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Research Article

Colonial and Morphological Characteristics of Some *Aspergillus* Fr.:Fr. Species Isolated from Vineyards in Manisa and İzmir Provinces (Turkey)

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Abstract: Five *Aspergillus* Fr.:Fr. species and 3 varieties were described from the point of view of colonial and morphological characteristics. These species and varietes are isolated from soil, grape and sultana raisins from vineyards in Manisa and İzmir provinces (Turkey). These are as follows: *A. flavofurcatus* Bat. & H.Maia, *A. heteromorphus* Bat. & H.Maia, *A. pulverulentus* (McAlpine) Wehmer, *A. unguis* (Emile-Weil & L.Gaudin) Thom & Raper, *A. viridinutans* Ducker & Thrower, *A. foetidus* Thom & Raper var. *pallidus* (Nakaz., Simo & A.Watan.) Raper & Fennell, *A. foetidus* Thom & Raper var. *acidus* (Nakaz., Simo and A.Watan.) Raper & Fennell and *A. nidulans* (Eidam) G.Winter var. *acristatus* Fennell & Raper. The final 2 are cited for the first time in Turkey.

Key Words: Mycoflora, *Aspergillus*, Manisa, İzmir, Turkey

Manisa ve İzmir Yörelerindeki Bağlardan İzole Edilen Bazı *Aspergillus* Fr.:Fr. Türlerinin Koloni ve Mikroskopik Özellikleri

Özet: Bu çalışmada beş *Aspergillus* Fr:Fr türü ve üç varyetesi koloni ve morfolojik özellikleri bakımından tanımlanmıştır. Bu tür ve varyeteler Manisa ve İzmir illerindeki sultani cinsi üzüm bağı toprakları ile yaş ve kuru üzümlerden izole edilerek tanımlanmışlardır. Bunlar; *A. flavofurcatus* Bat. & H.Maia, *A. heteromorphus* Bat. & H.Maia, *A. pulverulentus* (McAlpine) Wehmer, *A. unguis* (Emile-Weil & L.Gaudin) Thom & Raper, *A. viridinutans* Ducker & Thrower, *A. foetidus* Thom & Raper var. *pallidus* (Nakaz., Simo & A.Watan.) Raper & Fennell, *A. foetidus* Thom & Raper var. *acidus* (Nakaz., Simo & A.Watan.) Raper & Fennell ve *A. nidulans* (Eidam) G.Winter var. *acristatus* Fennell & Raper'dır. Belirtilen son iki tür Türkiye mikoflorası için ilk kez belirtilmektedir.

Anahtar Sözcükler: Mikoflora, *Aspergillus*, Manisa, İzmir, Türkiye

Introduction

Moulds are of great importance not only with respect to health and industry but also in terms of economics due to their metabolic properties. In industrial microbiology, the ability to produce organic acids such as citric acid and itaconic acid, some enzymes, pigments and antibiotics by moulds has been exploited (Topal, 1984). In addition, these organisms are important contaminants of food and agricultural products due to their presence in the soil and air. As well as their negative effects on food, moulds are capable of producing mycotoxins. One to their importance, the microfloristic study of moulds is well in progress around the world. The most common strains reported belong to *Penicillium* Link:Fr and *Aspergillus* Fr.:Fr. genera (Asan, 2000).

There have been many studies on the microfungus flora of Turkey. The majority of the studies were carried out to determine the microfungus flora of the soil in different parts of the country (Öner, 1970; Öner, 1974; Ekmekçi, 1975; Öner et al., 1977; Hasenekoğlu, 1985; Hasenekoğlu, 1987; Gür, 1991; Sülün & Hasenekoğlu, 1993; Asan & Ekmekçi, 1994; Haliki & Dizbay, 1997; Azaz & Hasenekoğlu, 1997; Asan, 1997a,b), others were undertaken on several kinds of agricultural products (Ulukuş & Sağır, 1982; Aran & Eke, 1987; Çolakoğlu, 1987; Çolakoğlu, 1991, Hasenekoğlu, 1988b) and foods (Alperden et al., 1982; Hasenekoğlu, 1988a; Birbir et al., 1995; Eltem & Öner, 1995, Güven et al. 1997).

A checklist showing the strains belonging to the genera *Aspergillus* and *Penicillium* Link:Fr. in Turkey has been in preparation since 1940 (Asan, 2000). According to published articles, there are 251 species belonging to these 2 genera that have been identified after isolation from different parts of Turkey.

The aim of the study is to contribute to the checklist of *Aspergillus* and *Penicillium* of Turkey. Some new strains of *Aspergillus* were isolated and identified from vineyard soils and grape and sultana raisin samples in our research.

Materials and Methods

The soil, grape and sultana raisin samples were taken from 62 different vineyards in Manisa and İzmir provinces (Figure 1) in 1998 and 1999. In the isolation of moulds from soil samples, the "soil dilution plate method" (Waksman, 1922) was used. In the isolation of moulds from fresh and dried sultanas the "pour plate method" (Brock & Madigan, 1991) was used. Rose-bengal chloramphenicol agar (Oxoid CM549) and dichloran-glycerol (DG18) agar base (Oxoid CM729) were used as isolation media.

The identifications of the isolates were made using Raper & Fennell (1965); Smith (1971), Domsch et al. (1980), Samson et al. (1981), Samson & Pitt (1990), Powell et al. (1994) and Samson & Pitt (2000). Czapex dox agar (CZ) (modified) (Oxoid CM97), malt extract agar (MEA) (Oxoid CM59) and Czapek yeast autolysate agar (CYA) were used as identification media. Citation of the authors of fungal names was performed according to Kirk & Ansell (1992).

Results

As a result of the survey, 772 moulds were isolated from soil, grape and raisin samples. The identifications revealed 39 *Aspergillus* species and varieties, and among them 2 varieties are new reports for the Turkish mycoflora. The descriptions of the most abundant 5 species and 3 varieties are given below.

