Chapter 4
Morphology-Based Characterization of *Trichoderma* Species

4.1 Introduction

The fungal kingdom is diverse, and it is estimated about 1.5 million species globally but only around 70,000 known species have been identified to date (Siddiquee 2014). Most of these species are classified as filamentous fungi. Several types of living organisms have been found from different substances that are utilized by humans for various applications. From these, an approximately 10% has been discovered and described until now. As morphology-based identification of fungi is still preliminary required for the given putative genus name. Hibbett et al. (2011) mentioned that still traditional morphology-based classification is authorized in the diverse classification of Fungi. The International Code of Nomenclature for algae, fungi, and plants (Melbourne Code) requires morphological Latin or English diagnosis for valid new fungal taxon descriptions (Norvell 2011). Microscopic observations are still a simple, economic, and efficient way to assay of morphological characters, and classification. The observations of the beauty of fungi are still enjoyable for many mycologists; they are very much excited to identify the anonymous species under microscope. In the past 10 years, new fungal species are identified averages of 1196 per year (Hibbett et al. 2011). Among these new fungal species are identified only morphology characters without prior DNA sequencing or molecular data from 1999 to 2009 (Hibbett et al. 2011). Combinations of physiological characteristics and microscopic observations still have practical and scientifical value in examining of fungi. Microscopic observations are commonly applied in fungal research and commercial laboratories.

Fungal species which belongs to the genus of *Trichoderma* (telemorph *Hypocrea*, Hypocreales, Ascomycota) was first introduced by Persoon in 1794. The genus of *Trichoderma* is found in soil, decaying wood, agricultural lands, prairie, forest, salt marsh, desert soils, manure and other form of plant organic matter throughout all climatic zones (Gams and Bissett 1998; Hoyos-Carvajal and Bissett 2011).

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The genus *Trichoderma* is easily grown with different media such as Potato Dextrose Agar (PDA), Blakeslee's Agar (BLA), Malt Extract Agar (MEA), Cornmeal Dextrose Agar (CMD), and Czapek Dox Agar (CDA) (Bissett 1991). The colony appearances of *Trichoderma* species are diverse according to the type of media used. According to Samuels et al. (2002), there are slight differences on the colony appearance of *Trichoderma* when they are grown onto PDA and also in CMD. They reported that *Trichoderma* species are white on rich media such as PDA and more transparent when grown on media such as CMD (Samuels et al. 2002; Shah et al. 2012). And they also found that the mycelia development and pigmentation can be better observed on PDA compared to other media (Samuels et al. 2002). Scattered blue-green or yellow-green pigment on the colony becomes observable when conidia are formed. Occasionally, they observed concentric rings made by these pigmentations by some *Trichoderma* species (Samuels et al. 2002). For example, *T. harzianum* formed 1–2 concentric rings with green conidial production when grown on PDA but no concentric ring has observed by *T. viride* and *T. pseudokoningii* (Shah et al. 2012). The green conidia are generally a diagnostic of the genus but green conidia also have been found in the unrelated genera such as *Penicillium* and *Aspergillus* (Jaklitsch et al. 2006). Some species are characterized by a complete lack of pigment in reverse, where reddish brown pigment occurs in reverse for some species (Gams and Bissett 1998). The colonies of some *Trichoderma* species on PDA are shown in Fig. 4.1. Furthermore, indistinct moldy or musty odors are commonly produced by different *Trichoderma* strains. Some species of *Trichoderma* such as *T. viride* is produced a sweet smell resembling "coconut" odor (Gams and Bissett 1998, 2002; Samuels et al. 2002).

Majority of *Trichoderma* species grown well at 25–35 °C, but some species grow well above 35 °C also (Samuels et al. 2002). Growth rates in culture can be useful to distinguish between morphologically similar species (Gams and Bissett 1998). For example, *T. harzianum* can be distinguished from the morphologically similar species of *T. aggressivum* and *T. atroviride* by growing at 35 °C (Samuels et al. 2002). After 4 days of incubation, neither *T. aggressivum* nor *T. atroviride* colony grew more than 5 mm while *T. harzianum* grew well and sporulated at 35 °C (Samuels 2004).

Trichoderma species are very diverse in their morphological characters. It produces numerous spores (conidia) which are mostly green depending on the cultural agar (Howell 2003). The backside of the colonies are often colorless, buff, yellow, amber of yellow-green and also many species produced thick-walled spores (chlamydospores) in immersed mycelium (Gams and Bissett 1998). They are decomposers of woody material due to its classification as the imperfect fungi (unknown sexual stage), fast-growing fungi with rapid growth rate, dominant in soil with a wide range of substrates, and are an aggressive competitor in nature (Howell 2003).

Microscopic observations such as spores and their arrangement, conidiophores, conidia, phialide, and chlamydospore are very important in the classification of species of *Trichoderma* isolates. The colonies of *Trichoderma* have the key characters such as growth rate, growth pattern, pigmentation, pustule formation, and odors that can be used to identify as *Trichoderma* species.

