جامعة جنوب الوادى
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KEY

For Identification OF Aspergillus& Penicillium And Other Genera

BY
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Identification

K E Y T O GROUPS
Based primarily on morphology

I. sterigmata strictly uniseriate

A - C onidial heads clavate with spore masses splitting at maturity, in blue-green shades; vesicles strongly clavate ..... A- clavatus group

B. Conidial heads radiate to columnar, variable in color; vesicles variable, from globose or nearly so to subclavate or turbinate

1. Conidial heads radiate, variable in size, in bluish green or olive green shades (brown in one species); osmophilic bright yellow cleistothecia abundant in most species .. .. A. glaucus group

2. C onidial heads radiate to very loosely columnar, comparatively large, in grayish or yellowish green to olive-br shades; white to purplish or olive cleistothecia produced in three species ..... A. ornatus group

3. C onidial heads radiate (short columnar in one species), small, in pinkish fawn shades; cleistothecia lacking ..... A. cervinus group

4. Conidial heads loosely to definitely columnar, often long, thin and twisted, in green shades; conidia cylindrical when young; osmophilic; cleistothecia lacking ..... A. restrictus group

5. conidial heads compactly columnar, in pale gray-green to dark blue-green shades; conidial not cylindrical when young; not osmophilic ..... A. fumigatus group

A. Cleistothecia lacking ..... A fumigatus series

B. Cleistothecia present, white to yellowish... fisheri series

II. Sterigmata biseriate, or uniseriate(the former predominat) or with both conditions in the same head

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II. Sterigmata biseriate, or uniseriate(the former predominat) or with both conditions in the same head

A. conidial heads usually globose when young, radiate or splitting in age, rarely losely columnar; vesicles globose to subglobose ..... or somewhat elongate; conidio-phores not constricted below the vesicle; sclerottia produced in many species

1. Conidial heads globose when young, sometimes remaining so but usually splitting into more or less well-defined columns at maturity

a. Conidial heads in yellow, buff, or ochraceous shades; conidiophores commonly roughened and often pigmented; cleistothecia in one species .....A. ochraceus group

b. Conidial heads in shades of black; conidiophores usually smooth and colorless or becoming pigmented below the vesicle ..... A. niger group

C. Conidial heads white or cream colored; conidiophores smooth and colorless .....A. candidus group

2. Conidial heads typically radiate with spore chains usually separate, sometimes forming poorly defined columns

A. Conidial heads in yellow-green to deep olive-brown shades; conidiophores usually roughened, colorless A. flavus group

B. Conidial heads in yellow-brown to dull buff shades; Condidiophores smooth or delicately

A. wentii Group

roughened, coloyless or lightly pigmented ..A. wentii group

B. conidial heads large, radiate; vesicles strictly globose; conidiophores definitely constricted below the vesicles; sclerotia lacking

1. Conidial heads of one type, buff-brown, pale yellow-green or blue-green; conidiophores usually colorless smooth; osmophilic; cleistothecia produced in two species

A. cremeus group

2- conidial sttructures of two types; large heads light gray, green , or olive-buff with conidiophores usually in brown shades and encrusted; fragmentary structures borne near or beneath the agar surgace .....A. sparsus group

III . Sterigmata strictly biseriate

A. Conidial heads typically in definite green shades; hulle cells usually globose but sometimes irregularly ovate to pyriform

I. Conidial heads typically radiate; becoming loosely columnar in some species: conidiophores colorless or light brown. commonly exceeding 300 u in length; vesicles variable elongate, subglobse, hemispherical, or only slightly expanded hulle cells sometimes abundant, more often limited or lacking .....A. versicolor group

a. Conidial heads uniformly pigmented, small or fragmentary structures sometimes present; hyphal masses or sclerotia occasionally produced .....A. versicolor series

b. Conidial heads not uniformly pigmented, both white and green heads present (at least on some substrates)

.....A. janus series

2. Conidial heads typically columnar, usually dark yellow-green but occasionally gray blue-green or brownish; conidiophores brown walled, commonly less than 300 u long; vesicles supglobose, hemispherical, or terminally fattened; hulle cells typically, produced, usually abundant, clustered, forming crusts, or enveloping ascocarbs; cleistothecia common, purplish at maturity; ascospores in orange-red to blue-violet shades

....A. nidulans group

B. Conidial heads in shades other than true green, hulle cells when present, elongate to strongly curved and twisted

I. Conidial heads radiate to broadly columnar, in drab, olive, or dull brown shades; conidiophores typically brown-walled; vesicles variable from globose to elongate or hemispherical; hulle cells elongate, often strongly curved or twisted .....A. ustus group

2. Conidial heads broadly to irregularly columnar white to avellaneous or vinaceous; conidiophores with walls brown or uncolored; vesicles subglucose to elongate; elongate hulle cells or heavy-walled hyphal elements prelent.A. flavipes group

3. Conidial heads compactly columnar, typically in cinnamon to orange - brown or pale buff shades ; conidiophores colorless; vesicles hemispherical ....A. terreus group

KEY TO GROUPS

Based primarily on color

A. Conidial heads showing some shade of green during development .....B.

AA. Conidial heads in some other color .....L.

B. Vesicles clavate or subclavate; sterigmata uniseriate ..C.

BB. Vesicles not clavate; sterigmata uniseriate or biseriate.D

C. Vesicles strongly clavate; conidial heads blue-green, becoming gray in age .....A. clavatus group

CC. Vesicles subclavate; sterigmata uniseriate; conidial heads yellow-green gray-green, or blue-green when youngdarkening in most species .....A. ornatus group

D. Conidial heads bright yellow-green when young, sometimes becoming brown in age, loosely radiate; sterigmata biseriate in most species. .....A. flavus group

DD. Conidial heads in other green shades; sterigmata uniseriate or biseriate .....E.

E.

E. Colonies mostly showing naked yellow cleistothecia and yellow or red encrusted hyphae .....A. glaucus group

EE. Colonies lacking naked yellow cleistothecia and yellow and red encrusted hyphae .....F.

F. Conidial heads definitely columnar.....G.

FF. Conidial heads globose, radiate, or loosely columnar.I.

G. Sterigmata uniseriate .....H.

GG. Sterigmata biseriate; globose to subglobose hulle cells common; cleistothecia in some species; ascospores orange red to violet.....A. nidulans group

H. Conidial heads columnar, long, narrow(often twisted) to irregular; conidia usually formed as cylindrical segments from the sterigmata; cleistothecia lacking; typically osmophilic.....A. restrictus group

HH. Conidial heads columnar, compact and typically uniform in diameter throughout; conidia not formed ar cylindrical segments; cleistothecia in some species; not typically osmophilic.....A. fumigatus group

I. Vesicles small, variable in shape.....J.

II. Vesicles large, strictly globose; conidiophores constricted below the vesicle.....K.

J. conidial heads blue-green, dull yellow-green, or gray blue-green, radiate to loosely columnar; hulle cells globose to subglobose .....A.versicolor group

JJ. Conidial heads olive, olive-gray, dark to light brown; radiate to broadly columnar; hulle cells elongate to twisted.....A.ustus group

K. Conidial heads graying in age from blue-green or olive-buff shades .....A. sparsus group

KK. Conidial heads pale yellow-green, blue-green, or buff-brown.....A. cremeus group see also A.wentii group)

L. Growth very sparse and sporulation poor on Czapek's agar

LL growth and sporulation usually abundant on CZ pek agar

ager .....M.

M. Heads loosely to compactly columnar.....N.

MM. Heads globose to radiate.....O.

N. Heads loosely columnar, white, flesh colored, or cream-buff.....A. flavipes group

NN. Heads compactly columnar, avellaneous to cinnamon.....A. terreus group

O. Heads persistently white; larger heads definitely globose or radiate.....A. candidus group

OO. Heads not white.....P

P. Heads in yellow, ochraceous or light brownish shades .Q.

PP. Heads in black or dark brown shades ..A. niger group

Q. Heads in sulphur yellow to ochraceous shades A. ochraceus group

QQ. Heads in yellow-brown to dull buff shades .....A. wentii group (also A. cremeus group in part)

Aspergillus clavatus Group

clavatus

group key

C onidial structures often I to 5 or more cm. in length.....A. giganteus Wehmer

Conidial structures not exceeding 4.0mm. in length .....A. clavatus Desm.

Conidial structures less than I.O MM in length .....A. clavato-nanica Batista, Maia, & Alecrim

Aspergillus glaucus Group

Group Key

A. Asc ospores lenticular 6u or less in long axis, including ridges or crestg

1. Ascospores with convex surfaces smooth or nearly so

a. Equatorial ridges lacking, furrow absent or showing, only as a trace

(I) Conldial heads large, radiate to loosely columnar, borne above the surface layer of cleistothecia and enveloping hyphae ...A. repens DeBary. *

(2) Conidial heads small, enmeshed with the cleistothecia in a felt of sterile hyphae ..A. peseudoglaucus Blochwitz

b. Equatorial ridges lacking but with furiow definite, appearing asa a narrow shallow depression; colonies yellow, not developing red pigmentation ..A. tonophilus ohtsuki

c. Equatorial ridges low and roundded, furrow broad and shallow colonies developing a strong red or orangered pigmentation ..A. rub er (Konig, spieckeimann Bremer) thom church .

d. Equetorial ridges thin and flexuous, crestlike (ascospore resemblingapulley) ..A. chevalieri (Mangin Thom& Church

2. Ascospores with convex surfaces rough

a. Conidia smali, smooth walled; ascospoies with definite crests .. A. chevalieri Ver. intermedius Thom & Raper

b. Conidia small, echinulate; ascospores without crests but with prominent V-shaped furrow flanked by irregularr ridges.

(I) Colonies predominantly cleistothecial ..A. amstelodomi (hangin Thomy chueck

(2) coloniespredominankly conieial ..A. monteyidensis Talice machkinnon

(I) Conidia small, not exceeding 5.5 u in diameter .....
.....A. cristatus n. sp.

(2) Conidia large, exceeding 5.5 u in diameter.

(a) Ascospores 6.5 to 7.5 u in long axis. A. mangini Thom & Raper

(b) Ascospores 7.5 to 8.5 u in long axis .....
.A. umbrosus Bainier & Sartory

(c) Ascospores 9.0 to I0.0 u in long axis.....
.....A. echinulatus (Delacr.) Thom & Church

B. Asci ripening more slowly, colonies favored by media containing 40 per cent sugar or more.

(I) Ascospores roughened in equatorial area with ridges and furrow.

(a) Ascospores 8.8 to 9.6 u by 6.0 to 6.8 u with ridges ~~relatively~~ thin and irregular; colonies developing brick red pigmentation on M 40y agar .A. medius to 7. 5u Meissner

(b) Ascspores5.5 u by 4.0 to 5.0 u (up to 9.0 by 7.0 u), with ridges low; colonies maize to buff yellow on Czapek agar with ca. 70 per cent sucrose.....
...A. halophilicus chistensen, papavizas,& Benjamin

(2) Ascospores usually without equatorial ridges and furrow...A. carnoyi (Biourge) Thom & Raper

-- [illegible] SPECIFICATIONS

2. Conidial heads white.....A. niveo-glaucus Thom & raper

II. Cleistothecia absent, but coiled ascogonia abundant

A. Ascogonia producing naked clustered asci; conidial heads light brown .....A. athecius n. sp.

B. Ascogonia never producing asci; conidial heads green pro

ASPERGILLUS ORNATUS GROUP

GROUP KEY

I. Conidial heads yellow-green to brownish green. radiate; conidia citriform to elliptical; cleistothecia tyically produced.

A. Cleistothecia at first white and parenchymatous throughout, becoming purplish at maturity; produced in dark incubated cultures.

1. Ascospores with multiple thin flexuous crests.....
.....A. ornatus Raper, Fennell & Tresner

2. Ascospores with a single equatorial ridge(two adherent crests?) ....A. citrisporus (Von Hohnel) Raper, Fennell Tresner

B. Cleistothecia white to olive, lacking a definite wall, surrounded by loosely interwoven fine hyphae; ascospores large, spiny, without equatorial furrow or ridges .....
.....A. spinulosus Warcup n. sp.

II. Conidial heads light grayish blue-green, loosely columnar or radiate; conidia elliptical; sclerotia or compact sclerotium-like masses of hulle cells typically present and produced more abundantly in dark-incubated cultures.

A. Sclerotia present; heads columnar; conidiophores and conidia delicately roughened..A. paradorus Fennell & Raper

B. Hulle cell masses present; heads usually radiate; conidiophores and conidia smooth ..A. raperi Stolk

III. Conidial heads at first dark blue-green then brownish black, radiate; conidia globose, strongly spinulose to irregularly warty.....A. brunneo-uniseriatus Singh & Bakshi

Aspergillus ceruinus Group

GROUP KEY

I. Heads radiate

A. Conidiophores exceeding IOO, erect to terminally recurved

I. Conidiophores usually IOO to 300 ,in length, walls very thick, I.5 to 2.0 ,; heads erect.....
....A. cervinus (Massee) emend. Neill

2. Conidiophores extremely variable, IOO to 800 u, in length; large heads usually erect, smaller heads often nodding ...A. kanagawaensis Nehira

B. Conidiophores not exceeding IOO in length; vesicles upright or borne at an angle ..A. parvulus Smith

II. Heads columnar

A. Conidiophores and vesicles strongly pigmented, vesicles borne at acute angles.....
.....A. nutans Mclennan & Ducker

Aspergillus restrictus Group

GROUP KEY

I. Heads columnar; vesicles small, flask shaped. domelike or only gradual enlargements of the conidiophore apices fertile on the upper surface only

A. Conlonies 4 to 5 cm. in diameter in 3 weeks at 26 C on standard Czapek'S agar ..A. caesiellus saito

B. Colonies less than I.5 cm. at 3 weeks on Czapek's agar

I Rapidly growing on M 40 y agar, dark olive green; columns long, often twisted, adherent in fluid mounts .....
...A. restrictus smith

2.less rapidly growingon M40y agar, light gray green,columns more delicate

a. conidia elliptical when first formed, often remaining so,mostly 4,5uby3,0to 3.5uchains adherent in fluid mounts.....A. conicus Blochwitz

b. conidia subglobose to pyriform, mostly 3.0 to 3.5u in diameter, chains not adherent in fluid mounts.....
... .A. gracilis Bainier

II. Heads radiate when young, tardily becoming loosely or irregularly columnar; vesicles subglobose to pear-shaped, fertile over the upper half to two-thirds.....
.....A. penicilloides spegazzini

Aspergillus Fumigatus Group

GROUP KEY

Cleistothecia absant .....A. fumigatus series

A. Conidial heads erect, compart, and strongly columnar; vesicles commonly 20to 30u in diameter, upright on the conidiophore

I. conidiophores 0.5 mm.or less; conidial heads dark green; conidia globose echinulate .....A. fumigatus Fresenius

2. Conidiophores exceeding 0.5 mm.; conidial heads light yellow-green; conidia elliptical, smooth or nearly so.....
.. ....A. fumigatus varellipticus, n.var.

B. Conidial heads often presenting a nodding appearance. smaller than the preceding and not consistently columnar; vesicles less than 20u in diameter

I. Vesicles upright but with sterigmata often borne in lateral or basal clusters to give a pseudonodding appearance..... ....A. unilateralis Thrower

2. Vesicles often borne at an angle to the conidiophore

a. Conidiophores thin walled, sinuous; vesicles uncolored and often strongly nodded, conidia in pale blue green shades.....A. viridi-nutans Ducker& Thrower

b. Conidiophores heavy walled; vesicles and sterigmata colored ; conidia in dark blue- green shades

(I) Conidia conspicuously echinulate; colony reverse uncolored or nearly so ....A. duricaulis, n.sp.

(2) Conidia finely spinulose; colony reverse in reddish brown to deep rose shades ...A. brevipes Smith

II. Cleistothecia present .....A. fischeri series

A. Cleistothecia and enveloping hybhae white to cream in color

I. Ascospores showing two distinct equatorial crests

a. Convex surfaces bearing anastomosing ridges.....

.....A. ficheri wehmer

b. Convex surfaces smooth (ornearly so) .....

..... A. ficheri var.glaber Fennell & raper

C. Convex surfaces spinulose or echinulate .....

....A. Ficheri var. spinosus, n. var.

2. Ascospores showing more than two equatorial crests

a. ascospores showing 4equatorial crests.....

.....A. Quadricinctus Guill

B. cleistothecia and enveloping hyphae in yellow, golden or orange shades

I. Colonies loose textured, growing rapidly on all media, cleistothecia large

a. Ascospores with prominent equatorial crests and

C convex surfaces conspicuously echinulate .....
.....A. aureolus Fennell & raper

2. C colonies close textured , growing very restrictedly on czapek's agar, cleistothecia small

a. ascospores with prominent equatorial crests and convex surfaces delicately echinulate.....
..... A. stramenius Novak & kaper, n. sp.

