Characterization of *Candida albicans* strains by molecular biology techniques

Presented by

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(Bacteriology, Immunology & Mycology)

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بسم الله الرحمن الرحيم

{ قالوا سبحانك لا علم لنا الا ما
علمتنا انك انت العليم الحكيم }

صدق الله العظيم

The aim of the work :-

The aim of the present work was to use both PCR and RAPD for the characterization and differentiation of various isolates of *C. albicans* recovered from various sources and to determine the degrees of genotypic similarities among isolates recovered from the different sources.

Material and methods

Material:

- **Samples:**

A total of one hundred and twenty samples were collected including the following:

Source of samples	Number of samples
Meat	50
Chickens ration	10
Milk	10
yoghurt	50
Total	120

All samples were collected in sterile containers and transported to laboratory as quickly as possible.

Methods:-

Isolation of Candida species from samples :

•Identification:

Microscopic identification of *C.albicans* :

Biochemical identification of *C.albicans* :

Sugar fermentation.

Sugar assimilation .

Physiological identification of *C. albicans.*

Adherence of Candida albicans on buccal epithelial cells .

Germ tube formation by C. albicans .

Characterization of different strains of *Candida albicans* by polymerase chain reaction (PCR) :

Differentiation of different strains of *Candida albicans* by randomly amplified polymorphic DNA (RAPD) :

Results

Prevalence of yeasts in various animal samples .

Samples	Prevalence of yeasts		
	No of samples	No of +ve	%
Meat	50	19	38
Chickens ration	10	5	50
Milk	10	3	30
yoghurt	50	31	62
Total	120	58	48