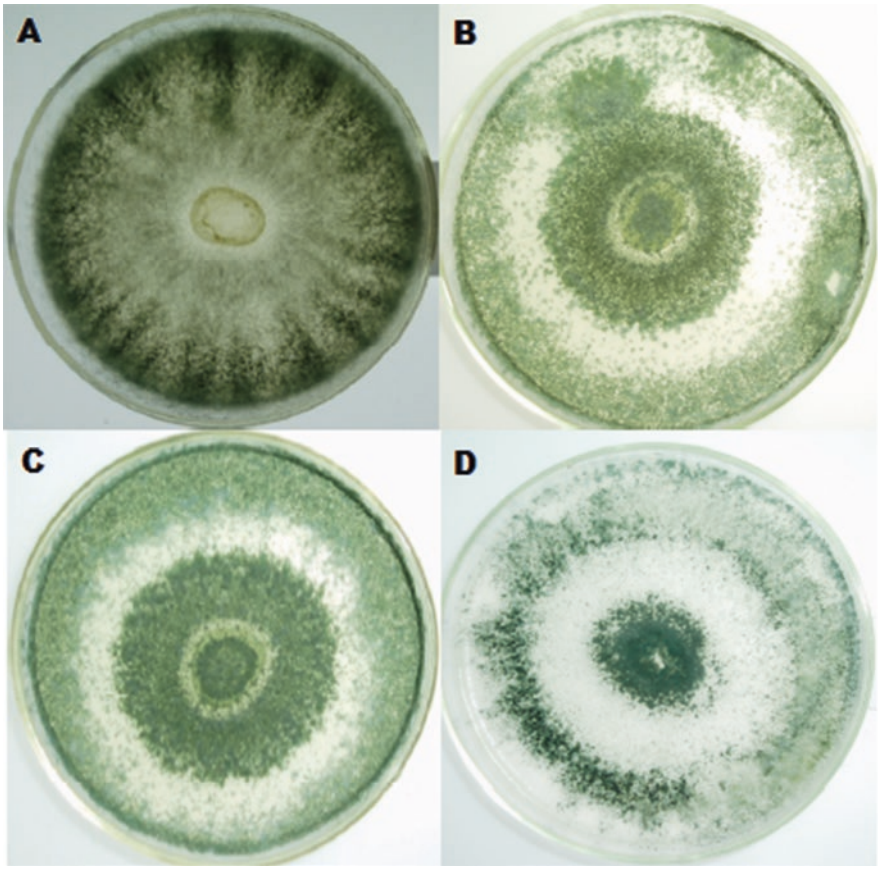

Fig. 4.1 Colony appearance of *Trichoderma* species are grown on PDA for 7 days where picture (**a**) is *T. atroviride*, (**b**) is *T. longibrachiatum*, (**c**) is *T. virens*, and (**d**) is *T. harzianum* (Source: Zhang and Wang 2012)

4.2 Definitions and Taxonomic Value of Morphological Traits

The following phenotype descriptions are mandatory to know the terminology definitions as *Trichoderma* mycologists; all terminology definitions descriptions are included in this book even through previously defined by Jaklitsch (2009):

4.2.1 Teleomorph

Asci: are not diagnostic traits, their size is mostly dependent on ascospore size; the length of the stipe is measured. Asci can see much wider in thin KOH mounts shortly before drying out.

Ascospores: tiny in size, however, some individual species can be defined using this character. Green ascospores turn brown in KOH, and appear often too brown in photographs taken from mounts in KOH; the color in KOH is also dependent on the thickness of mounts, i.e., in thinner mounts ascospores appear more distinctly brown.

Ostiolar dots/regions/areas: are measured in dry stromata. They include the ostiolar opening and a part of the surrounding cortex of the stroma, which appears darkened using a hand lens. Sometimes perithecial contours are parts of ostiolar dots. Ostiolar dots are commonly inconspicuous in teleomorphs of *Trichoderma* sect. *Trichoderma*. True ostioles, i.e., the ostiolar openings, are colorless, generally minute, inconspicuous or invisible. The apical width of ostioles in section refers more correctly to the ostiolar width at the stroma surface; ostioles may be attenuated at the true apex; the width includes the wall of the ostioles, except where noted. Ostiolar dots are of limited value in species delimitation, although typical, fresh material of certain species can often easily be identified using this character: e.g., fine, plane dots densely set in large numbers as in *H. strictipilosa* vs. *large*, projecting dots in small numbers as in *H. sinuosa*.

Perithecia: are immersed in a single layer in the upper part of the stroma. The perithecial height measured in median section includes the apex located at 1/3–1/2(−2/3) of the lower part of the ostiolar canal. Perithecia appear larger in crush mounts than in sections. There is considerable variation in the size of perithecia, but ranges are overlapping. The perithecial wall may be colorless or pigmented or change its color in KOH (e.g., *H. thelephoricola* vs. *H. sinuosa*).

Stroma: is a more or less pseudoparenchymatous fungal tissue containing usually more than one perithecium; highly conserved in *Hypocrea*, i.e., not particularly informative in many species. Stroma is used in a broad sense, i.e., including also entirely prosenchymatous ("hyphal") stromata, more correctly described as subicula that form usually widely effused mats.

Stromata, fresh/dry, immature/mature: Stromata in nature may be fresh, dry, or rewetted. They may be immature, mature or overmature. Their appearance is often strongly dependent on climate conditions. Fresh stromata are soft. Their sizes only estimates in the field. They often shrink considerably upon drying. The mature state of stromata may be indicated by green, white or yellow, floccose, granular or filiform ascospore deposits. These should not be mistaken for white or green floccules caused by conidiophores of the anamorph often present on immature, sometimes also (over-)mature stromata.

Stromata, colors: are variable and difficult to define, but are useful for preliminary identification of species. Color changes upon drying of fresh stromata can be diagnostic. Drying in nature poses difficulties, e.g., originally yellow stromata may be already reddish brown at the time of collection. In addition, the presence of only one developmental state of stromata in a specimen makes even preliminary identification difficult. Usually some uncertainty remains, particularly in species with yellow, pulvinate stromata, or reddish brown to brown, pulvinate stromata.

Stromatic tissues: are studied in vertical section. The morphology of the several parts of the stroma may be diagnostic, particularly the cortical tissue, e.g., pseudoparenchymatous in *H. aeruginea*, prosenchymatous in *H. danica* and *H. spinulosa*.

4.2.2 Cultures

Autolytic activity: is a semiquantitative estimation of usually circular colorless excretions at tips or less commonly at septa of mainly superficially growing hyphae; often they are accompanied by death of hyphal segments. In some species, autolytic excretions and dying hyphae become yellow, reddish or brown.

Coilings: are circular-oriented parts of aerial hyphae or vegetative hyphae on the agar surface. These structures are absent to abundant, commonly more abundant on PDA (sometimes on Spezieller nahrstoffarmer agar, SNA) than on other media.