B. Ascospores with low equatorial crests and convex surfaces finely reticulate or spinulose..A. auratus Warcup. n. sp.

Aspergillus Ochraceus Group

GROUP KEY

I. C Conidial heads in pale pure yellow shades

A. Sclerotia cream to pale yellow, produced in a dense layer, 300 to 450 u in diameter; conidial heads loosely radiate, spore chains adherent into narrow divergent columns.....A. sulphureus(Fres.) Thom & Church

B. Sclerotia white to cream to pale pink, produced singly, I.0 to I.5 mm. in diameter; conidial heads hemispherical to loosely columnar, or split into two or more compact .....
.. columns..A. sclerotiorum Huber

II. C Conidial heads in bright golden yellow shades

A. Sclerotia black at maturity, vertically elongate,I to 3mm. high (at several months containing multiple cleistothecia); conidia smooth, oval to subglobose,2.5 to 4.0 bu 2.0 to 3.5u; c... h, conedial heads changing. to cinnamon buff in age...A. alliaceus Thom & C hurch

B. Sclerotia orange to rufous, globose to subglobose, 500 to 700 u in diameter, conidia heavy walled, smooth , elliptical or ovate, 3.3 to 4.4 u by 2.5 to 3.0u;

Conidial heads remaining bright in age ...A. auricomus (Guéguen) saito

III. Conidial heads in dull yellowish cream, buff, or ochraceous shades

A. Sclerotia produced in most strains

I. Sclerotia abundant, small, commonly 400 to 500u

a. Sclerotia pure yellow then brown; conidia globose, subglobose or elliptical, 2.75 to 3.5 u or 3.0 to 3.3 u by 2.5 to 2.8 u .....A. melleus Yukawa

2. Sclerotia scattered, developing late, large, commonly 500 to I000 u

a. Sclerotia pink to vinaceous purple when mature, globose, ovate to cylindrical; conidia globose to subglobose, mostly 2.5 to 3.0 u ...A. ochraceus Wilhelm

b. Sclerotia cream to buff or clay colored, globose to ovate; conidia elliptical to pyriform 4.0 to 5.0 u by 3.0 to 3.5 u .....A. ostianus Wehmer

c. Scmerptoa wjote tp cream, ovate to discoid; conidia ovate to elliptical, mostly 3.2 to 4.0 u by 2.8 to 3.2 u.....A. elegans Gasperini

B. Sclerotia unknown

I. Colonies [illegible] close textured, sporulating slowly conidial heads pinkish buff; conidia subglobose ovate or elliptical, mostly 3.0 to 4.0 u by 2.5 to 3.0 u..... .....A. petrakii Voros

Aspergillus niger Group

GROUP KEY

I. Sterigmata in two series

A. Colonies (conidial heads) on Czapek's agar appearing carbon black to the naked eye

I. Conidia 6 to IO u in diameter at maturity ..A. carbonarius (Bainier) thom

2. Conidia 5.0 u or less in diameter at maturity

a. Conidiophores not exceeding 4.0 mm. in length

(I). Colonies spreading rapidly on Czapek's agar.....A. ficuum (Reich.) Hennings

(2). Colonies growing mores slowly on Czapek's agar

(a). Conidia at maturity horizontally flattened, mostly 3.0 to 3.5 u in diameter, with longitudinal color bars or striations ....A. phoenicis (Cda.) Thom

(b). Conidia at maturity globose, mostly 4.0 to 5.0 u irregularly roughened with conspicuous ridges and echinulations not arranged as longitudinal striations.....A. niger V. Tiegh .

b. Conidiophores commonly exceeding 5 mm. and reaching I cm. but also commonly with shorter stalk bearing diminutive heads .....A. pulverulentus (Mcalp.) Thom

B. Colonies (Conidial heads) grayish olive brown or deep olive brown when young; usually becoming reddish brown to brownish black, but with olive or grayish colors often persistent.

(I). Heads quickly dark black-brown or reddish brown a.

a. Conidia under 5.0 u in diameter, horizontally flattened, and appearing striate at maturity

(I) Heads quickly dark black- brown; colony reverse uncolored; conidiophores mostly 2 to 3 mm. but up to 5.0 mm. long; conidia mostly 3.0 to 3.5 u in diameter.....A. tubingensis(Schober) Moss.

(2) Heads quickly reddish brown; colony reverse in similar shades; conidiophores mostly I.0 to 1.5 mm. long; conidia mostly 4.0 to 4.5 u in diameter..... .....A. awamori Nakazawa

b. Conidia 6.0 to 8.0 u in diameter, globose to subglobose, coarsely tuberculate ....A. flavo-furcatis Batista and Maia (see A. flavus group)

2. Heads persistently dark grayish brown or olive-brown

a. Conidia at maturity elliptical, conspicuously echinulate, 5.0 to 5.5 u by 3.3 to 3.8 u.....A. ellipticus, sp . nov.

b. Conidia at maturity globose or nearly so, sometimes elliptical when young

(I) Conidia at maturity conspicuously spinulose..... .....A. heteromorphus Batista and Maia

(2) Conidia at maturity irregularly and finely roughened

(a) Conidial heads generally small, in age on malt agar splitting into fairly numerous compact divergent columns....A. foetidus (Naka.) Thom and Raper

(b) Conidial heads large, columns few and poorly defined on malt agar

(I) Basal mycelium on malt agar uncolored or only faintly yellow..A. foetidus(Naka.) T. & R. var. N ., S.,&W.

(2) basal mycelium on malt agar bright golden yellow .....A. foetidus (Naka.) T.& R.var.acidus .N S.,& W.

II. Sterigmata uniseriate

A. Conidia globose to subglobose, conspicuously echinulate; vesicles commonly 20 to 35 u but ranging from I5 to 45 u .....A. japonicus saito

B. Conidia subglobose to definitely elliptical, conspicuously echinulate; vesicles commonly 60 to 80 u ranging from 35 to 100 u ...A. A. aculeatus Iizuka

Aspergillus flavus Group

GROUP KEY

I. Conidial heads in pale to intense yellow or yellow-green shades when young

A. Colonies not shifting to brown on Czapek's agar; conidia definitely echinulate

1. Sterigmata either single or double with the latter predominant; heads radiate or very loosely columnar ..... A. flavus Link

2. Sterigmata typically in a single series

a. Heads colunar; sterigmata usually uniseriate..... A. flavus var. columnaris, N. var.

b. Heads radiate; sterigmata uniseriate..... A. parasiticus Speare

B. Colonies shifting to light brownish green in age on Czapek's agar; conidia irregularly roughened or smooth

1. Conidia large, mostly 4.5 to 7.0 u but up to 8.0 u or 10.0 elliptical at first, then globose to subglobose, smooth to irregularly roughened

a. Conidiophores borne primarily from the substrate ..... A. oryzae(Ahib.) Cohn

b. Conidiophores borne primarily as short branches from aerial hyphae.... A. oryzae(Ahlb.) Cohn Var. effusus (Tiraboschi) Ohara

2. Conidia small, oval to elliptical, mostly 3.0 to 3.5u by 2.4 to 3.0 u smooth or nearly so

a. Growth negligible on Czapek's agar; conidial structures abundant, zonately arranged on ~~mall agar conidiophores~~ smooth or nearly so ... A. zonatus Kwon & Fennell, n. sp.

b. Growth spreading on both Czapek's and malt agars; conidial structures often forming coremiform clusters; conidiophores conspicuously roughened.....A. clavato-
.... flavus, N. sp

II. Conidial heads in deep yellow-green to olive-brown shades when young; conidia conspicuously verruculose

A. Conidial heads at first deep yellow-green, shifting to brownish green or brown on czapek's agar .....
.....A. tamarii Kita

B. Conidial heads quickly olive'brown then dark brown..
....A. Lavo-furcatis Batista& Maia

III. Conidial heads in pale yellowish olive or grayish olive shades; conidia smooth or nearly so

A. Conidiophores conspicuously échinulate...A. subolivaceus,n.sp.

B. Conidiophores smooth or nearly so..A.avenaceus smith

Aspergillus wentii Group

GROUP KEY

I. Conidiopheres smooth or granulose

A. Conidial heads large, up to 500 u or more in diameter, on smooth or slightly granular stalks which may reach several millimeters in length...A. wentii Wehmer

B. Conidial heads smaller and borne on shorter stalks

I. Sterigmata mostly double, few single; both single and double sterigmata often observed in the same head

a. Conidia mostly 5.5 to 6.5 u coarsely echinulate...
.....A. terricola Marchal

b. Conidia mostly 4.5 to 5.5 by 3.8 to 5.0 u, rugulose.
.....A. terricola var. americana Marchal

2. Sterigmata almost entirely single, rarely double, and then at the base of the vesicle in otherwise uniseriate heads ....A. terricola var. indicus(Mehrotra&Agnihotri) n. comb .

II. Conidiophores conspicuously echinulate.....
.....A. thomii Smith

Aspergillus cremeus Group

GROUP KEY

I. Conidial heads in light green shades
A. Sterigmata biseriate; cleistothecia present .....
....A. cremeus Kwon & Fennell,n. sp.
B. Sterigmata initially uniseriate. becoming biseriate progressively from the base of the vesicle upward; dark stroma'like hyphal masses present..... ...
.....A. stromatoides, n. sp.
C. Sterigmata uniseriate; dark hyphal masses and cleistothecia lacking...A. itaconicus Kinoshita

II. Conidial heads in light brown shades
A. Sterigmata biseriate; cleistothecia present .....
.....A. chrysellus Kwon & Fennell, n. sp .
B. Sterigmata mostly uniseriate, occasionally biseriate; cleistothecia absent...A.flaschentraegeri Stolk

Aspergillus sparsus Group

GROUP KEY

I. Sterigmata in two series
A. Conidia usually of one type
1. Conidia in large heads light yellow'green to olive-buff, subglobose to elliptical, delicately roughened.....A. sparsus Raper & Thom

2. Conidia in large heads darker green, globose, conspicuously echinulate; similar conidia produced from fragmentary structures at agar surface ..A.biplanus, spnov .

B. Conidia of two types

I. Conidia in large heads gray , slightly elliptical, smooth or nearly so; conidia from low level fragmentary structures, green,globose, conspicuously roughened .....A. diversus, sp. nov.

II. Sterigmata in a single series

A. Conidia in heads borne on long stalks at first elliptical and smooth, those subsequently formed subglobose to globose, brown and coarsely roughened as are the conidia of short stalked and fragmentary heads ....A. funiculosus Smith

Aspergillus versicolor Group

GROUP KEY

I. conidial heads of one color....A.versicolor series

A. Vesicles globose to somewhat elongate, fertile over most of the vesicular surface; globose to subglobose hullecells often present; compact hyphal masses and sclerotia lacking

I. Mature conidia not exceeding 4.0 u consistently globose to subglobose

a. Conidiophores uncolored to faintly yellowish

(1) Conidial heads variable in color, light yellow-green, buff to orange'yellow, or occasionally flesh colored..... A. versicolor(Vuill. tiraboschi

(2) Cnidial heads always blue'green when young.....
....A. sydowi(Bain.&Sart.) Thom and Church

b. Conidiophores definitely brown

(I) Conidial heads radiate, very dark yellow-green; conidiophore walls smooth; Hulle cell masses conspicuous.....A. silvaticus Fennell & Raper

(2) Conidial heads radiate; very dark blue-green; conidiophore walls definitely rough; hulle cells lacking... ..... .A. pulvinus Kwon & Fennell. n. sp.

(3) Conidial heads variable in shape, often loosely columnar; conidiophore walls smooth but with knobby encrustments on malt agar...A. speluneus. n. sp.

2. Mature conidia usually exceeding 4.0 u globose, subglobose, or elliptical

a. Conidiophores colored in brown shades

(I) Conidia strictly globose, echinulate

(a) Conidial heads pale gray-green, globose to somewhat elongate Hulle cells in small colorless clusters .: .....A. granulosus Raper & Thom

(b) Conidial heads dark yellow-green globose to elliptical hulle cells in conspicuous hyaline to purplish masses.....A. caespitosus Raper& Thom

(2) Conidia of two types; elliptical and smooth; globose and rough .....A. asperescens Stolk

b. Conidiophores uncolored in wet mounts; conidia 4.0 to 5.0 u in long axis ...A. varians Wehmer

B. Vesicles turbinate, spathulate, or merely slight expansions of the conidiophore apices, fertile on the apex only; hulle cells lacking, or if present, pyriform to elongate; compact hyphal masses or sclerotia present

I. Soft masses of white to cream-colored, compacted, thin-walled or heavy-walled cells present, sometimes in lim-

a. Stalks long, 1 to several cm.; smooth; conidia mostly oval to elliptical, smooth or very slightly roughened; abundant sclerotium-like masses composed of globose, elongate, or pear-shaped elements resembling hulle cells ....A . malodoratus Kwon & Fennell. n. sp.

b. Stalks less than 400 u delicately roughened; conidia globose, 4 to 7 u in diameter with long echines; sclerotium-like masses sparsely produced, composed of thin-walled cells and surrounded by short sterile hyphae..... .....A. crystallinus Kwon & Fennell. n. sp.

2. True sclerotia present, cream to buff; vesicles turbinate, often borne at a slight angle to the conidiophore

a. Conidial heads dark yellow-green; conidiophores up to 600 u long; conidia globose, minutely asperulate, mostly 2.5 to 3.0 u in diameter ...A. peyronelii sappa

b. Conidial heads gray-blue-green, conidiophores up to 350u long; conidia globose to subglobose, smooth or nearly so, mostly 2.2 to 2.8 u in diameter ...A. arenarius,n.sp.

II. conidial heads of two colors; green or white ....A. JANUS series

A. Vesicles of green and white heads dissimilar

1. Vesicles of white heads conspicuously clavate, 45 to 60u by 15 to 18 u borne on conidiophores usually exceeding 2 mm. in length ....A. janus Raper& Thom

2. Vesicles of white heads not conspicuously clavate,20 to 25 u by 14 to 18 u borne on conidiophores less than 2.0 mm. in length.....A. janus var. brevis Raper& Thom

B. Vesicles of green and white heads essentially similar

I. Conidia from both white and green heads smooth walled and of similar dimensions ....A. allahabadii Mehrotra & Agnihotri

2. Conidia from green heads rugulose and larger than the smooth conidia of white heads

a. Colonies on malt agar producing cushion-like overgrowths of thick-walled hyphal elements .....
.....A. ambiguus Sappa

b. Colonies on malt agar notproducing overgrowths .....
.....A. microcysticus Sappa

Aspergillus nidulans Group

GROUP KEY

I. Ascospores present

A. Ascospores orange-red in color

1. Equatorial crests two in number, rarely lacking. not exceeding 2.0 u in width

a. Convex walls smooth

(1) Equatorial crests lacking ....A. nidulans var. acristatus Fennell& Raper
.....

(2) Equatorial crests present

(a) Conidial stage dark yellow-green, promiment on malt agar, generally arising from submerged mycelium.

(1.) Coarse, encrusted, spicular hyphae absent

(1.) Crests entire, 0.5 to 1.3 u wide ...A. nidulans (Eidam) Wint

(2.) Crests entire, 1.5 to 2.0 u wide

(i') Crests entire, 1.5 to 2.0 u wide

(i") Cleistothecial envelope in dull shades consisting of hulle cells only .....A. nidulans var, latus Thom & Raper

(2.) Cleistothecial envelope consisting of hulle cells associated with abundant mycelium in bright yellow to

red- orange shades.....A. heterothallicus Kwon, Fennell,& Kaper, n. sp .

(c) Crest dentate.....A. nidulans var. dentatus Sandhu& Sandhu

(2) Coarse, encrusted, spicular hyphae present; cleistothecia rare .....A. unguis(Emile-weil& Gaudin) Thom & Kaper

(b) Conidial stage green , inconspicuous on malt agar, generally arising from aerial mycelium

(1) Cleistothecia borne in mycelial tufts with few accompanying hulle cells

(a) Cleistothecia 50 to 100 u associated mycelium heavily encrusted, silvery in apperance .... A. fruticulosus n. sp.

(b) Cleistothecia less than 50u associated mycelium mostly unbranched, not encrusted ....A. parvathecius, n.sp.