Two strains of *A. flavofurcatus* Bat. & H.Maia were identified, one from the soil and another from dried fruit samples. Two strains of *A. foetidus* Thom & Raper var. *acidus* (Nakaz., Simo & A.Watan.) Raper & Fennell, one from grape and another from raisin samples, were found. In *A. foetidus* Thom & Raper var. *pallidus* Nakaz., Simo & A.Watan., 29 samples were identified in soil, 79 in fresh grapes and 59 were from raisins, making a total of 167 strains. In *A. heteromorphus* Bat. & H.Maia a total of 9 strains were identified, 5 from fresh and 4 from dried grapes. For *A. nidulans* (Eidam) G.Winter. var. *acristatus*

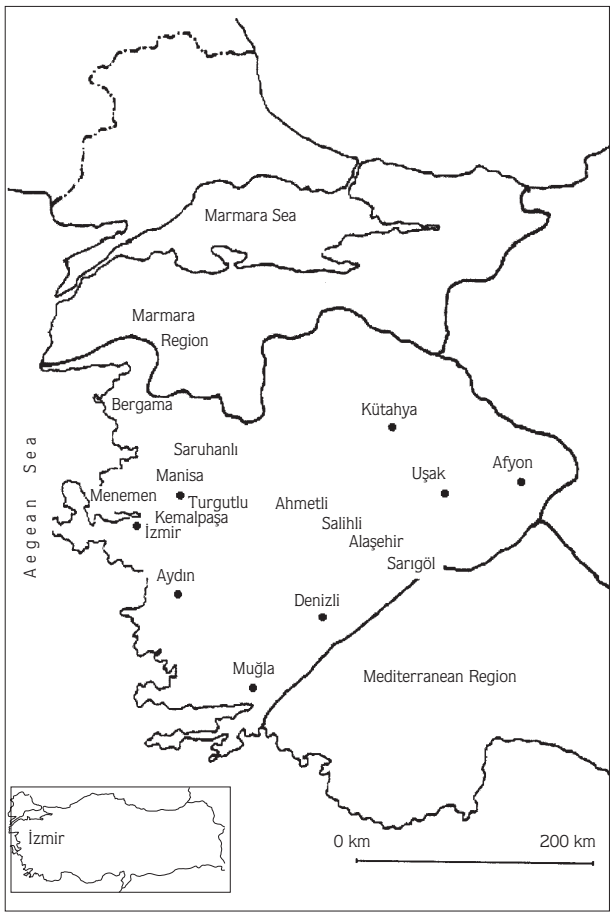

Figure 1. Map of investigation area.

Fennell & Raper 4 strains were determined from soil samples only. In *A. pulverulentus* (McAlpine) Wehmer 1 strain in grapes, in *A. unguis* (Emile-Weil & L.Gaudin) Thom & Raper 3 strains in raisins and in *A. viridinutans* Ducker & Thrower 2 strains from soil and raisin samples were found.

Aspergillus flavofurcatus Bat. & H.Maia, *Anais Soc Biol Pernambuco* 13: 94-96 (1955).

Colony Characteristics: This species, in 10 days, at 25 ºC on CZ, produced colonies 4 cm in diameter. Colony surface is green at margins and brown in the centre and around, the reverse is colourless. There are colourless exudates. The colony is 2.0-3.0 mm deep because of long stipes. Conidial heads are globose formerly, then radiate and 200-400 µm in diameter. Colonies on MEA are 5.5-6.0 cm in diameter and dark brown-black. More or less zonate, basal mycelium thin. On the colony, there are white hyphae.

Microscopic Characteristics: Vesicles are globose or subglobose and 20-70 µm in diameter. Phialide biseriate, metulae 10-12 x 4.0-6.5 µm, phialide 9.0-20 x 3.0-6.5 µm. Stipes are smooth and pigmented, 10-20 µm wide, 1.5-2.1 mm long. Conidia are slightly rough, globose, yellow-brown and 5.0-8.0 µm in diameter.

Aspergillus foetidus Thom & Raper var. ***acidus*** (Nakaz., Simo & A.Watan.) Raper & Fennell, Gen. *Aspergillus* 326 (1965).

Colony Characteristics: On CZ, at 27 ºC, in 2 weeks, colonies are 4.5-6.0 cm in diameter. Texture is lanose, margin white, centre yellowish. On basal mycelium sporulation is not dense. Sporulation is more at colony margin and centre. Conidial heads are blackish brown. Reverse bright yellow. Odour not distinct. On MEA at the same physical conditions, colonies are 4.0-5.0 cm in diameter. There is zonation and mycelium is golden yellow.

Microscopic Characteristics: On CZ conidial heads are 550-650 µm, stipes are 700-1000 x 15-27.5 µm, vesicles are 40-70 µm, phialide biseriate, metulae 18-22 x 4.5-5.0 µm, phialide 7.0-10 x 2.0-3.0 µm. Conidia are globose, 4.0-4.5 µm and wavy.

Aspergillus foetidus Thom & Raper var. ***pallidus*** (Nakaz., Simo & A.Watan.), Raper & Fennell, Gen. *Aspergillus* 325 (1965).

Synonym: *Aspergillus aureus* var. *pallidus* Nakaz, Simo & A.Watan. *J Agr Chem Soc Japan* 12: 961-962 (1936).

