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Stenotrophomonas maltophilia virulence and phenotypic properties of clinical relevance

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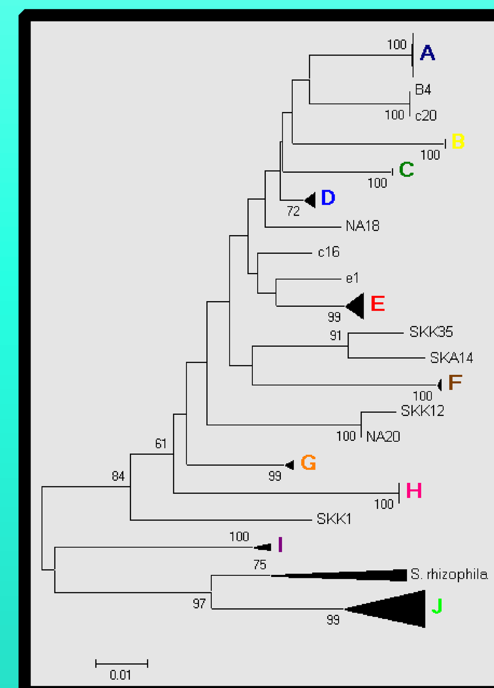
Introduction

Stenotrophomonas maltophilia is a genetically highly versatile bacterial species, belonging to the γ-β subclass of *Proteobacteria.* It is ubiquitously distributed in the environment but recently gaining evidence as causal agent of nosocomial infections [1]. According to their *gyrB* gene sequences the *S. maltophilia* isolates could be classified into ten different genomic subgroups (*gyrB* gene phylogenetic tree as seen below). To have a look at the *S. maltophilia* physiological properties, we used 51 isolates of clinical and environmental origin representative for the genetic groups A-J, and some isolates belonging to none of the groups. Phenotypic properties of clinical relevance were tested, thereby major focus lied on the establishment of an assay with *Dictyostelium discoideum* and *Acanthamoeba castellanii* as host organisms to measure bacterial virulence.

Conclusions

Stenotrophomonas maltophilia was shown to be a highly versatile species, on a physiologic level. Most phenotypic properties could neither be ascribed to the appearance in clinical or environmental surroundings nor to their genetic subgroups. We were able to show that **virulence in an amoeba model could be correlated to their genetic groups.** Genetic groups including respiratory tract isolates from cystic fibrosis patients thereby tend to show no virulence to the amoebae.

