

Analysis of the degree of genetic diversity of *Aspergillus fumigatus* strains isolated in the area of a municipal landfill site

Krzysztof Frączek, Dariusz Ropek

Department of Microbiology and Biomonitoring, University of Agriculture in Krakow, Poland



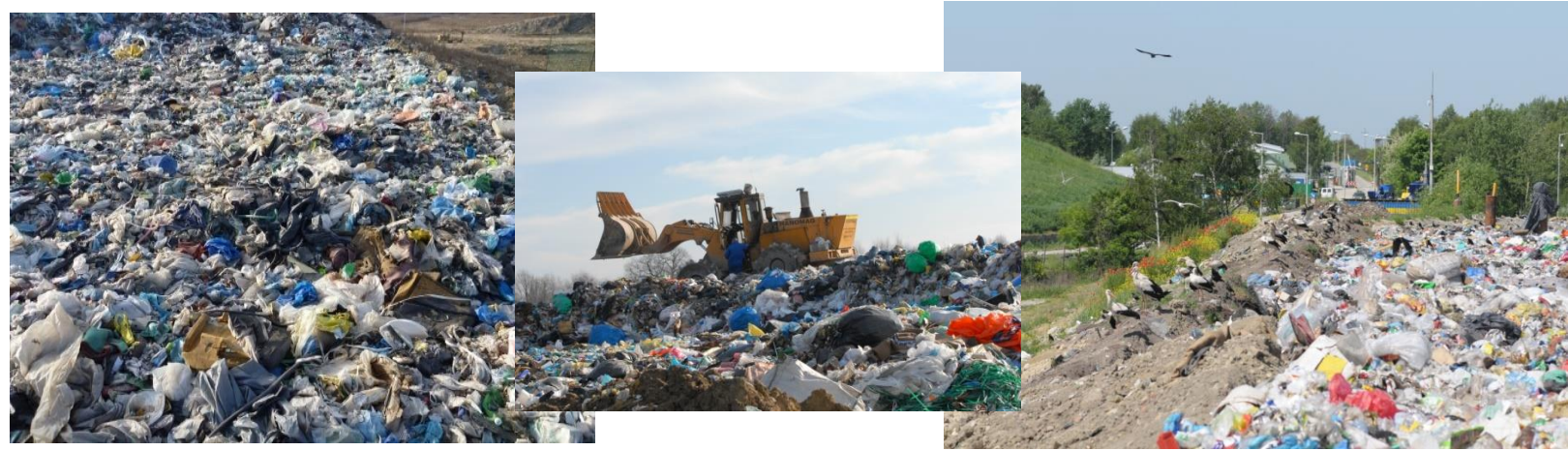
INTRODUCTION

The process of collecting municipal waste in landfills increases environmental pollution and thus has a harmful effect on human health, caused by the effect of microorganisms spreading in the form of bioaerosols. Municipal waste landfills are also a place of permanent or temporary residence of many organisms (birds, insects, rodents), and thus they become environmental vectors of pathogenic bacteria and fungi, allowing them to colonize the environment in nearby areas. In order to select an indicator of the colonization of the environment around the municipal landfill site, strains of fungus *Aspergillus fumigatus* were selected for testing from the reservoir of microorganisms. This selection was based on the fact that this species most frequently colonises the landfill waste and that insects and birds act as environmental vectors of this microorganism.



THE AIM OF THE STUDY

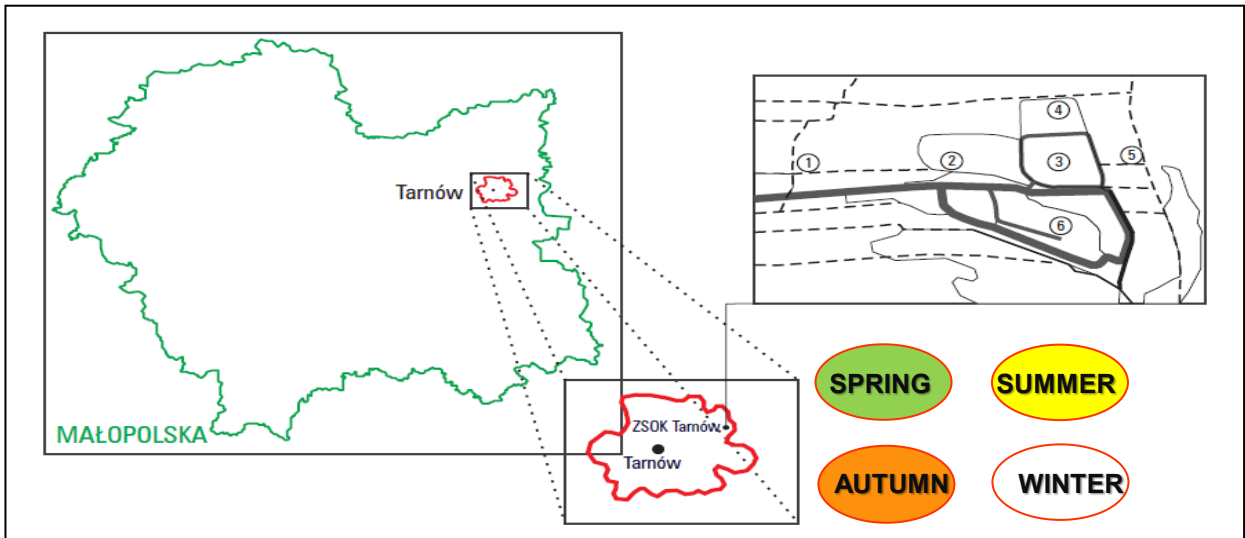
The aim of the study was to select a marker of fungal colonization of the environment around the municipal waste landfill and the workers employed in its operation.



