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A Research Note

Heat-resistant Fungi in Soil Samples from Northern Nigeria

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ABSTRACT

A total of 156 soil samples collected from parts of Northern Nigeria were examined for heat-resistant fungi (HRF) which could cause spoilage of heat processed fruits and fruit products. Each sample was subjected to 70°C for 1 h before plating on potato sucrose agar. Approximately 77% of all the samples contained HRF which were identified as *Neosartorya fischeri, N. fischeri* var. *spinosus, N. quadricincta* and *Aspergillus fischeri*. Other HRF which occurred infrequently were *Aspergillus fumigatus* and *Penicillium* spp. The fungal counts in the positive samples generally ranged from 18-300 propagules/10g of soil.

Heat-resistant fungi (HRF) are those which survive certain heat treatments *(3, 8)* used in food processing, and subsequently grow in the finished products. The fungi are important in the spoilage of fruits and fruit products which are processed at mild temperatures to avoid flavor damage. Among the genera so far known, *Byssochlamys* seems to be the most important, having been implicated in spoilage of canned and bottled fruits *(6)*. Since its first isolation in England, HRF have been shown to occur widely in continental Europe, Canada and parts of the United States *(5, 9)*. It appears that the soil is the natural habitat of the fungi and the major source of fruit contamination *(9)*. This knowledge has probably led to possible improvements in sanitation during handling of fruits before processing.

Two reasons have prompted our current interest in the occurrence of HRF in Nigerian soils. The first is the isolation of *Byssochlamys* from an imported spoiled canned fruit juice *(2)* and the inadvertent disposal, by laboratory personnel, of the isolate which could thus initiate growth of the organism locally. The second reason arose from relatively recent progress in production, processing and marketing of fruits and fruit products in Nigeria. Among the advances are the increasing use of local fruits (to supplement imported concentrates) in juices, purees and pastes production and the exportation of locally grown fruits to foreign countries. Information on predominant HRF of the local environment would be useful to local processors and to foreign consumers of Nigerian fruits and fruit products.

MATERIALS AND METHODS

Collection of soil samples

A total of 156 samples were collected between January 1985 and December 1986, in sterile polythene bags, from gardens, orchards and farmlands in six towns (Table 1) in Northern Nigeria. Metallic spoons were used for the collection, and they were sterilized before and between collection of different samples, by dipping in alcohol followed by flaming. Samples were brought to the laboratory within 2 h of collection, or sometimes after 48-96 h, depending on the source. In Zaria samples were collected from the botanic and other gardens and from experimental farms in the main campus of Ahmadu Bello University where the work was done. The samples from Kano were collected from a farmland in the outskirts of the town and those from Jos were obtained from a garden in the main campus of the University of Jos. Other samples were collected from appropriate places by volunteers who were acquainted with relevant aseptic and other techniques. At each location sampling of soil was at random, but an approximate distance of three yards was kept between any two sampling sites. Samples were collected from the soil surface and maximum depth did not exceed 2-4 cm.

Isolation of heat resistant fungi from soil samples

20 g of each sample was weighed out aseptically and transferred into 50 ml of sterile distilled water in a medicine bottle which was then shaken vigorously by inverting it up to ten times. The contents of the bottle were then heated in a water bath at 70°C for one h *(9)*; a thermometer was inserted into the bottle to ensure maintenance of the temperature. After the interval, the bottle and its contents were allowed to cool slowly to room temperature. It was then shaken again and 10 ml portions of the contents were pipetted into duplicate petri dishes. Approximately equal volumes of double strength potato sucrose agar (PSA) containing tetracycline (30µg/ml final concentration) were poured into the petri dishes, mixed with the samples and allowed to set. All the agar plates were incubated at room temperature (26-35°C depending on the season) for 3-5 d.

After the incubation period, representative types of fungal colonies were counted, purified on other PSA plates and then preserved on slants of the same medium. Agar plates which showed no fungal growth were incubated for another 5 d before they were considered negative and discarded.

TABLE 1. *Heat-resistant fungi in Nigerian soil samples.*

Source of sample	Fraction contaminated by HRF[a]	Mean count of propagules of HRF/10g of soil[b]	Range of viable propagules of HRF/10g	Predominant genera of HRF[c]
Zaria	92/120	71	0-300	A, N, P
Bauchi	1/2	50	0-75	N
Bida	9/10	21	0-50	A, N
Jos	9/10	39	0-105	N
Kano	3/13	46	18-53	A, N
Maiduguri	5/11	42	0-233	N
Total	119/156 (76.9%)			

[a] No. of positive samples / Total no. of samples

[b] Sum of the no. of HRF/10g in positive samples / Total no. of samples

[c] N includes: *Neosartorya fischeri* (Wehmer) Malloch & Cain, *N. fischeri* var. *spinosa* (Raper & Fennel) Malloch & Cain and *N. quadricincta* (Yuill) Malloch & Cain
A includes *Aspergillus fischeri* Wehmer and *A. fumigatus*
P = *Penicillium* spp.