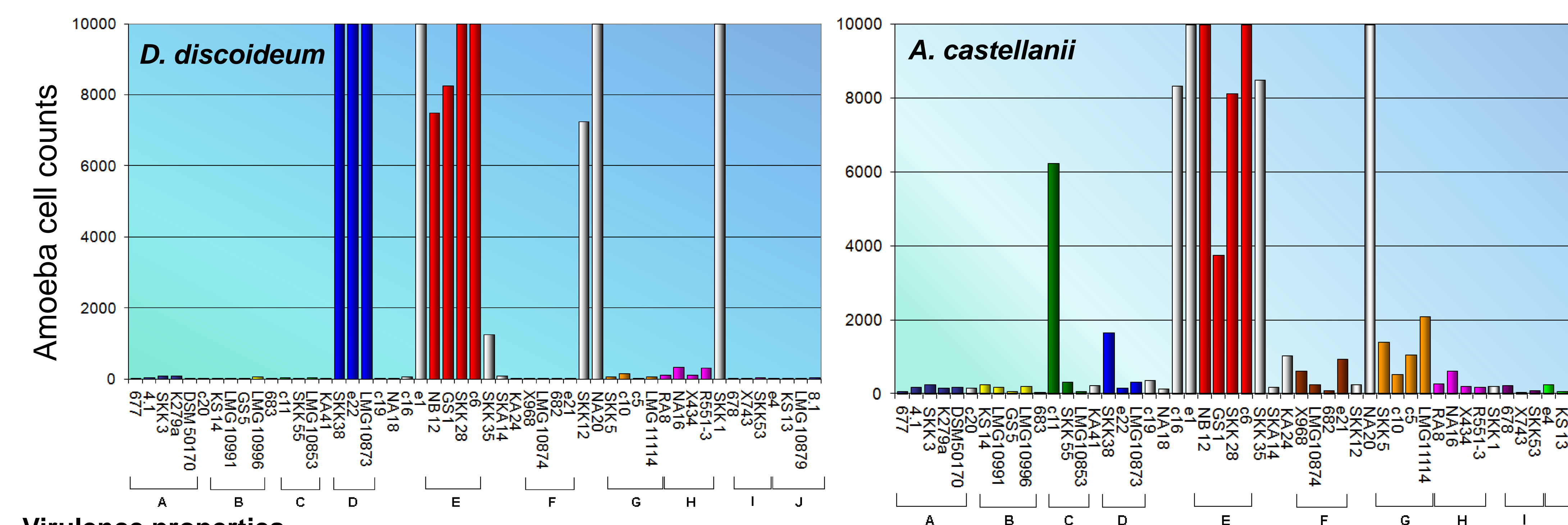


Neighbour Joining tree of partial *gyrB* sequences

Virulence assayed by amoebae as host organisms

Plate killing assay

In order to assess bacterial virulence the amoebae *Dictyostelium discoideum* and *Acanthamoeba castellanii* were used as model organisms in a plate killing assay. 5 µl droplets containing defined numbers of amoebae (10.000, 5.000, 2.500,…,5) were spotted on a bacterial lawn. Plates were incubated at 30°C for 3 days with *A. castellanii*, and for 5 days at 22,5°C with *D. discoideum.* After that, bacterial virulence was measured as the count of amoebae necessary to form a plaque on the bacterial lawn.

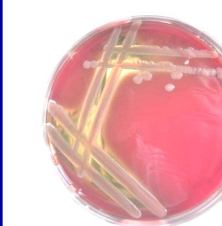


Virulence properties

The high virulence for **all group E isolates** and **for some single strains** was demonstrated in both amoeba models. However, for some isolates, like SKK12, and SKK1 and all isolates from group D, virulence occurred only with *D. discoideum* but not with *A. castellanii.* Vice versa, some strains showed virulence properties only with *A. castellanii*, namely strains c11 and group G strains. Groups A, B, C, F, H, I, and J were non-virulent, or contained only one or two isolates of low virulence. These results suggested that some groups have an increased pathogenic potential compared to others.

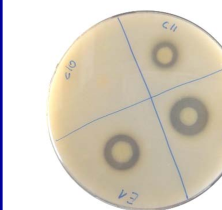
The amoeba model is still insufficient to elucidate all pathogenicity pathways for *S. maltophilia.* A lot of clinical strains, known to have already caused infections in humans, were characterized as non-virulent for the amoebae. For example, the pathogenicity of *S. maltophilia* strain K279a, which was isolated from an infected patient, was previously proved in a nematode model [2].

Exotoxins/Exoenzymes



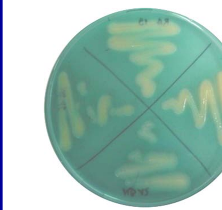
Hemolysin production was determined on sheep blood agar plates. Bacterial strains were incubated for 72 to 120 hours at 30°C . A clear zone around the bacterial colonies indicated production of hemolysins.

Hemolysis of erythrocytes was observed for 45 of the 51 tested isolates. Five strains that did not produce hemolysins originated from infected patients. Thereby, no specific genetic group or isolation site was preferred.



Protease production on skim milk containing Muller-Hinton agar was determined as clearing zone around a bacterial colony after 48 and 72 hours.