Color of colony reverse: is determined on white background. Usually no or little discoloration of CMD (cornmeal agar plates supplemented with 2% (w/v) D(+)-glucose-monohydrate (CMD)), and SNA (synthetic nutrient-poor agar), are caused by growth of *Hypocrea*/*Trichoderma*, with few exceptions, e.g., green color by *H. aeruginea*. Pigments are more frequently formed on PDA; typically yellow, brown, less commonly reddish colors are distinguished. However, formation may vary among isolates and may be absent in old cultures.

4.2.3 Growth Media

The compatibility with studies is selected by Samuels et al. (2006) in below:

CMD: has provided good growth, long-term vitality, and good conidiation, but conidiation often degenerates and may be absent after several subsequent transfers.

PDA: has provided good growth, usually abundant mycelium, but conidiation is frequently effuse and ill defined, often remaining colorless or white in green-conidial species. However, this medium often provides diagnostic macroscopic growth/conidiation patterns.

SNA: often comparable with CMD, it yields cultures of low biomass, with hyphae degenerating soon. However, the main advantage of SNA over CMD is a more reliable and transfer resistant conidiation. Conidiation morphology on SNA and CMD is usually in accordance with that found in nature.

4.2.4 Growth Plates/Colonies

Growth plates/colonies: centre of the colony = area around the inoculation plug (called plug in descriptions); proximal area = area behind the plug, i.e., short growth distance to the margin of the petri dish; distal areas = opposite proximal areas, i.e., long growth distance to the margin of the petri dish; middle of the colony = centre of the plate. Lateral areas = areas close to the lateral margins of the petri dish. Margin stated without further specification denotes the distal colony margin.

Hyphae, primary: are the first hyphae leaving the inoculation plug, beginning the principal hyphal network. These are often conspicuously wider than secondary hyphae.

Hyphae, secondary: are branches originating from primary hyphae; these are usually narrower than or of similar size as primary hyphae. Relative differences in hyphal width are characteristic for many species. The value of this trait is limited by the large number of *Hypocrea*/*Trichoderma* species.

Odor of colonies: is of limited use for identification. Coconut like odor on CMD and PDA, caused by the antifungal antibiotic 6-pentyl-α-pyrone, is typical for some species of sect. *Trichoderma*. Other odors may be formed on PDA, but experience is required for their detection, because odors are difficult to quantify and most people are neither able to perceive specific odors nor link them to known odors.

4.2.5 Growth Plates/Conidiation

The following scenarios are found in *Trichoderma*:

1. Pustulate conidiation only, i.e., complex branching in tufts, pustules, shrubs, or granules
2. Pustulate conidiation preceded by effuse conidiation
   (a) Effuse conidiation on conidiophores with less complex branching than in pustules, otherwise similar to pustulate conidiation, with phialides and conidia being in the same range or phialides tending to be slightly longer and narrower than in pustules.
   (b) Effuse conidiation on simple, mostly verticillium-like, conidiophores different from pustulate conidiation in shape, size and arrangement of phialides, terminal branches and/or conidia (“synanamorph”) (Chaverri and Samuels 2003)
3. Effuse conidiation only, often starting shortly after the onset of growth (typical for *T.* sect. *Hypocreanum*).

4.2.6 Conidial Heads, Wet Versus Dry

Conidial heads, wet vs. dry: wet heads occur in many species, often they can be only seen on plates with the lid attached by parafilm, and removal of the lid may result in immediate drying of heads.

4.2.7 Conidiophores/Trees, Regular

Conidiophores/trees, regular: means a tree-like or more or less pyramidal shape (such as *Picea abies*), attenuated upwards, branches mostly paired and increasing in length from the top down, branches substituted by phialides at and near the top. The term trees is mostly used for side branches or ends of conidiophores having this shape as is common in *Trichoderma*. In pustules, conidiophores or trees are referred to the terminal branching system on the periphery of the pustule.

4.2.8 Conidiophore Terminology (Source: Jaklitsch 2009)

The following standard characters were noted and measured: type of anamorph (*Acremonium*- see Fig. 4.2, *verticillium-, gliocladium-, pachybasium-, or trichoderma* like; see Fig. 4.3) previously described by Jaklitsch (2009)

1. ***Acremonium-like*:** is the least complex, short, effuse type of conidiation in *Trichoderma*. The conidiophores consist of a stipe of one to a few cells, with one or few phialides originating directly from this stipe on a single level. This conidiation structure is typical for sect. *Hypocreanum*, where conidiation is initially acremonium-like but usually develops into a verticillium-like structure bearing long phialides.

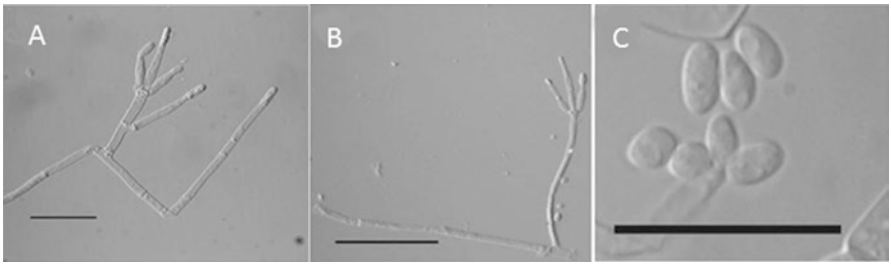

Fig. 4.2 Selected examples are showed the conidiosphore (**a**, **b**) and conidia (**c**) of *Trichoderma* species which is *Acremonium*-like

