PN-AAA-515

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D C 20523

DATE: 11/18/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C5-322
An. Rept Jan 88 - Dec 88

Attachment

3554

ANNUAL REPORT JANUARY 1988 - DECEMBER 1988

US - ISRAEL CDR PROGRAM PROJECT No. C5-322
CONTRACT No. DPE-55-44-G-DD 6009-00

" Development of a simple and rapid biochemical test for the accurate identification of fungi and its application in epidemiological study in LDC."

Principal Investigator: Dr. Itzhack Polacheck, Department of Clinical Microbiology, The Hebrew University - Hadassah Medical School and Hadassah University Hospital, P.O. Box 12 000 Jerusalem 91120, Israel.

ANNUAL REPORT JANUARY 1987-NOVEMBER 1988.

During this year of the grant the final form of the diagnostic kit for the rapid identification of yeast was developed. This kit in its present form can identify the 11 most common yeasts which cause infections (see Results).

Using this kit we started to conduct a study on the prevalence of yeast mycoses in the hospital of the University Clinique in Kinshasa Zaire. This study revealed, to our surprise, that the second most prevalent yeast species in positive clinical specimens was Cryptococcus neoformans. In Israel Cryptococcus neoformans is only the eighth. most prevalent yeast; in Zaire it is the second most popular yeast after Candida albicans, the frequency of the other yeast species were similar (Figure 1). The next step was to determine which variety and serotype of Cryptococcus neoformans were commonly isolated. We found that only one isolate out of the 16 tested belonged to variety gattii while the other 15 were of variety neoformans as detected by Canananine-Glycine-BromThymol blue (CGB medium) (Figure 2). Further analysis revealed that all the isolates of variety neoformans were of serotype "A" and the unique isolate of variety gattii was of serotype"B". This data surprised us because it was reported by Dr. Kwon and Dr. Bennett that variety gattii Serotype "B" is the most prevalent isolate in tropical areas including Central Africa.

In our study 12 out of the 15 cryptococcal isolates were from documented AIDS patients, although according to WHO definition, in countries where diagnosis is poor, any cryptococcsis case is considered an indication of the presence of AIDS. According to the literature all the cryptococcal isolates from AIDS patients are of "A" serotype, this includes tropical areas were "B" and "C" are normally more prevalent. This fact might reflects a sensitivity of AIDS patients to serotype "A".

This study was the subject of a lecture that I presented in the 10th congress of the international society for human and animal mycology which took place in Barcelona in June 27th, 1988 (see enclosed abstract).

During the next year we plan to continue with the epidimiolgical study in Kinshasa and to evaluate the kit with isolates that will arrive to both laboratories.

However, since there are a poor communications between both countries (sometimes its take about 4 months to recieve mail), we would like to apply for an extension of an extra year in order to complete our objectives.

