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Advantages of X-Ray Microanalysis in the Field of Medical Mycology

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Abstract

A new apparatus, which combines an electron microprobe and a scanning electron microscope, has been used. This apparatus enabled to conduct a systematic elemental analysis of two species among Aspergillaceae. All elements in the periodic table whose atomic numbers lie between boron and uranium can be detected.

Introduction

Microanalysis by means of X-ray spectrometry allows to detect in a sample all elements of MENDELEEV's classification comprised between boron and uranium. We deemed it interesting to apply this method to the study of some human pathogenic fungi.

Materials and Methods

1 – Basis of the Analytical Method

The basis of X-ray microanalysis is as follows: the X-ray spectrum emitted under the impact of an electron beam consists on the one hand of a continuous spectrum and on the other hand of a number of characteristic lines that depend on the atoms constituting the anticathode. The small area bombarded in the sample plays the part of the anticathode and sends out, in all directions, X-rays whose spectral analysis is effected by means of spectrometers.

2 — Techniques

The cultures of *Aspergillus clavatus* Desmazieres and *Aspergillus oryzae* (Ah1b) Cohn (both 12 days old) were completely cleared of their food material (Sabouraud infusion). The fragments of mycelium were neither fixed, stained nor embedded. After quick washing in sterile demineralized water, in order to eliminate any traces of nutrient medium, the samples were dried between two sheets of blotting paper and flattened with a weight. The surface of the samples must be as even as possible. The cultures were placed on aluminium cylinders and treated with silver lacquer so as to secure the conductivity of electrons. Then they were placed in a vacuum evaporator where they were coated with a film of aluminium. A coating from 10 nm is generally a desirable range.

For microanalysis, we used the Camebax unit, composed of a modular apparatus for scanning and X-ray microanalysis. This apparatus combines in the same assembly an electron microprobe with three wavelength X-ray spectrometers and a scanning electron microscope. The analysis is performed with the help of an inclined spectrometer that allows to use samples up to 1 mm in diameter.

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The cultures are analyzed through the combined process of scanning electron microscopy and X-ray spectrometry. After obtaining secondary electron images by means of scanning electron microscopy, some elements belonging to MENDELEEV's periodical classification are investigated by means of wavelength dispersive spectrometry. The X-rays emitted are analyzed with the crystal detectors of spectrometers: pentaerythritol (PET), acid potassium phthalate (KAP) and lithium fluoride (LIF). The conditions required for detection are: accelerating voltage 20 KV, current delivered 50 nA.

Results

The study of *Aspergillus clavatus* enabled to detect seven elemental chemical components. With the PET crystal detector appeared the specific spectra of the following elements: calcium (Kα line, traces), potassium (Kβ and Kα lines), chlorine (Kα line), sulphur (Kα line), and phosphorus (Kα line). The Ag Lγ, Lβ and Lα lines came from the silver lacquer used to stick the samples on the supports. With the KAP crystal detector, the Kα lines of magnesium (traces) and sodium appeared. The Al Kα line came from metallization.

The analysis of *Aspergillus oryzae* showed the presence of eight elements. With the PET crystal detector appeared the specific spectra of calcium (Kβ and Kα lines), potassium (Kα line), chlorine (Kβ and Kα lines), sulphur (Kα line) and phosphorus (Kα line). With the KAP crystal detector, silicon (Kβ and Kα lines), magnesium (Kα line) and sodium (Kα line) were detected. The aluminium Kβ and Kα lines came from metallization.

In the two species, the LIF crystal detector allowed to detect no element. No other elements of MENDELEEV's periodical classification were detected. But the natural components to be found in living tissues, namely carbon, hydrogen, oxygen and nitrogen, were not investigated. As to the negative results, this technique may not prove useful for infinitesimal quantities.

Discussion

The history of electron probe microanalysis started with the pioneer work of MOSELEY (1913), who showed that it was possible to detect all elements in the periodic table from their characteristic X-ray emission