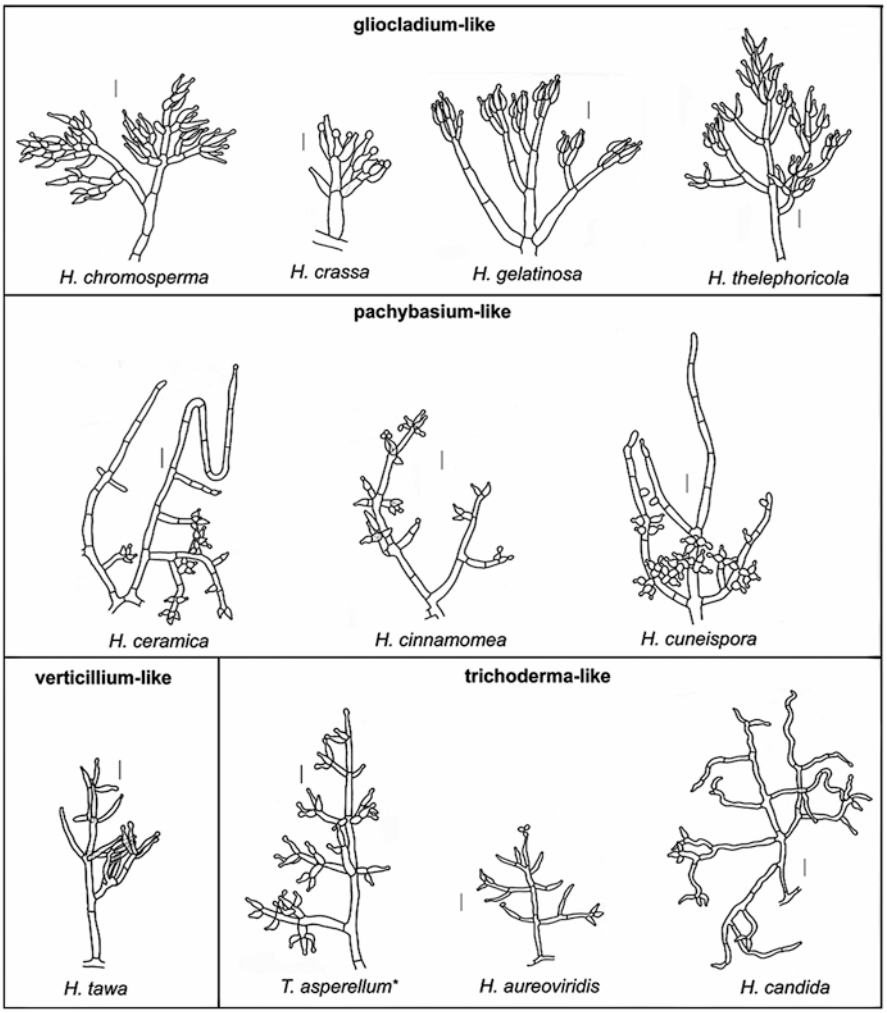

Fig. 4.3 Selected examples are showed the five pattern types of conidiosphore of *Trichoderma/Hypocrea* species which are *Acremonium*-like, *Gliocladium*-like, *Pachybasium*-like, *Verticillium*-like and *Trichoderma*-like pattern (Chaverri and Samuels 2003)

2. ***Verticillium-like*:** unbranched or slight branched erect conidiophores with verticils or whorls of distinctly divergent phialides on several levels; phialides within a whorl originate on the same level around the main axis. Contrary to true *Verticillium*, the term is used for conidiophores with secondary branches having similar arrangements.
3. ***Gliocladium-like*:** unbranched or more or less intensely branched conidiophores with penicilli of densely packed and appressed, more or less parallel, phialides at the top.

4. ***Pachybasium-like*:** conidiophores typically densely branched and stout, with small ampulliform or lageniform phialides. Part of the conidiophores are often ending in simple or branched, sterile or fertile elongations.
5. ***Trichoderma-like*:** is difficult to circumscribe, due to the lack of a strict borderline to *verticillium*-like. It comprises narrow, flexible and richly branched conidiophores that may be irregular, with distinct curvatures or terminally pyramidal with branches often at right angles. The phialides are typically lageniform, in irregular clusters, often bent, sometimes repetitive. Frequently, *verticillium*-like structures are also present on *trichoderma*-like conidiophores.

4.2.9 Effuse Conidiation (Synanamorphs)

Effuse conidiation (synanamorphs): conidiation without formation of distinct, macroscopically visible units. They are usually made up of more or less evenly distributed simple erect conidiophores, macroscopically only visible if densely arranged and forming green conidia. Effuse conidiation may also occur in macroscopically visible, downy or hairy areas on long aerial hyphae. Conidia of effuse conidiation are typically formed in wet heads.

4.2.10 Pustules

Pustules: are in their extreme form distinct dense opaque conidiation structures, often pulvinate with circular outline, originating on a single stipe, a thick aerial hypha from which primary branches emerge. Pustules may grade into transparent, loose structures, i.e., tufts.

4.2.11 Shrubs, Granules

Shrubs, granules: are small conidiation units (usually larger single conidiophores) spread on the agar like sand (granules), often scarcely visible macroscopically; 0.2–0.5 mm diam. They may constitute the final stage of conidiation or develop between effuse and pustulate conidiation.

4.2.12 Tufts, Also Called "Fluffy Tufts"

Tufts, also called "fluffy tufts": are conidiation structures appearing macroscopically as loose, cottony, often confluent masses or aggregations of conidiophores with variable outlines, usually not circular. In some species pustules develop from tufts.

4.3 Macroscopic Analysis

Several groups of *Trichoderma* colonies are analysed by morphological characteristics for identification of *Trichoderma* isolates. This procedure is allowed large number of samples making in a small timeframe and cost effective. Slants culture of *Trichoderma* isolates are aseptically recultured onto a new PDA agar in petri dish and incubated for 4–5 days at room temperature condition of 22 ± 2 °C (12 h dark and 12 h light). *Trichoderma* isolates are formed several groups using the following macroscopic criteria: growth rate, mycelium density and appearance, conidiation color, pattern and coloration of the medium. Some *Trichoderma* isolates have ability to produce sweet coconut odor.

4.3.1 Trichoderma asperellum

The macroscopic and microscopic observations of *T. asperellum* are shown in Figs. 4.4 and 4.5.