(2) Cleistothecia obscured by a nearly continuous layer of hulle cells ...A. aurantiobrunneus (A., H., & K.)n. comb.

b. Convex walls not smooth

(1) walls echinulate .... A. nidulans var. echinulatus Fennell & Kaper

(2) Walls coarsely rugulose .....A. rugulous Thom & Kaper

2. Crests two in number, 3.0 u or more in width.

a. Crests dissected, stellate.....A. variecolor(B&B.) Thom & Kaper

b. Crests entire .....A. variecolor var. astellatus Fennell & Kaper

A. Stellatus

3. Crests four or more in number,

a. Crests four in number, narrow .....A. quadrilineatus Thom & Kaper

b. Crests multiple, sometimes irregularly arranged and

giving the impression of striations .....A. striatus Kai. Tewari & Mukerji

B. Ascospores blue-violet in color, with convex surfaces echinulate .....A. violaceus Fennell& Raper

II. Ascospores not present

A. Conspicuous coarse encrusted spicular hyphae present; hulle cells lacking except in one unique strain which has been known to produce occasional cleistothecia .... .A. unguis (E.W.&.G.) Thom & Raper

B. Conspicuous spicular hyphae absent

I. Hulle cells absent or limited in number

a. Hulle cells absent, yellow mycelium prominent

(i) Conidiophores short, straight; conidial heads loosely columnar to somewhat divergent; arising from a yellow submerged mycelium .....A. aureolatus Hunt. Cvet.& Bata

(2) Conidiophores short, curved or coiled;conidial heads columnar, enmeshed in a prominent yellow aerial mycelium ....A. recurvatus, n. sp.

b. Hulle cells very limited in number, scattered, seldom exceeding IO to I2 u in diameter ...A. speluneus, n. sp. (see A. versicolor group

2. Hulle cells aggregated in scattered and irregular masses suggestive of cleistothecia; not produced on malt agar .....A. caespitosus Raper & Thom (see A. versicolor group)

3. Hulle cells a bundant, scattered throughout the mycelial felt; conidiophores very short ...A. subsessilis, n . sp.

4. Hulle cells abundant, massed to form continuous crusts on malt agar

a. Hulle cell crust blue-gray, colonies restrictedly growing ....A. crustosus,n. sp.

b. Hulle cell crust reddish purple, colonies rapidly spreading .....A. multicolor sappa

c. Hulle cell crust bronze .....A aeneus sappa

d. Hulle cell crust golden yellow ..A. silvaticus Fennell & Raper (see A. versicolor group)

e. Hulle cell crust white to creamy white, conidial heads few in number and developing tardily ....A. eburneo-cremeus sappa

Aspergillus ustus Group

GROUP KEY

======

I. Vesicles upright on the conidiophores

A. Conidial heads in olive-gray to drab or red-brown shades

1. Conidial heads variable, radiate when young to loosely or broadly columnar at maturity

a. Hulle cells typically present, scattered throughout the colonuY or forming irregular masses not associated with pigmented mycelium ...A. ustus(bain.) Thom & church

b. Hulle cells abundantly produced, forming conspicuous masses associated with brightly pigmented yellow mycelium .....A. puniceus Kwon & Fennell. n. sp.

2. Conidial heads persistently radiate

a Conidial heads reddish brown (near wood brown); hulle cells elongate, twisted, in tufts of red mycelium ....A. panamensis Raper & Thom

b. Conidial heads buffy olive; hulle cells elongate, seldom bent, associated with yellow mycelium ....A. conjunctus Kwon & Fennell, n . sp .

II. Vesicles borne at a sharp angle to the vertical axis of the conidiophore .....A. deflectus Fennell& Raper

Aspergillus flavipes Group

GROUP KEY

I. Cnidiophores definitely pigmented in yellow to light brown shades

A. Conidial heads usually white to very pale buff, in occasional strains darker near avellaneous .....
.....A. flavipes (Bain.& sart.) Thom& church)

II. Conidiophores unpigmented or very faintly yellowed

A. Conidial heads persistently white ... A. niveus Blochwitz

B. Conidial heads at first white, becoming vinaceous fawn ..;.....A. carneus(V. Tiegh.) Blochwitz

Aspergillus terreus Group

GROUP KEY

I. Colonies velvety; conidial heads long, compactly columnar, in cinnamon to orange-brown or brown shades; borne on short conidiophores

A. Sclerotium-like masses of swollen, relatively heavy-walled cells lacking on malt and agar....A. terreus Thom

B. Sclerotium-like masses present on malt agar .....
.....A. africanus Fennell & Raper

II. Colonies floccose, aerial mycelium conspicuously golden yellow, conidial heads small, compactly columnar, cream to buff; borne on conidiophores to 500 or more long.....A .....A. terreus var. aureus Thom & Raper

Practical physiology of fungi

For 4th year of B.Sc. students

Prepared by

Prof. Dr. Abdelrahman Saleem

Culture media

Classification of culture media

Criteria used for classifying culture media includes their chemical composition, physical properties and their use. Every culture medium is designed for a definite use and hence its physical and chemical characteristics depend on its application and function.

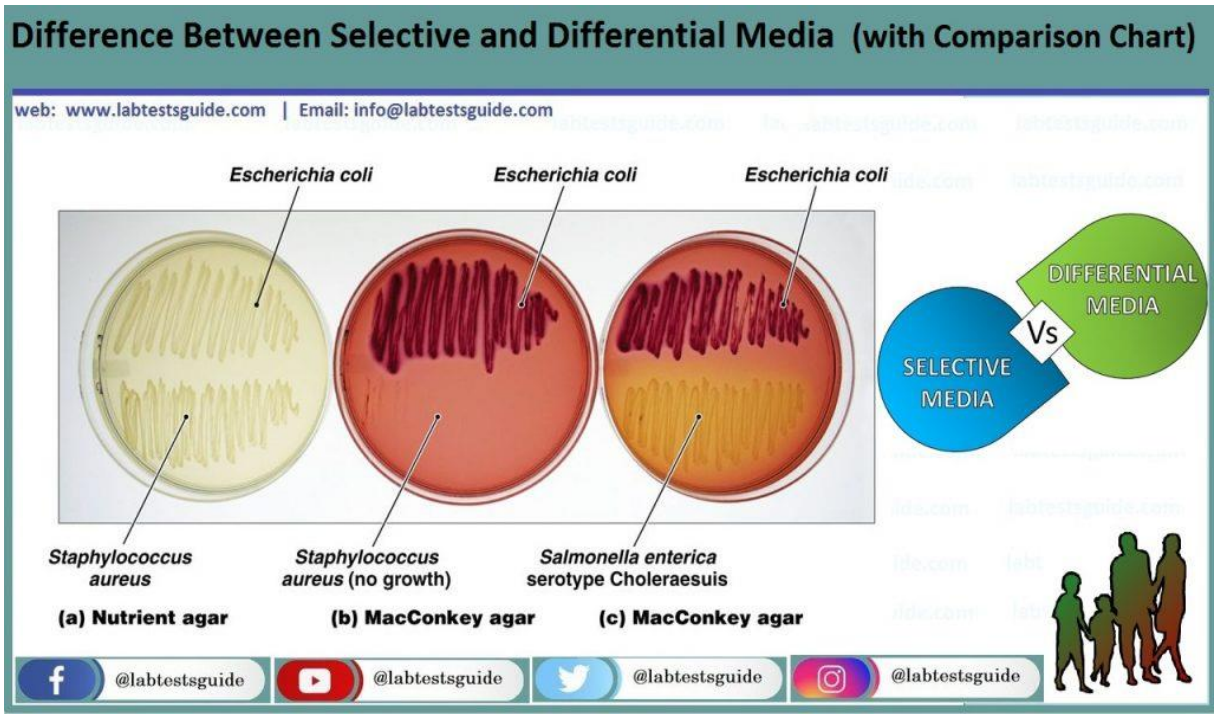
I- Classification of culture media according to their use

According to their use culture media are divided into the following types:

1- **Routine laboratory media**: These media contain certain complex raw materials of plant or animal origin such as yeast extract, malt extract, peptone etc., and are employed for routine cultivation and maintenance of a wide variety of fungi.

2- Enriched media: These media are prepared by supplementing the routine laboratory media with some specific substances such as vitamins and amino acids to meet the nutritional requirements of more fastidious of fungi and are employed for their cultivation.

3- Selective media: These media facilitate the isolation of a particular group or species of microorganisms from mixed cultures. Such media contain substances which inhibit microorganisms except the desired group or species.



4- Differential media: These media are supplemented with certain reagents or chemicals for differentiating between various kinds of microorganisms on the basis of visible differences in their growth patterns. Such type of media is used more often in bacteriological studies.

5- Assay media: These type of media is specifically employed for the assay of some metabolites such as enzymes, vitamins, amino acids, antibiotics, disinfectants etc., and are of definite composition.

6- Biochemical media: These media are generally used for the differentiation of microorganisms on the basis of their biochemical activities, and are helpful in the study of their metabolic processes.

II- Classification of culture media according to their chemical composition: According to their chemical composition media are classified into the following types:

1- Natural media: The natural medium comprises entirely complex natural products of unknown composition. The raw material of a natural medium may be

of plant or animal origin, and some of the common ingredients employed for this purpose include extracts of plant and animal tissues, e.g., fruits, vegetables, egg, milk, blood, body fluids, yeast, malt and manure extracts etc. Obviously, the chemical composition and concentration of a natural medium is not well defined. On account of their complex nature, these media are able to support a variety of organisms, and hence are quite useful for routine laboratory cultures of fungi.

2- Semisynthetic media: These media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources with unknown composition. The chemical composition of a semisynthetic medium is partly known. The medium is a best serve as a routine medium and sometimes for physiological studies. Potato dextrose agar (PDA) is one of the popular media.

3- Synthetic media: These are chemically defined media of known composition and concentration. The media are exclusively composed of pure chemical substances. However, absolute purity of the ingredients is achieved, although substances of only analytical reagent quality are used for such purposes. One account of their known composition as well as being in solution, these media are quite useful for nutritional and metabolic studies of fungi. The composition of these media may be amended as per requirement and as such they may be simple or complex in composition. A simple synthetic medium contains a single carbon and energy source, a nitrogen source, generally as ammonium salt, some Sulphur and phosphorus sources and various minerals. All these ingredients are dissolved in a buffered aqueous base. However, for more fastidious organisms, a complex synthetic medium is designed by incorporating some additional factors such as certain vitamins, amino-acids, purines, pyrimidines etc., or by employing a multitude of carbon and nitrogen sources together.

C- According to their physical states: Media are classified into the following types:

1- Solid media: Media in solid state are in use since the beginning of laboratory studies of fungi. The first laboratory culture of fungi was obtained on a solid media such as fruit slices. Some common examples of such media are nutrient impregnated slices of potato, carrot, sugar-beet etc. and coagulated egg or serum. However, with the advent of agar as a solidifying agent, such media have largely been replaced by agar media. Use of fruits and vegetable slices in the cultivation of fungi is now more or less restricted to the baiting technique employed for isolation of some specific organisms.

2- Solid-reversible to liquid media: Such reversible media were first introduced by Koch (1881) who observed that addition of 2 to 5 percent of gelatin to the commonly employed media rendered them a semi-solid consistency. However, gelatin could not find a wide application on account of its low melting point (37˚C), and also because it is hydrolyzed by many proteolytic bacteria at ordinary temperature. The use of agar for solidifying culture media was also initiated the same year and in the same laboratory.

3- Semi-solid media: These are media with gelatinous consistency and are employed for specific purpose. They contain a small amount of agar or some other solidifying agent like corn meal. These media are sometimes used for the study of motile reproductive structures of fungi.

4- Liquid media: These are media without any solidifying agent, and are indispensable for most of the quantitative studies of fungi. Nutritional and metabolic studies of fungi, as well as microbiological assays are invariably carried on liquid media. Some of the advantage of liquid media is that they permit the cultures to be aerated, the mycelium to be weighed and the metabolic products to be analyzed easily. However, with respect to routine studies, liquid media have some distinct disadvantages. Growth in liquid media does not manifest the morphological characteristics of microorganisms. They are also difficult to handle

without disturbing the culture. Moreover, liquid media are least helpful in the purification of microorganisms from a mixed culture. For an even distribution of nutrients and for providing uniform aeration to growing fungus, the liquid cultures are sometimes put to constant mechanical shaking.

Sterilization

Sterilization refers to the process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses and spore forms etc.) from a surface, equipment, foods, medications, or biological culture media.

Sterilization can be achieved through application of heat, chemicals, irradiation and filtration.

There are three main methods for sterilization:

1- Physical methods

2- Chemical methods

3- Mechanical methods

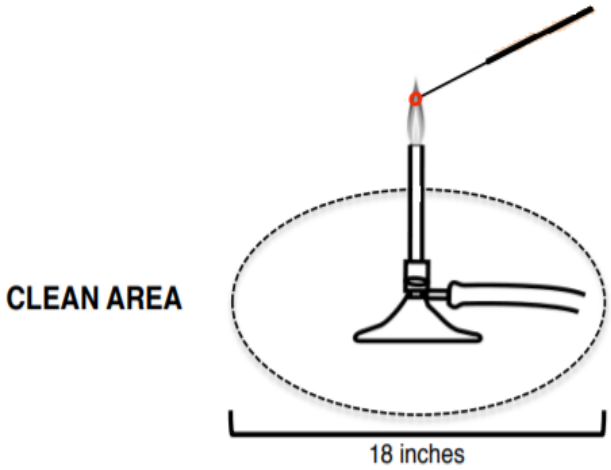
1- Physical methods

Sterilization by heat

Heat may be utilized for sterilization either in dry or moist form. However, moist heat is much more effective and requires both shorter duration and lower temperature. Sterilization by moist heat generally is complete at 121°C for 15-30 minutes of exposure. On contrast, sterilization by dry heat requires a temperature of 160°C for 60 minutes. The two kinds of heat treatments kill the microorganisms by coagulating and denaturing their enzymes and other proteins.

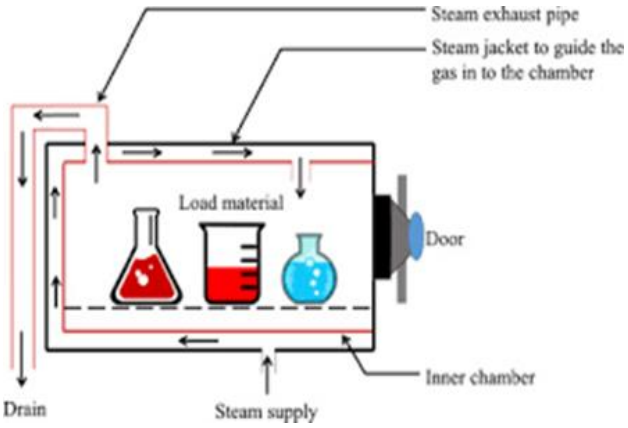
Application of dry heat

a- Flaming



b- Hot-air oven

Oven



radiation

c- Radiation (Infra-red or Ultra violet)

Application of moist heat

The use of the Autoclave for sterilization

2- Chemical methods

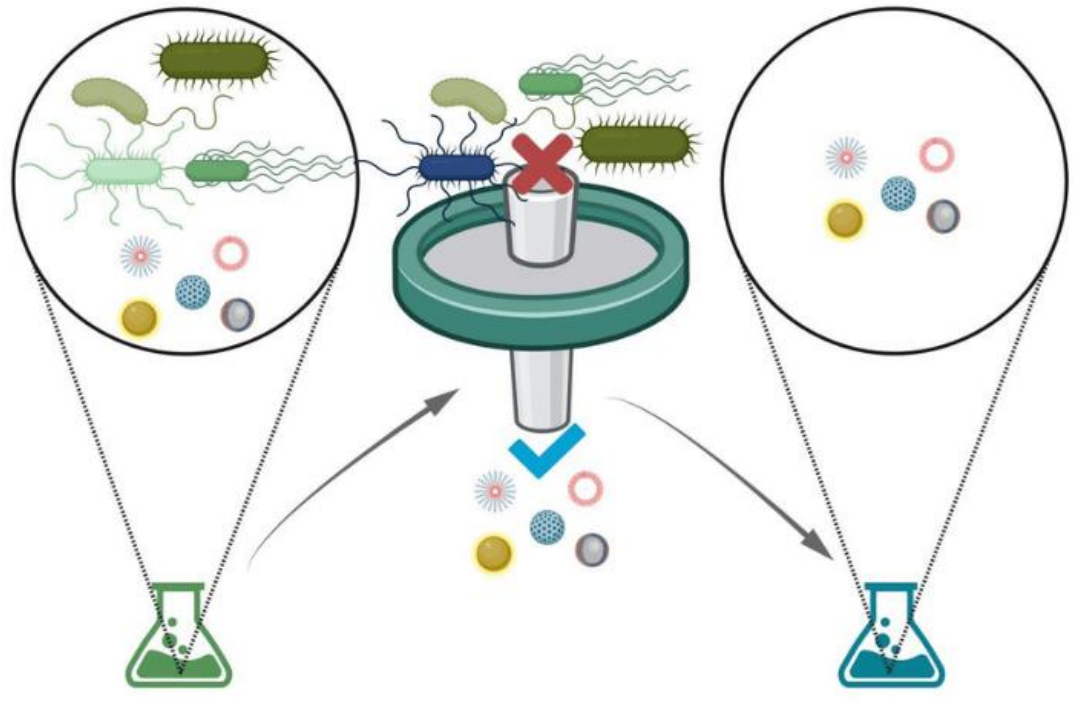
Using of chemical substances as agents, like chloroform, mercuric chloride, formaldehyde and ethyl alcohol.

