

**CONCLUSIONES**

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De los resultados obtenidos a modo de conclusiones podemos destacar:

1. Las especies del género *Aspergillus* se aíslan frecuentemente en los substratos estudiados, en especial en piensos. Su recuento parcial en este tipo de muestras representa más del 50% del recuento fúngico total.
2. De las especies productoras de OA aisladas en el presente estudio, la frecuencia de aislamiento de *Aspergillus niger* es superior a la de *A. ochraceus*.
3. Las cepas tipo de las dos especies propuestas en el agregado *A. niger*, *A. niger* CBS 554.65 y *A. tubingensis* CBS 134.48 están muy próximas en términos filogenéticos, por lo que podría tratarse de una misma especie.
4. La digestión con *Rsal* del fragmento 5.8S-ITS rDNA amplificado mediante PCR es un sistema fácil, práctico y rápido para clasificar cepas del agregado *A. niger* en las dos especies propuestas.
5. Las cepas de los dos grupos N y T son morfológicamente indistinguibles. Las pequeñas diferencias observadas no constituyen una característica práctica para diferenciar ambos grupos.
6. La temperatura óptima de crecimiento de las cepas del agregado *A. niger* es de 35°C, no desarrollándose por debajo de 10°C, ni por encima de los 50°C. Al considerar la división del agregado en los grupos N y T, las cepas tipo T presentan una temperatura óptima de 35°C y las tipo N de 30-35°C.
7. Las cepas tipo T se diferencian de las tipo N por desarrollarse más rápidamente a la temperatura más baja (10°C) en la que se obtuvo el desarrollo de las especies del agregado.

8. El crecimiento de las cepas del agregado *A. niger* se ve favorecido a concentraciones bajas de cloruro sódico, observándose una mayor tolerancia a 25°C que a 35°C. Los grupos de cepas N y T presentan una respuesta similar al NaCl.
9. El método de extracción de OA a partir de los bocados de cultivos en YES agar o CYA, y su detección mediante TLC ha resultado ser un buen método de criba para detectar cepas productoras de OA, debido a su sencillez y bajo coste de realización.
10. Todas las cepas ocratoxigénicas del agregado *A. niger* con patrón de RFLP conocido pertenecen al grupo N, por lo que los aislamientos del grupo T parecen no ser capaces de producir OA.
11. Las dos grupos de cepas N y T no presentan entidad suficiente para ser considerados como pertenecientes a especies distintas.