Confirmation of heat-resistance of the isolates

When each isolate on PSA slant was determined (visually or microscopically) to have sporulated, the spores were dislodged as much as possible by means of a sterile inoculating wire loop and a few drops of sterile 0.5 M phosphate buffer pH 6.0. More sterile buffer was then added to the slope, the contents were shaken vigorously and later emptied into a screw-capped test tube containing 8-10 ml of the sterile buffer (i.e. 0.5 M phosphate buffer).

A thermometer was inserted into the test tube in a water bath which was then heated for 1 h at 70°C *(9)*. After cooling, portions were plated out as usual with double-strength PSA and incubated at room temperature for 1 week. Mycelial growth which occurred after this period was purified on PSA plates and finally transferred to plates of Czapek's Dox agar for identification and on slants of the medium for preservation. Identification was carried out by reference to Smith *(6)* and by comparing of the isolates with an authentic culture of *Byssochlamys nivea* which was identified at the Commonwealth Mycological Institute, Kew.

RESULTS AND DISCUSSION

Out of a total of 156 soil samples examined in this study, 119 samples (approx. 77%) contained HRF (Table 1). The range of viable propagules of HRF per 10 g of soil in the positive samples was 18-300. In all the positive samples, regardless of their origin, the predominant types of fungal colony which developed on the isolation medium appeared similar in gross morphology. All of them had a granular appearance on the agar plates. Microscopy revealed the granules to be composed of cleistothecia. Some of them formed yellow or orange or pink pigments which were readily lost on subculture. The isolates were identified in this laboratory as strains of *Neosartorya* spp. When seven randomly chosen representatives were sent to CM1, Kew, Surrey, they were described as *Neosartorya fischeri* (represented by 2 isolates from Zaria and Maiduguri samples), *N. fischeri* var. *spinosus* (1 from Bida), *Aspergillus fischeri* (1 from Zaria), *Neosartorya quadricincta* (3 from Zaria, Maiduguri and Bauchi samples). Since *A. fischeri* is the anamorph of *N. fischeri (10)* it is likely that our isolate failed to produce cleistothecia when it was subcultured at Kew. Since the samples examined in this study were obtained from towns separated by distances ranging from 150-800 km, the frequent occurrence of *Neosartorya* spp. indicates that it is an important genus of HRF in soils of Northern Nigeria. The predominance of *Neosartorya* is especially noteworthy in Zaria in view of the large number of samples examined; all the positive soil samples (77%) from Zaria contained *Neosartorya* spp. Other HRF identified in this study were *Aspergillus fumigatus* which occurred in approximately 25% of Zaria samples and in all the 10 and 3 samples from Bida and Kano respectively, and an unidentified *Penicillium* spp. which was detected in two samples form Zaria.

The occurrence of *Neosartorya* spp. as the commonest HRF in Northern Nigeria soil differs from the reported predominance of *Byssochlamys* spp. in temperate countries *(5, 9)*. *Neosartorya* spp. have not been extensively reported as HRF in surveys conducted in temperate lands just like *Byssochlamys* and its anamorph *Paecilomyces* was not found in this study. However, it is interesting that the anamorphs of *N. fischeri* and *N. fischeri* var. *spinosus* (namely *Aspergillus fischeri* and *A. fischeri* var. *spinosus* respectively), *A. fumigatus* and *Penicillium* spp. have been consistently reported as HRF by various workers *(5, 9)*. In an earlier survey of Nigerian soil fungi, neither *Neosartorya fischeri* nor its imperfect form *Aspergillus fischeri* was recorded *(4)*. That was surprising because the samples analyzed in that survey were obtained from the campus of the University of Jos (the same location where ten samples almost all (9/10) of which were positive of HRF were collected for this study (Table 1), and also because the workers steamed the soil samples for 4 to 10 min at 100°C. Although their technique facilitated the isolation of *Ascomycetes*, it is likely that the condition (100°C for 4-10 mins.) was more lethal to *Neosartorya* than 70°C for 60

min. (which was used in this survey). The importance of using specific techniques for isolating specific fungi from soil is obvious.

The ability of *Byssochlamys* to cause spoilage of thermally processed fruits and fruit products has been attributed to, among others, its heat resistance (due to its ascospores) and its pectinolytic enzymes *(7)*. There is evidence that strains of *Neosartorya fischeri* can exhibit extraordinary heat resistance in some fruit products; a D-value of 4.2-16.2 min at 88°C has been reported for the organism in some products *(1)*. Further work will be needed to determine the ability of the organism to cause spoilage of tropical fruits and fruit products.

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