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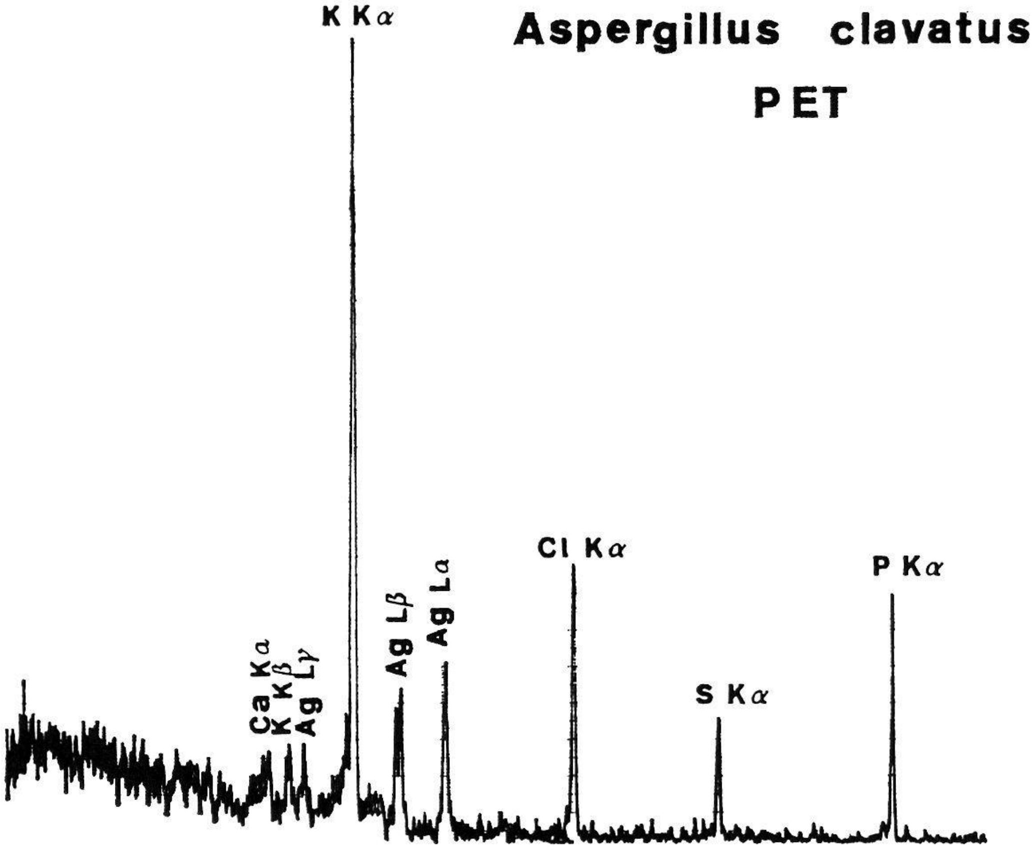
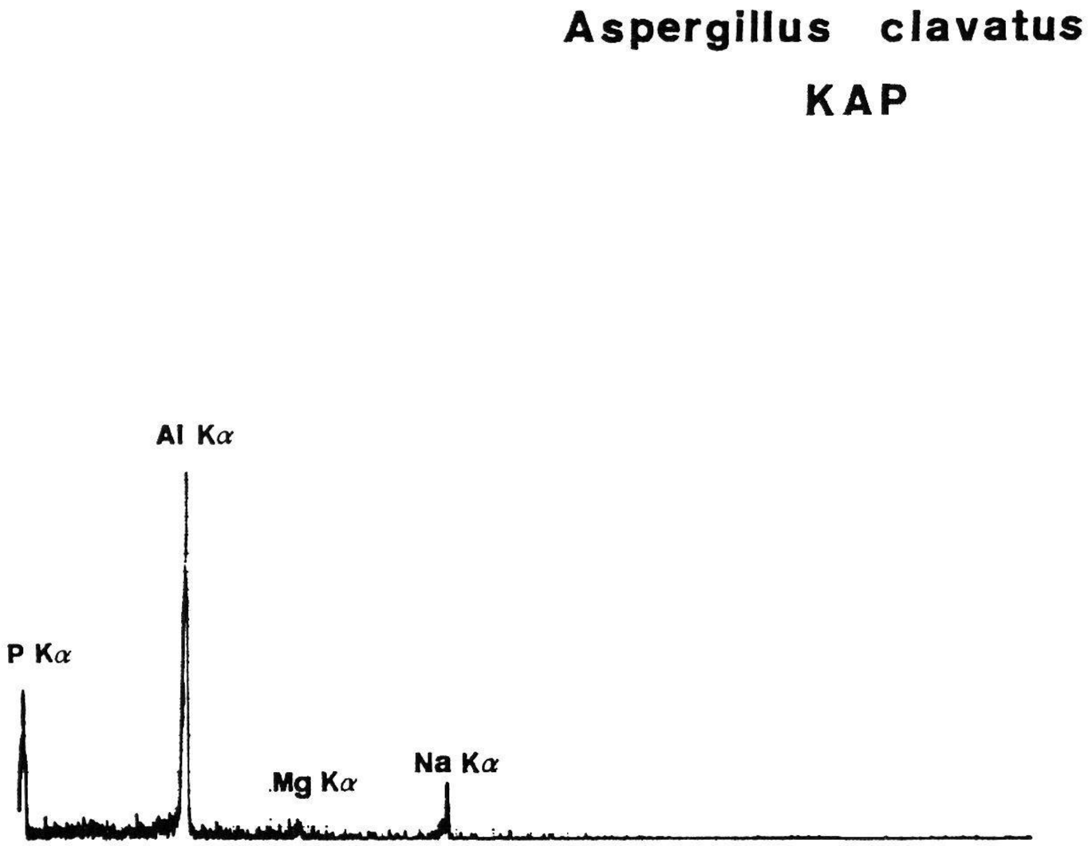
Fig. 1. Analysis of *Aspergillus clavatus* by means of wavelength dispersive spectrometry (Camebax system). With the PET crystal detector are identified: calcium (traces, Kα line), potassium (Kβ and Kα lines), chlorine, sulphur and phosphorus (Kα lines). The Ag Lγ, Lβ and Lα lines come from the silver paint used to stick the specimens on the supports.