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**ANEXOS**



## **ANEXO I. Abreviaciones.**

**ATCC**, American Type Culture Collection, Rockville, MD, EEUU.

**BEN**, Balkan Endemic Nephropathy (nefropatía endémica de los Balcanes).

**CBS**, Centraalbureau voor Schimmelcultures, Baarn, Holanda.

**CFI**, Concentración mínima inhibitoria.

**CYA**, Czapek Yeast extract Agar (agar Czapek extracto de levadura).

**ETS**, Externally Transcribed Spacer (espaciador transcrito externamente).

**FAO**, Food and Agricultural Organisation.

**FDA**, Food and Drug Administration.

**GRAS**, Generally Regarded As Safe.

**IAC**, Immunoaffinity Columns (columnas de inmutuafinidad).

**HPLC**, High Performance Liquid Chromatography (Cromatografía líquida de alta eficacia).

**IARC**, International Agency for Research on Cancer.

**IGS**, Inter-Genic Spacer (espaciador intergénico).

**IMI**, International Micological Institute, Egham, Reino Unido.

**ITS**, Internally Transcribed Spacer (espaciadores transcritos internamente).

**LLP**, Liquid – Liquid Partition (partición líquido – líquido).

**MEA**, 2% Malt Extract Agar (agar extracto de malta al 2%).