3- Mechanical methods

Sterilization by filtration

This technique employs special type of filters having pores so small that ordinary bacteria are arrested. This method is particularly useful for sterilizing heat sensitive materials, such as culture media containing serum, antibiotic solutions, culture filtrates etc. The most common filters are Seitz filters and Cellulose membrane filters.


Flask of sample
Cap
Membrane filter
Cotton plug in vacuum line ensures sterility
Sterile filtrate
Vacuum line



Methods for measuring fungal growth

Growth may be defined as the orderly increase in cell components leading to an increase in biomass (Prosser, 1995). The growth form of filamentous fungi is complex; extension of individual hyphae is localized at the tip, whereas biomass synthesis supporting that growth may take place throughout the mycelium. The growth of a fungus can be measured in various ways such as increase in colony diameter, increase in dry weight, rate of production of different type of metabolites, etc. The mechanisms involved in the control and regulation of mycelial growth are better studied on solid medium than in submerged cultures, as fungi are adapted to growth on solid substrates.

Direct methods

Some of the direct methods to determine fungal growth are stated afterwards:

Growth of hyphal extension

Measure of hyphal extension rate is measured microscopically on solid medium as an increase in length, e.g. measure of the increase in radii of circular colonies, daily or every other fixed period of time, after inoculating the mold in an agar plate and incubate it at the appropriate conditions. This is probably the most common technique for estimation of growth of filamentous fungi on solid media. A growth rate function can be derived by plotting colony diameter against time and measuring the slope of the straight part of the line. Analysis is now greatly facilitated by the increased availability of image analysis systems which enable automated measurement of hyphal lengths and subsequent kinetic analysis of data (Wiebe and Trinci, 1990; Gray and Morris, 1992).

Measure of fungal biomass

Molds are usually grown on the surface of a cellophane membrane, overlaying the agar, from which the biomass can be washer or otherwise removed for the determination of the dry weight. This measure is also possible in liquid cultures. In both solid and liquid media, separation of biomass from the growth medium is slow, tedious and requires relatively large amounts of biomass for accuracy.

Turbidimetric techniques are less reliable due to the heterogeneous nature of liquid cultures of filamentous fungi.

Indirect methods

Ergosterol

Fungal plasma membranes are similar to mammalian plasma membranes, differing in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. The plasma membrane regulates the passage of materials into and out of the cell by being selectively permeable. Membrane sterols provide structure, modulation of membrane fluidity, and possibly control of some physiologic events. Fungal growth and biomass could therefore be estimated by measuring this specific component of fungi. Quantifying ergosterol production in foods has proved more difficult. Since now, ergosterol content has been mainly assayed in cereal samples. The determination of ergosterol is also valuable in correlating metabolites such as aflatoxins and OTA (Gourama and Bullerman, 1995; Saxena et al. 2001).

Impedimetry and conductimetry

Metabolites produced by growth of microorganisms in liquid media alter the medium's impedance and conductance. The use of changes in these properties has been used to estimate fungal growth. A major problem of these techniques involves the selection of suitable media, but when the method is set up, this method results rapid and effective.

Adenosine triphosphate (ATP)

Another measure of microbial biomass is the measure of the bioluminescence emitted by the molecules of fungal ATP. However, living plant cells contain also high levels of ATP and fungi are often very difficult to separate from food materials.

Pectinesterase

The fundament of this technique is that gas liquid chromatography is used to determine the amount of methanol released from pectin by the fungal enzyme

pectinesterase. This is considered a rapid method for detecting viable spores of spoilage fungi, but it needs some improvements before practical application.

Fungal volatiles

It consists in measuring the effects of fungi on foods. Fungi produce chemical volatiles during growth and particular chemicals can be detected and therefore measure fungal growth in an indirect way. Several commercial gas sensor array instruments are now available on the market covering a variety of chemical sensor principles, system design and data analysis techniques. A series of different detection principles can be used in chemical gas sensors: heat generation, conductivity, electrical polarization, electrochemical activity, optical properties, dielectric properties and magnetic properties. In principle, the results obtained from a gas-sensor array represent qualitative and quantitative information of the composition of the headspace gas mixture of a sample. The technique should therefore have a great potential in a number of applications related to food. Numerous **electronic nose** studies related to food already have been published, but the electronic nose technology applied on food must be regarded as being in its early stage. A goal of this technology is to explore the use of an electronic nose for rapid detection of food spoilers and pathogens via development of a standard curve of some potential volatile compounds that can be used to develop some specific aroma-labeled substrates.

Immunological techniques

Fungal cell wall proteins produce antigens, which can be detected by immunological methods. Some antigens are derived from components common to a wide range of fungi, and hence are indicative of general fungal growth, while others are genus or even species specific.

Molecular methods

They are based on nucleic acid sequences that are specific to the target fungi. The most known method is called **nucleic acid hybridization** and it involves the selection, cloning and chemical labelling of sequences specific to the target

organism. These are then used as probes to detect RNA or DNA of the pathogen in extracts of the substrate. DNA may be specific at almost any taxonomic level. In some instances the detection and identification of the causal agent(s) may be secondary to other consideration. For example, it may be more important to quantify the amount of pathogen present rather than just determine its identity. Several approaches have been taken to develop diagnostic assays, and are divided into immunological and DNA-based systems, this last generally being **polymerase chain reaction** (PCR). In contrast to hybridization, PCR-based assays for detecting mycotoxins in fungi have been widespread in the last years. PCR is an extremely sensitive technique and involves the enzymatic amplification of a target DNA sequence by a thermostable DNA polymerase.

Experiment 1

1- Isolation of fungi from natural sources

- Preparation of culture media and sterilization.
- Isolation of fungi on the suitable media for fungal growth.
- Identification and preservation of fungi

Table 1: …….

……..…….

Fungi	Counts	%	OR

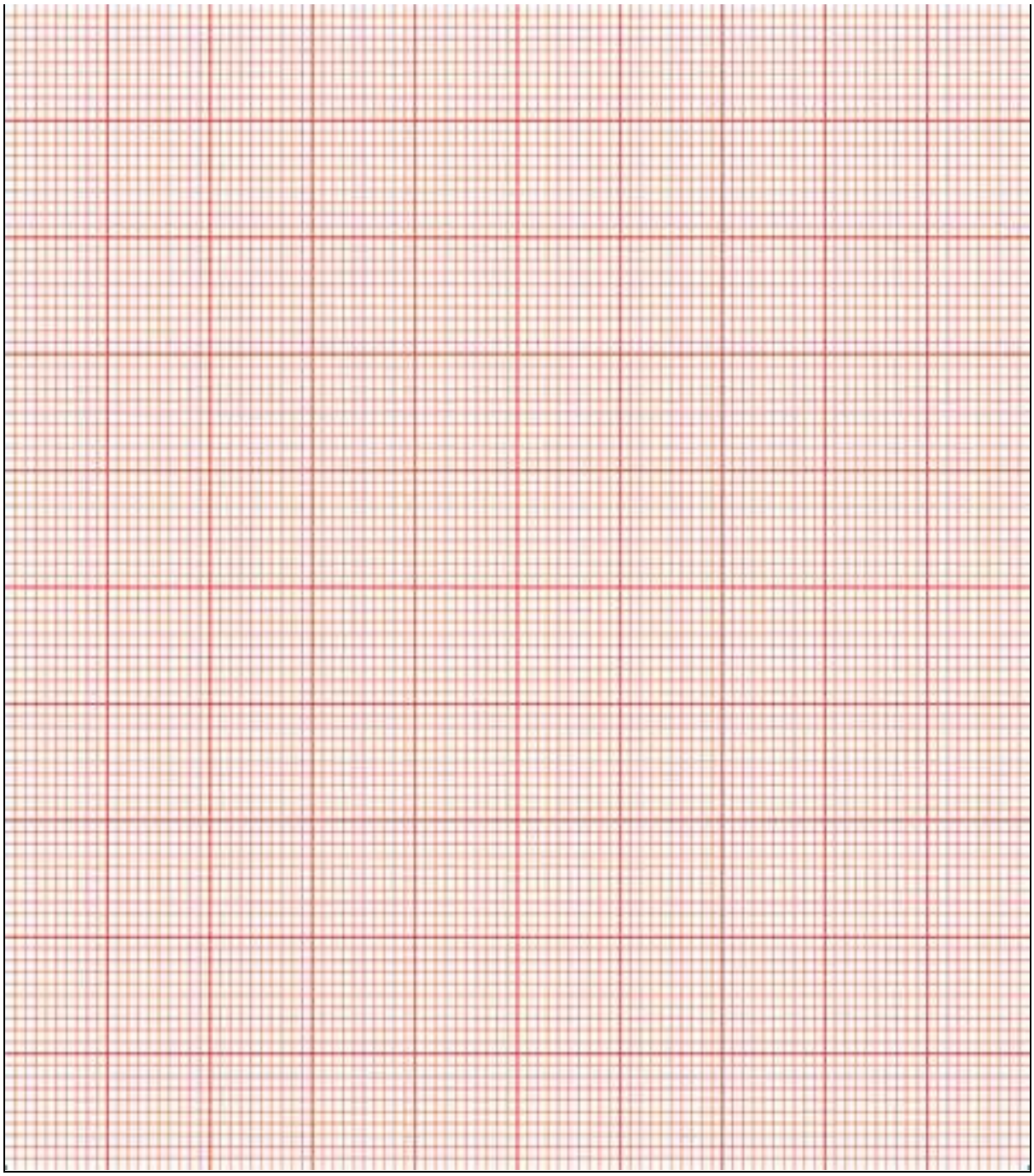


Figure 1: ……..

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Comment

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2- Factors affecting fungal growth and metabolism

Fungi have in the course of evolution diversified to exploit a wide variety of habitats. Different species hence require different conditions for optimal growth. Microbial metabolism is significantly influenced by the physical and chemical environment. Thus, toxin-producing fungi may invade food at pre-harvesting period, harvest-time, during post-harvest handling and in storage. According to the site where fungi infest food, toxinogenic fungi can be divided into three groups (Suttajit, 1989).

- Field fungi, includes species of plant pathogenic fungi, usually with high requirements of water, such as *Alternaria*, *Cladosporium* and *Fusarium*.
- Storage fungi, with lower requirements of humidity, are principally the genus *Aspergillus* and *Penicillium*.
- Advanced deterioration fungi, normally do not infest intact food, but easily attack damaged one and require high moisture content. Some examples are some other aspergilli species, *Chaetomium*, *Scopulariopsis*, *Rhizopus*, *Mucor* and *Absidia*.

The main factors that influence growth of fungi include temperature, pH and moisture. But apart from environmental factors, chemical and biological factors clearly play a role (Figure 49). Under some circumstances these effects are additive. Under others, the implication is that synergistic interactions lead to a combined effect of greater magnitude than the sum of constraints applied individually. This has been described by Leistner and Rödel (1976) as the 'hurdle concept.' Moreover, hurdles are frequently combined to minimize the impact of processing on the quality and to improve the safety of ready-to-eat foods.

Environmental factors

The large and diverse group of microscopic foodborne yeasts and molds includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile, environmental requirements. There

are several major parameters governing fungal invasion, growth and production of mycotoxins:

Experiment 2

Temperature

Temperature is an important environmental factor affecting growth and mycotoxin production by molds. Fungi are capable of surviving under the full range of temperatures normally experienced in environments in which they live. The temperature range usually reported for fungal growth is broad (10-35°C), with a few species capable of growth below or above this range. Fungi can be divided according to their tolerance to temperature in psychrophilic, mesophilic, and thermophilic fungi.

Procedures

1- Preparation of culture media and sterilization

2- Cultivation of fungi

3- Incubation of cultures under various temperatures

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under various temperatures.

4- Illustrate the data and write a comment.

Table 2:……..……..…

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Temperature	*Aspergillus*	*Fusarium*	*Rhizopus*
15			
20			
25			
30			
35			
40			

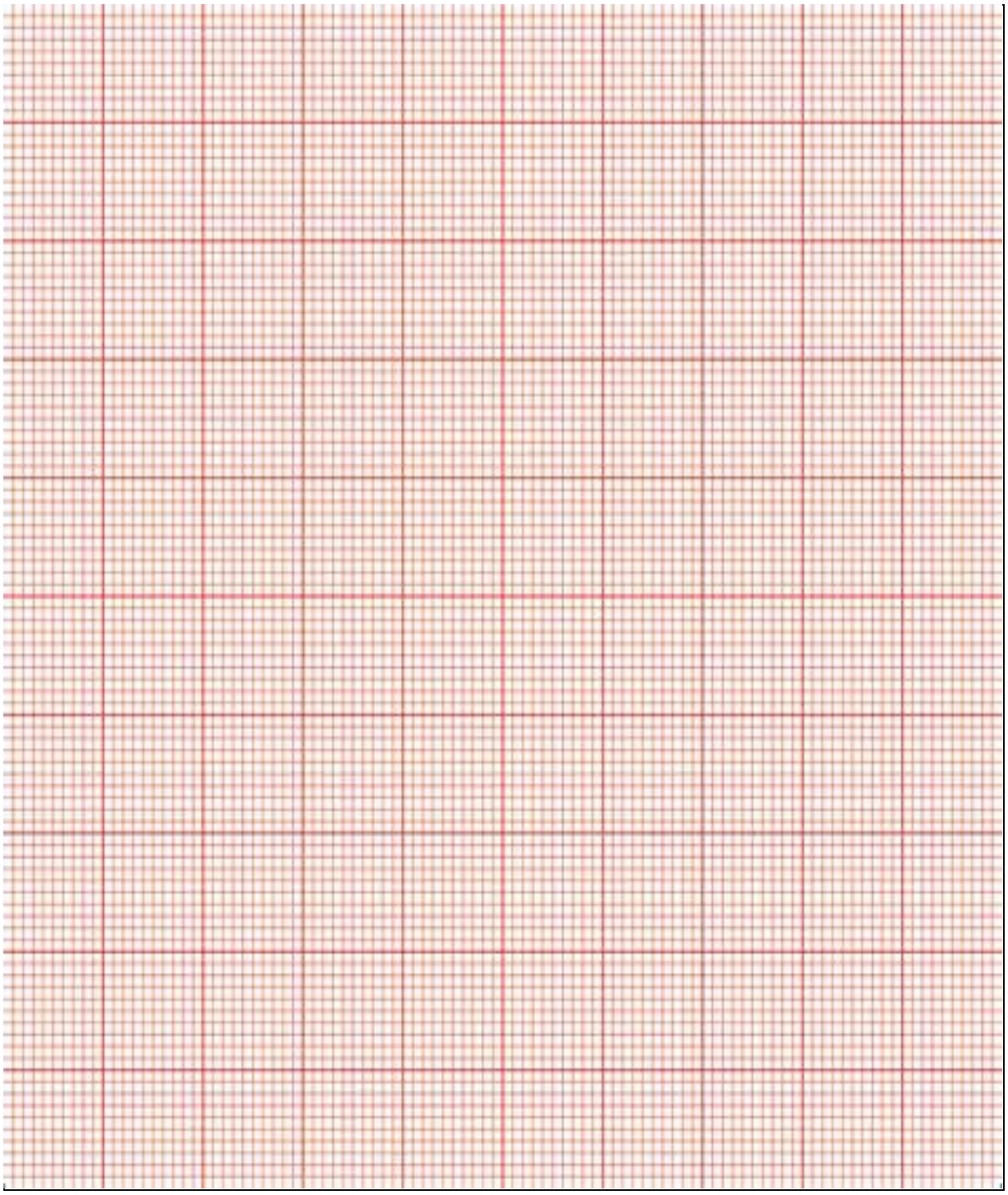

Figure 2: ……..