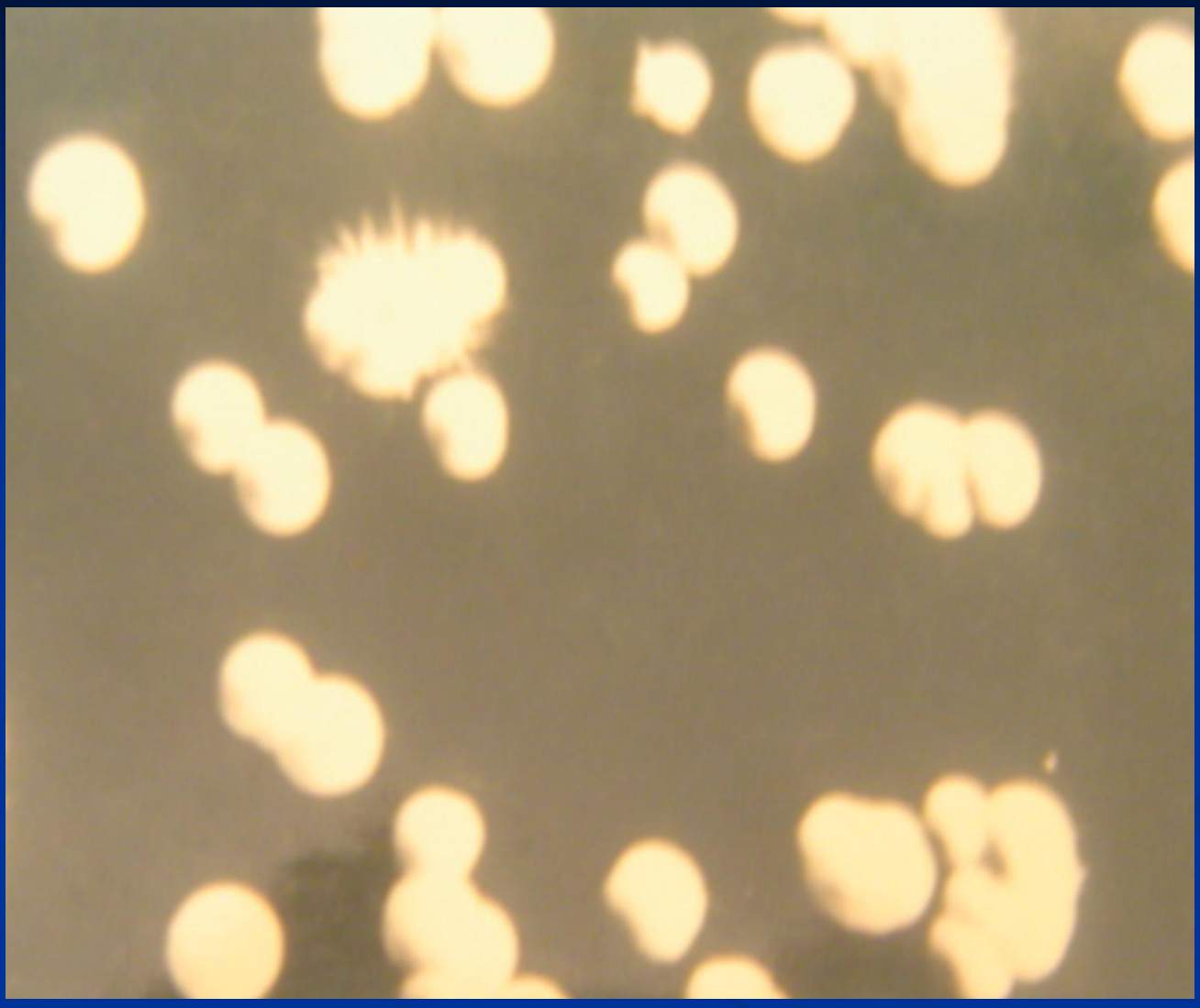
Total samples examined = 120

Candida species isolated from animal samples

Strains	Samples									
	Meat (50)		Ration (10)		Milk (10)		Youghrt (50)		Total (120)	
	No +ve	%	No +ve	%	No +ve	%	No +ve	%	No +ve	%
C.albicans	14	28	5	50	3	30	31	62	53	44.2
C. krusei	4	8	0	0	0	0	0	0	4	3.3
C.Pseudo - tropicalis	1	2	0	0	0	0	0	0	1	0.8

The total % was calculated by division of +ve samples to the total number of samples examined.