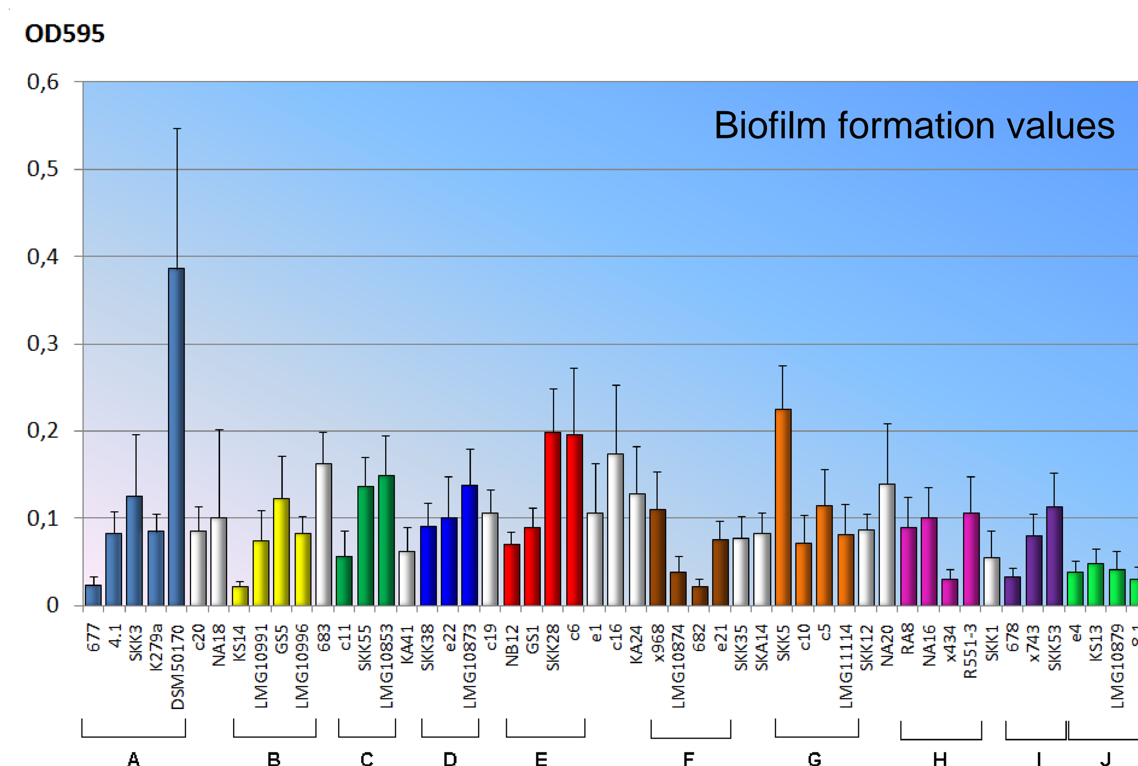
Of the 51 tested strains 48 showed proteolytic activities, three of them only little proteolytic activity. No specific preference for proteolysis and genetic grouping was observed.



Siderophore production was determined by decolourization of a CAS (chrome-azurole-S)-iron-complex on agar plates. Bacteria were grown over night at 30°C on nutrient agar supplemented with CAS and Fe(II).

For all tested strains of *S. maltophilia* decolourization due to siderophore production could be observed. Hence, siderophore production is not a useful factor to virulence determination and/or phenotypic differentiation of *S. maltophilia* strains.

Biofilm formation and Motility



Biofilm formation was monitored after 24 hours growth at 30°C in polystyrene microtiter plates. The optical density was measured at 595 nm of the crystal violet stained biofilm diluted 1:10 in 70% ethanol.

Biofilm formation values varied between 0.023 and 0.386. 40% of the strains were able to form a moderate or strong biofilm with values above 0.100. 38% were weak biofilm formers and 22% did not form any biofilm.

The measured values **did not correlate with** the previously defined **genetic groups**.

Cell motility was tested as **twitching and swimming motility** on agar plates according to Bonaventura *et al.*, 2007 [3]. The mean of at least three experiments was determined for each strain. 17 out of 51 strains showed no twitching motility, the other isolates showed a twitching range from 3.2 to 9.5 mm in diameter after 24 hours at 30°C.
Of the tested strains,15 showed no swimming motility. For 36 isolates swimming motility ranged from 5.3 to 20.1 in diameter after incubation at 30°C for 24 hours.
A correlation between swimming and twitching motility could be observed (Pearson's r=0.609, p<0.0001).
Motilities did not correlate with either the ecotype or the biofilm formation potential.

Antibiotic resistance

Antibiotic resistance (MIC= minimum inhibitory concentration) to Vancomycin, Gentamicin, Tetracycline, Norfloxacine, and Co-trimoxazole was determined for the 51 *S. maltophilia* isolates as described by the CLSI standards for broth microdilution [4].
All tested strains displayed a very **high resistance** towards **Vancomycin**, with MIC values from 32 to >512 µg/ml.
Most *S. maltophilia* strains (43 isolates) were resistant to Gentamicin with MIC values ranging from 32 to > 512 µg/ml, eight strains had MICs of 8 µg/ml and below.
Differences in the resistance profiles were shown for **Tetracycline.** 41 strains showed MICs of 16 to 32 µg/ml, seven strains had MICs of 8 µg/ml or lower, while two strains had a MIC of 64 µg/ml.
Differentiated resistance profiles were obtained for **Norfloxacine**, 25 strains had MICs in the intermediate range of 16 to 32 µg/ml, for five strains the MIC was 64 µg/ml and 21 strains were susceptible to Norfloxacine.
The only antibiotic combination tested for which overall susceptibility could be observed was **Co-trimoxazole**. **All strains tested were susceptible** with MICs of ≤0,125/2,375 µg/ml. For only two strains a MIC of 2/38 µg/ml and 0,5/9,5 µg/ml was obtained.

Antibiotic resistances were quite alike for almost all *S. maltophilia* strains, independent of the isolation source, the potential for biofilm formation or virulence potential.

Literature

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