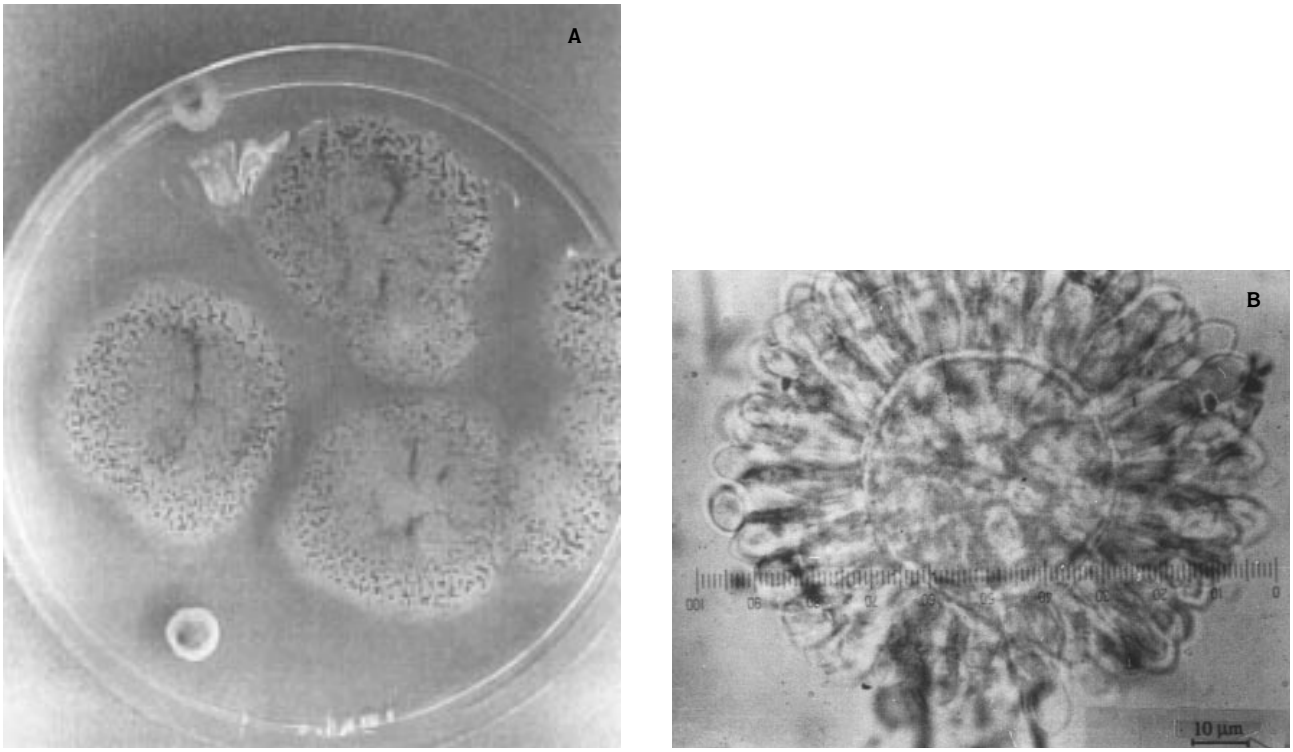

Figure 2. *A. flavofurcatus* on MEA (A), microscopic appearance of conidial head (B).

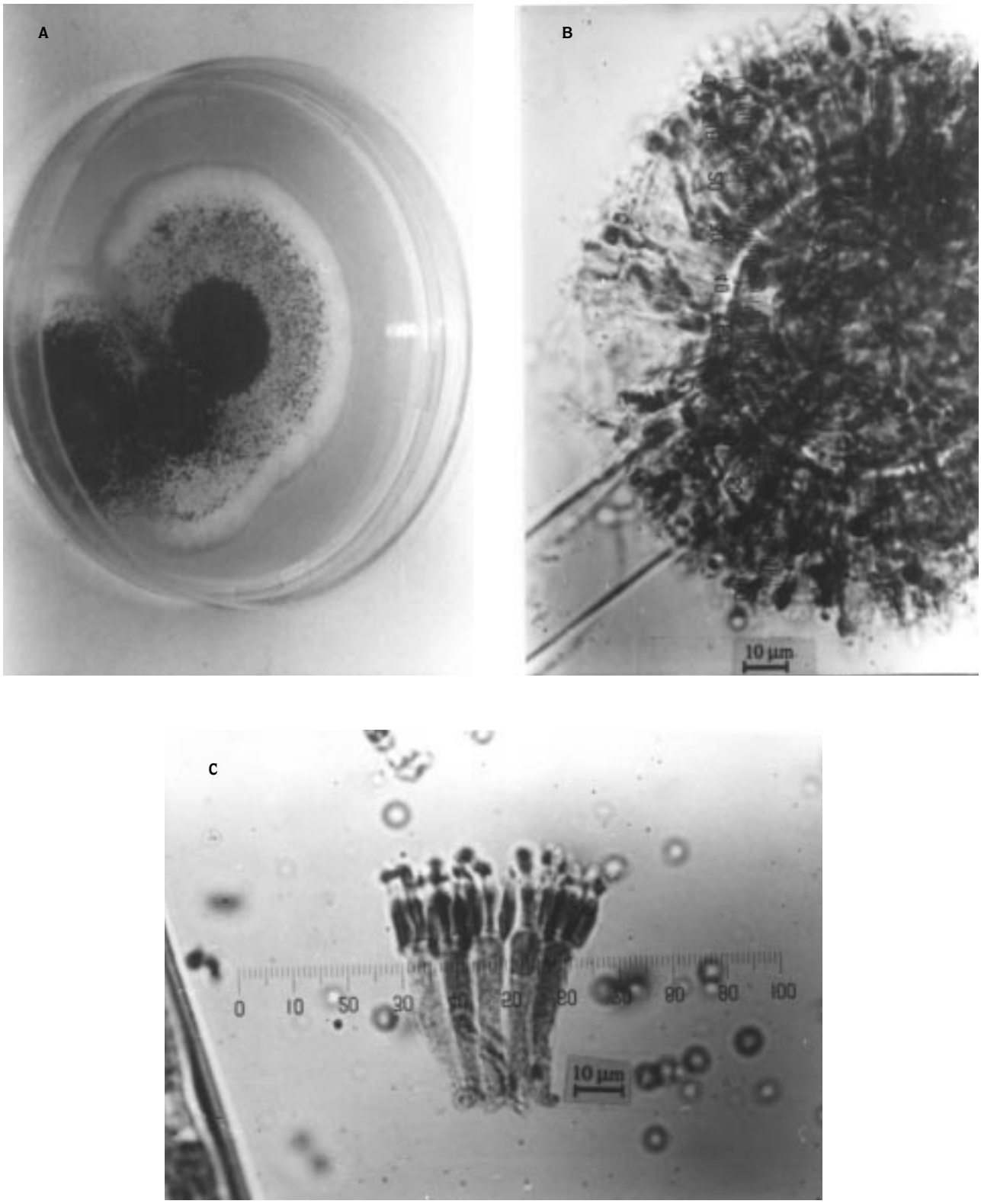

Figure 3. *A. foetidus* var. *acidus*. on MEA (A), microscopic appearance of conidial head (B) and phialide (C).

Colony Characteristics: This species develops colonies 2.5-4.0 cm in diameter on CZ. Colony surface is white or cream formerly, then turns to dark olive green-brown. Reverse colour of colony is colourless or cream, changes brown; later odour is mouldy, no zonation. There are colourless exudates on colony surface. Conidal heads are in olive green, radiate and 200-600 µm in diameter.

Microscopic Characteristics: Stipes are smooth, brown pigmented, 1.0-2.5 µm x 10-20 µm. Vesicles are globose generally 50-80 µm in diameter. Phialide biseriate, metulae 10-20 x 3-5 µm, phialide 10-20 x 3-5 µm. Conidia are globose or subglobose, obvious and rough, 2.5-5.0 µm in diameter.