Culture of *Candida albicans* on Sabouraud dextrose agar

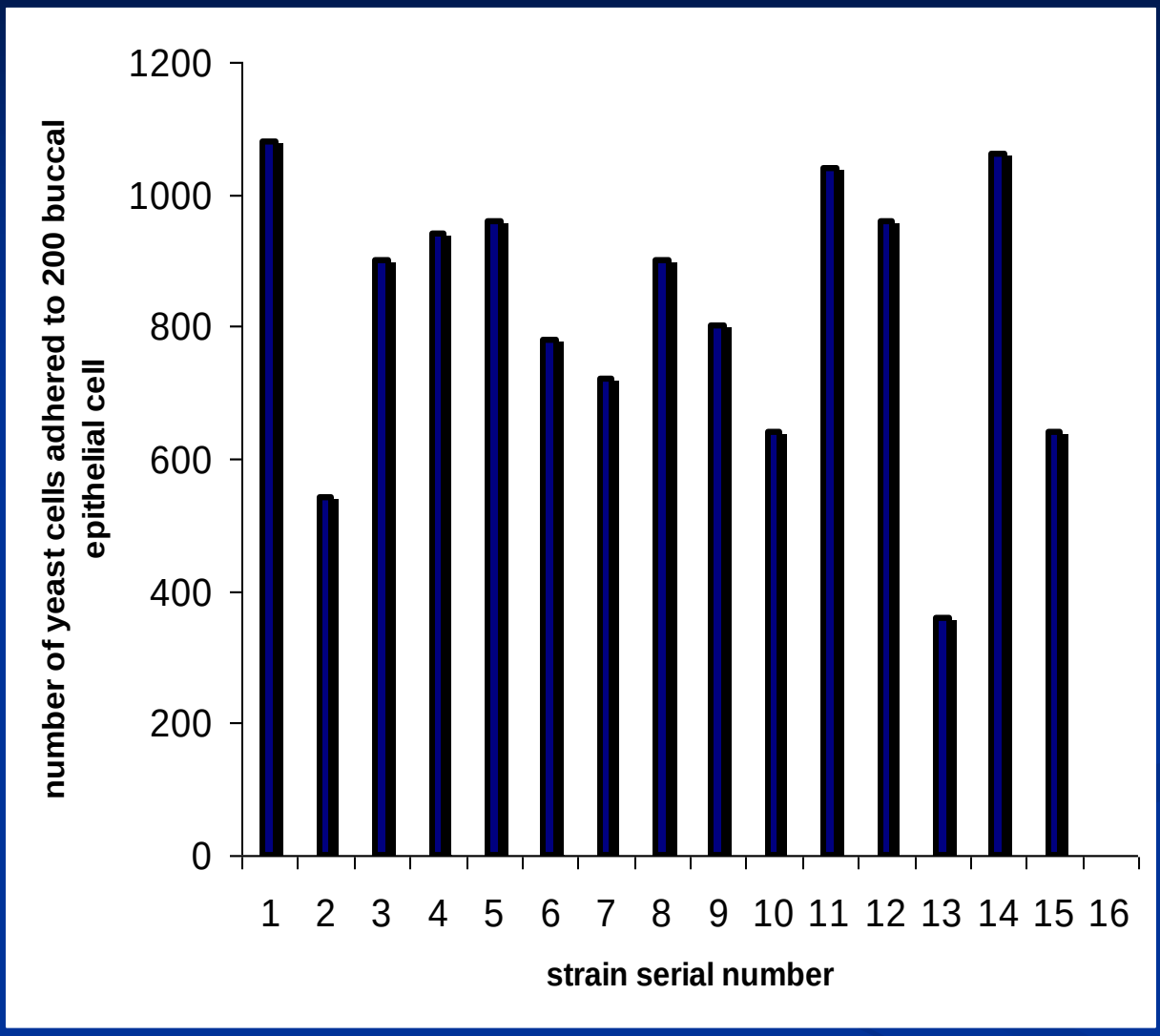


single colonies of *Candida albicans*

- **Physiological identification of *Candia albicans***

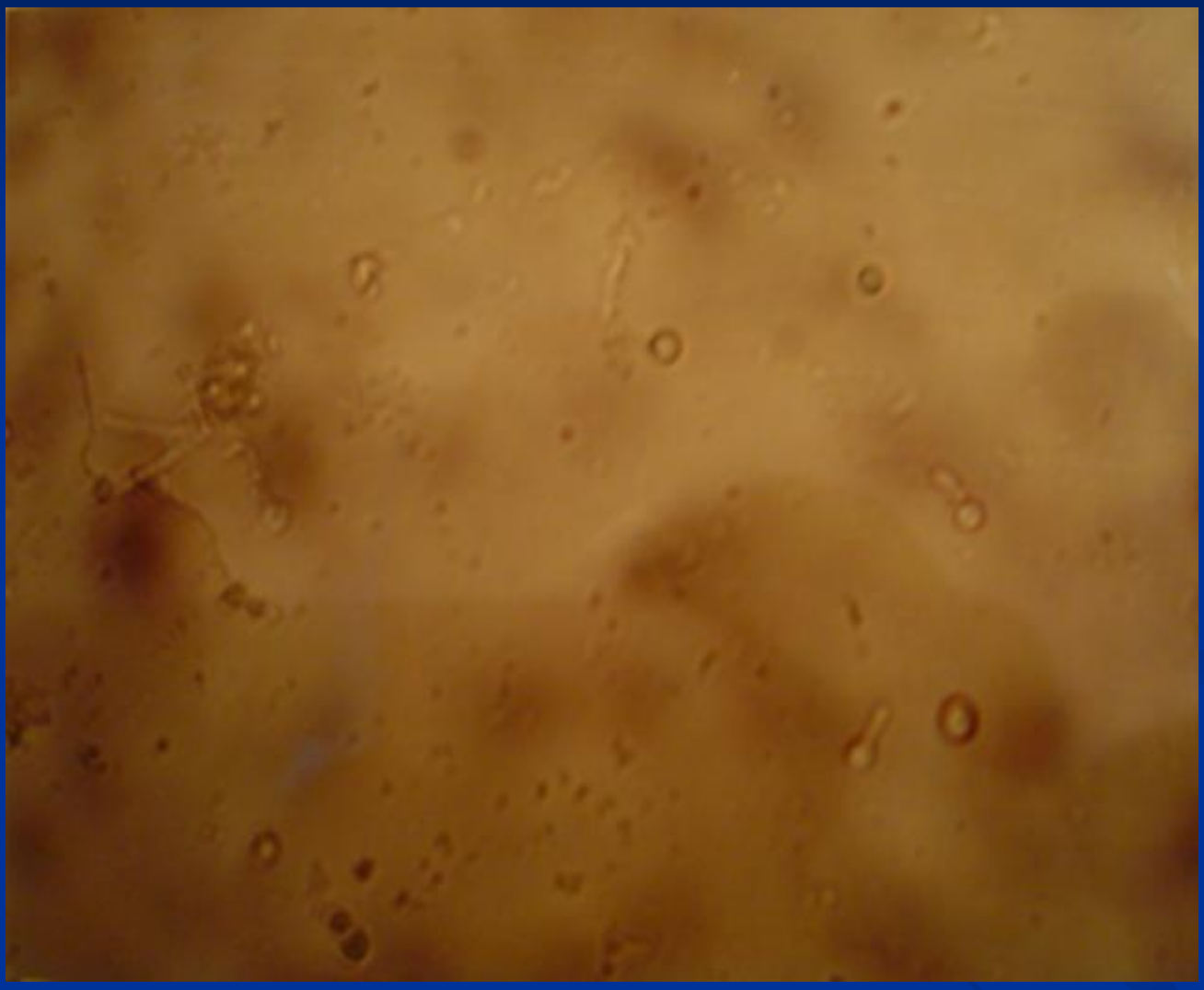
1. Adhesion to buccal epilthelial cells of *Candida albicans* isolates.

Strain serial number	Strain number	Source	No. of cells adhered to 200 buccal epithelial cells
1	3	Meat	1080
2	26	Meat	540
3	55	Meat	900
4	49	Meat	940
5	58	Meat	960
6	2	Chickens ration	780
7	4	Chickens ration	720
8	5	Chickens ration	900
9	1	Milk	800
10	8	Milk	640
11	12	yoghurt	1040
12	13	yoghurt	960
13	22	yoghurt	360
14	38	yoghurt	1060
15	40	yoghurt	640