Macroscopic characteristics

- Colonies of *Trichoderma* isolates are grown on PDA for 5 days at 28 ± 2 °C in darkness with brief exposure to ambient fluorescent light (8 h) forming one to two concentric rings near the inoculum zone with a dense conidial production, with white conidia toward the green centre (Fig. 4.4(a)).
- For the colony reverse, it is creamy in color and often folded or convoluted.
- No color diffusion or pigment is observed throughout the PDA plate (Fig. 4.4(b)).
- A lack of aerial mycelium.
- Absence sweet coconut odor.

Microscopic characteristics

- The conidiosphores are highly branches and arrangement in symmetric order.
- The conidiosphores primary branches arising below the tip often paired with same length and projecting at nearly 90 degree to the main axis.
- The phialides typically produced at the tips of the primary, secondary and tertiary branches, rare straight along the length of the branches, typically in whorls of 2–4 phialides, straight, ampuliform, slightly enlarged in the middle with 8 μm long in average.
- The phialides slightly enlarged in the middle with 1.0–2.0 m width.
- Intercalary phialide has not found in these isolates.
- In addition, the dark green conidia which are globose to subglobose or ovoidal in the size of 3–4 μm observed.
- Within 1 week, the chlamydophores are formed either terminal or frequently intercalary, on immersed hyphae, subglobose to ovoidal, smooth and pale green.

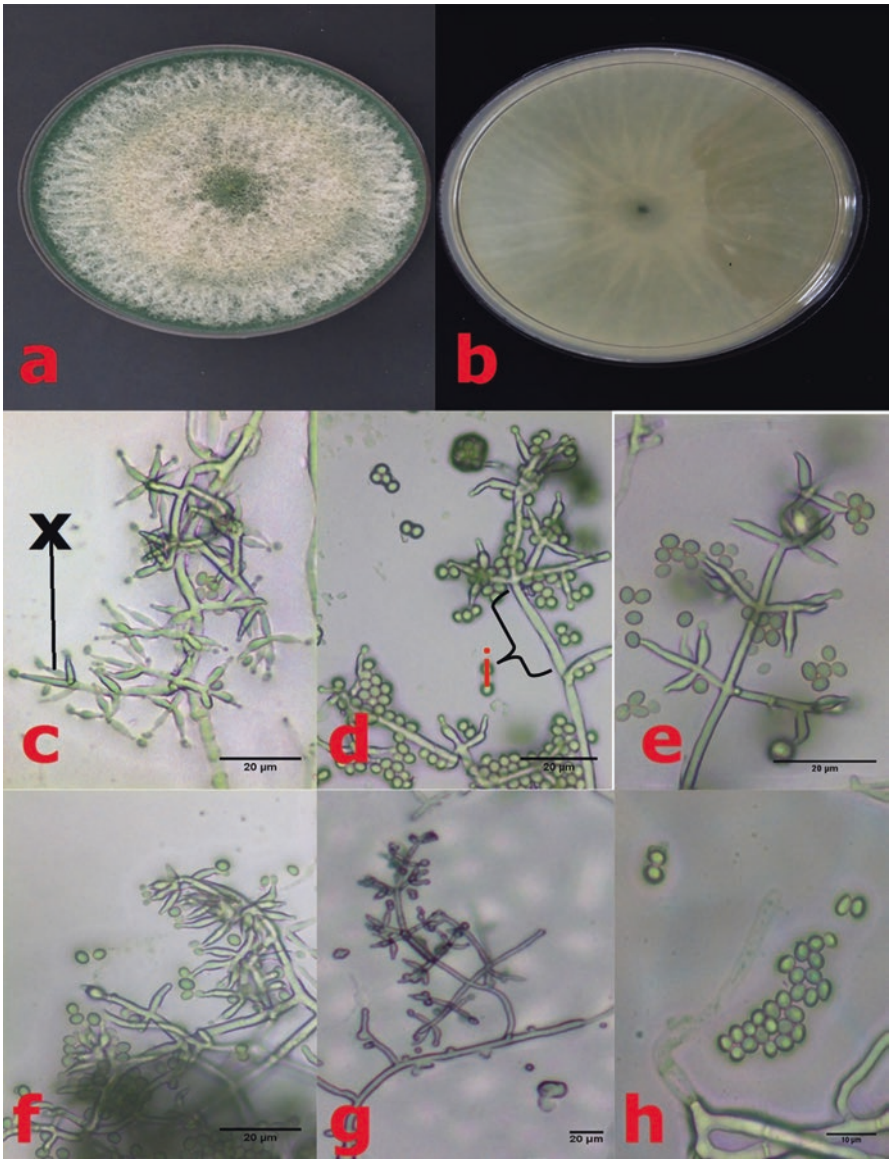

Fig. 4.4 A representative morphological characteristics of *Trichoderma asperellum*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–g**). Conidiosphores; (**h**) Conidia; X. Phialides. **c–f** and **h** were observed with 400× magnification while **g** was observed with 100× magnification