**mtDNA**, DNA mitocondrial.

**NRRL**, Northern Regional Research Laboratory, Peoria, IL, EEUU.

**NTS**, Non-Transcribed Spacer (espaciador no transcrito).

**OA**, Ocratoxina A.

**PCR**, Polimerase Chain Reaction (reacción en cadena de la polimerasa).

**PDA**, Potato Dextrose Agar (agar patata glucosado).

**rDNA**, DNA ribosomal.

**RAPD**, Random Amplified Polymorphic DNA (polimorfismos de DNA amplificado aleatoriamente).

**RFLP**, Restriction Fragment Length Polimorphism (polimorfismos de longitud de fragmentos de restricción).

**SEM**, Scanning Electron Microscope (microscopio electrónico de barrido).

**SFW**, Suero fisiológico con tween 80 al 0,05%.

**SPE**, Solid Phase Extraction (extracción de fase sólida).

**TLC**, Thin Layer Chromatography (cromatografía en capa fina).

**UFC**, Unidades formadoras de colonias.

**YES**, Yeast Extract Sucrose (extracto de levadura sacarosa).

## **ANEXO II. Colores.**

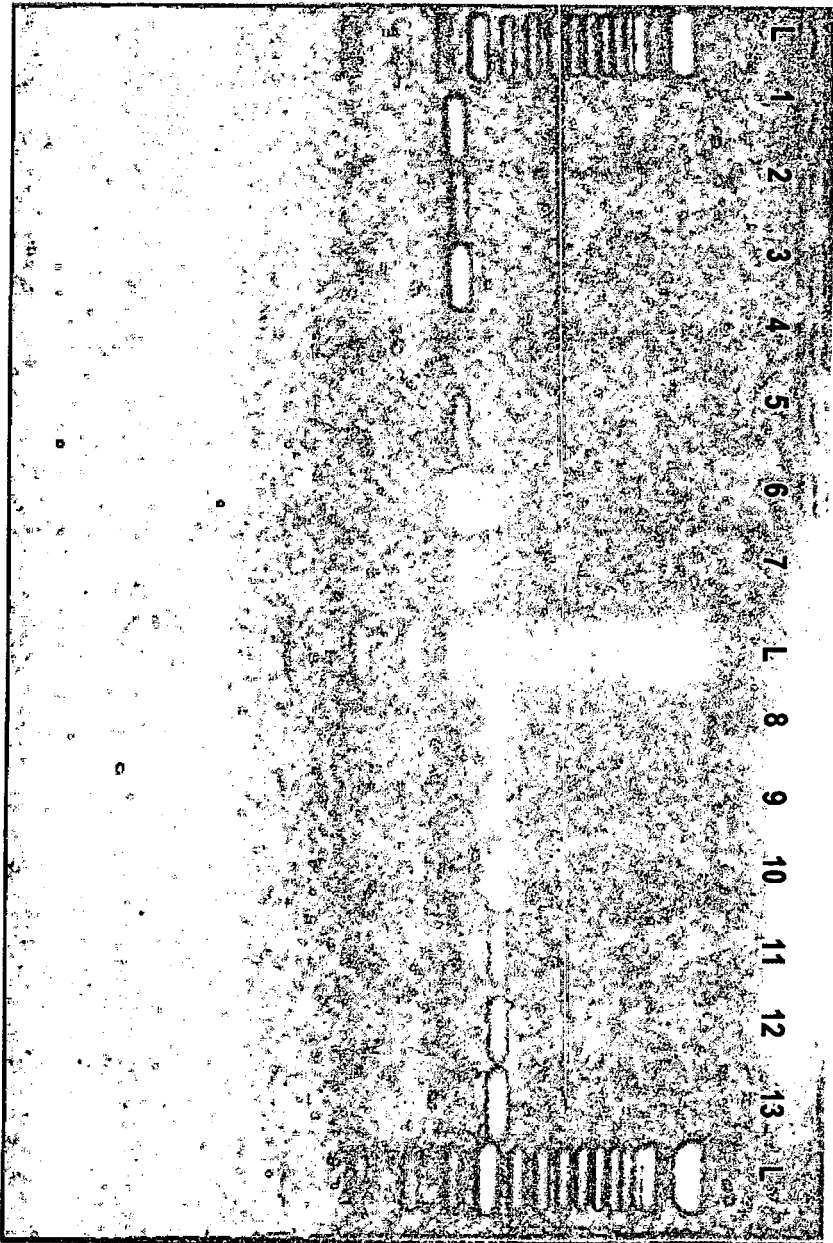
En el presente anexo se relacionan los colores mencionados en el presente estudio con los números de referencia de la carta de identificación de colores del *Royal Botanic Garden Edinburgh* (1969).