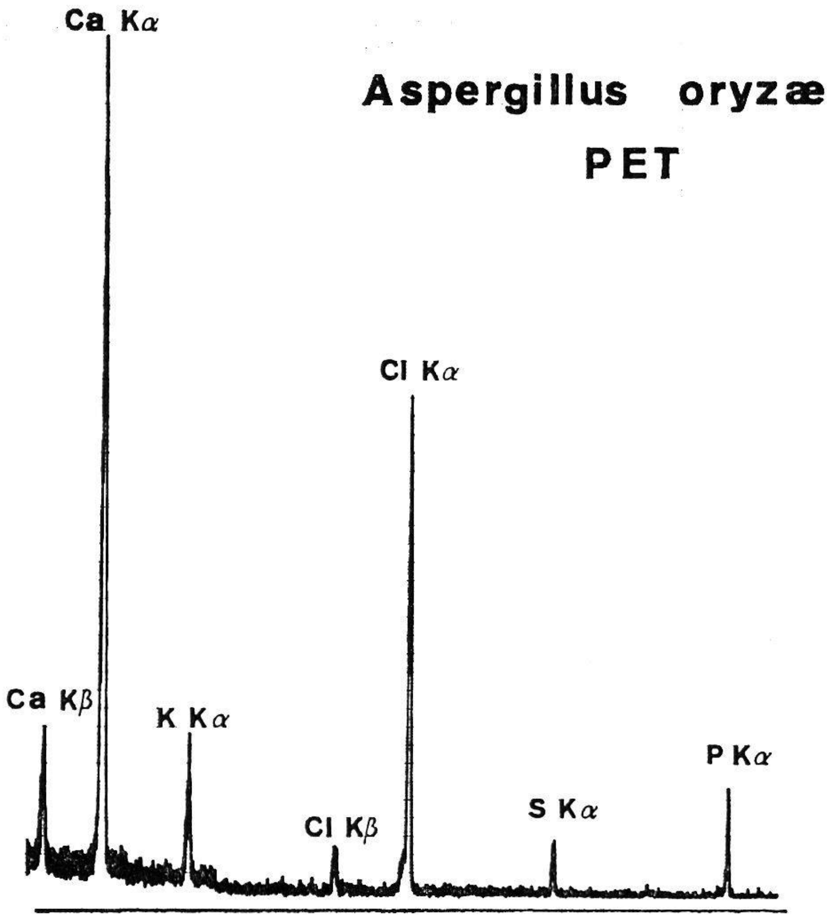
Fig. 2. Aspergillus clavatus. Analysis with the KAP crystal detector. The aluminium Kα line comes from metallization. Presence of magnesium (traces) and sodium.