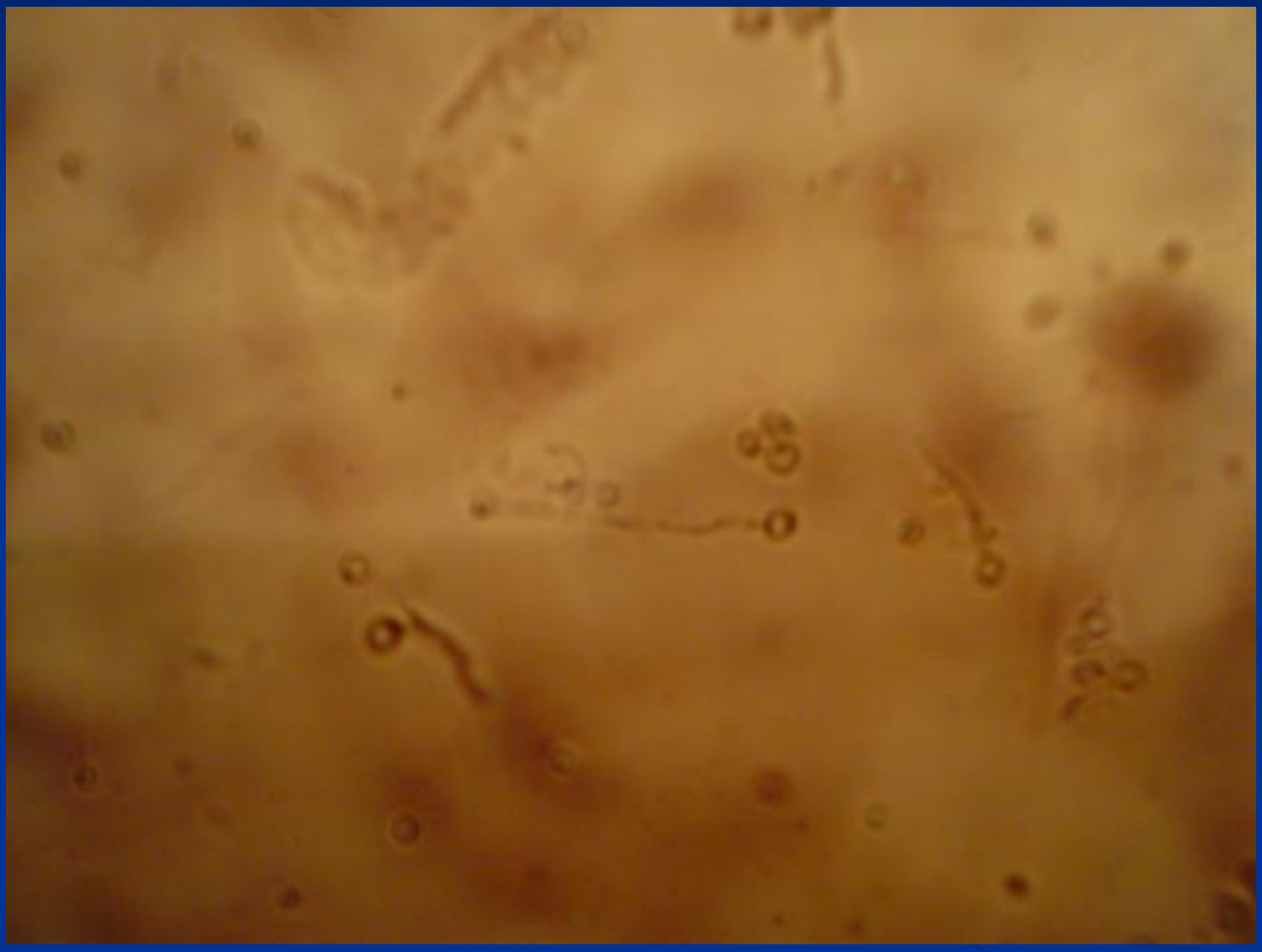


Adhesion of *C. albicans* to human buccal epithelial cells.

2. Germ tube formation by *C. albicans.*



Germ tube formation by *C. albicans*



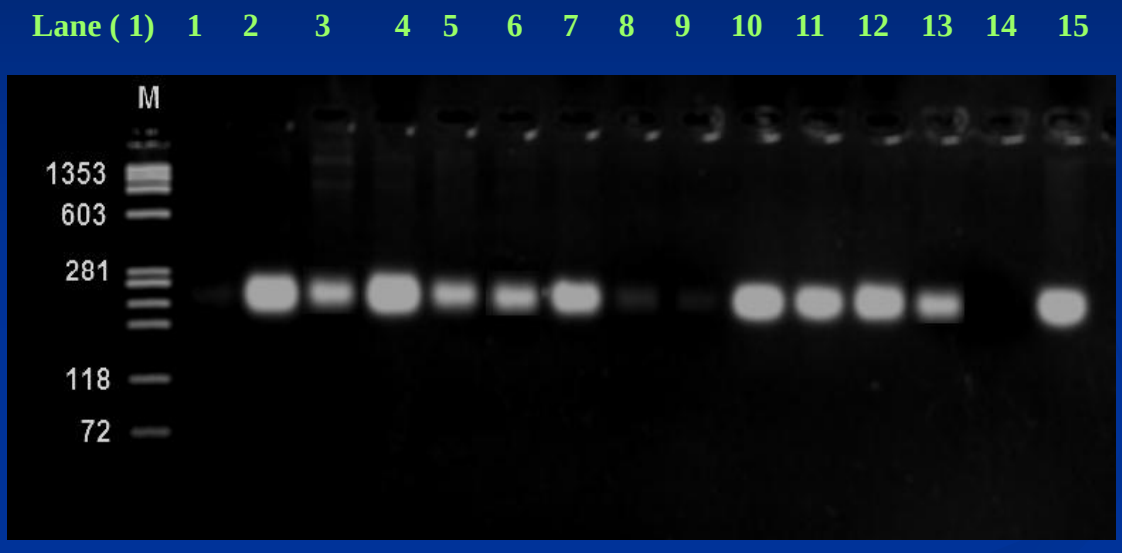
Germ tube and beginning of pseudomycelium formation by *Candida albicans* .