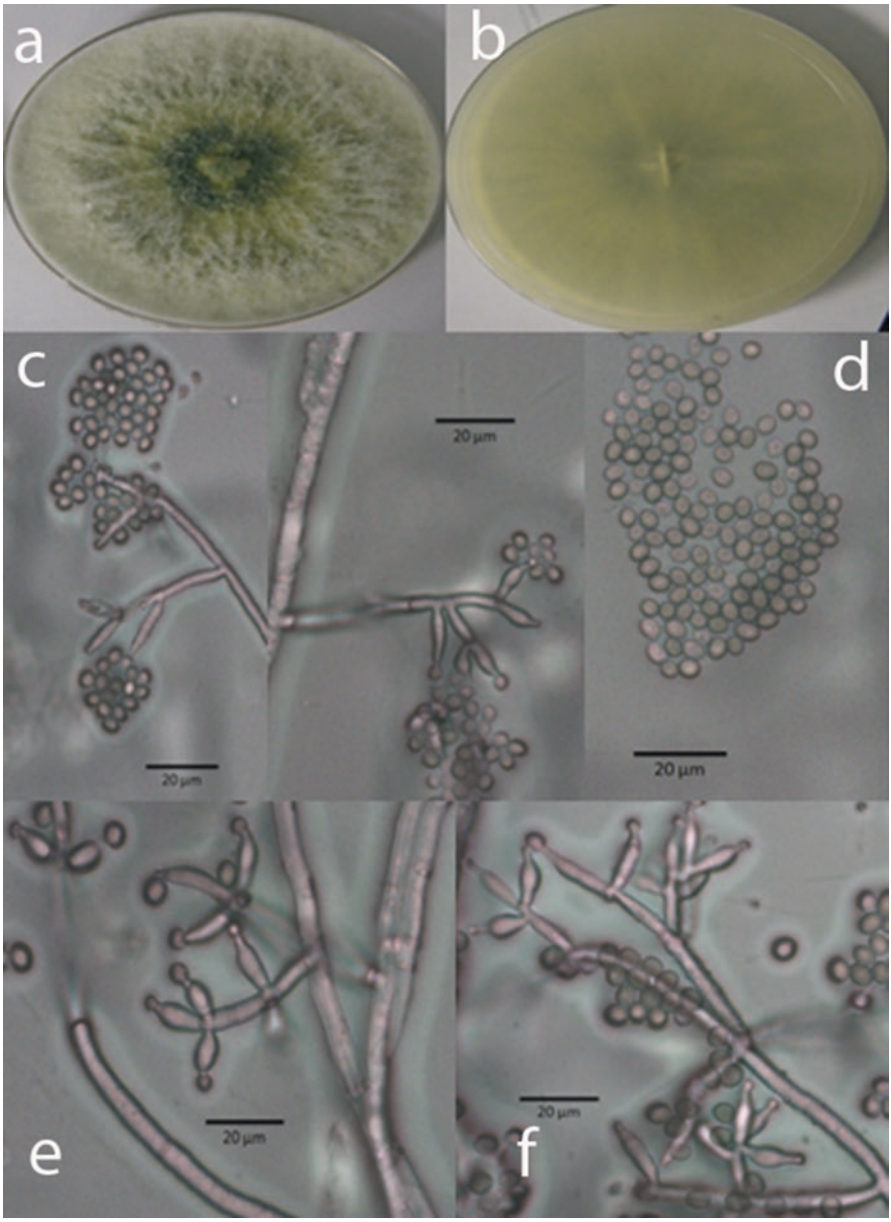

Fig. 4.5 A representative morphological characteristics of *Trichoderma asperellum*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c**) Phialides with condia; (**d**) Conidia; and (**e**, **f**) Conidiosphores were observed with 400× magnification

4.3.2 *Trichoderma harzianum*

The macroscopic and microscopic characteristics of *T. harzianum* are shown in Figs. 4.6 and 4.7.

Macroscopic characteristics

- Colonies of *T. harzianum* isolates usually formed 1–2 concentric rings with green conidial production in mature colonies (Figs. 4.6 (a) and 4.7 (a)) during incubation for 5 days at 28 ± 2 °C.
- It has observed to cause intense coloration of the agar from yellow to dark brown pigments in mature colonies (Fig. 4.6(b)).
- The mycelium is initially smooth, watery white in color and sparse, until floccose aerial mycelium has produced.
- Conidiation has predominantly effused with pustules typically merged into large irregular masses with powdery surface appearance.
- Indistinct sweet coconut odor produced.

Microscopic characteristics

- The conidiosphores are spread to the top in pyramidal fashion and highly branches (Fig. 4.6(c–g)). The main branches mostly in groups of three or four are obtained.
- Branches of the conidiosphores are typically paired.
- Phialides are flask shaped and typically short and broad in the middle with the average length of 4–6 μm.
- The phialides are observed often terminates in a narrow neck and arising mostly in crowded and have whorls of 3–5; the whorls are typically flask-shaped and enlarged in middle.
- The length of phialides has tended to be shorter than other *Trichoderma* species with the average length of 6.0–10.00 μm.
- The conidia [Figs. 4.6 and 4.7(b)] tend to be globose to subglobose in shape with the average length of 2–3 μm and pale green in color.
- Pustules are not formed or poorly formed.

4.3.3 *Trichoderma hamatum*

The macroscopic and microscopic observations of *T. hamatum* are shown in Fig. 4.8.

Macroscopic characteristics

- The colonies grow moderately rapid, close to the agar, and entire the whole petri dishes within 3 days during incubation period at 28 ± 2 °C.
- It produced some aerial mycelium which is white in color with some yellow conidiation in the centre of the plate and grown densely (Fig. 4.8(a)).
- Isolates never cause discoloration of the agar (Fig. 4.8(b)) and odorless.

