Karlsruhe Institute of Technology

Institute of Functional Interfaces
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Phenotypic properties of *S. maltophilia* isolates in relation to their genetic subgroups

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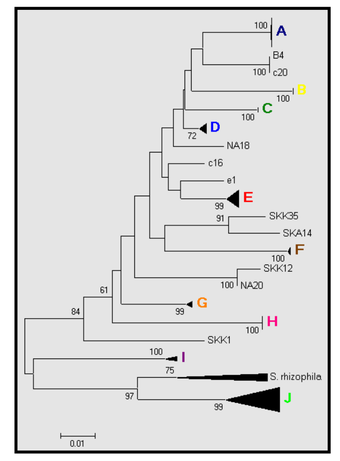
Virulence assayed by amoebae as host organisms

Introduction

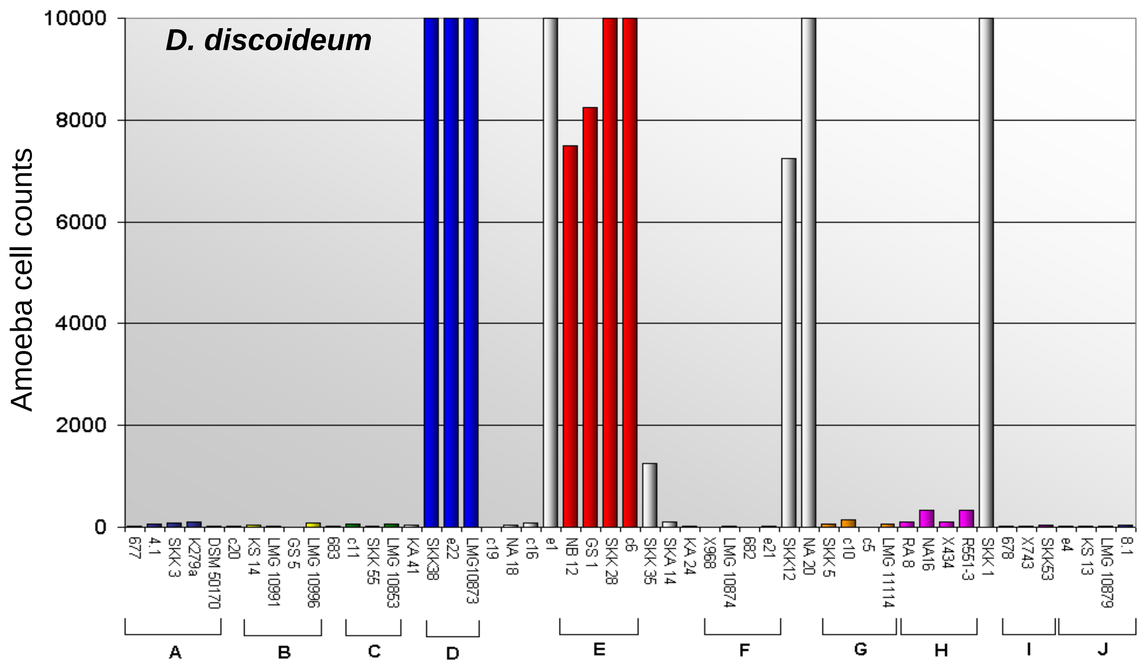
Stenotrophomonas maltophilia is a highly versatile bacterial species, belonging to the γ-β subclass of *Proteobacteria*. It is ubiquitously distributed in the environment but recently gaining evidence as causal agent of nosocomial infections [1]. According to their *gyrB* gene sequences the *S. maltophilia* isolates could be classified into ten different genomic subgroups (*gyrB* gene phylogenetic tree as seen on the left). 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.