AREA OF MUNICIPAL LANDFILL SITE

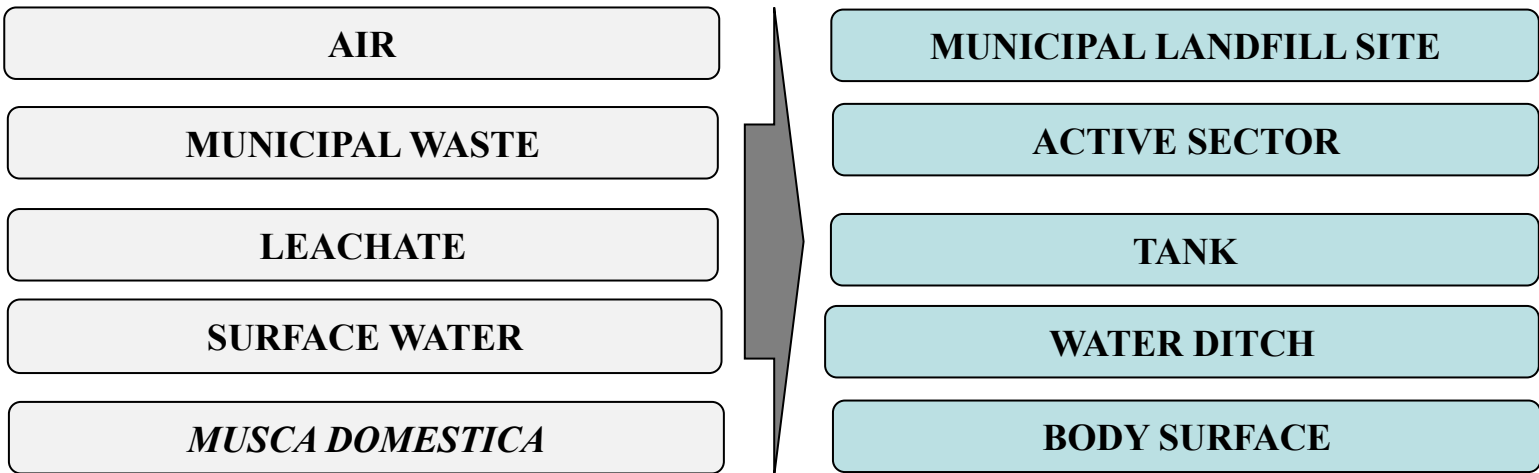
MATERIALS AND METHODS

The strains were isolated from air samples, the deposited wastes, and surface and leachate waters, as well as from the body surface of a housefly. Genetic differentiation of the isolated fungal strains was performed using two genetic typing methods: RAPD and PCR-MP.