Aspergillus heteromorphus Bat. & H.Maia in *Anais Soc Biol Pernambuco* 15: 200 (1957).

Colony characteristics: On CZ in 2 weeks, at 25 ºC, colonies are 3.0-3.5 cm in diameter. Greenish black, at the margins there is submerged mycelium 2.0-3.0 mm in width. Conidial heads at margin are small and yellow, thin, smooth, richly sporulated, in centre more sporulated. Reverse is colourless, no distinct odour, no exudate. On MEA under the same physical conditions, colonies are 6.0-7.0 cm in diameter, plane, radiate, velvety; there are brown, globose or subglobose sclerotia 100-150 µm in diameter.

Microscopic Characteristics: On CZ, at the marginal and submarginal areas stipes are 800-900 x 8.0-12 µm, conidial heads are dark green, globose and radiate, 180-200 µm in diameter becoming columnar with age. Vesicles are reddish brown, 35-45 µm, phialide biseriate, metulae 10-12 x 3.5-4.0 µm, phialide 6.0-8.0 x 2.5-3.0 µm. Conidia 3.0-3.5 µm, verruculose, sclerotia are 300-500 µm in diameter. Heads are radiate, 100-3000 µm, colony surface granular, more or less zonate, heads are darker in centre and margins, odour not distinct, no exudate, reverse colourless.

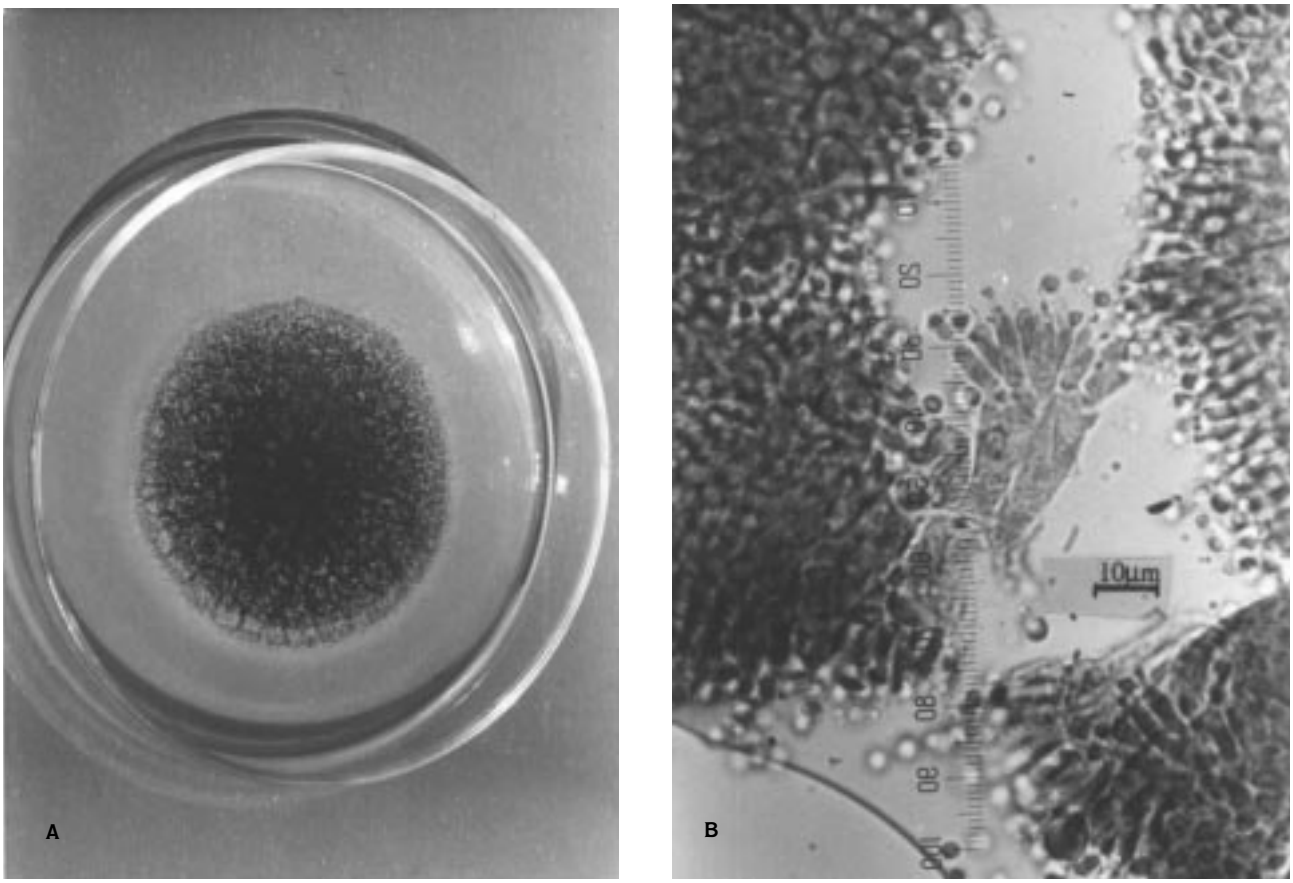

Figure 4. *A. foetidus* var. *pallidus* on CZ (A), microscopic appearance of phialide and conidia (B).