FIGURE 1

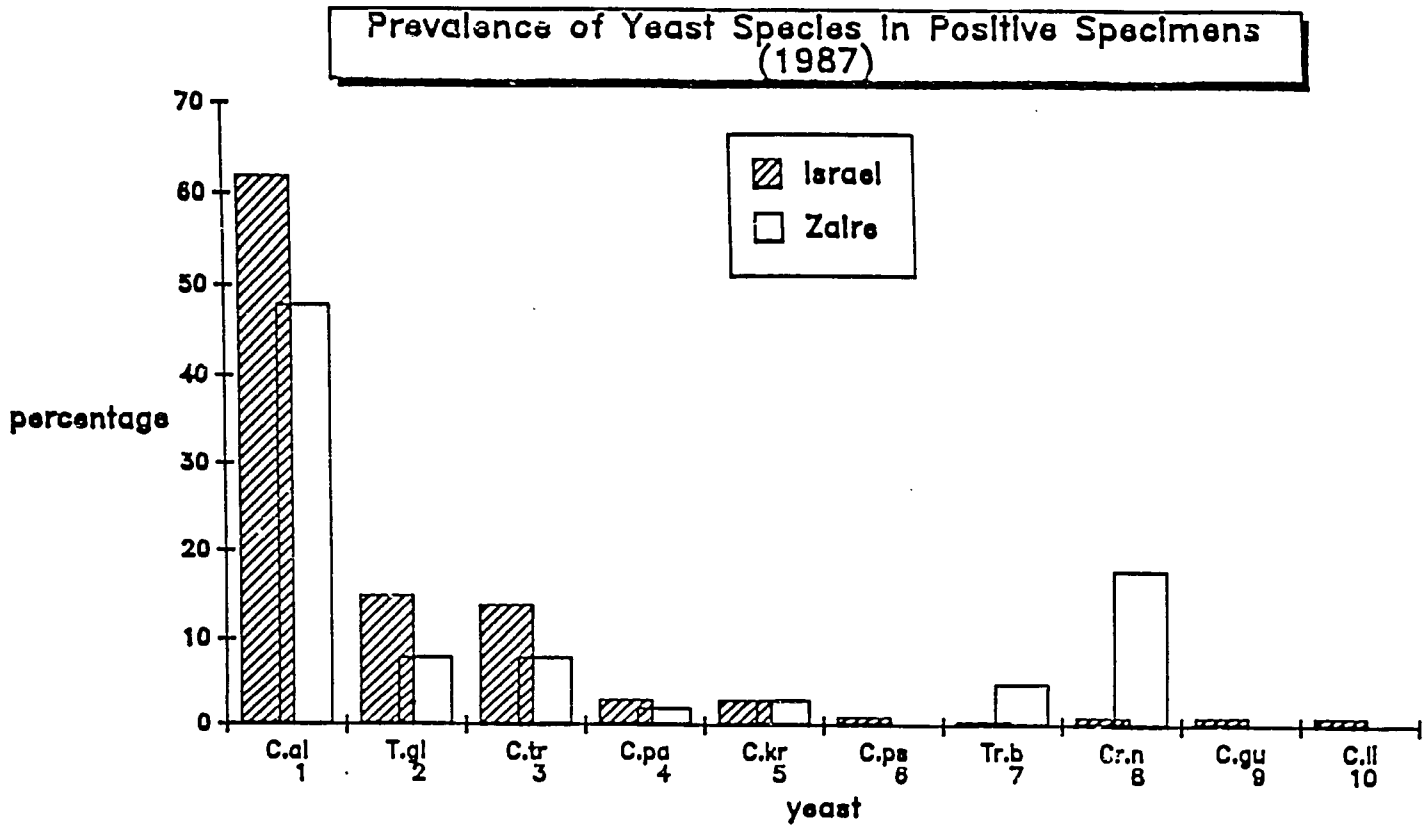
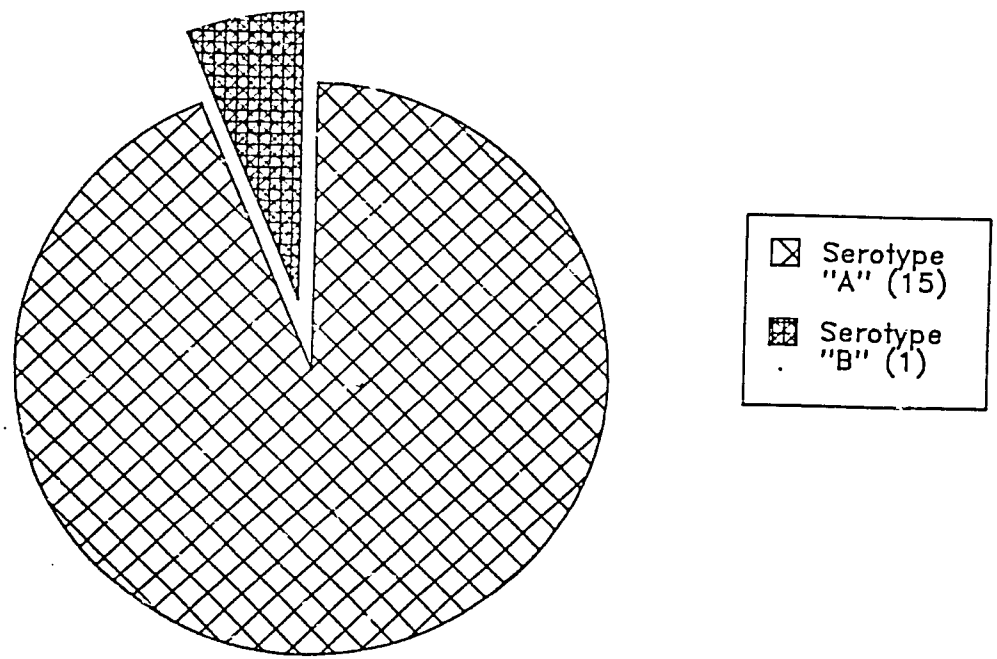

