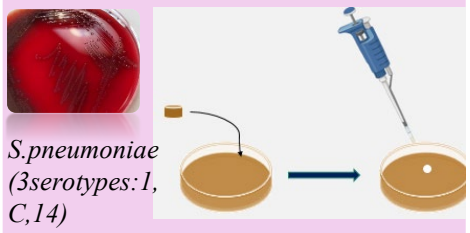
1-Isolation of actinomycetota

I- Lichens sampling from 9 Moroccan ecosystems → II- Isolation of lichen associated Actinomycetota using endophytic and epipphytic technics



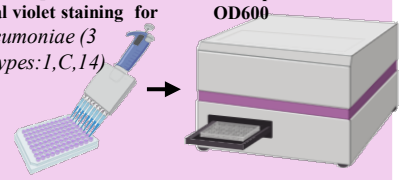
2-Screening vs *S.pneumoniae*

Antibacterial screening using agar disc and well methods against *Streptococcus pneumoniae*



3- Biofilm production

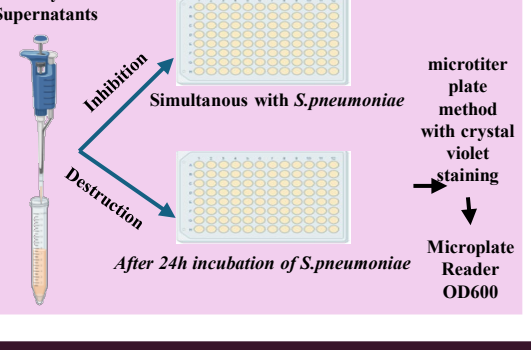
microtiter plate method with crystal violet staining for *S.pneumoniae (3 serotypes:1,C,14)* → Microplate reader OD600



OD value	Biofilm production
<0.120	Non- biofilm producer
0.120-0.240	Moderate biofilm producer
>0.240	Strong biofilm producer

3- Antibiofilm assay

Actinomycetota Supernatants



Results

Sampling and isolation results

Table 1 : Sampled lichens and their stations

Sample	Lichen source	Sampling position
L1	Xanthoria sp	High atlas, Toufliht
L2	Heterodermia sp	
L3	Ramalina sp	
L4	Pseudovernia furfuracea	
L7	Non identified sp	
L5	Ramalina lacera	High atlas, Essaouira road
L8	Seirophora lacunosa	
L6	Xanthoria sp	High atlas, Ourika road
L9	Xanthoria sp	Rif, El houceima town
Total	41	

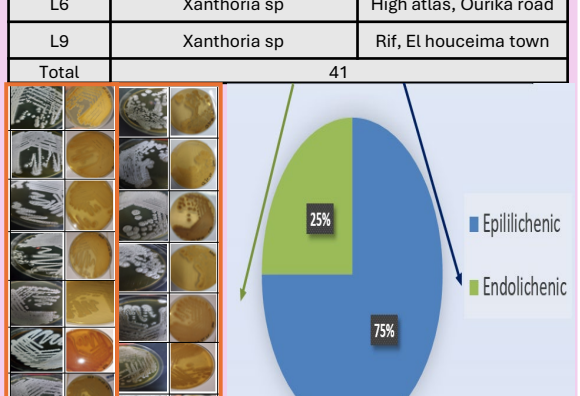
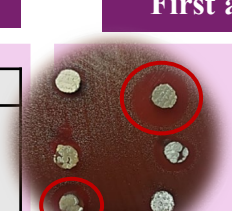


Figure 1 : pourcentage of endolichenic and epilechnic isolates

First and second screening results



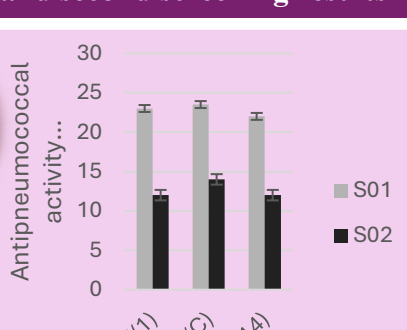


Figure 2 : Antipneumococcal activity using Disc diffusion method

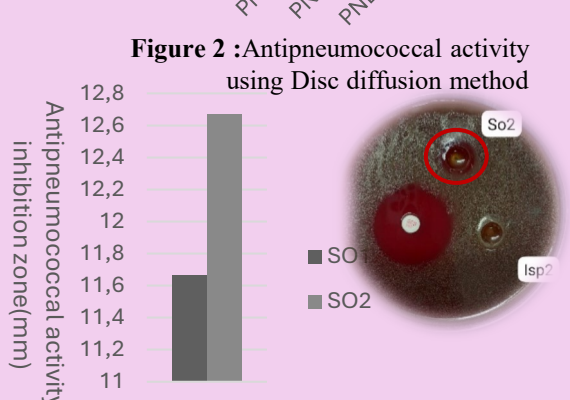
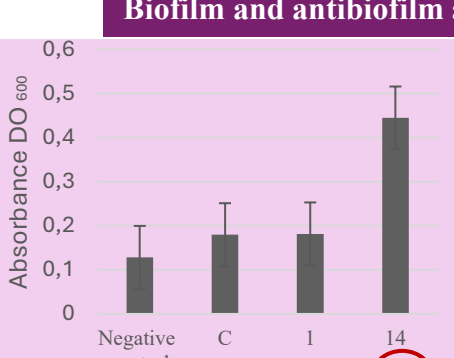


Figure 3 : Antipneumococcal activity using Disc diffusion method

Biofilm and antibiofilm assay results



S.pneumoniae serotype 14 strain was a strong biofilm producer with a OD_{600} of 0.286 whereas stereotype C and 1 had OD_{600} values below 0.120 classifying them as non-biofilm producers

Figure 4 : Biofilm production assay

The two *Actinomycetota* isolates designated SO1 and SO2 demonstrated activity in both inhibition and destructing biofilm formation with antibiofilm efficacy decreasing from inhibition (98.90% and 72.04%) to destruction (91.3% to 64.5%).

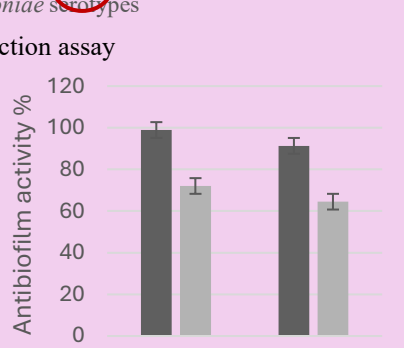


Figure 5 : The antibiofilm activity pourcentage of bioactive actinomycetota

Conclusion

These findings highlight the potential of lichen associated *Actinomycetota* isolated from underexplored Moroccan ecosystems as a valuable source of bioactive metabolites for medical applications, offering potential solutions to combat multi-drug resistance of biofilm-forming *S.pneumoniae* strains. Further molecular analysis are underway to visualise the biofilm formation and destruction under Scanning Electron Microscopy (SEM) and to identify the promising strains.