K Kα
Aspergillus clavatus
PET
Cl Kα
P Kα
Ag Lβ
Ag Lα
Ca Kα
K Kβ
Ag Lγ
S Kα

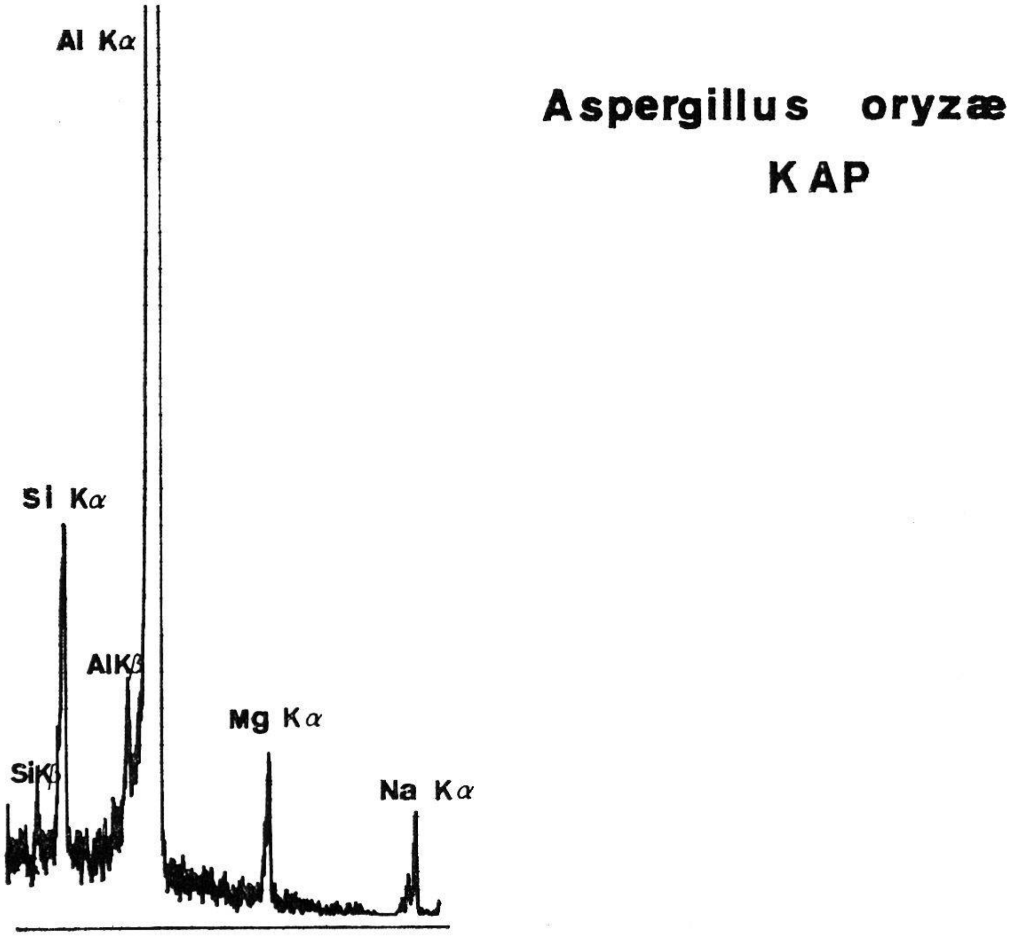
1


Aspergillus clavatus
KAP
Al Kα
P Kα
Mg Kα
Na Kα

2


Ca Kα
Aspergillus oryzæ
PET
Cl Kα
Ca Kβ
K Kα
P Kα
S Kα
Cl Kβ

3


Al Kα
Aspergillus oryzæ
KAP
Si Kα
AlKβ
Mg Kα
SiKβ
Na Kα

4

excited by the impact of an electron beam. The application of X-ray spectrometry was developed by CASTAING (1951). In the biological area, it was used notably by GALLE (1965).

In the field of animal biology, X-ray microanalysis was used to detect the distribution of some metals in insects and calcium in the thyroid gland of some mammals (MARTOJA, 1971). Electron microprobe analysis was used to study the cytoplasmic granules in *Tetrahymena pyriformis* (COLEMAN et al., 1972). The refractive bodies in *Amoeba proteus* were studied by means of X-ray spectrometry.

In plant biology, the study of silica in developing epidermal cells of *Avena* internodes was made using electron probe analysis (KAUFMAN et al., 1969). The entry and distribution of aluminium in *Zea mays* was analysed by the same technique (RASMUSSEN, 1969). The localization of aluminium in the cortex cells of bean and barley roots was studied by microanalysis (WAISEL et al., 1970). The structured granules in *Fischerella ambigua* have been investigated by this method (THURSTON & RUSS, 1971). Although X-ray spectrometry has long been used for the elemental analysis of biological specimens, it was not used until recently in medical mycology. The method has been applied to the study of some pathogenic fungi for the detection of their elemental composition (THIBAUT et al., 1974). Thanks to this technique, relevant differences in the natural chemical contents of a number of species of Aspergillaceae were observed (THIBAUT & ANSEL, 1973, 1975).

Conclusion

Microanalysis by means of X-ray spectrometry enables to obtain chemical information concerning specimens which have not been submitted to fixation, dehydration and embedding and without resorting to a variety of staining methods or different instruments. It makes it possible to study the distribution of periodical elements in biological tissues during the examination of samples by scanning electron microscopy. The analytical electron microscopy enables to observe some differences in the elemental chemical composition of closely related species, for instance *Aspergillus clavatus* and *Aspergillus oryzae*.

Fig. 3. Aspergillus oryzae. Spectra of calcium (Kβ and Kα lines), potassium (Kα line), chlorine (Kβ and Kα lines), sulphur and phosphorus (Kα lines). PET crystal detector.

Fig. 4. Aspergillus oryzae. X-ray spectra of silicon (Kβ and Kα lines), magnesium (Kα line) and sodium (Kα line). The aluminium Kβ and Kα lines come from metallization. KAP crystal detector.

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