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Comment

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Experiment 3

Hydrogen ion concentration (pH)

In general, there is a lack of information on the effect of pH on fungal growth parameters, in spite of a considerable literature on growth in relation to the initial pH of media. This data is of limited value, since fungal metabolism alters pH during their evolution. Hydrogen ion concentration in a medium could affect growth either indirectly by its effect on the availability of nutrients or directly by action on the cell surfaces. The acid/alkaline requirement for growth of all yeasts and molds is quite broad, ranging from pH 3 to above pH 8, with optimum around pH 5, if nutrient requirements are satisfied. In general, Aspergillus species are more tolerant to alkaline pH while *Penicillium* species appear to be more tolerant to acidic pH (Wheeler et al., 1991). It is seen that in situations near neutral pH, fungi must compete with bacteria for niches, and at higher aw values most fungi are not competitive in mixed culture. However, where a w is below 0.90, fungi become dominant irrespective of pH. In specialized niches where bacteria do not appear to have a role as pathogens, specific Fusarium and *Penicillium* species are dominant even at neutral pH and high a_w (Wheeler et al., 1991).

Procedures

1- Preparation of culture media with variable pH values and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under various pH values.

4- Illustrate the data and write a comment.

Table 3:……..……..…

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pH values	*Aspergillus*	*Fusarium*	*Rhizopus*
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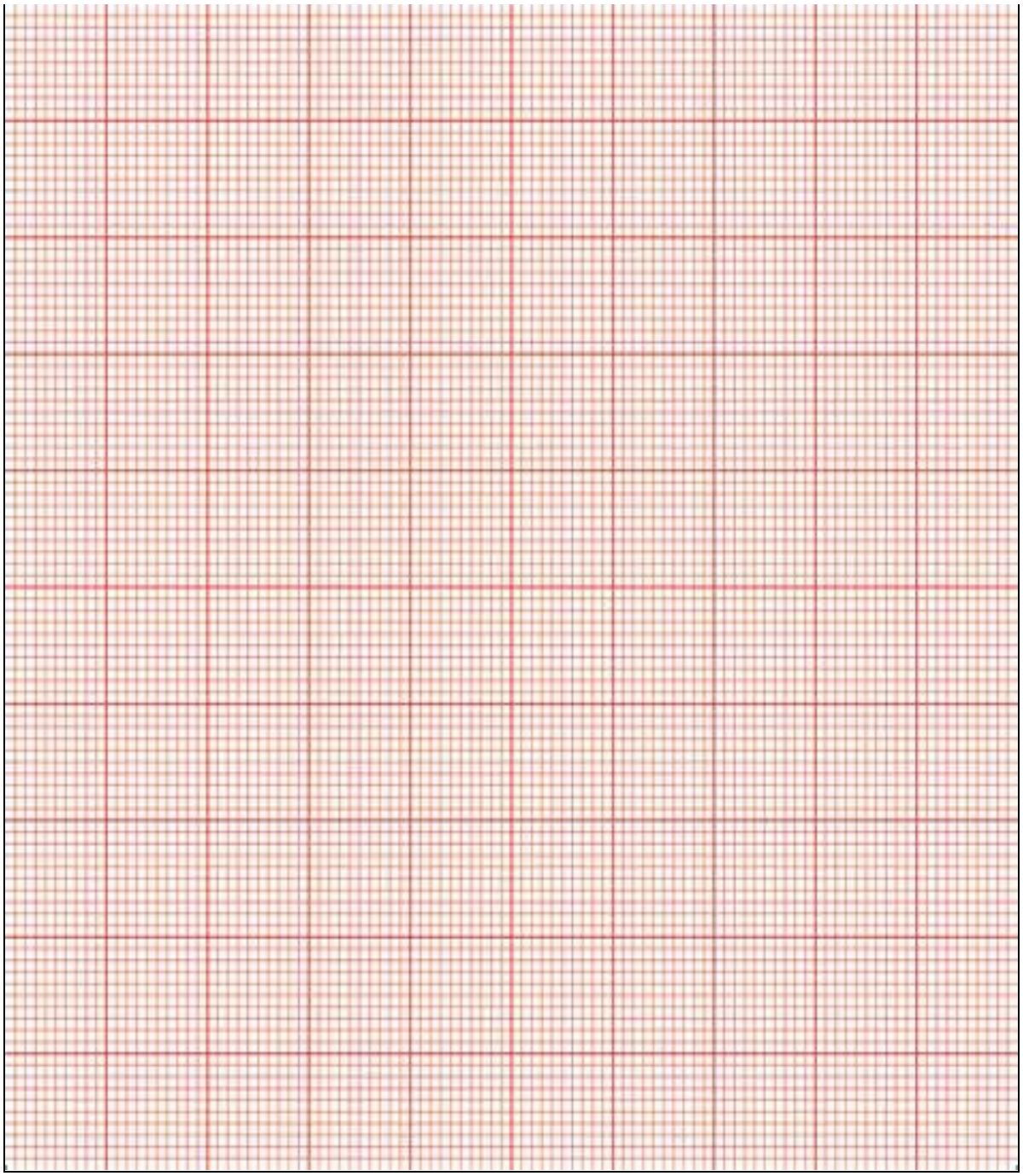

Figure 3: ……..

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Availability of water

Living organisms consist largely of water. Hence if an organism is to grow has to take up water from the environment. Whether water enters or leaves a cell depends on the difference between the water potential of the cell and that of the surrounding medium, water moving from a region of high to one of lower water potential (Carlile and Watkinson, 1996). In microbiology, three measures of the water availability have been used: water potential (ψ), water activity (a_w), and relative humidity (R.H.).

Water potential is the sum of numerous components, of which the most important are osmotic, matric and turgor potential, and is measured in units of pressure.

Water activity is a fundamental property of aqueous solutions, and by definition is the ratio of the vapour pressure of the water in the substrate (P) to that of pure water at the same temperature (P0):

$$a_w = P/P0$$

p0 p aw = Water activity ranges from zero (water absent) to 1.0 (pure water). For an ideal solution aw is independent of temperature, and in actual practice, the aw of a given solution varies only slightly with temperature within the range of temperature permitting microbial growth. The relationship between water potential and water activity is given by the next equation, where the value of k depends on temperature and is, for example, 1.37 at 25ºC and 1.35 at 20ºC.

$$\psi \text{ (Mpa)} = k \ln a_w$$

Not only is the availability of water in the surrounding liquid phase of importance to fungi, but the water content of the adjacent gas phase. The water content of the atmosphere is expressed in terms of **relative humidity**, the ratio of the water vapour pressure of the gas phase being considered, to that of a saturated atmosphere at the same temperature. It is hence the same ratio as water activity but expressed as a percentage. In most of the studies presented in this thesis, aw was used to describe the status of the water in solution or substrate in preference

to R.H., which applies more strictly to the surrounding atmosphere. Under equilibrium conditions the two terms are interchangeable. A required aw in the environment of a fungus may be obtained either by fixing the water content or the solute concentration in the culture substrate or by keeping the substrate in equilibrium with an atmosphere of controlled R.H. Moisture requirements of foodborne molds are relatively low; most species grow at a 0.85 a w or less, although yeasts generally require a higher water activity. 0.60 aw is considered the limit for cell growth, but spores of Aspergillus and *Penicillium* for example, are able to survive at lower aw for several years (Carlile and Watkinson, 1996). Moisture control is the best and most economical means to control the environment to prevent mold growth and mycotoxin production.

Light

There are some reports that illumination will increase or more commonly reduce the rate at which fungi spread across an agar surface. Such effects are sometimes due to the photochemical destruction of components of the medium but in other instances a direct effect on metabolism seems likely. The biosynthesis of pigments, mainly carotenoids, as consequence of light action has been demonstrated.

Availability of oxygen

Organisms can obtain energy by oxidative (respiratory) metabolism or by fermentation. The implications for oxygen requirements of the occurrence of respiration, fermentation or both in a fungus divided them in obligate aerobes, facultative anaerobes and obligate anaerobes (Carlile and Watkinson, 1996): Food spoilage molds, like almost all other filamentous fungi and yeasts, have an absolute requirement for oxygen. However, many species appear to be efficient oxygen scavengers, so that the total amount of oxygen available, rather than the oxygen tension, determines growth. The concentration of oxygen dissolved in the

substrate has a much greater influence on fungal growth than atmospheric oxygen tension (Pitt and Hockings, 1997). The most oxygen demanding molds will colonize the surface of the food, while the less exigent could be found inside the food. Although probably not economically feasible, one sure way to prevent mycotoxin contamination of cereals and other food, is to store them under anaerobic conditions, e.g. CO_2 or nitrogen. For instance, this could be done in large airtight silos. The molds would not grow, but this type of environment control is sometimes understandably unrealistic.

Chemical factors (**Nutritional factors**)

Experiment 4

Effect of different Carbon sources on fungal growth

All forms of life, including molds, require exogenous materials to build into biomass. As heterotrophs, the molds require organic compounds for both the synthesis of biomass (anabolic metabolism) and to produce the energy to drive these reactions (catabolic metabolism). These aspects of metabolism are frequently referred to as primary metabolism (Smith and Moss, 1985). Fungi can use a number of different **carbon sources** to fill their carbon needs for the synthesis of carbohydrates, lipids, nucleic acids and proteins. Oxidation of sugars, alcohols, proteins, lipids, and polysaccharides provides them with a source of energy. Differences in their ability to utilize different carbon sources, such as simple sugars, sugar acids, and sugar alcohols, are used, along with morphology, to differentiate the various yeasts.

Procedures

1- Preparation of culture media with different carbon sources and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature and pH

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under different carbon sources.

4- Illustrate the data and write a comment.

Table 4:……..……..…

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Carbon sources	*Aspergillus*	*Fusarium*	*Rhizopus*

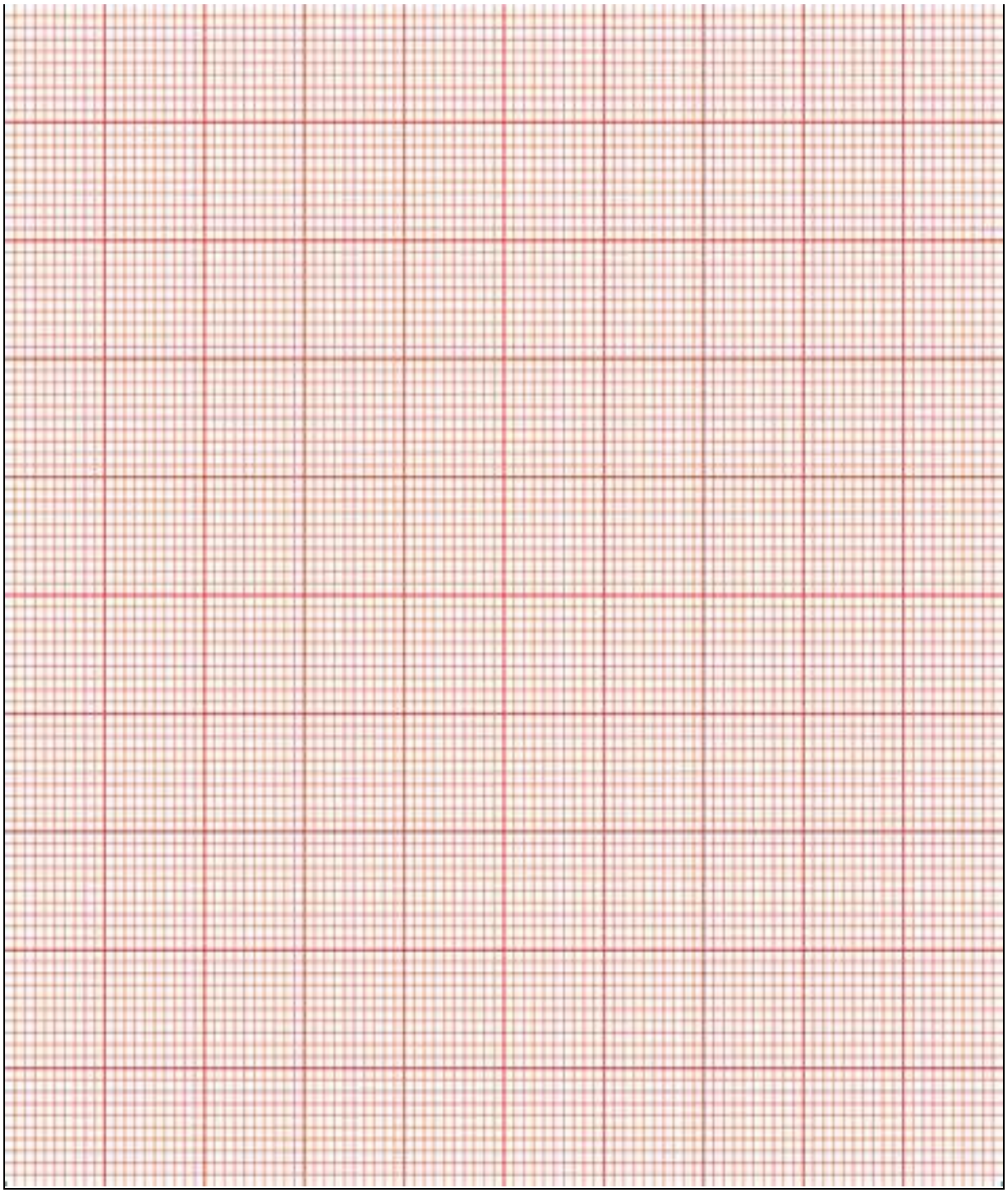

Figure 4: ……..

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Experiment 5

Effect of different Nitrogen sources on fungal growth

Fungi require a source of **nitrogen** for synthesis of amino acids for proteins, purines and pyrimidines for nucleic acids, glucosamine for chitin, and various vitamins. Depending on the fungus, nitrogen may be obtained in the form of nitrate, nitrite, ammonium or organic nitrogen as no fungus can fix nitrogen. Most fungi use nitrate, which is reduced first to nitrite and then to ammonia. Therefore, availability and type of nutritional factors such as carbon source and nitrogen source can also affect both mycotoxin production and morphological differentiation. Other major nutrients for fungi are sulphur, phosphorus, magnesium and potassium, which can be supplied to most fungi as salts. Trace elements like iron, copper, manganese, zinc and molybdenum are required by nearly all fungi as cofactors for enzymes. But in high amounts, some trace elements can become toxic for some fungi. For example, OTA production by *A. ochraceus* strains varied with the different concentrations of yeast extract (0-4%) and sucrose (0-4%) in a laboratory medium (Atalla and El-Din, 1993).

Procedures

1- Preparation of culture media with different nitrogen sources and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature and pH

3- At the end of the incubation period, filtration of cultures and determination of mycelial growth that grown under different nitrogen sources.

4- Illustrate the data and write a comment.

Table 5:……..……..…

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Nitrogen sources	*Aspergillus*	*Fusarium*	*Rhizopus*

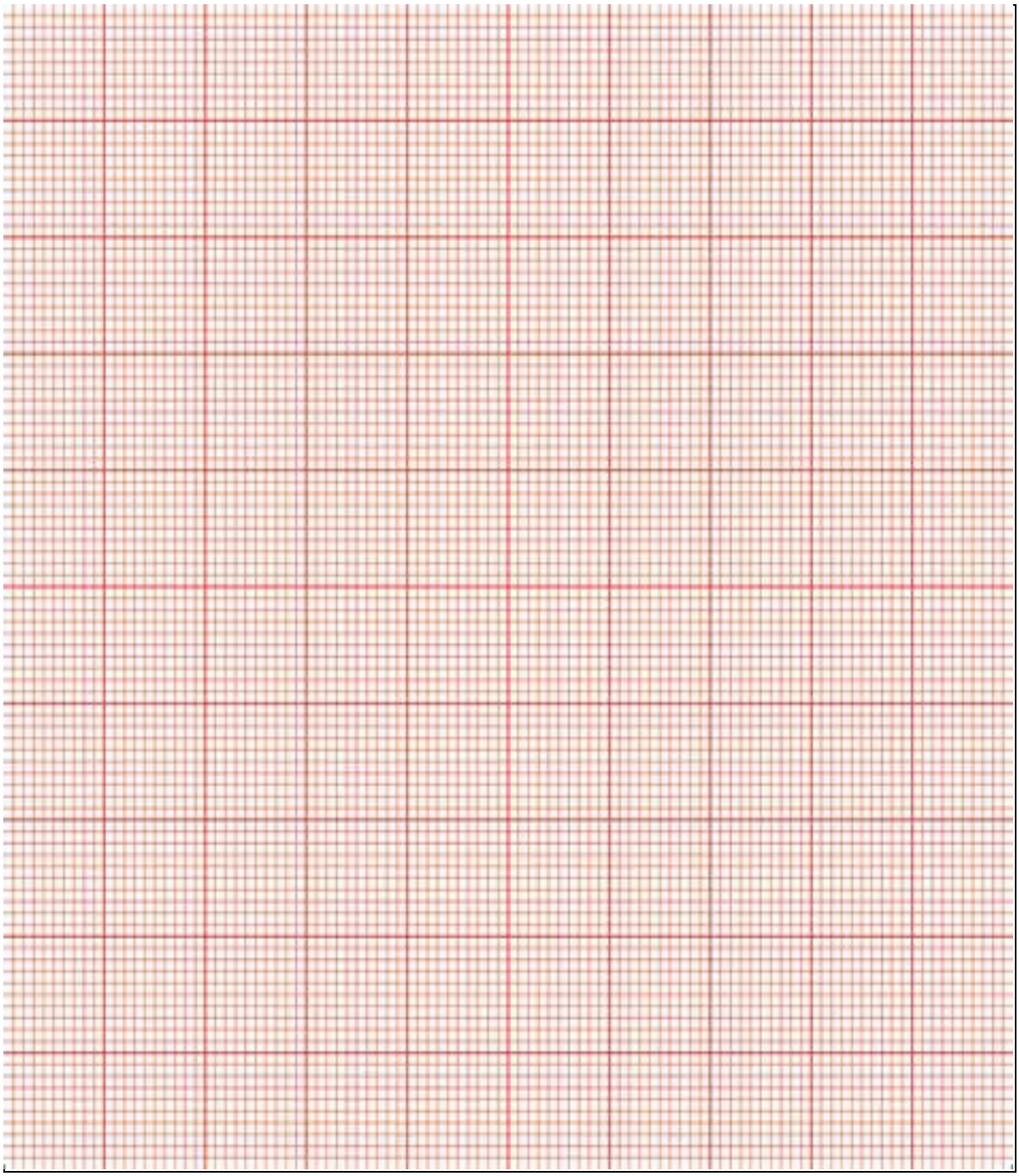

Figure 5: ……..