<b>A:</b> amarillo	54
<b>AC:</b> amarillo claro	50
<b>AG:</b> amarillo grisáceo	55
<b>B:</b> blanco	1
<b>BE:</b> beige	4
<b>C:</b> caqui	64
<b>CR:</b> crema	2
<b>G:</b> gris	34
<b>GN:</b> gris negruzco	37
<b>M:</b> marrón	24
<b>MC:</b> marrón claro	17
<b>MG:</b> marrón grisáceo	33
<b>MN:</b> marrón negruzco	36
<b>MO:</b> marrón oscuro	16
<b>MR:</b> marrón rojizo	23
<b>N:</b> negro	38
<b>NA:</b> naranja	48
<b>V:</b> verde	62
<b>VC:</b> verde claro	68
<b>VO:</b> verde oscuro	65

**ANEXO III. Láminas.**

## Lámina I.

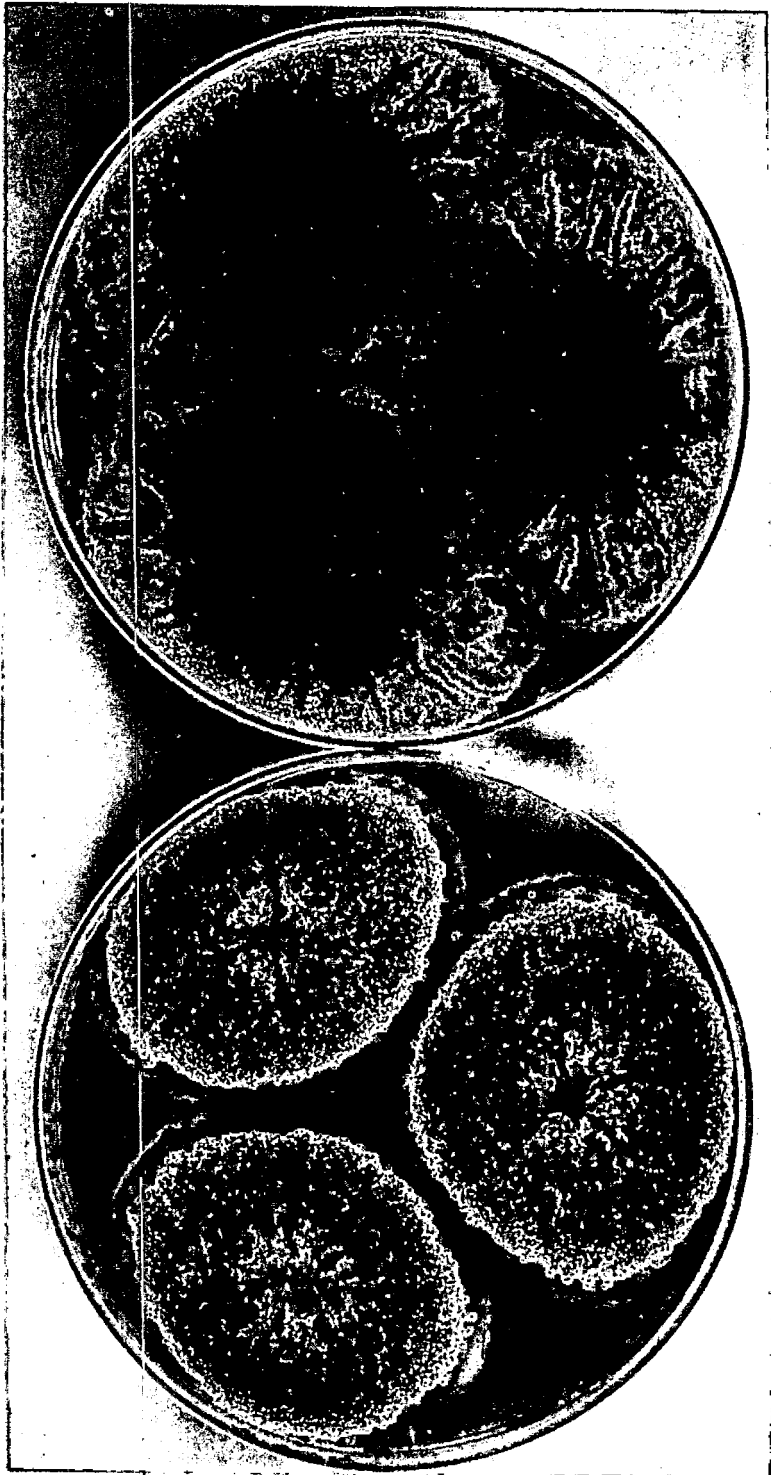
Patrones de RFLP N y T obtenidos al digerir con *RsaI* los fragmentos 5.8S ITS-rDNA amplificados mediante PCR. Patrón N: dos fragmentos de 519 y 76 pb. Patrón T: Un fragmento de 595 pb. Carrera L: marcador 100-bp DNA ladder (Gibco BRL); carreras 1-7: patrón N; carreras 8-13: patrón T. Carrera 1, CBS 554.65; carrera 2, CBS 126.49; carrera 3, NRRL 3122; carrera 4 ATCC 22343; carrera 5, CBS 618.78; carrera 6, IMI 211394; carrera 7, CBS 118.35; carrera 8, CBS 134.48; carrera 9, CBS 117.32; carrera 10, IMI 172296; carrera 11, IMI 63764; carrera 12, CBS 558.65; carrera 13, ATCC 26036. El fragmento de 76 pb es demasiado pequeño para observarse con claridad.



**Lámina II.**

Aspecto de las colonias de *Aspergillus niger* var. *niger* (A220) desarrolladas en MEA (A) y CYA (B) a los siete días de incubación a 25°C.