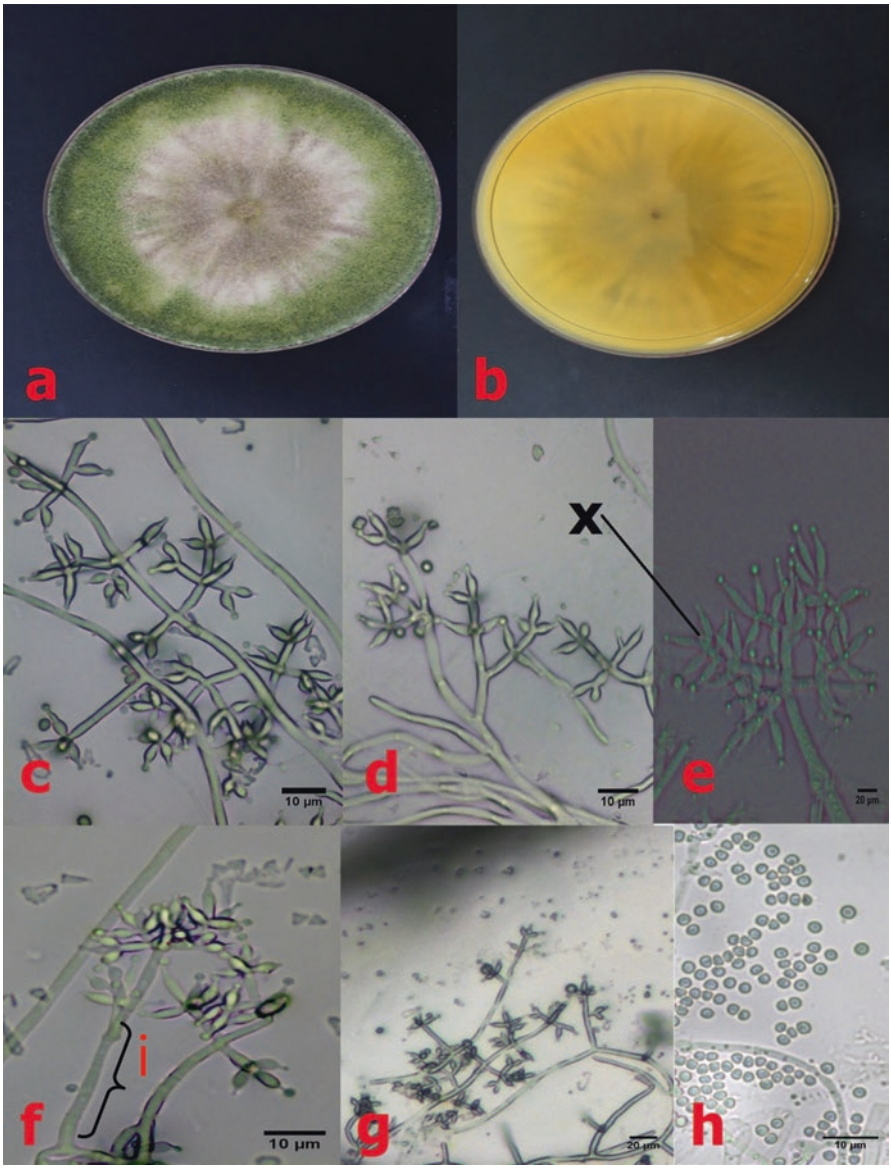

Fig. 4.6 A representative morphological characteristics of *Trichoderma harzianum.* (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; **c–g** and X. Phialides; **h** Conidia and **c–g** and i Conidiosphores were observed with 400× magnification while **g** and **h** was observed with 100× magnification

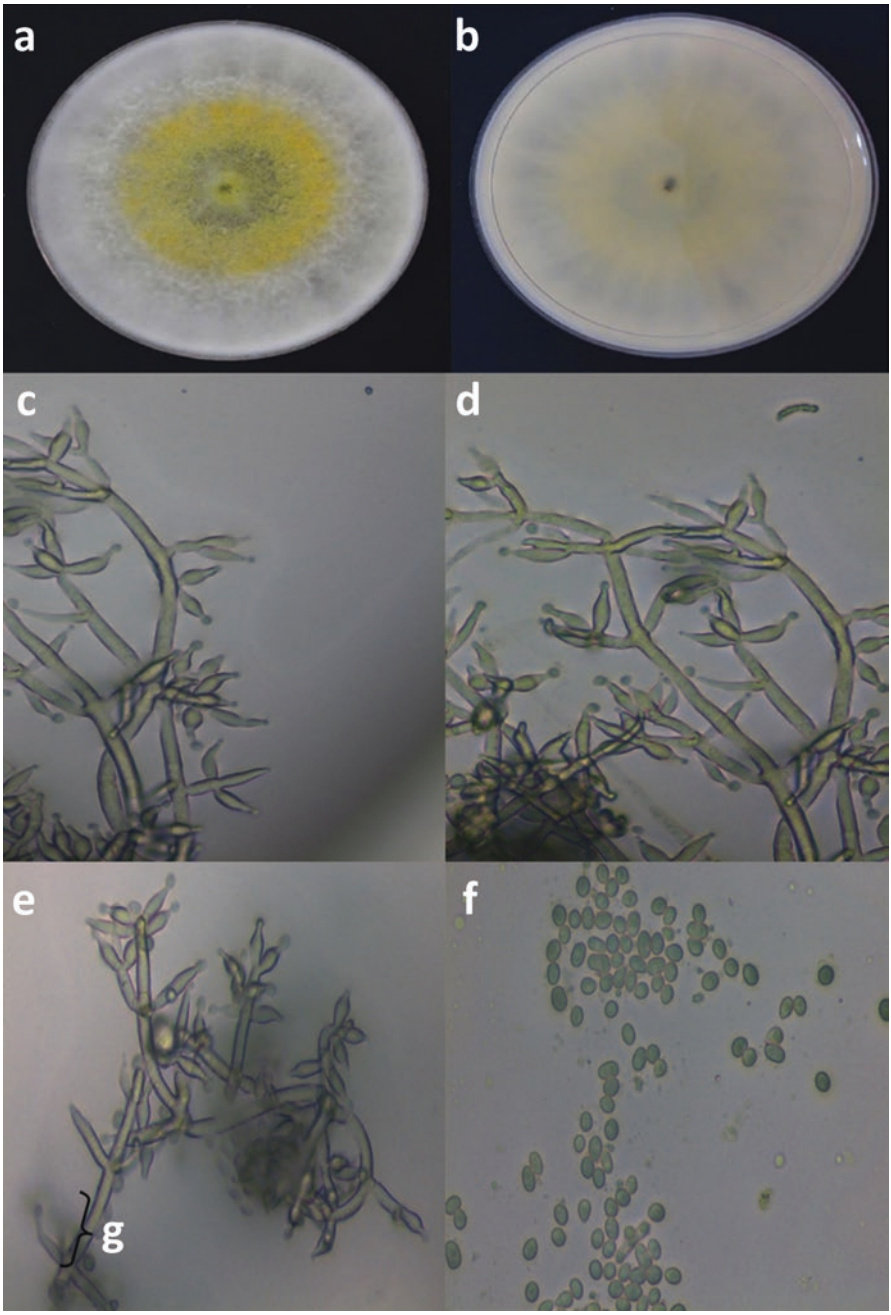

Fig. 4.7 A representative morphological characteristics of *T. harzianum.* (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–e**) Phialides; (**g**) Conidiosphores; and (f) Conidia were observed with 400× magnification while **f** was observed with 100× magnification