Location of measurement points at the municipal landfill site

Sample collection points for strain isolation of *Aspergillus fumigatus*



RESULTS

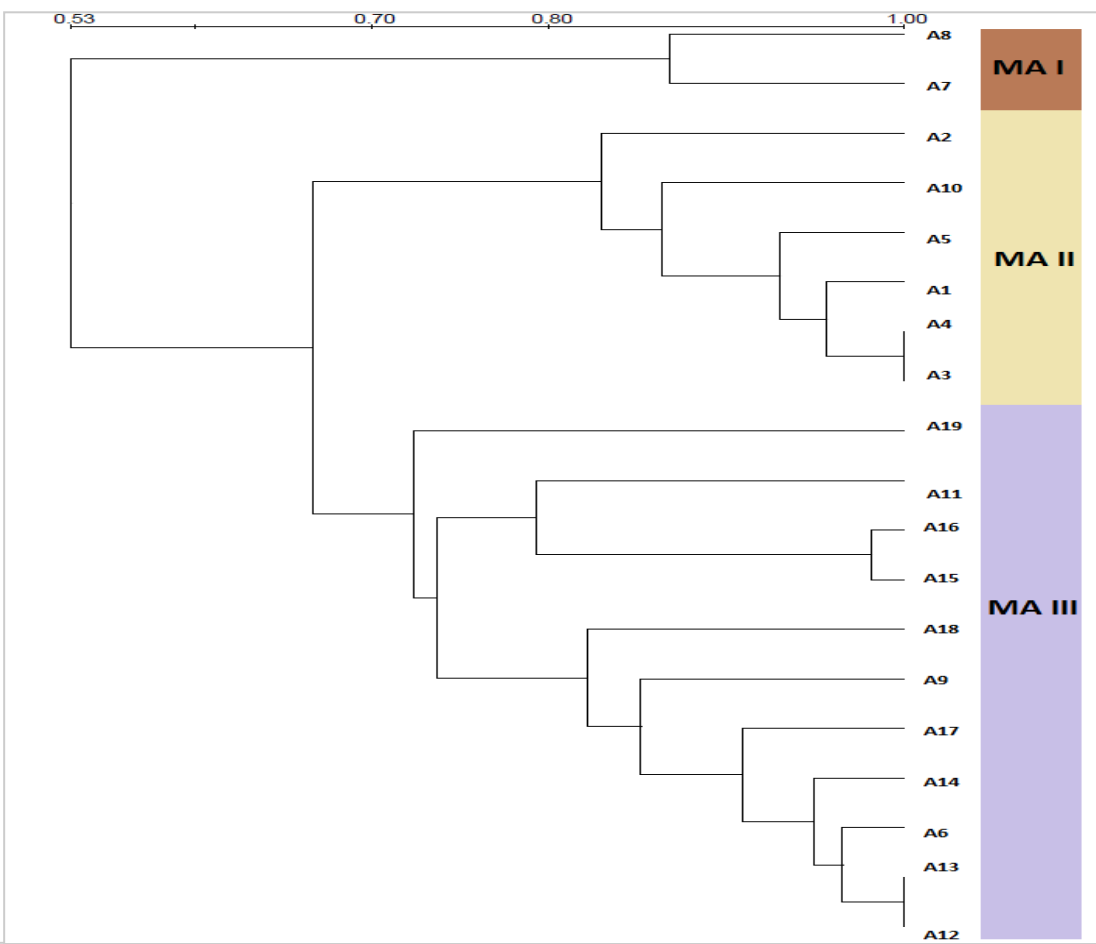


Fig. 1. Dendrogram of genetic similarity of *A. fumigatus* strains determined using the Dice coefficient and cluster analysis method based on the PCR-MP genotyping results

The overall genetic similarity of the tested strains was 53%. Based on the statistical analysis of genetic similarity, three genotypic groups were distinguished: MA I, MA II and MA III (similarity level min. 72%). Within the genotypic groups, the level of similarity between different genotypes did not exceed 90.9%. In the tested group, 10 clonally dependent strains (with similarity level $\geq 92\%$) were identified, with two different genotypes: A1/A3/A4/A5 ($\geq 93\%$) and A15/A16 (98.2%).

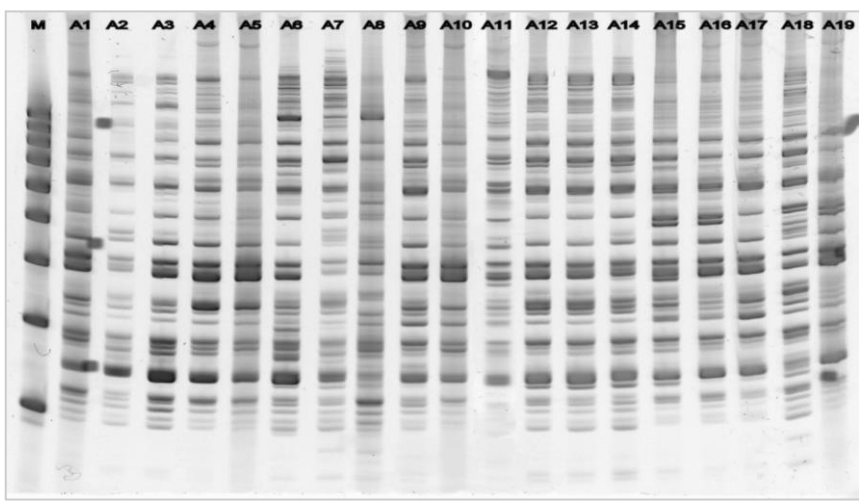


Fig. 2. The results of *A. fumigatus* strains' genotyping using the PCR-MP technique. M – marker IDEAL (visible bands: 3653, 2555, 1827, 1241, 900, 700 bp)