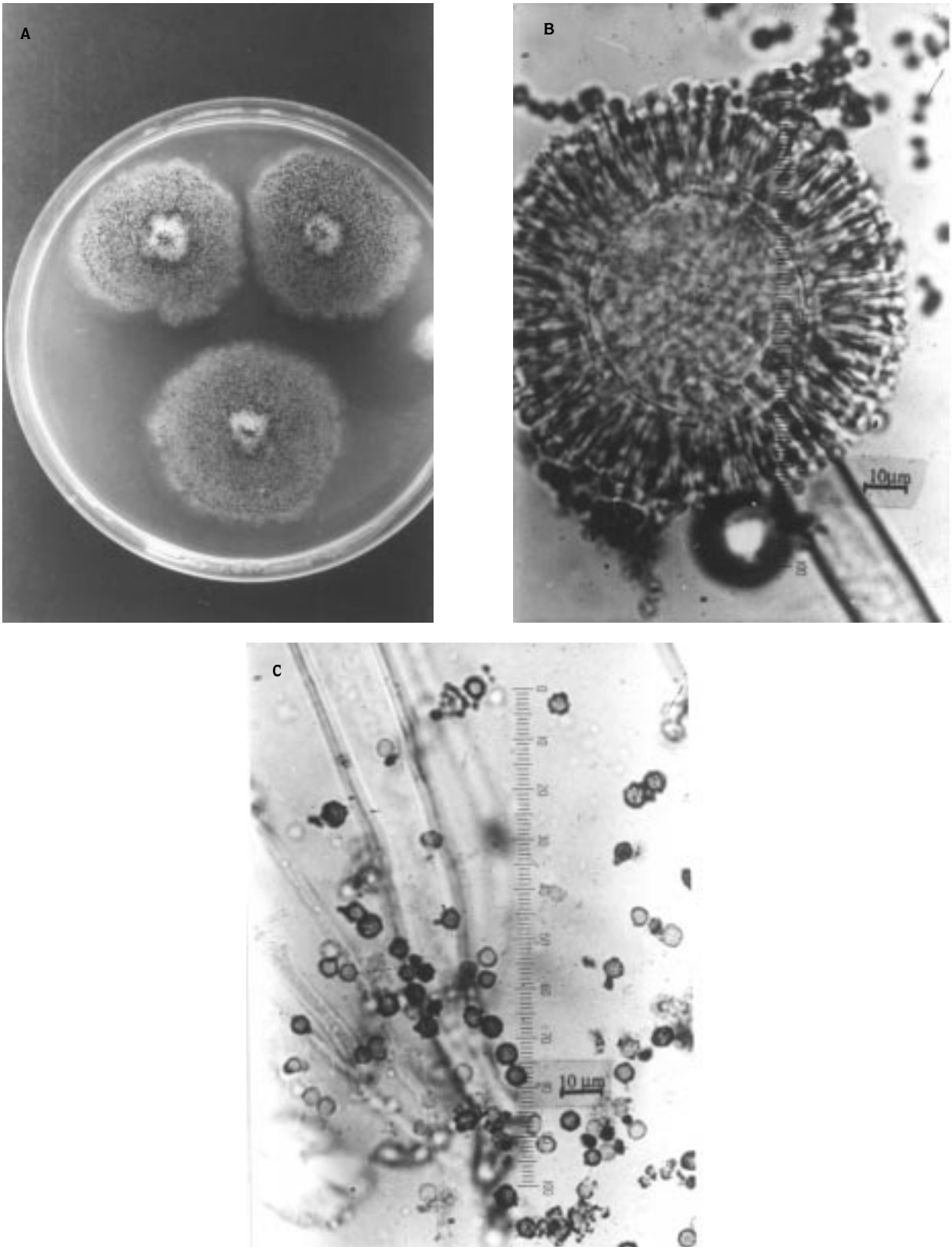

Figure 5. *A. heteromorphus* on CZ (A), microscopic appearance of conidial head (B) and conidia (C).

Aspergillus nidulans (Eidam) G.Winter var. ***acristatus*** Fennell & Raper, *Mycologia* 47 (1): 79 (1955).

Synonym: *Emericella acristata* (Fennell & Raper) Y.Horie in *Trans Brit Mycol Soc Japan* 21: 491 (1980).

Colony Characteristics: Colony diameter is 2.5-3.0 cm in 10 days at 25 ºC on CZ. Texture lanose, at the beginning of development colony surface is white then ochre, centre of colony lightly raised. Reverse is dark purple like eggplant. Colourless exudates on the surface. Conidial heads are short and columnar.

Microscopic Characteristics: Stipes are brown and smooth, 75-150 x 4.0-5.0 µm. Vesicles are subglobose and 8.0-11 µm in diameter. Phialide biseriate, metulae 5.0-6.0 x 2.0-3.0 µm, phialide 5.0-6.0 x 2.0-2.5 µm. Conidia are globose, smooth, 3.0-4.0 µm in diameter. Cleistothecia are globose, 60-180 µm. Ascospores are orange-red, 4.0-5.0 x 3.0-4.0 µm in diameter. Hulle cells are globose, 25-30 µm.

Aspergillus pulverulentus (McAlpine) Wehmer, *Centralbl Bacteriol* 2. Abth. 18: 394 (1907).

Basionym: Sterigmatocystis pulverulenta McAlpine, *Agric Gaz New South Wales* 7: 302 (1897).

Colony Characteristics: This species developes quickly on CZ in 7 days, at 25 ºC, produces colonies 2.5-4.0 cm in diameter. Texture is velvety, surface grey-black, reverse white-cream. Basal mycelium is white, odour mouldy, exudates are small and colourless. Conidial heads are blackish grey, characteristically radiate, usually 500-600 µm in diameter. Colonies on MEA developed rapidly and reached 5.0 cm in diameter at the same temperature in 7 days. Texture is velvety, surface brown and black, reverse colour white; colourless exudates and zonation are present.