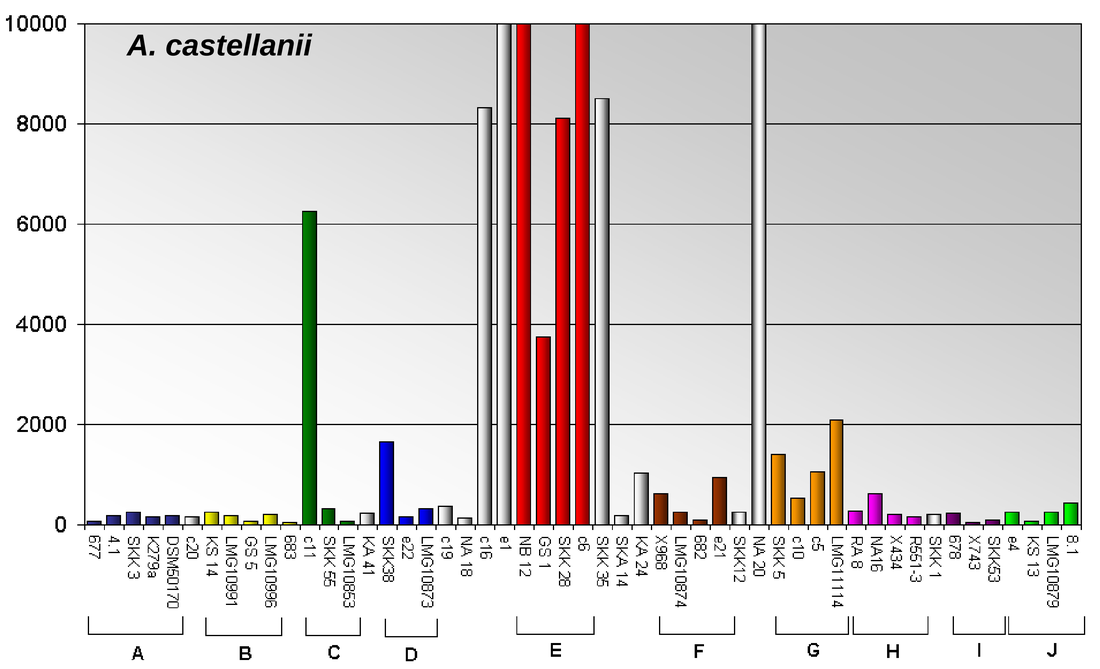


Neighbour Joining tree of partial *gyrB* sequences

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 amoeba necessary to form a plaque on the bacterial lawn.





Virulence properties

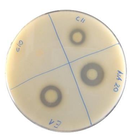
The high virulence for **all group E isolates** and **for some single strains** was demonstrated in both amoeba models. However, for some isolates, like , SKK12, and SKK1 and all isolates from group D, virulence occurred only with *D. discoideum* but not with *A. castellanii.* Vice versa, some strains 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].

Phenotypic properties compared to genetic groups or virulence

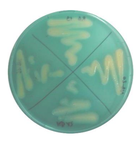
Hemolysin production was determined on sheep blood agar plates. Bacterial strains were incubated for 72 to 120 hours at 30 °C . A clear zone around the bacterial colonies indicated production of hemolysins.

Haemolysis 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 Müller-Hinton agar was determined as clearing zone around a bacterial colony after 48 and 72 hours.

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



Siderophore production was determinded 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 FeII.

For all tested strains of *S. maltophlia* 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.

Conclusions

Three factors described to contribute to virulence of bacteria were tested for *Stenotrophomonas maltophilia*. Most strains, independent of its clinical or environmental origin, were able to produce hemolysins, proteases, and siderophores, but none of these factors correlated with virulence properties tested for amoeba or genetic subgroups described earlier.

Acknowledgements

We thank A. Becker, E. Kniel, A. Wolf, M. Dow, A. van der Haström for kindly providing bacterial strains. Amoebae were provided by R. Gräf and J. Overhage. This research was funded by the German Research Council (DFG)

Literature

[1] Looney *et al. Lancet Infect Dis* (2009) 9 pp. 312–23
[2] Fouhy *et al.* (2007*) J. Bacteriol* 189 (13) pp. 4964 - 68

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