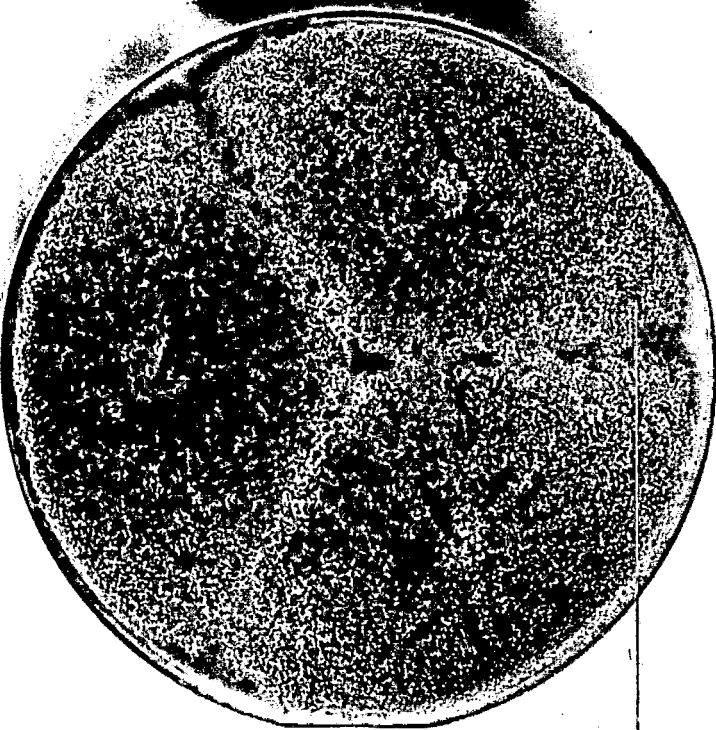
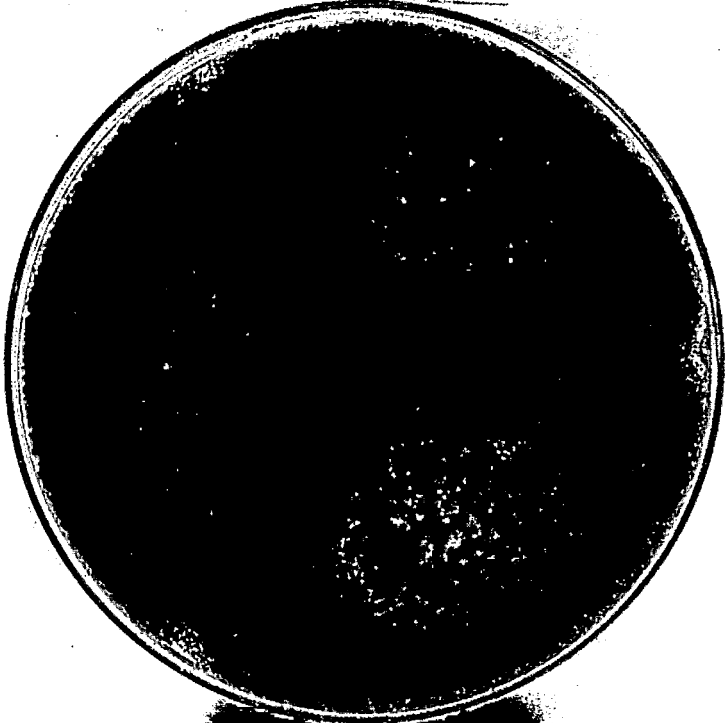




**Lámina III.**

Aspecto de las colonias de *Aspergillus niger* var. *niger* (A220) desarrolladas en CYA (A) y CYA20S (B) a los siete días de incubación a 37°C y a 25°C, respectivamente.