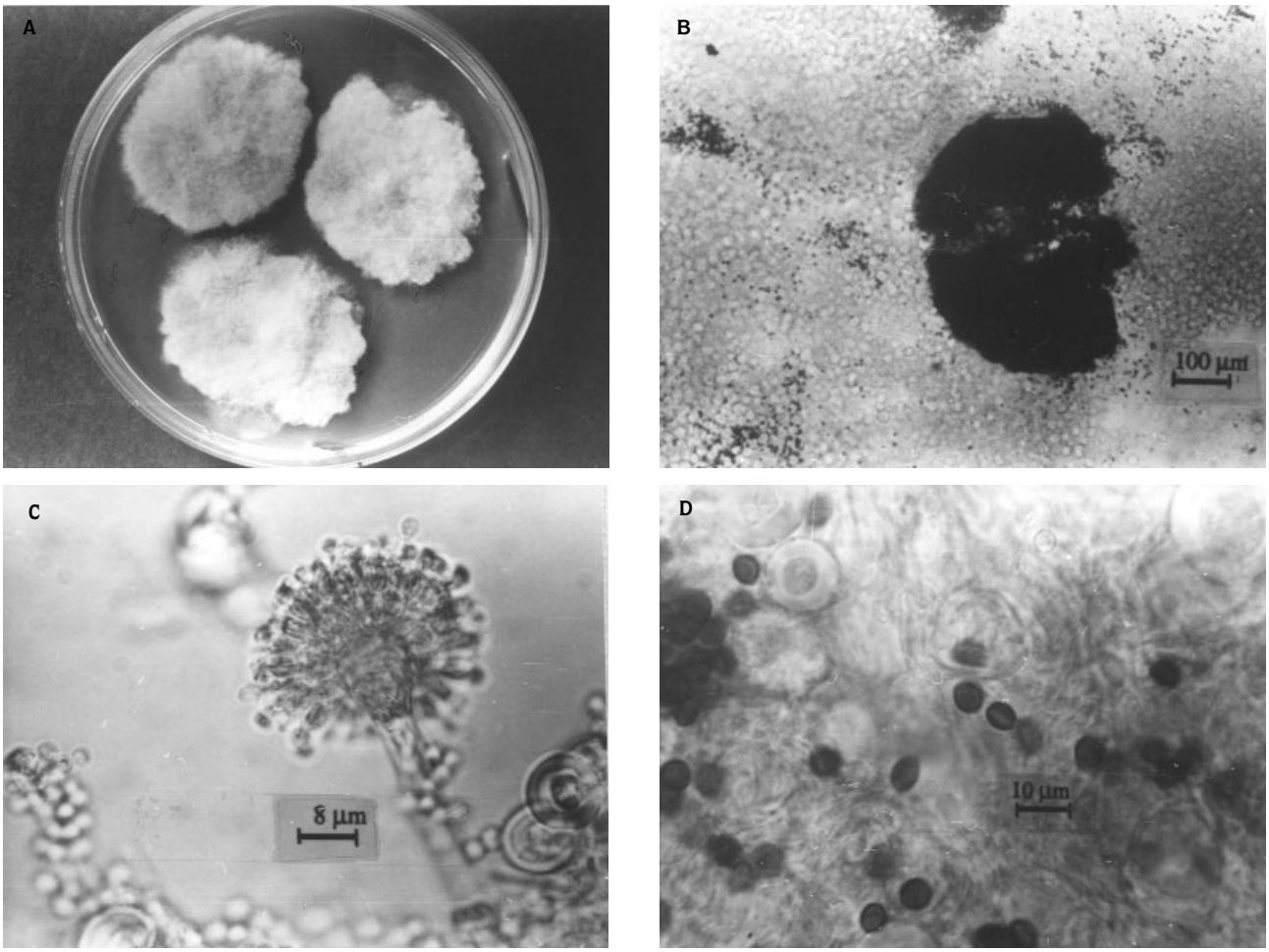

Figure 6. *A. nidulans* var. *acristatus* on CZ (A), cleistothecium (B), microscopic appearance of conidial head (C) and ascospores and hulle cells (D).

Microscopic Characteristics: Stipes are smooth, colourless, upper parts are light brown, 15-20 µm in width, 1000-3000 µm sometimes 5000 µm in length. Foot cells are 35-45 µm in length. Vesicles are globose, 60-75 µm in diameter. Phialide biseriate, metulae 20-30 x 3.0-3.5 µm in young individuals and 40-50 x 3.0-3.5 µm in adults. Conidia are globose-subglobose, verruculose or granular and 4.5-5.0 µm in diameter. Measurements of colonies on MEA are similar to those on CZ, but conidial heads are more columnar.

Aspergillus unguis (Emile-Weil & L.Gaudin) Thom & Raper, *Mycologia* 31: 667 (1939).

Basionym: *Sterigmatocystis unguis* Emile-Weil & L.Gaudin, *Arch Med Exp Anat Pathol* 28: 463 (1918).

Teleomorph: *Emericella unguis* Malloch & Cain, *Can J Bot* 50: 62 (1972).

Colony Characteristics: On CZ in 10 days at 25 ºC bright yellow in centre, darker and green around. Colony centre is raised and after day 13 becomes wrinkled. Reverse is nearly orange. At first conidial heads radiate, but later turn columnar. On the MEA at the same physical conditions, colonies are sage green and 3.0-4.0 cm in diameter.

Microscopic Characteristics: On CZ, stipes are smooth and yellow-brown, 45-60 x 3.0-3.5 µm at the foot cells, walls are thick and roughened, there are sterile spicular hyphes. Vesicles are hemispherical and 7.0-12 µm in diameter. Phialide biseriate. Metulae 5.0-6.0 x 2.5-3.0 µm, phialide 6.0-7.0 x 2.5-3.0 µm. Conidia are globose or subglobose in shape and rough, 2.5-3.0 mm in diameter. Conidia chains are 150 µm in length. On MEA, conidial structures are similar to those on CZ.

Aspergillus viridinutans Ducker & Thrower, *Aust J Bot* 2: 355 (1954).

Colony Characteristics: This species produces colonies which are 3.0-4.5 cm in diameter in 14 days at 25 ºC on CZ. Colony is white at first and then turns green in centre and cream along margins. Reverse is light