Characterization of *Candida albicans* isolates from different sources by Polymerase Chain Reaction (PCR) :

Bands of PCR amplification products of *Candida albicans* strains obtained with primers pairs SAP2 and SAP3.

Strain serial number	Strain number	source	DNA bands produced by SAP2 primer showed at 258 bp respectively	DNA bands produced by SAP3 primer showed at 172 bp respectively
1	3	Meat	Abscent	Abscent
2	26	Meat	Present	Present
3	55	Meat	Present	Present
4	49	Meat	Present	Present
5	58	Meat	Present	Present
6	2	ration	Present	Present
7	4	ration	Present	Present
8	5	ration	Abscent	Abscent
9	1	Milk	Abscent	Abscent
10	8	Milk	Present	Present
11	12	Yoghurt	Present	Present
12	13	Yoghurt	Present	Present
13	22	Yoghurt	Present	Present
14	38	Yoghurt	Abscent	Abscent
15	40	Yoghurt	Present	Present

Strains serial numbers of *C. albicans* lanes (1-15)

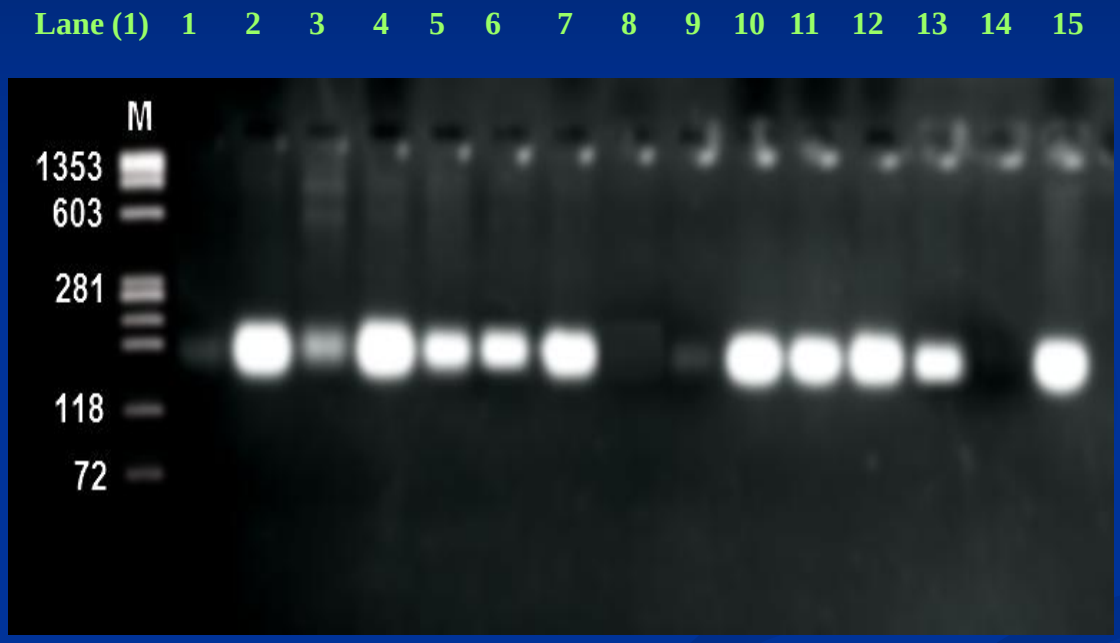


PCR for SAP2 gene of *Candida albicans* (DNA bands showed at 258 bp respectively).

bp : base pairs .
Lane (1): molecular size marker (in base pairs).
M : molecular size marker .

Strains serial numbers (1-5) isolated from meat.
Strains serial numbers (6-8) isolated from ration.
Strains serial numbers (9-10) isolated from milk.
Strains serial numbers (11-15) isolated from yoghurt.

Strains serial numbers of *C. albicans* lanes (1-15)