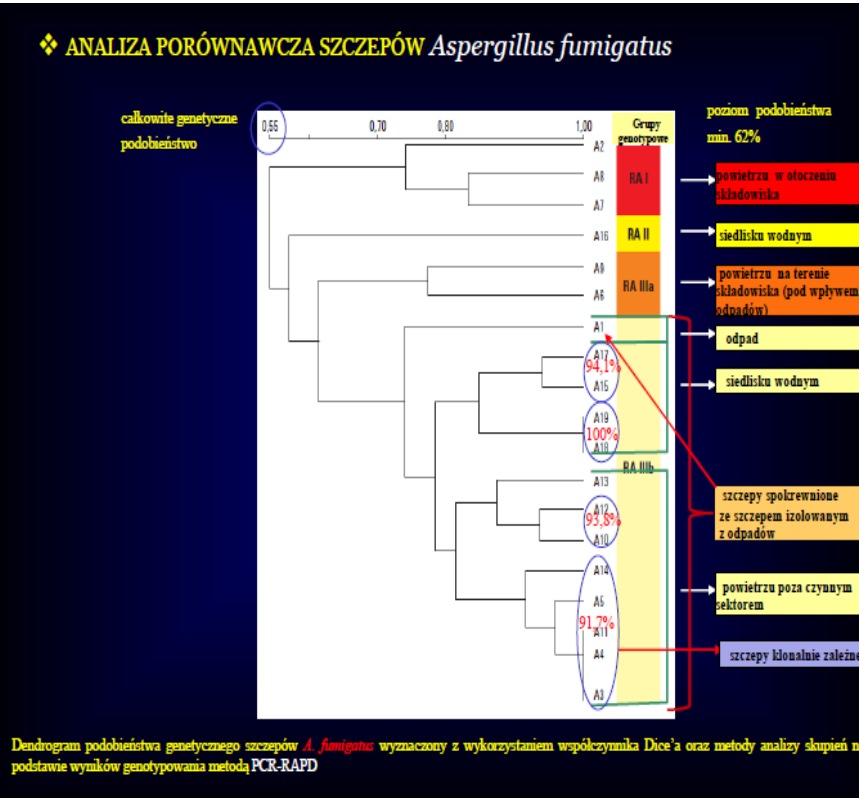


Fig. 3. Dendrogram of genetic similarity of *A. fumigatus* strains determined using the Dice coefficient and cluster analysis method based on the RAPD genotyping results

Z różnicowania metodą PCR-MP uzyskano 17 różnych profili elektroforetycznych (podobieństwo $<100\%$). Zidentyfikowano 10 szczepów klonalnie zależnych o dwóch różnych genotypach.

The first genotype group (MA I) is the most different from the others, which indicates that the strains belonging here (A7 and A8) are a natural component (fungal background) of the air in this region. The second genotype cluster (MA II) includes strains that are approximately 65% related to the MA I group, i.e. strains that have undergone gradual genetic modification towards the ability to colonize the waste. This is evidenced by the close similarity of the A1 strain from the waste and the others present in the air at various sites (1, 2 and 5) outside the active sector. The third cluster (MA III) includes strains that seem to be of waste origin, 72% related to the A19 strain isolated from the waste. These related strains were found both in the leachate and in the water of the ditch located in the vicinity of the landfill. The insects were also colonized by a fungal strain (A14), closely related to the strains found in the air of the active sector and the surroundings, forming a clonally dependent group.

CONCLUSIONS

- The analyzed genotypic methods distinguish *A. fumigatus* strains that are a natural component occurring in the landfill environment from those that occur in the waste or occur in habitats directly influenced by the waste.
- The obtained results indicate that genetic differentiation of strains of this fungus species occurs in the area of the landfill so that it is possible to find a waste type, dependent subtypes occurring in the aquatic habitat, in air and on insects feeding on waste, and finally types originating from the environment adjacent the landfill and adapting to it.
- The results of molecular analyses indicate that methods based on the analysis of genetic material can be used to study the extent of microbial colonization of the environment and humans in the zone of impact of a municipal landfill site. They not only allow the selection of a marker of fungal colonization of the environment around the landfill, but also provide the possibility to determine the source of microorganisms and track the routes of their dissemination in the area around the landfill, as well as to predict the extent of human health exposure to them.