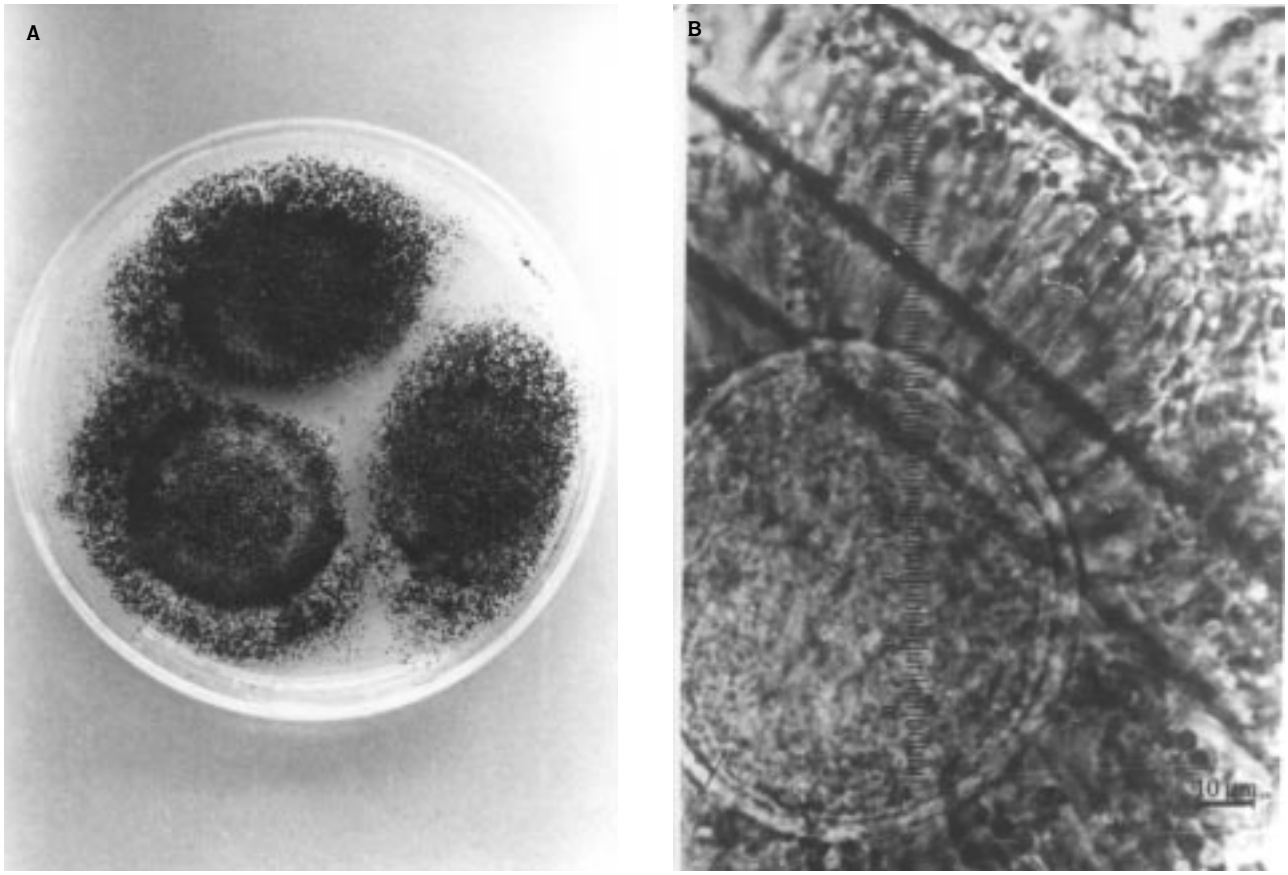

Figure 7. *A. pulverulentus* on CZ (A), microscopic appearance of conidial head (B).

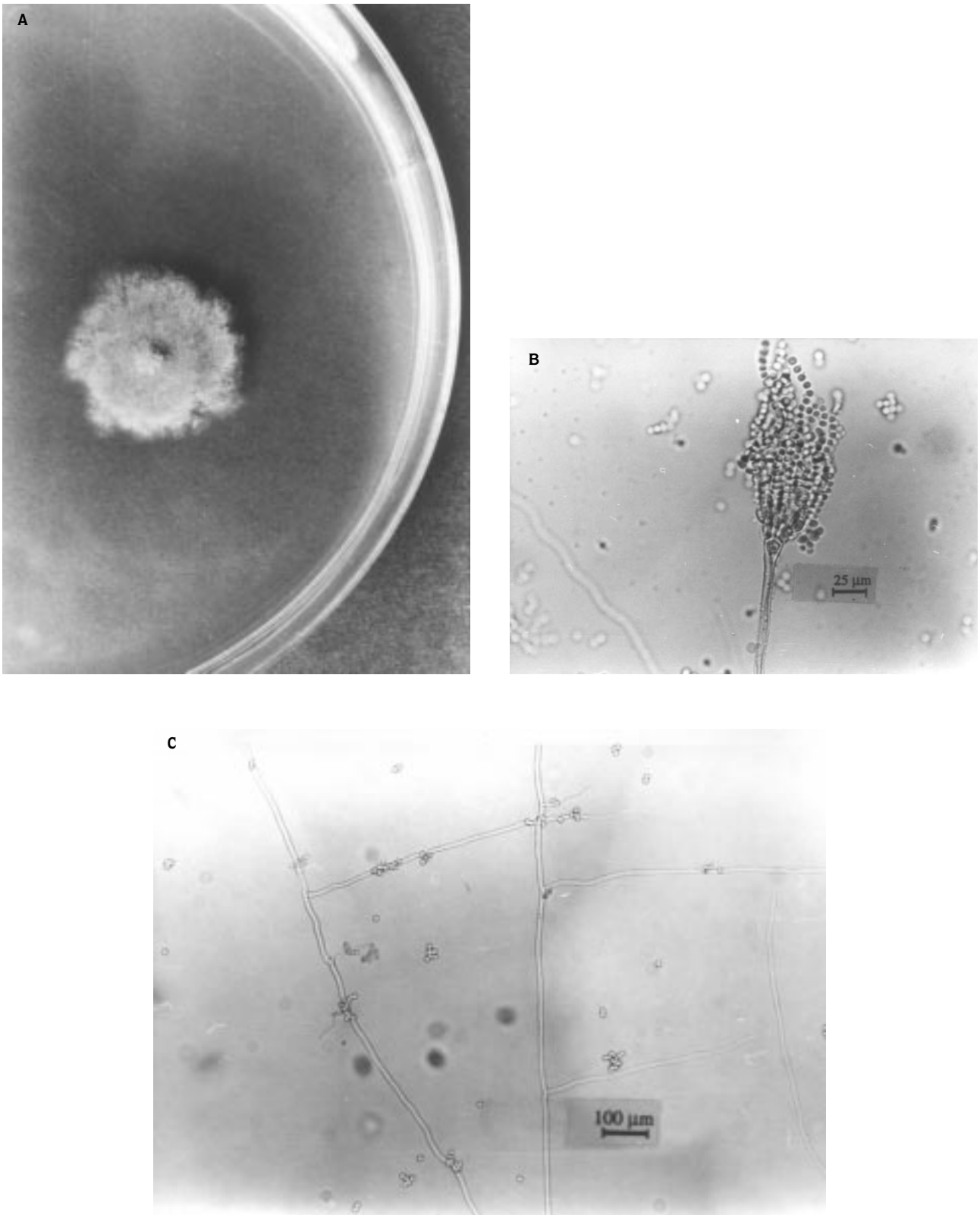

Figure 8. *A. unguis* on CZ (A), microscopic appearance of conidial head (B) and sterile hyphae (C).

brown. Exudates are small and colourless with no zonation. Conidal heads are columnar and dark, 50-60 x 20-25 µm. Colonies on MEA 6.0-6.5 cm in diameter under the same conditions. Surface is light green-white. Colourless exudates are present. Texture is funiculose-floccose. Reverse is colourless.

