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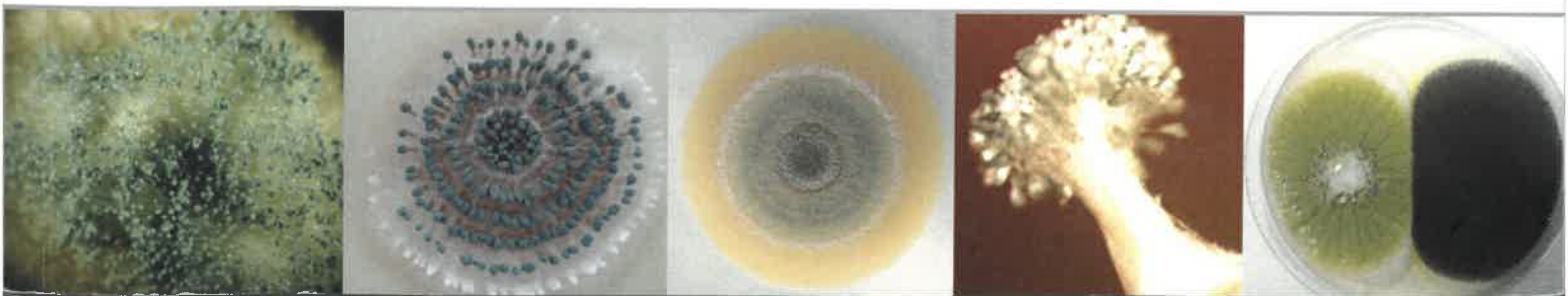

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Analysis of genetic characteristics and the aflatoxin formation of different *Aspergillus* strains

Alexandra Schamann[1], Sebastian T. Soukup[1], Rolf Geisen[1], Sabine E. Kulling[1], Markus Schmidt-Heydt[1*]

[1]Max Rubner – Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany

*corresponding author: Markus.Schmidt-Heydt@mri.bund.de

The contamination of food and feed with carcinogenic aflatoxins produced mainly by *Aspergillus flavus* and *A. parasiticus* is a serious problem, especially in countries with warm and humid climates such as Kenya, where outbreaks of acute aflatoxicosis have been regularly reported in the past (Azziz-Baumgartner *et al.* 2005). Highly aflatoxigenic *A. minisclerotigenes* strains have been identified as cause for the severe aflatoxin contamination of home-grown maize. For analysis of aflatoxigenic *Aspergillus* strains from Kenyan maize fields, fungal strains were isolated from maize kernels and soil samples and aflatoxigenic strains were identified, confirming the high prevalence of the *A. minisclerotigenes* species. A selection of specific strains including two strains of *A. minisclerotigenes*, which showed high genetic similarity to the strains that appeared to cause the aflatoxicosis outbreaks, was sequenced using short-read and long-read sequencing, and hybrid assemblies were generated. Further, their aflatoxin formation was measured on laboratory media using a validated UHPLC-MS method. Anomalies in their aflatoxin biosynthesis, such as the inability of an *A. parasiticus* strain to form aflatoxin B_1 and G_1, were explained by various deletions and mutations within the aflatoxin gene cluster. In addition, the biosynthesis of M-group aflatoxins and interesting aflatoxin precursors such as the rarely described aspertoxin by *A. flavus*, *A. minisclerotigenes* and *A. parasiticus* was detected. Since the formation of M-group aflatoxins by fungi is poorly investigated, *A. flavus* was incubated on autoclaved maize kernels for up to 10 days and the biosynthesis of B- and M-group aflatoxins as well as aflatoxin precursors was measured. Relatively high levels of O-methylsterigmatocystin and aspertoxin were detected, raising the question of the toxicological importance of these aflatoxin precursors. Further, the formation of aflatoxin M_1 and M_2 by *A. flavus* on cereals was confirmed, for which no maximum level exists in the EU regulation. Thus, the monitoring of the occurrence of toxicologically relevant precursors and M-group aflatoxins in food is recommended. In conclusion, the multidisciplinary combination of genetic and chemical analyses provided a holistic and very profound presentation of aflatoxigenic *Aspergillus* strains and their relevance to food safety aspects.

Azziz-Baumgartner E, Lindblade K, Gieseker K, Rogers HS, Kieszak S, Njapau H, Schleicher R, McCoy LF, Misore A, DeCock K, Rubin C, Slutsker L, Aflatoxin Investigation Group (2005). Case–control study of an acute aflatoxicosis outbreak, Kenya, 2004. Environ health perspect 113:1779–1783. https://doi.org/10.1289/ehp.8384.