A



B

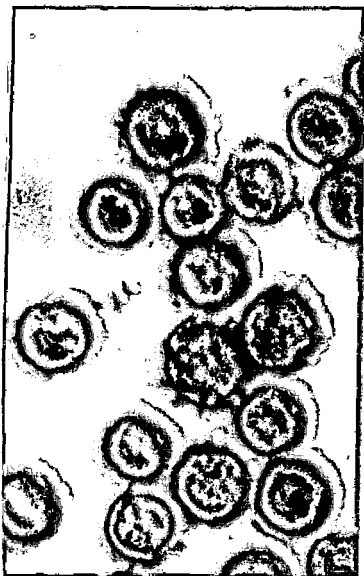
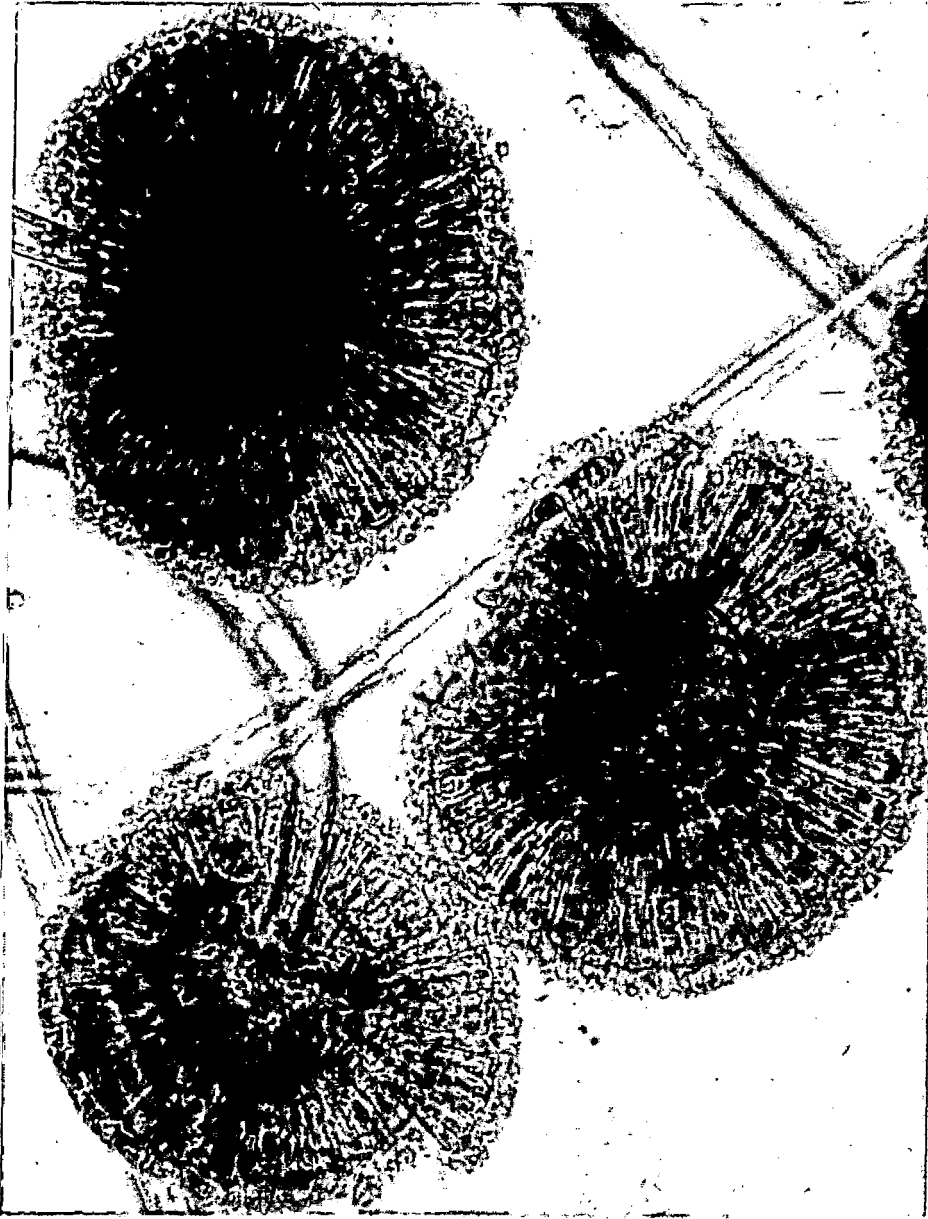
**Lámina IV.**

Aspecto de las cabezas conidiales de *A. niger* var. *niger* (A266).

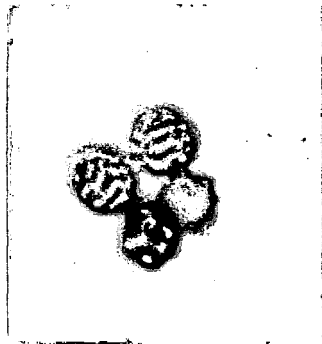
Detalle de la ornamentación de los conidios:

A: ornamentación formada por estrías longitudinales (A645).

B: ornamentación formada por protuberancias (IMI 211394).