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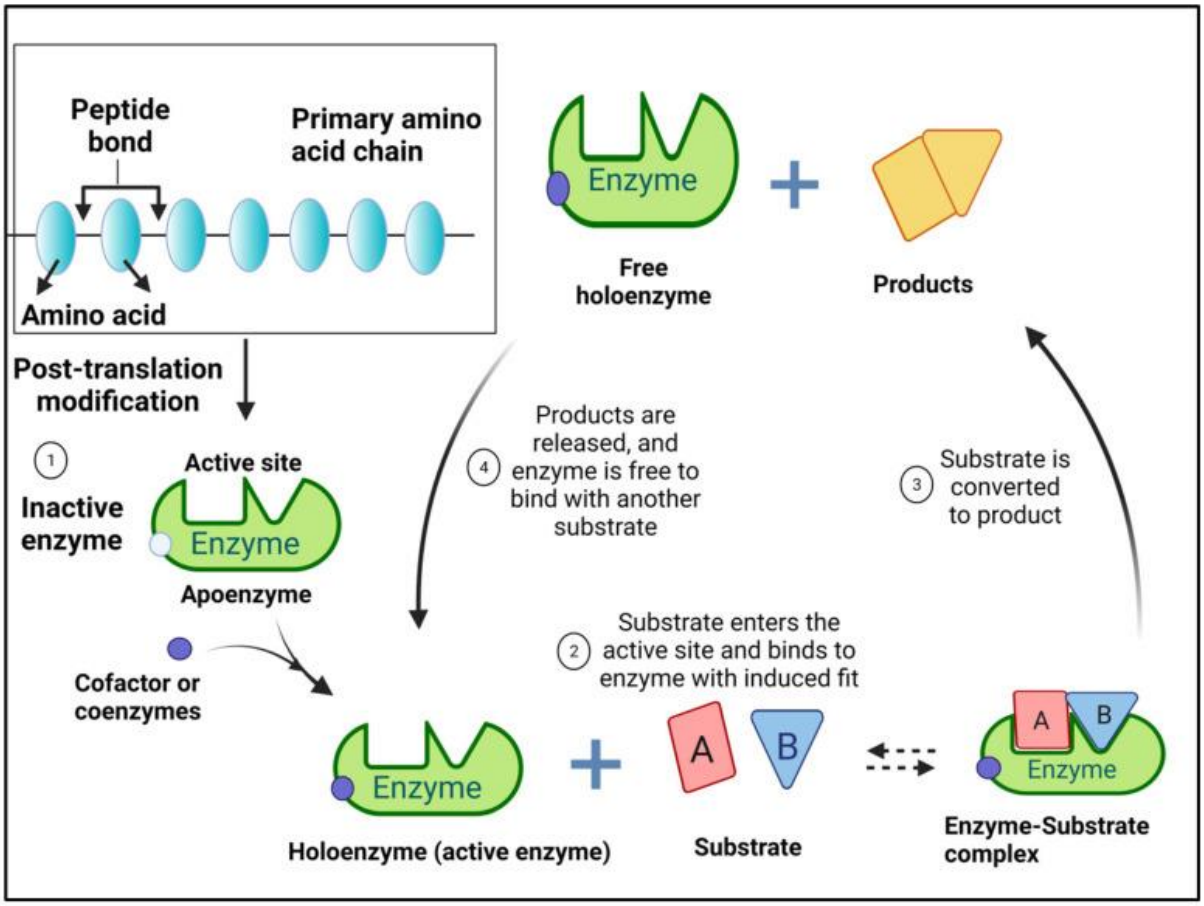
Experiment 6

Fungal enzymes

Enzymes have played an important role in different types of biological systems for various applications. They are proteins that break down and convert complicated compounds to simple products. Fungal enzymes are compatible, efficient, and proper products for many purposes such as medicinal uses, industrial processing, bioremediation process, and agricultural applications. Fungal enzymes have been used in many industries, including baking, brewing, cheese making, antibiotics production, and commodities manufacturing, such as linen and leather. Furthermore, they also are used in other fields such as paper production, detergent, the textile industry, and in drinks and food technology in products manufacturing ranging from tea and coffee to fruit juice and wine. Recently, fungi have been used for the production of more than 50% of the needed enzymes. Fungi can produce different types of enzymes extracellularly, which gives a great chance for producing in large amounts with low cost and easy viability in purified forms using simple purification methods. Hydrolases are the most extensively studied groups of enzymes; they catalyze the hydrolysis of their substrate through the addition of water. Hydrolases represent the most commercially marketed enzymes due to their wide application in different industrial sectors. Fungal amylases, proteases, lipases, and cellulases represent the most commercially demanded enzymes.



Schematic illustration for enzyme structure, activation, and steps of enzyme and substrate interaction.

Amylases

Amylase enzymes are used for commercial application and was firstly applied medicinally in treating digestive disorders. Amylases could be classified into α, β, and γ-Amylases depending on the attaching site in the starch molecules and the nature of the resulting products. α-Amylases are calcium-dependent metalloenzymes that act randomly on the starchy substrates yielding maltose and maltotriose from amylose or glucose and dextrin from amylopectin. β-Amylases hydrolyze 1,4-glycosidic bonds in the carbohydrate chain, yielding one maltose unit. They are extensively important in plants, especially in the seed ripping process, but they are also reported from the microbial origin. γ-Amylases resemble the other two types of amylases in hydrolysis activity toward 1,4-

glycosidic linkages, unlike the two forms characterized with 1,6-glycosidic linkages hydrolysis activity and preferring acidic environment pH 3. *Aspergillus niger* is considered the potent commercial α-Amylase producer among all filamentous fungi. Many other fungi were reported for their capacity to produce different types of amylases, including *Aspergillus oryzae*, *A. terreus*, *Fusarium solani*, and *Penicillium citrinum*.

Lipases

Lipases are a group of hydrolytic enzymes that act by hydrolysis of triacylglycerol yielding fatty acid and glycerol. Lipases also catalyze the reverse reaction by esterification of glycerol and fatty acid. Fungal lipases are produced by several fungi including *Aspergillus niger*, *Penicillium verrucosum*, *Fusarium solani*, *Arthrographis curvata*, and *Rhodosporidium babjevae*. Lipases are implemented in vast commercial applications, including detergents and cosmetics additives, fine chemical production, medical application, paper pitching, leather de-fating, wastewater treatment, and biodiesel production. The application of lipase in biodiesel production, as an ecofriendly alternative for traditional fuel, intensifies the research in diminishing the production cost and enhancing the enzyme efficiency.

Proteases

Proteases play an important role in fungal physiology to digest extracellular large peptides and also in defense mechanisms against attaching pathogens. Based upon the amino acid in the enzyme active site, proteases could be categorized into different types, including serine, asparagine, cysteine, aspartic, and metalloproteases. Serine and metalloprotease are the most studied types among all proteases and are usually produced from microbial origins. Filamentous fungi, especially that of *Aspergillus* sp. are characterized by their high capacity for protease production. Other fungal genera also reported for their potency regarding proteases production, including *Penicillium* sp., *Fusarium* sp., and *Pichia farinosa*.

Cellulases

Cellulose, hemicellulose, and lignin are the main components of most agricultural wastes. Most fungi have the complete enzymatic system (Endoglucanases, Cellobiohydrolases, β-glucosidases, and Xylanases) to degrade this complex cellulosic material for nutrition. *Trichoderma reesei* is widely applied for the commercial production of cellulases, other fungi also represent potent cellulase producers, including *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Aspergillus brasiliensis*. Xylan, a complex polysaccharide, is also a major component of hemicellulose; hence, xylanases play an important role in the efficient hydrolysis of plant cellulolytic material. Regarding the diverse and complex structure of Xylan, its hydrolysis required a group of synergistically working enzymes (xylanolytic system) for complete degradation. Filamentous fungi are characterized by the required xylanolytic system for complete xylan degradation, especially that of *Trichoderma reesei*. *Aspergillus oryzae*, and *Aspergillus flavus*.

Detection of amylase produced by fungi

Procedures

1- Preparation of culture media for amylase production and sterilization

2- Cultivation of fungi

3- Incubation of cultures under suitable temperature and pH

3- At the end of the incubation period, filtration of cultures and assay for the enzyme activity.

4- Illustrate the data and write a comment.

Table 6:……..……..…

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Fungi	Amylase activity

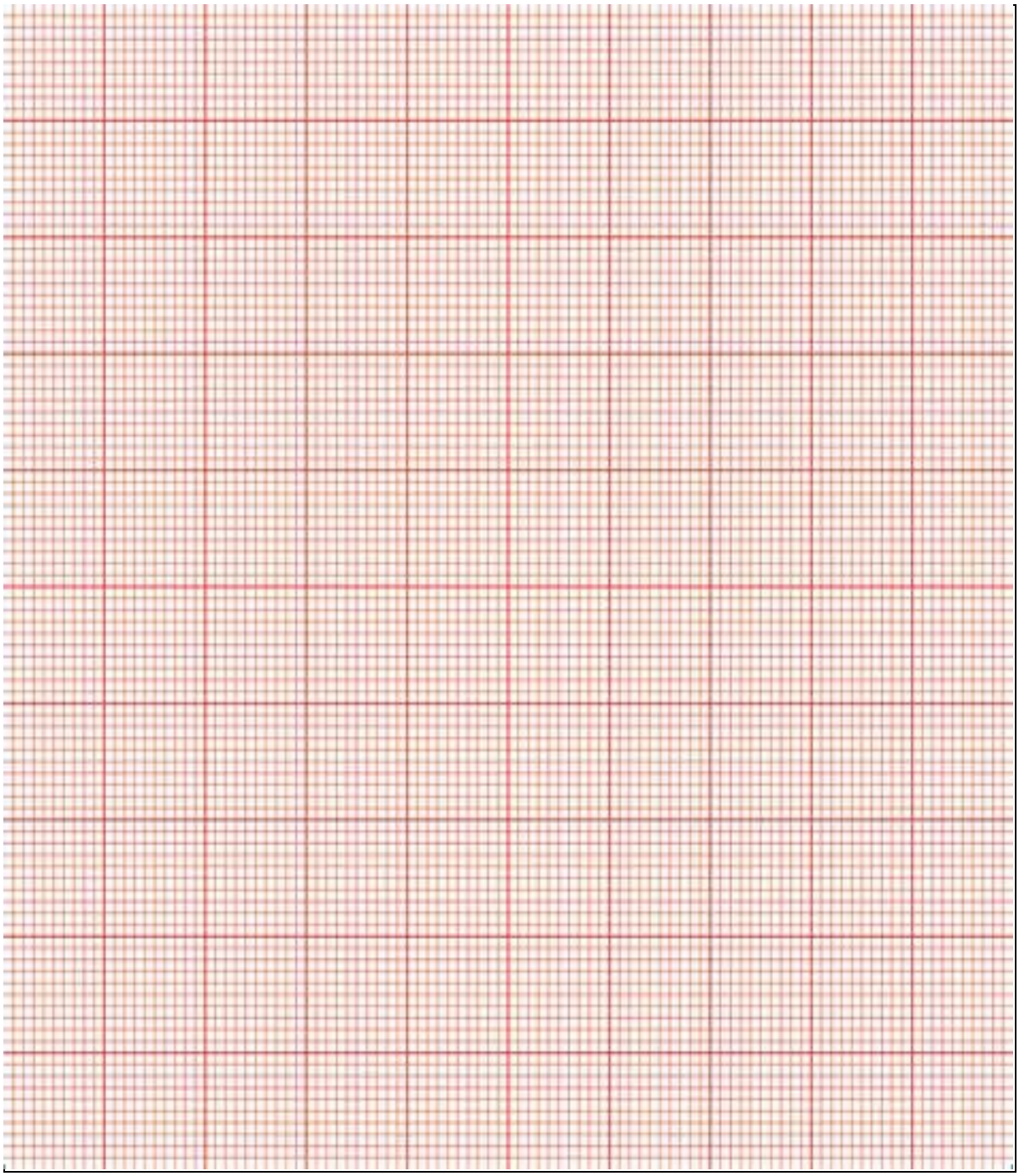

Figure 6: ……..

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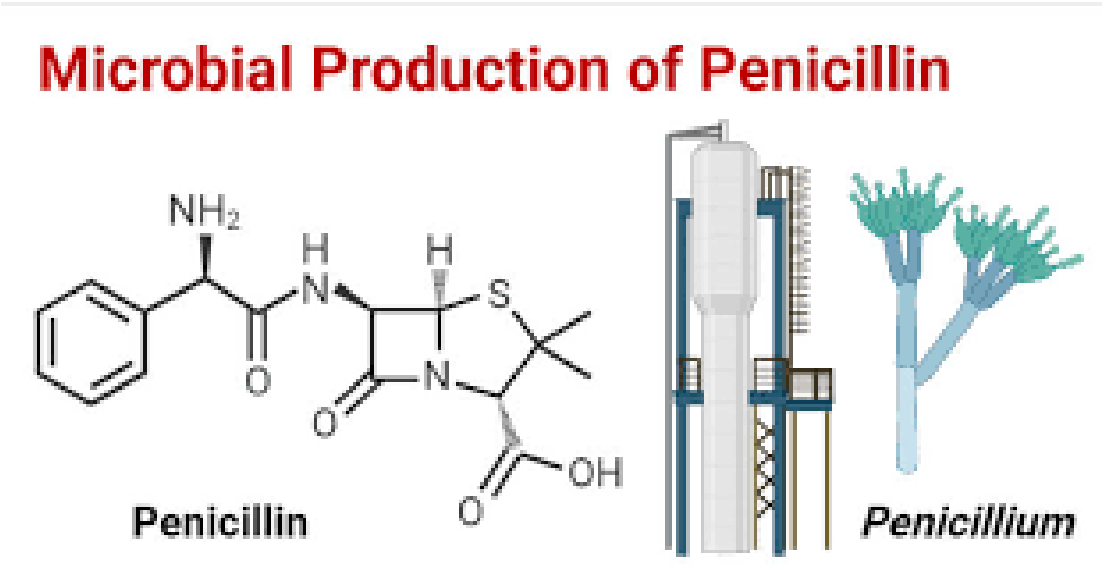
Experiment 7

Antifungal agents

A wide range of antifungal agents are used in combating biodeterioration and in preventing or treating fungal diseases of plants. In these contexts, they are commonly referred to as **fungicides**. Others are used for treating disease in animals and man, and are simply referred to as **antifungal agents.** Antimicrobial agents produced by means of a microbial fermentation, called **antibiotics**, by the plant on which the mold is growing, or added as biocides during crop management, are other factors interacting with the growth and metabolism of a mold. Antifungal agents differ widely in their chemical nature and in their properties and mode of action (Carlile and Watkinson, 1996). The effect of pesticides is interesting as they are largely used to control several diseases in plants. The correct use of fungicides to diminish fungal mycoflora could lead to a diminution in the amount of mycotoxins produced. But certain number of studies showed that the use of sub-lethal concentration could favour the production of the toxins (Moss and Frank, 1987). It is also possible that the pesticide decreases the synthesis of the mycotoxins without affecting the fungal growth (Draughton and Ayres, 1978, 1982).