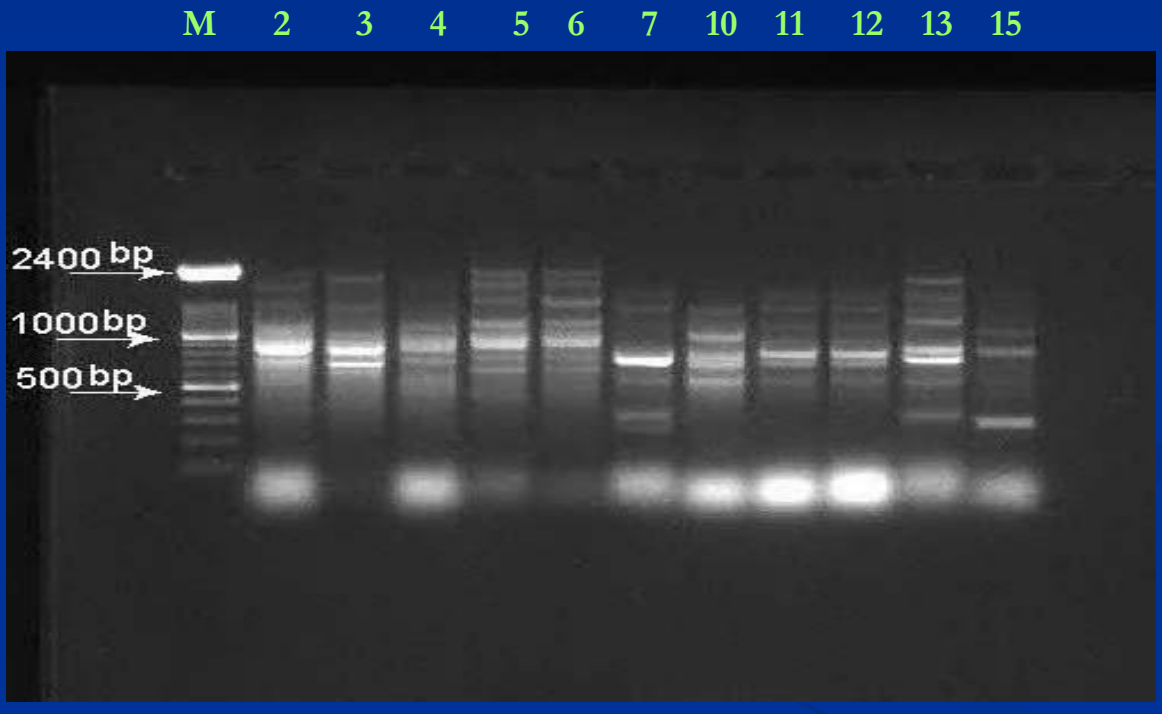
PCR for SAP3 gene of *Candida albicans* (DNA bands showed at 172 bp respectively) .

bp: base pairs .
Lane (1): molecular size marker (in base pairs).
M: molecular size marker.

Strains serial numbers (1-5) isolated from meat.
Strains serial numbers (6-8) isolated from ration.
Strains serial numbers (9-10) isolated from milk.
Strains serial numbers (11-15) isolated from yoghurt.

Differentiation of different strains of *Candida albicans* by randomly amplified polymorphic DNA (RAPD) using primer (1).

Strains serial numbers of *C. albicans* lanes (2-15)



RAPD patterns for *Candida albicans* strains obtained with primer (1) Lane M , molecular size marker (in base pair) ; lanes 2 to 15, *Candida albicans strains*.

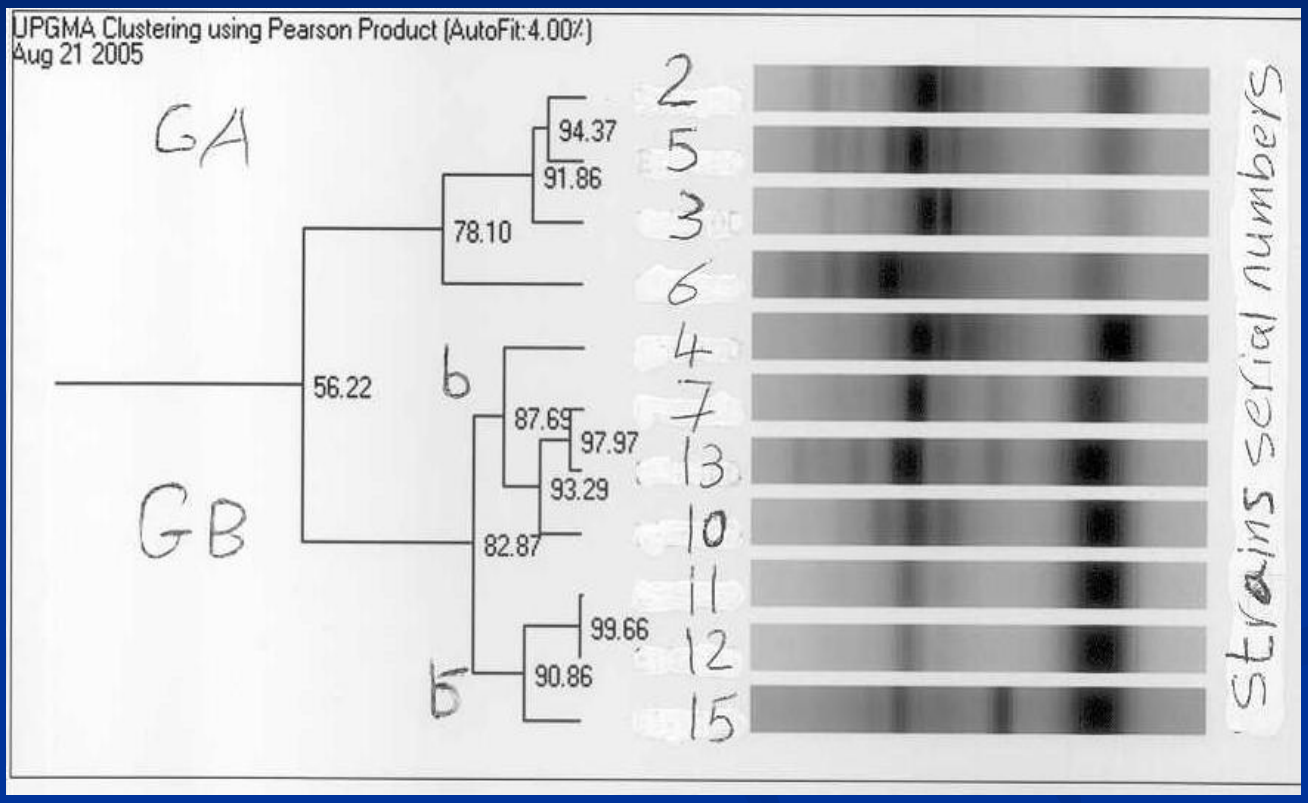
bp: base pairs.
M: molecular size of marker.