Microscopic Characteristics: On CZ, vesicles are globose or hemispherical and 3/4 of it is fertile. Diameter of vesicles is 8.0-13 x 9.0-12 µm. Vesicles are bent. Phialide uniseriate and 5.0-8.0 x 2.5-3.0 µm. Stipes are 80-100 µm in length, 3.0-4.0 µm in width and smooth. Conidia are globose or subglobose, rough, 2.5-3.0 µm in diameter.

Discussion

In studies carried out in Turkey on mycoflora, *Aspergillus* and *Penicillium* have been the dominant genera isolated from soil, agricultural and food commodities. In one examination of mycoflora in Turkey 82 *Aspergillus* species and varieties were determined (Asan, 2000). *A. flavofurcatus*, *A. pulverulentus*, *A.* unguis, *A. viridinutans* and *A. foetidus* var. *pallidus* were cited for the first time in Turkey by Eltem et al. (2001). In addition, *A. heteromorphus* was cited for the first time by Azaz & Pekel (2002). In our work 2 varieties (*A. foetidus* var. *acidus* and *A. nidulans* var. *acristatus*) are reported for the first time increasing the total number to 91.

In Turkey, the most widespread species is *A. niger* van Tieghem which is followed by *A. flavus* Link, *A. fumigatus* Fres., *A. versicolor* (Vuill.) Tiraboschi, *A. ochraceus* Wilhelm, *A. terreus* Thom and *A. wentii* Wehmer. It is thought that these species are more adapted to the prevailing ecological conditions (Asan, 2000).

Newly reported *Aspergillus* varieties in the Turkish mycoflora as well as other reported species were isolated and identified from the sultana vineyards in Manisa and İzmir provinces in the Aegean Region by Eltem et al. (2001).

A morphological examination of species for the purposes of identification was made first with the naked eye or by using a low magnification microscope and thereafter detailed examinations were performed according to Gams et al. (1987) by measuring the

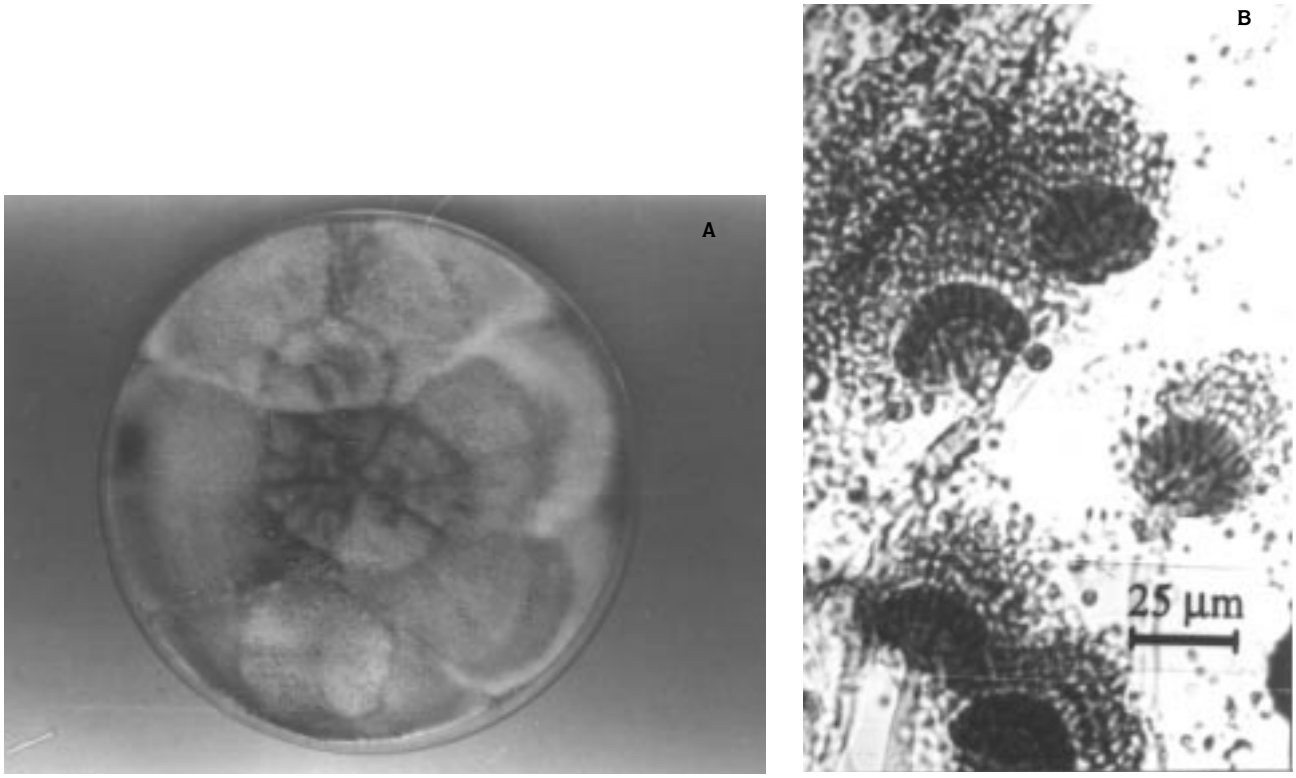

Figure 9. *A. viridinutans* on MEA (A), microscopic appearance of conidial head (B).

dimensions of the microbiological structures, photographing the microscopic structure and using relevant literature as references.

In the examinations, some colony and microscopic characteristics of the species were found to be different from those stated by Raper & Fennell (1965). For example, the colony diameter of *A. flavofurcatus* on CZ was smaller. It was determined that the colony diameter of *A. foetidus* var. *pallidus* ranged between 2.0 and 4.0 cm and the diameter of the vesicles varied up to 80 µm. The most distinguishing property of *A. foetidus* var. *acidus,* a golden yellow mycelium in MEA as stated by Raper & Fennel (1965), was observed in both of the strains.

Öner (1973) and Smith & Moss (1985) report that *Aspergillus* species are the most dominant in the soil of mild climatic zones, confirming our results. Eltem et al. (2001) reported that the genus *Aspergillus* is dominant in vineyards and that one of the most widespread fungi species is *A. foetidus* var. *pallidus;* 545 *Aspergillus* isolates and 167 *A. foetidus* var. *pallidus* strains were identified. In conclusion, the descriptions of some *Aspergillus* species are given in this paper.

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