Production of Penicillin by *Penicillium chrysogenum*

Procedures

1- Cultivation of fungi on a suitable media for penicillin production.

2- Incubation of cultures at suitable conditions (Temperature, pH, etc.).

3- At the end of the incubation period, filtration of cultures.

4- Extraction of penicillin using a suitable solvent.

5- Collection of solvent with the antibiotic.

6- Concentration of solvent by rotary evaporation.

7- Collection of solvent and dissolve of penicillin in methanol.

8- Analysis of penicillin for detection and concentration by TLC, HPLC, etc.

9- Illustrate the data and write a comment.

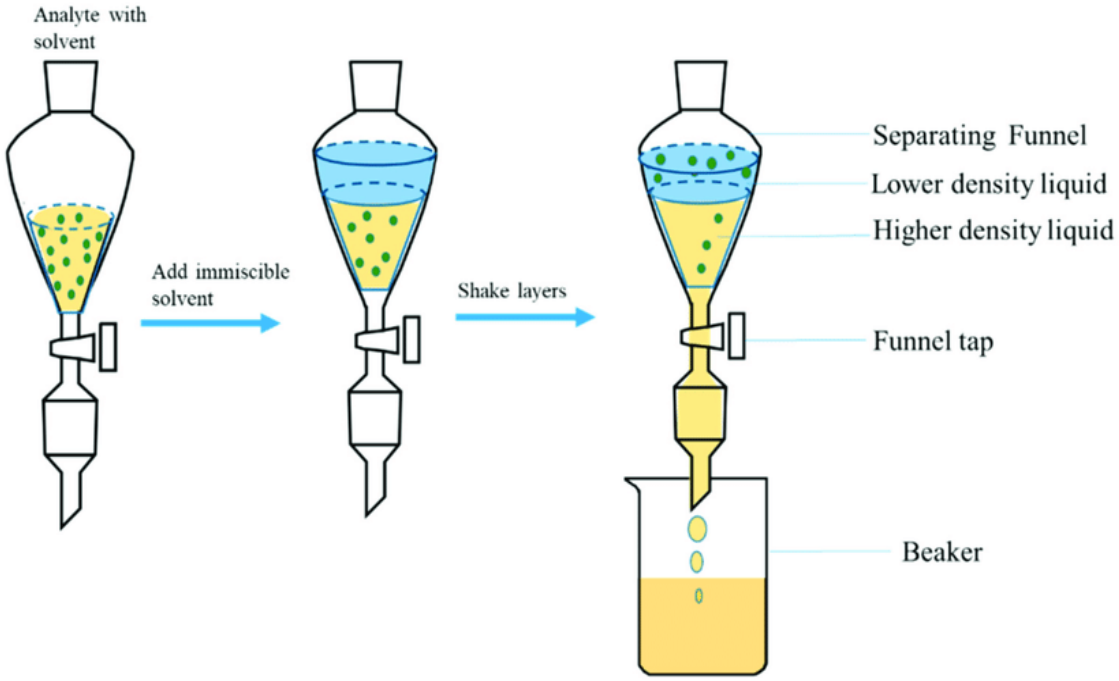


Table 7:……..……..…

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Penicillium strains	Penicillin activity
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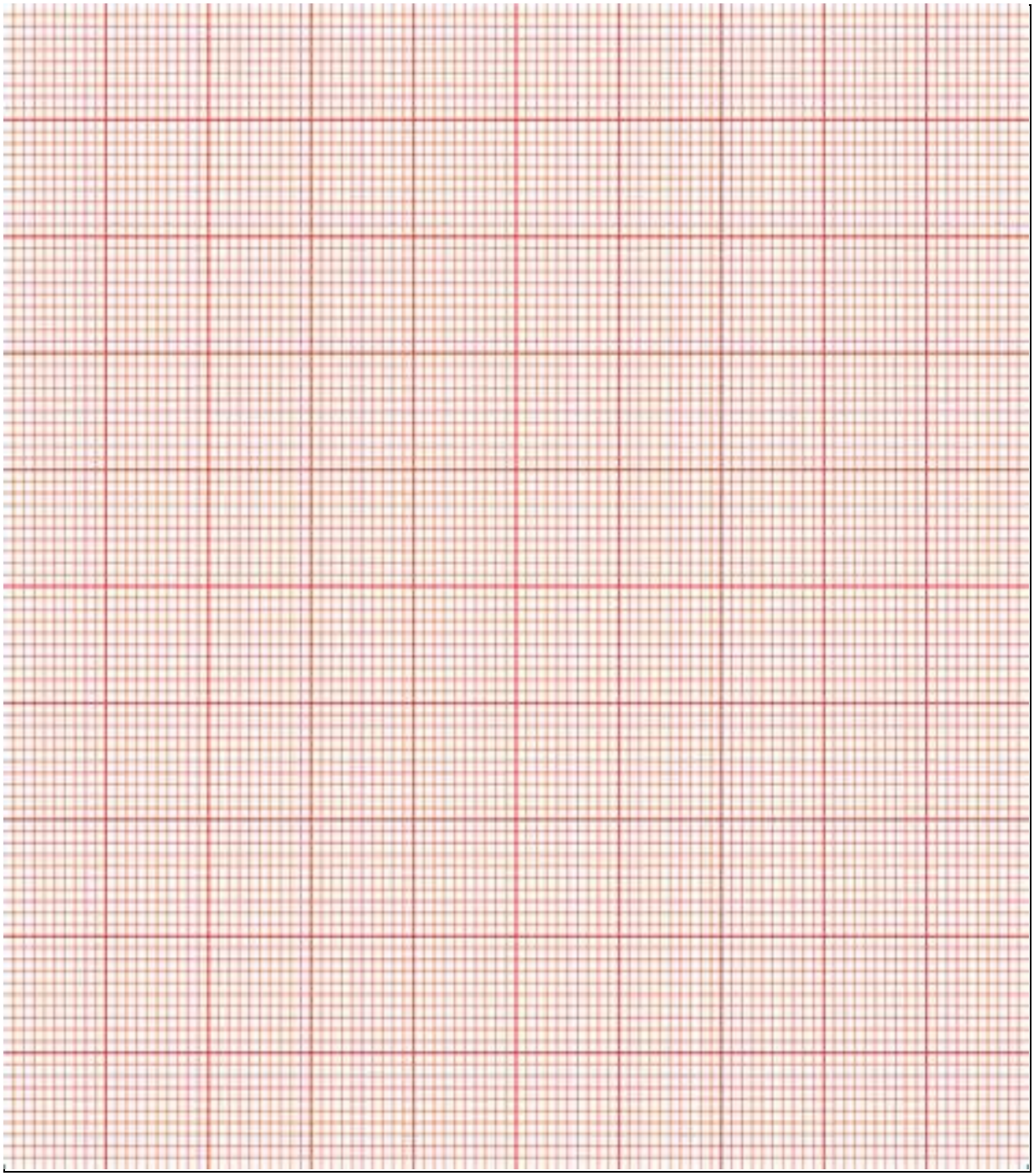

Figure 7: ……..

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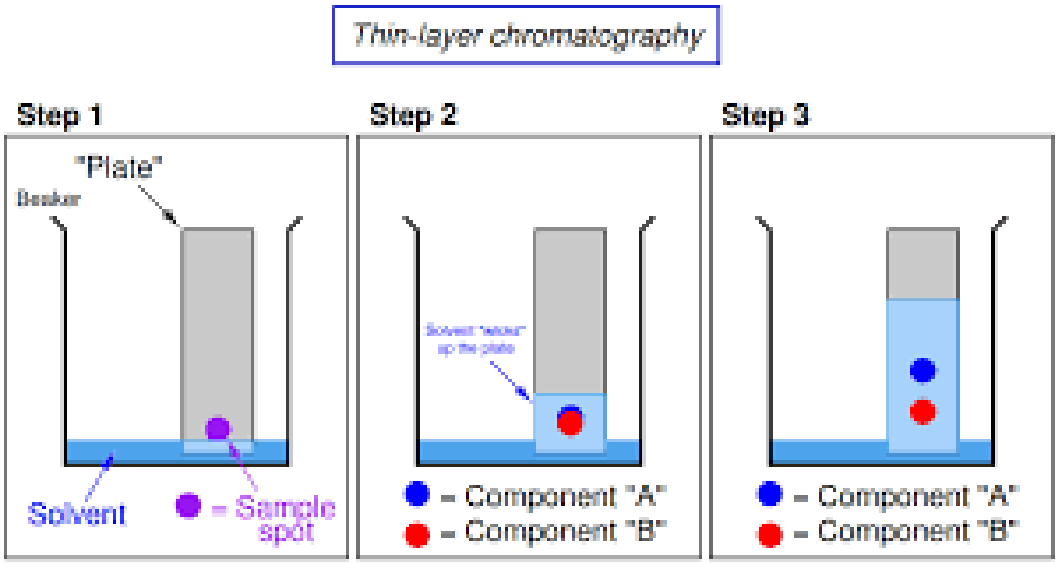
Methods for the analysis of antibiotics

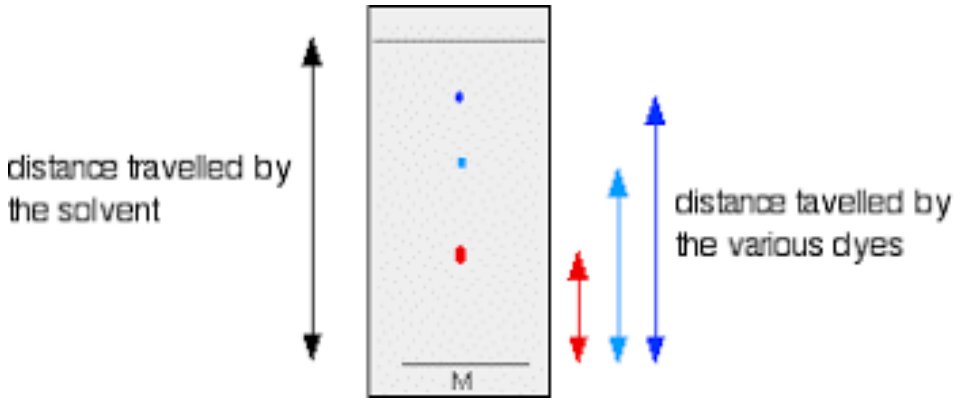
The different stages involved in the analytical process of antibiotics detection are sampling, extraction, clean-up, separation, detection and confirmation. Although many interfering compounds may be partially removed during the extraction sequence, further clean-up of the extract is normally necessary. The traditional clean-up systems generally involved either solvent portioning and/or open column chromatography on silica adsorbent. The development of solid phase extraction (SPE) cartridges containing packing with various surface chemistries allowed more rapid and efficient clean-up process. However, the introduction of the immunoaffinity columns (IAC) in which specific antibodies are bound to a solid matrix, has allowed an even more specific clean-up process. Classical analytical separation methods for antibiotics include TLC, HPLC, gas chromatography (GC) and MS. Mass spectrometry offers the ideal confirmatory technique via the detection of molecular ions at specific chromatographic retention times and via the generation of a compound specific fragmentation pattern.

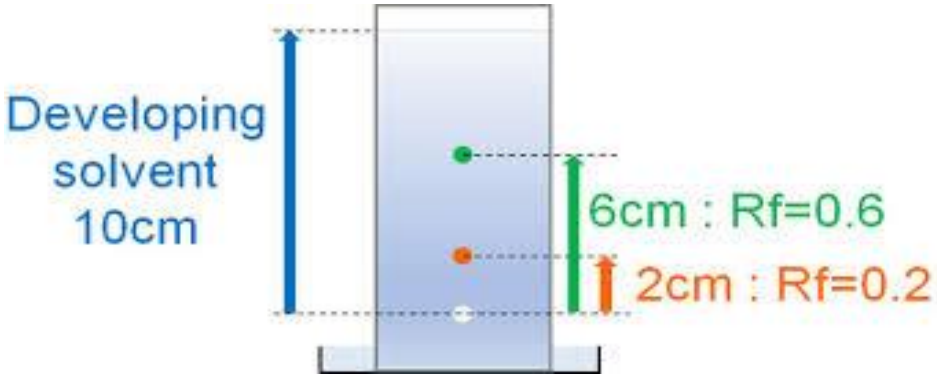
Thin Layer Chromatography (TLC) analysis

Thin layer chromatography, or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC is a sensitive technique - microgram (0.000001 g) quantities can be analyzed by TLC. TLC consists of three steps: spotting, development, and visualization. First the sample to be analyzed is dissolved in a volatile (easily evaporated) solvent to produce a very dilute (about 1%) solution. Spotting consists of using a micro pipet to transfer a small amount of the dilute solution to one end of a TLC plate, in this case a thin layer of powdered silica gel that has been coated onto a plastic or glass sheet. The spotting solvent quickly evaporates and leaves behind a small spot of

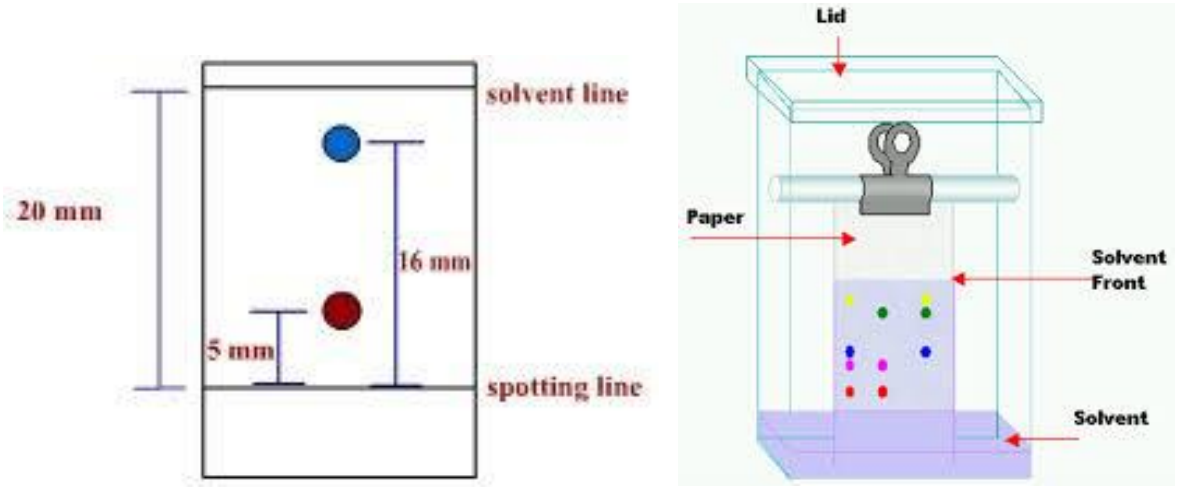
the material. Development consists of placing the bottom of the TLC plate into a shallow pool of a development solvent, which then travels up the plate by capillary action. As the solvent travels up the plate, it moves over the original spot. A competition is set up between the silica gel plate and the development solvent for the spotted material. The very polar silica gel tries to hold the spot in its original place and the solvent tries to move the spot along with it as it travels up the plate. The outcome depends upon a balance among three polarities - that of the plate, the development solvent and the spot material. If the development solvent is polar enough, the spot will move some distance from its original location. Different components in the original spot, having different polarities, will move different distances from the original spot location and show up as separate spots. When the solvent has traveled almost to the top of the plate, the plate is removed, the solvent front marked with a pencil, and the solvent allowed to evaporate. Visualization of colored compounds is simple–the spots can be directly observed after development. Because most compounds are colorless however, a visualization method is needed. The silica gel on the TLC plate is impregnated with a fluorescent material that glows under ultraviolet (UV) light. A spot will interfere with the fluorescence and appear as a dark spot on a glowing background. While under the UV light, the spots can be outlined with a pencil to mark their locations. A second method of visualization is accomplished by placing the plate into iodine vapors for a few minutes. Most organic compounds will form a dark-colored complex with iodine. It is good practice to use at least two visualization techniques in case a compound does not show up with one particular method. The Rf value is used to quantify the movement of the materials along the plate. Rf is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always between zero and one.







$$Rf = \frac{\text{distance moved by the compound}}{\text{distance moved by the solvent}}$$