Prevalence of Yeast Species in Positive Specimens
(1987)
Israel
Zaire
percentage
70
60
50
40
30
20
10
0
C.al 1
T.gl 2
C.tr 3
C.pa 4
C.kr 5
C.ps 6
Tr.b 7
Cr.n 8
C.gu 9
C.ll 10
yeast

FIGURE 2

Prevalence of Cryptococal Serotypes in Zaire (1987)



RESULTS

We developed a kit for the identification of the 11 most common yeasts which cause infections. The kit is based upon the enzymatic liberation of a chromogenic group, from a colorless substrate. The chromogenic groups used in this test are para-nitrophenyl and β-naphthylamide. These groups are attached to different proteins, lipids and sugars and the enzymatic profile of the different yeasts is determined according to them.

During the developmental stage of the kit the optimal conditions of the test and its final structure were determined. We found that a two hour incubation period, at a temperature of 30°C, was long enough to produce strong colors, which made clear the definition between positive and negative results. There was no advantage in adding buffer to the kit. Therefore, the tested yeasts were suspended in double distilled water, at a pH of between 5.0 - 6.0.

From a total of 117 tested substrates, 17 were chosen for use in the kit. These substrates were tested with different yeast concentrations, in order to negate the dependence of the test on the quantity of yeast cells being tested. The final concentration chosen for use in the test was 1×10^6 cells/ml. This concentration produced clear test results.

After completing the development of the diagnostic kit, the following tests were made in order to evaluate it:

1) Testing of additional yeasts, less common as pathogenic yeasts:
An additional 23 species were tested to ascertain that they didn't produce identical results to one of the 11 species for which the test was developed.

2) Testing of the culture collection:
More than 100 isolates from the laboratory culture collection were tested to ascertain that the isolate used during the developmental stage was typical of the species. The kit results were changed where necessary in accordance with results of the test.

The diagnostic kit is rapid, and the test takes only 2 hours. As it is not based upon the growth of the fungus, it is therefore not effected by different growth conditions. The reading of the results is simple and easy, demanding no particular skill from the tester. The accuracy of the test as determined by the different tests - is high.

The Final Diagnostic Test

Each of the following substrates (Table 1), were incubated with 0.1 ml of $1x10^8$ cells per ml, of the most common yeast species, encountered in the clinical laboratory, at temperature of 30°C. Detergent (at a final concentration of 0.2%) was added to few substrates prior to the incubation. The final results are summerized in table 2. In addition to the 11 medically most important species of yeast tested, 23 more species that are rarely encountered in the clinical laboratory were tested under the same conditions, to obtain different results to those presented in Table 2. This eliminates the possibility of false identification of the most important species. Results are summerized in Table 3.

Table 1:

substrate	amount(μg per well)
1) PNP-N-acetyl-β-D-galactosaminide	240
6) PNP-β-D-cellobioside	480
11) PNP-α-D-galactopyranoside	120
16) PNP-α-D-glucopyranoside	240
17) PNP-β-D- glucopyranpside	30
20) PNP-α-D-maltoside	120
21) PNP-β-D-maltoside	120,240
22) PNP-α-D-mannopyranoside	60
99) PNP-phosphpryl-cholin	400
34) L-Alanin-BNA	240
49) L-Glutamine-BNA	60
51) γ-N-L-Glutamyl-BNA	240
66) Hydroxy-L-proline-BNA	240
67) L-Isoleucyl-BNA	240
69) L-Leucyl-glycyl-BNA	240
81) L-Proline-BNA	120