Dendogram analysis of RAPD patterns of *Candida albicans* isolated strains

Groups of similarity		Primer complementary (pc1)	
		Compared stains serial numbers	Degree of similarity
Group A		2&5	94.37%
		2,3&5	91.86%
		2, 3,5 &6	78.10%
Group B	Class b	7&13	97.97%
		7, 10&13	93.29%
		4,7, 10 &13	87.69%
	Class b^-	11&12	99.66%
		11, 12 &15	90.86%
	Class b& Class b^-	4,7, 13, 10, 11, 12 & 15	82.87%
Group A & Group B		2, 5, 3, 6, 4, 7, 13, 10, 11, 12 & 15	56.22%

Strains serial numbers (1-5) isolated from meat.
Strains serial numbers (6-8) isolated from ration.
Strains serial numbers (9-10) isolated from milk.
Strains serial numbers (11-15) isolated from yoghurt.

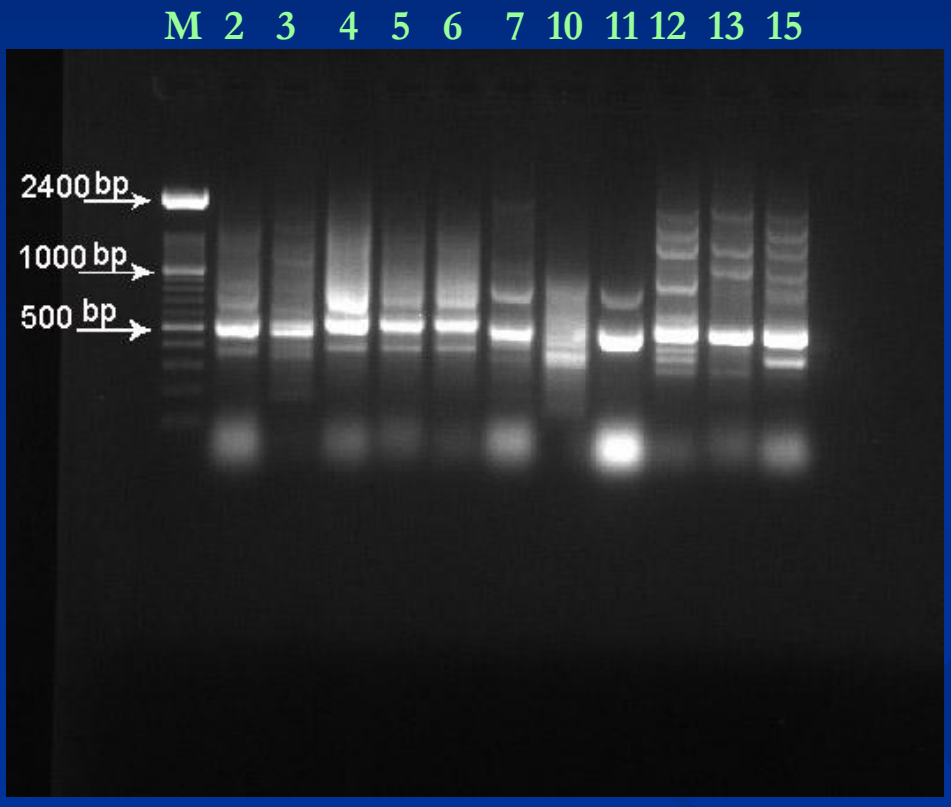
Percentage of similarity (%)



Dendogram analysis of RAPD patterns of *Candida albicans* isolated strains using primer (1) showed the (%) of similarity between strains .

Differentiation of different strains of *Candida albicans* by randomly amplified polymorphic DNA (RAPD) using primer (2) .

Strains serial numbers of *C.albicans* lanes (2-15)



RAPD patterns for *Candida albicans* strains obtained with Primer (2) Lane M, molecular size marker (in base pair); lanes 2 to 15, *Candida albicans* strains.