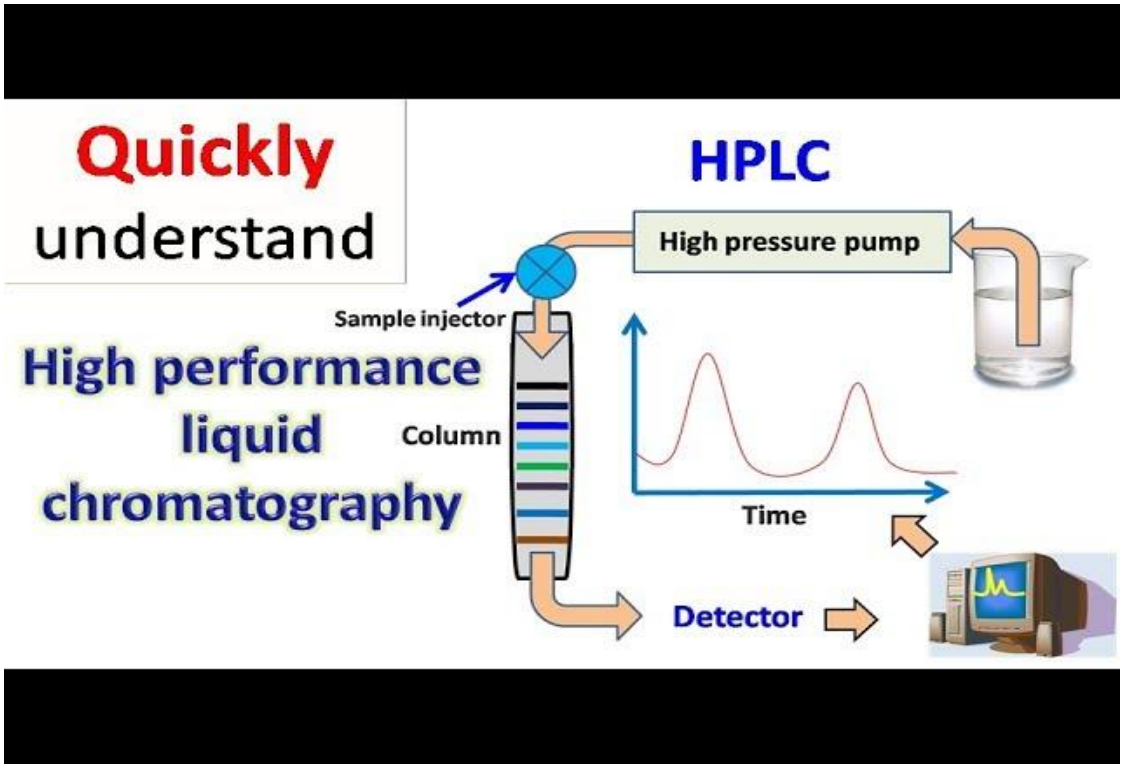


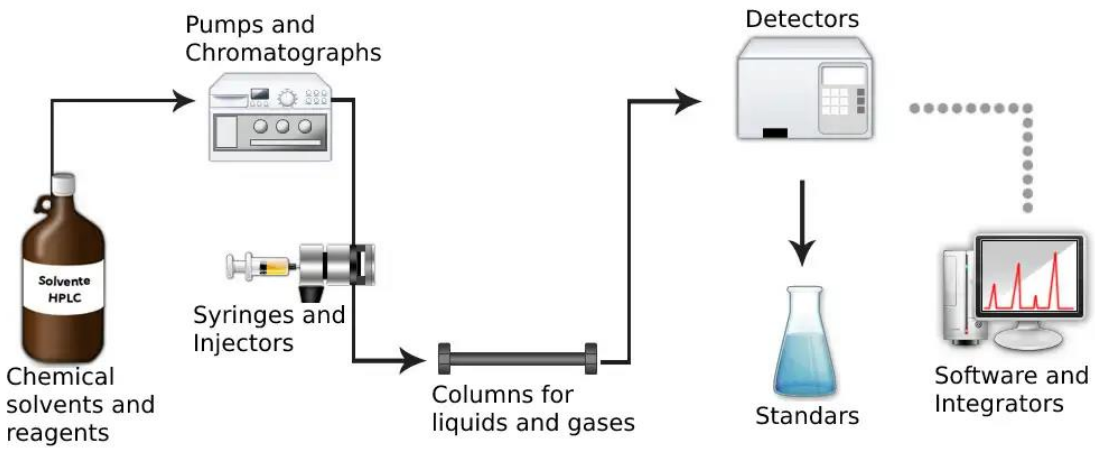
High-performance liquid chromatography (HPLC) analysis

High-performance liquid chromatography (HPLC), formerly referred to as high-pressure liquid chromatography, is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. HPLC relies on pumps to pass a pressurized liquid and a sample mixture through a column filled with adsorbent, leading to the separation of the sample components. The active component of the column, the adsorbent, is typically a granular material made of solid particles (*e.g.*, silica, polymers, etc.), 2–50 μm in size. The components of the sample mixture are separated from each other due to their different degrees of interaction with the adsorbent particles. The pressurized liquid is typically a mixture of solvents (*e.g.*, water, acetonitrile and/or methanol) and is referred to as a "mobile phase". Its composition and temperature play a major role in the separation process by influencing the interactions taking place between sample components and

adsorbent. These interactions are physical in nature, such as hydrophobic (dispersive), dipole–dipole and ionic, most often a combination.

The schematic of an HPLC instrument typically includes a degasser, sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis. Some models of mechanical pumps in an HPLC instrument can mix multiple solvents together in ratios changing in time, generating a composition gradient in the mobile phase. Various detectors are in common use, such as UV/V is, photodiode array (PDA) or based on mass spectrometry. Most HPLC instruments also have a column oven that allows for adjusting the temperature at which the separation is performed.





High-performance liquid chromatography (HPLC)

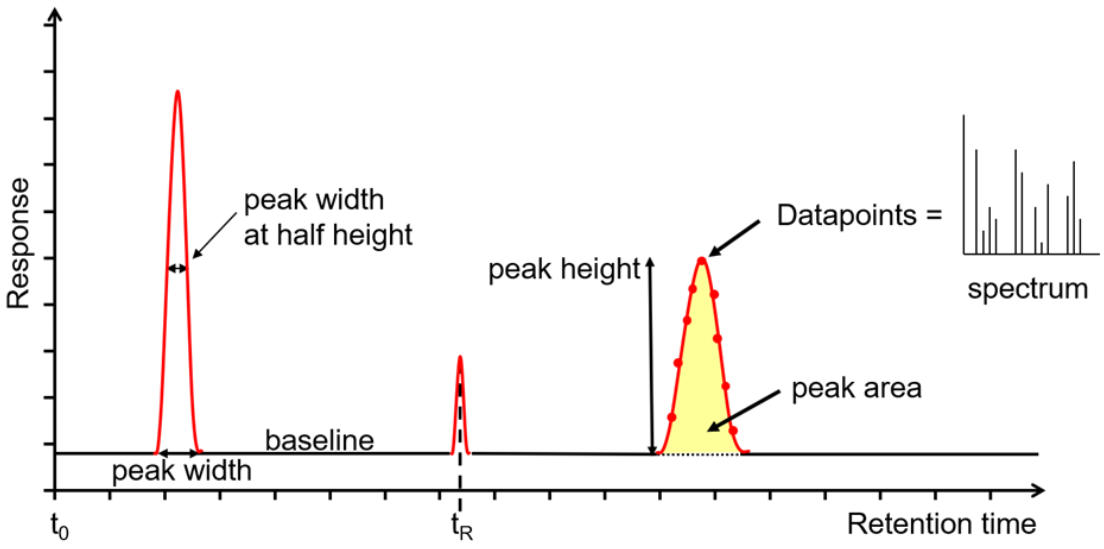
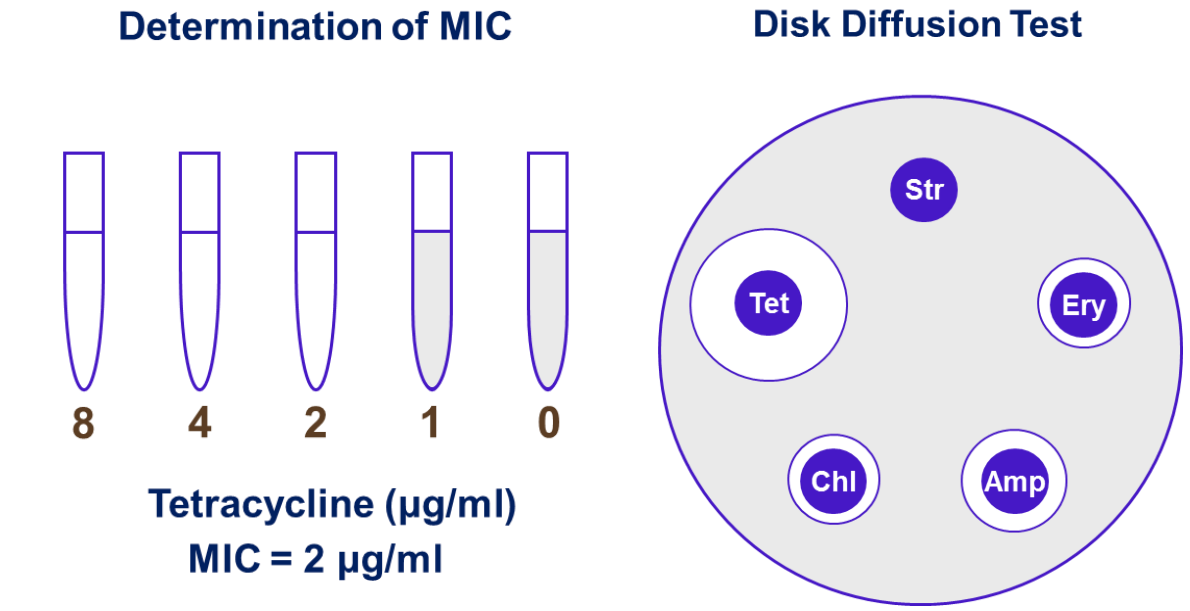
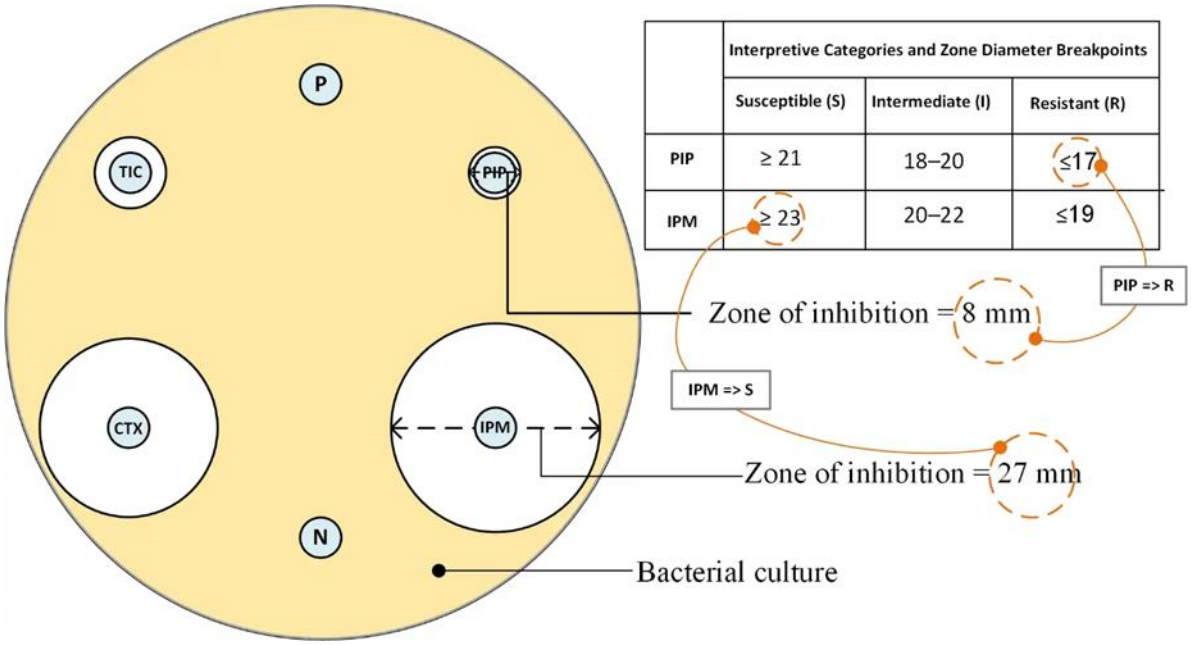


Chart of HPLC analysis

Susceptibility testing of antibiotics (Minimum inhibitory concentration, MIC).





	Interpretive Categories and Zone Diameter Breakpoints		
	Susceptible (S)	Intermediate (I)	Resistant (R)
PIP	≥ 21	18–20	≤17
IPM	≥ 23	20–22	≤19

Procedures

1- Preparation of suitable medium for antibiotic testing.

2- Cultivation of bacteria.

3- Placed of discs on the Petri-dish and added different antibiotics or different antibiotic concentrations.

4- Incubation of dishes for 24 or 48 hours.

5- Measure the inhibition zones around the discs and calculate the averages.

6- Determine the activity and MIC of the antibiotics.

7- Illustrate the data and write a comment.

Table 8:……..……..…

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Antibiotics	Inhibition zone (mm)	MIC

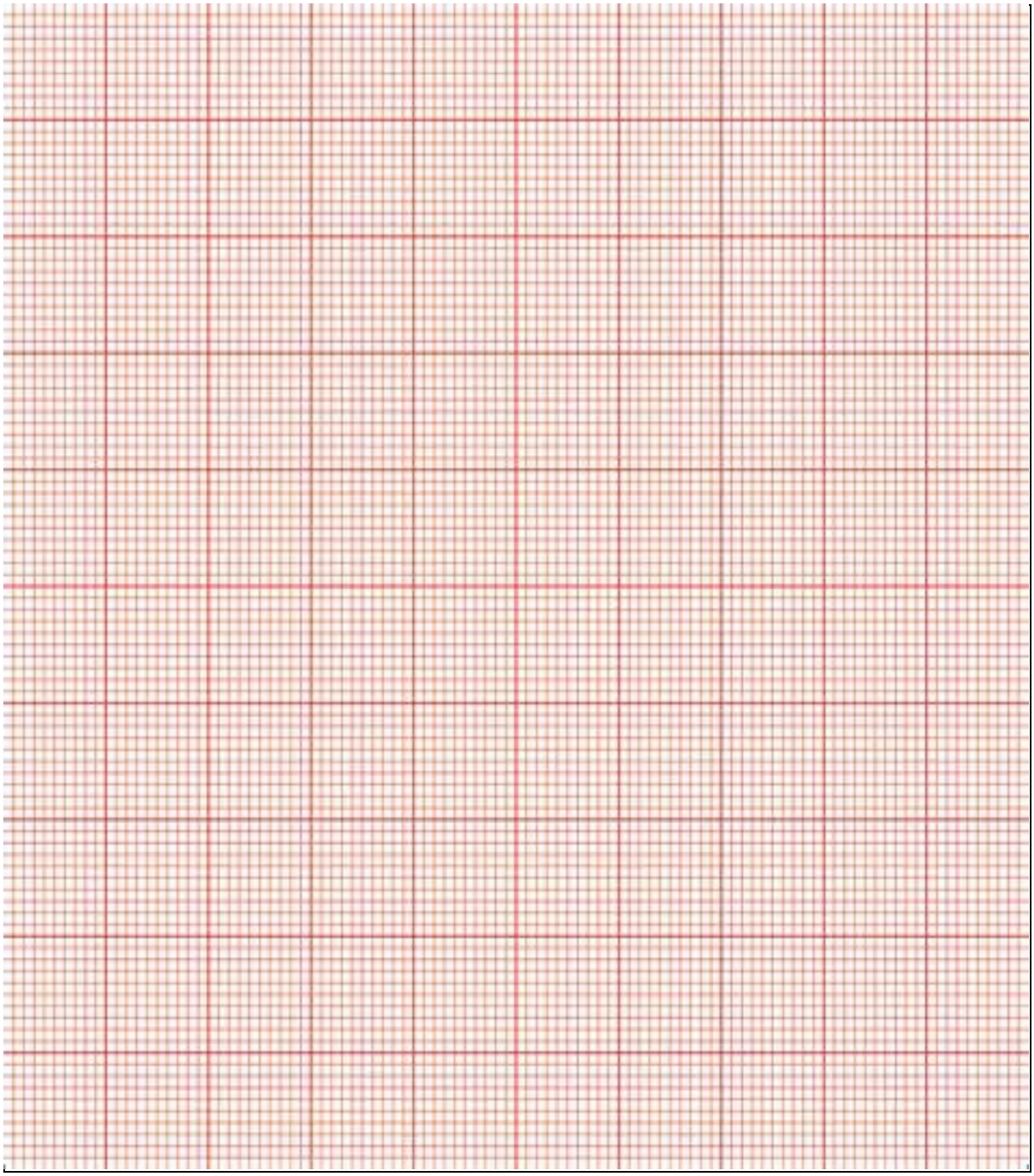

Figure 8: ……..

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