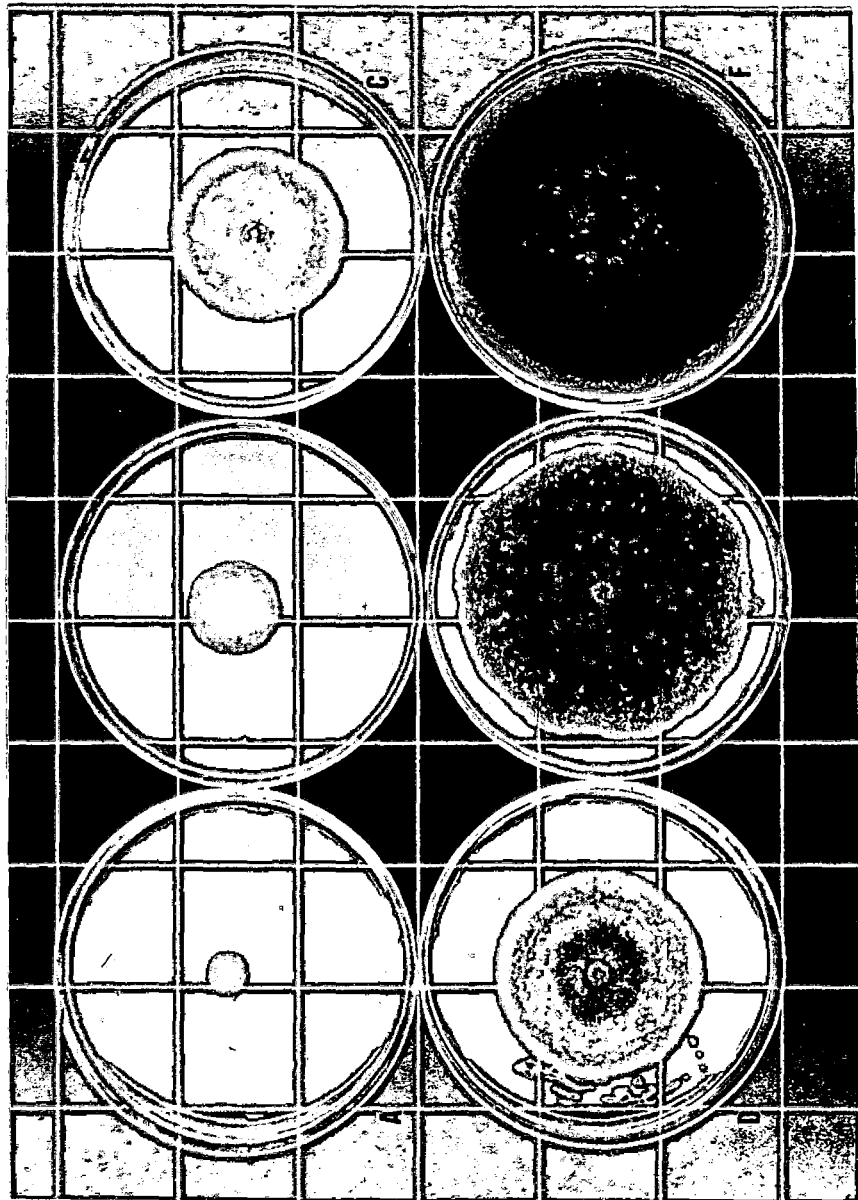
B



A

**Lámina V.**

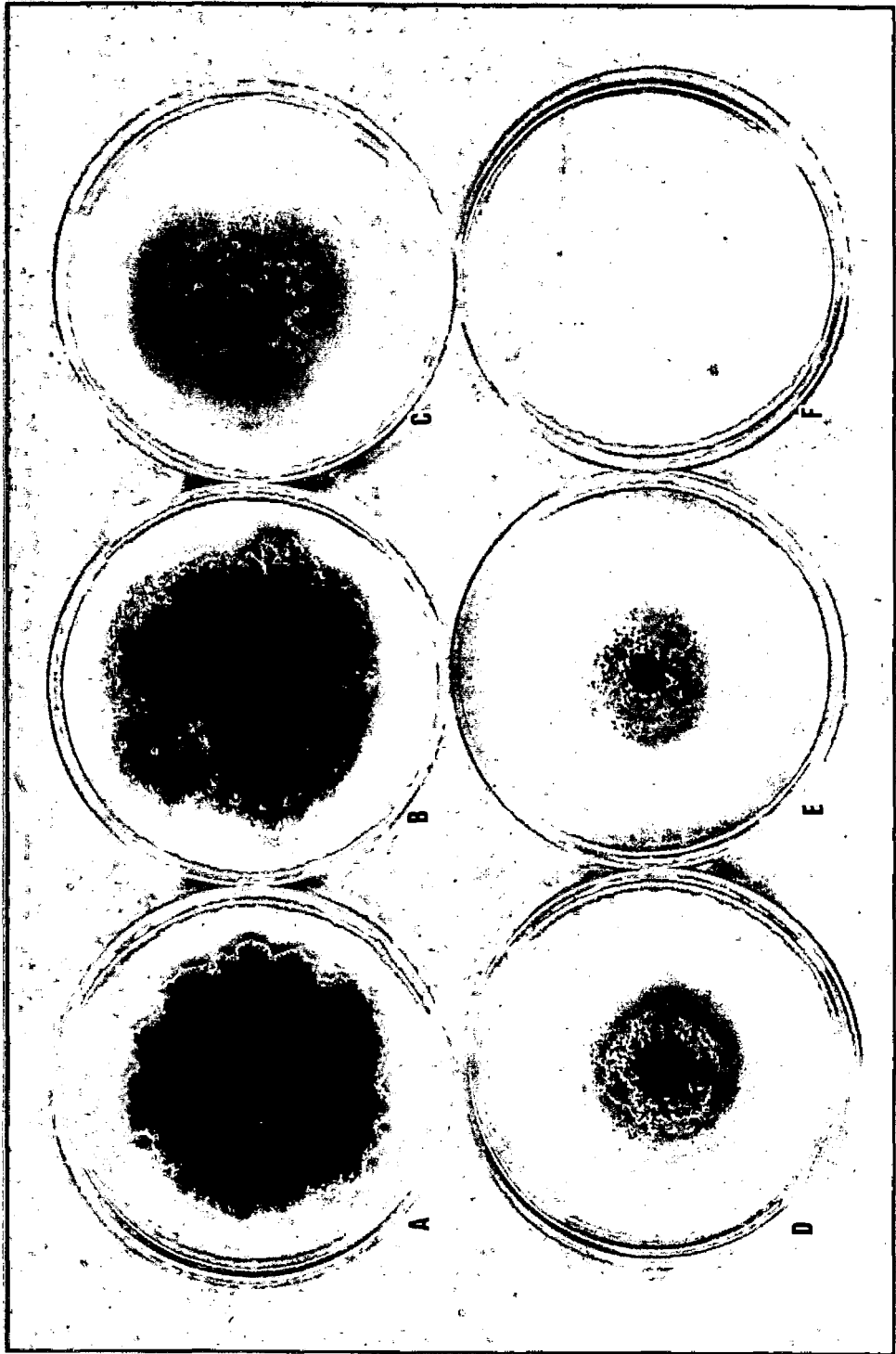
Crecimiento de *A. niger* var. *niger* (A81) a las temperaturas de 10°C (A), 15°C (B), 20°C (C), 25°C (D), 30°C (E) y 35°C (F).



## Lámina VI.

Crecimiento de *A. niger* var. *niger* (A656) a distintas concentraciones de NaCl: 0% (A), 2% (B), 4% (C), 6% (D), 8% (E) y 10% (F) a 35°C.





**Lámina VII.**



Alteraciones morfológicas inducidas por elevadas concentraciones de NaCl:

A: heteromorfismo (A88).

B: presencia de hifas retorcidas (607JC).