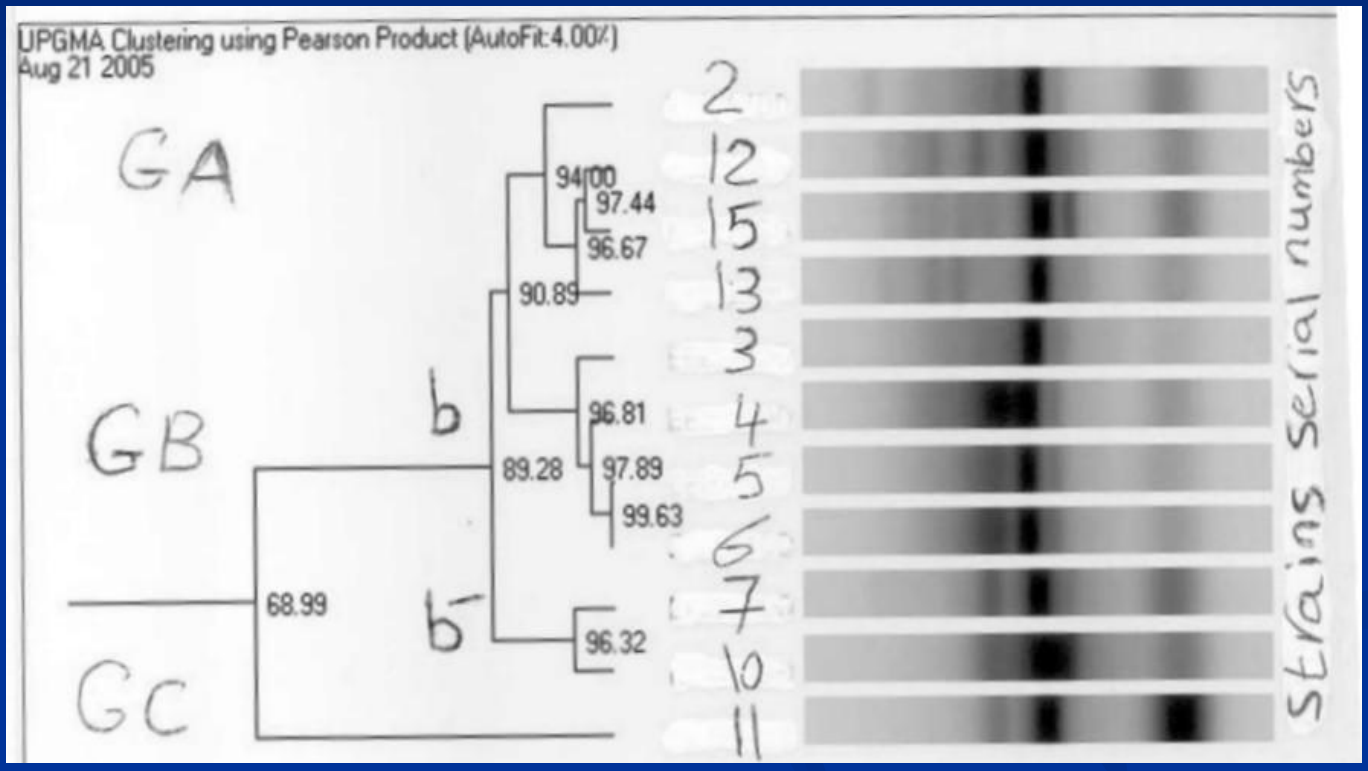
bp : base pairs.
M: molecular size marker.

Dendogram analysis of RAPD patterns of *Candida albicans* isolated strains.

Groups of similarity		Primer complementary (pc2)	
		Compared stains serial numbers	Degree of similarity
Group A		12& 15	97.44%
		12, 13 & 15	96.67%
		2, 12, 13 & 15	94.0%
Group B	Class b	5 & 6	99.63%
		4, 5& 6	97.89%
		3, 4, 5 & 6	96.81%
	Class b^-	7 & 10	96.32%
Group A& Class b		2, 12, 15, 13, 3, 4, 5, 6	90.89%
Group (A&B)		2, 12, 15, 13, 3, 4, 5, 6, 7& 10	89.28%
Groups (A, B& C)		2, 12, 15, 13, 3, 4, 5, 6, 7, 10& 11	68.99%

Strains serial numbers (1-5) isolated from meat.
Strains serial numbers (6-8) isolated from ration.
Strains serial numbers (9-10) isolated from milk.
Strains serial numbers (11-15) isolated from yoghurt.

Percentage of similarity (%)



Dendogram analysis of RAPD patterns of *Candida albicans* isolated strains using primer (2) showed the (%) of similarity between strains.

Conclusion :-

The importance of approaches of PCR and RAPD in laboratories researches is the quick and accurate identification of yeasts and other fungal diseases. This allows the delivery of the most effective drugs and the use of the proper dose of drugs for any particular infection. Molecular methods can give definitive identification and the results with in the same day. Also,they provide valuable information to physicians for correct animal management and health. Otherwise the field of molecular biology is the proper way for correct diagnosis and therapy of humans and animals.

THANK YOU