TABLE No 2

THE YEAST	SUBSTRATE No.																
	34	67	*49	69	*17	*22	*51	66	81	16	*21	1	20	21	6	*11	*99
C. albicans	+	+	+	+	V	+	+	+	+	+	V	+	+	V	–	–	–
C. stellatoidea	+	+	+	V	+	+	+	–	–	+	+	+	+	–	–	–	–
C. tropicalis	+	+	+	+	+	+	+	–	–	+	+	–	–	V	–	–	V
C. parapsilosis	+	+	+	+	+	+	+	+	+	–	–	V	–	–	–	–	+
C. lipolytica	+	+	+	+	+	+	+	+	+	–	–	–	–	–	+	–	–
C. quilliermondii	+	+	+	+	+	V	–	+	+	+	–	–	–	–	–	V	+
C. rubra	+	+	–	+	+	+	–	+	+	–	–	–	–	–	–	–	–
C. krusei	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
T. glabrata	+	+	+	+	V	+	–	–	–	–	–	–	–	–	–	–	–
C. pseudotropicalis	+	–	+	V	+	–	V	–	–	–	–	–	–	–	–	–	V
C. neoformans	–	–	–	–	+	–	V	–	–	V	+	–	V	V	V	–	V

With detergent

TABLE NO 3

THE YEAST	SUBSTRATE No.																
	34	67	*49	69	*17	*22	*51	66	81	16	*21	1	20	21	6	*11	*99
B.capitatum	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+
C.ciferrii	+	+	+	+	+	+	−	−	−	−	+	+	[illegible]	−	−	−	+
C.intermedia	+	−	+	−	+	+	−	+	+	−	−	−	−	−	−	−	−
C.lambica	−	−	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−
C.paratropicalis	+	−	+	+	+	+	−	−	−	+	+	+	−	−	−	−	+
C.albidus	+	−	−	−	+	+	−	−	−	+	+	−	−	+	+	−	−
C.laurentii	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+	−
C.uniguttulatus	+	−	−	+	+	+	+	+	+	−	+	−	−	−	−	−	+
G.candidum	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−
G.penicillatum	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−
Hs.quilliermondii	+	−	V	−	+	+	−	−	−	−	−	−	−	−	+	−	−
Hs.valbyensis	+	−	+	−	+	+	−	−	−	+	+	−	−	−	+	−	−
H.anomala	+	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	−
K.lactis	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
K.vanudenii	+	V	+	+	+	+	−	−	V	V	−	−	−	−	−	−	+
P.ohmeri	+	−	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−
Pr.wickerhamii	+	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	+
Pr.zopfii	+	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−
R.minuta	+	+	+	+	+	+	+	+	+	−	V	−	−	−	+	−	−
R.pilimanae	+	+	+	+	−	+	−	+	+	−	−	−	−	−	−	−	−
Ts.rosei	+	+	+	+	+	+	−	+	+	−	+	−	−	−	+	−	−
T.candida	+	−	−	−	V	+	V	+	+	−	−	−	−	−	V	−	−
Tr.beigeiii	+	−	+	−	+	+	−	−	+	+	−	+	−	−	−	+	+

With detergent *

THE PREVALENCE OF CRYPTOCOCCAL SEROTYPES IN ZAIRE

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An epidemiological study conducted during the last year in Zaire showed that Cryptococcus neoformans is the second most prevalent yeast in clinical specimens from Kinshasa, Zaire. Out of 57 yeasts, a total of 15 were C. neoformans. Of these, 14 were C. neoformans var neoformans and only 1 was C. neoformans var gattii, as detected by the Canavanine Glycine Bromthymol blue medium. Conventional serotyping indicates that all the variety neoformans isolates were of serotype A while the one isolate of variety gattii was of serotype B. Most of the strains (10 out of 15) were isolated from AIDS patients, among them 9 were of serotype A and 1 of serotype B. This data suggests that although a prevalence of erotype B is reported in Central Africa, serotype A is dominant among African AIDS patients. This is similar to that reported in Western AIDS cases. The question of whether this is due to a serotype specific virulence factor will be discussed. A correlation between cryptococcal serotype and chromosomal number as measured by electrokaryotyping will also be discussed.

This work was supported by AID grant CDR project C5-322.