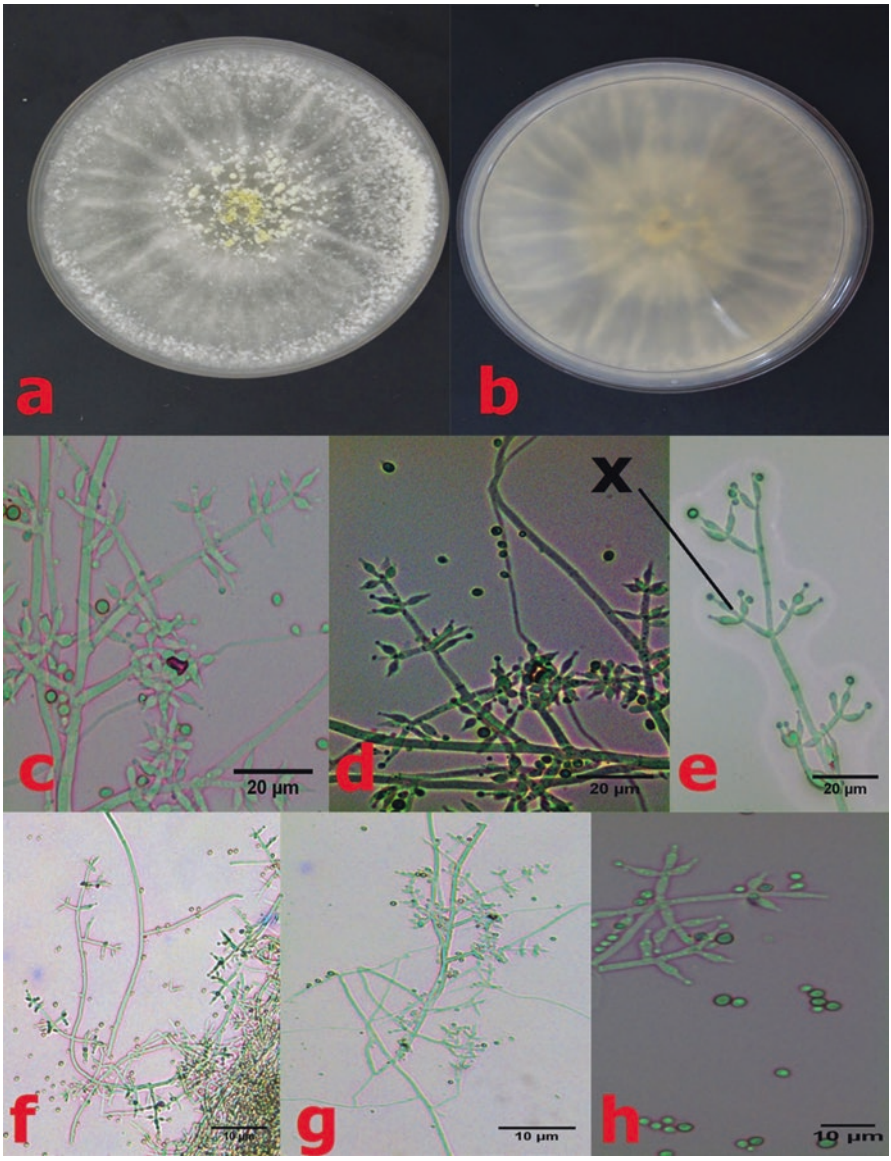

Fig. 4.8 A representative morphological characteristics of *Trichoderma hamatum*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–g**) Conidiosphores; (**h**) Conidia; X. Phialides. **c–e** and **h** were observed with 400× magnification while **f** and **g** were observed with 100× magnification

- The colonies produced dispersed cushion shaped, compact tufts of conidiosphores with some color variation in these isolates ranging from pale yellow, greenish yellow to greyish green.
- The conidia tend to form in concentric rings.

Microscopic characteristics

- The conidiosphores tend to aggregate in fascicles or pustules and highly branches in irregular pattern (Fig. 4.8(c–g)).
- The lateral branches usually comprised one or few broad cells with phialides arising at the tip and along the length. While the secondary branches may arise as single cells which phialides.
- The phialides are formed from short lateral branches at the base of the elongation, mostly ellipsoidal to ovoidal with an average length of 5–7 μm long, 3–4 μm wide at the widest point.
- Phialides arising in 1–3 whorls and densely clustered on board.
- Intercalary phialides are absent in these isolates.
- The conidia have a smooth walled, ellipsoidal, and green in color.
- The average length of the conidia obtained from 4 to 5 μm (Fig. 4.8(h)).
- Absence of chlamydospores.

4.3.4 Trichoderma reesei

The macroscopic and microscopic observations of *T. reesei* are shown in Fig. 4.9.

Macroscopic characteristics

- The colonies grow rapidly on PDA in room temperatures condition of 28 ± 2°C and produce an intense diffusing yellow pigment and fewer yellowish green conidia as they tend to form on the centre of the plate (Fig. 4.9(a, b)).
- One or two concentric rings may appear (usually in old culture), one near the margin and the other around the inoculum point.
- Apart from, cottony aerial mycelium is not forming.
- No distinctive odor has noticed from the isolates.

Microscopic characteristics

- The conidiosphores are sparingly branches (Fig. 4.9(c–g)). The primary branch is long while the secondary branch usually short and paired branching systems are rare.
- Each branch terminating in one or two phialides which are arising singly from the main axis and intercalary phialides are commonly observed in Fig. 4.9(f).
- Phialides are cylindrical or slightly inflated with an average length of 5–8 μm.
- The conidia are smooth walled, pale green in color and oblong or ellipsoidal in shape with an average length of 3–5 μm (Fig. 4.9(h)).

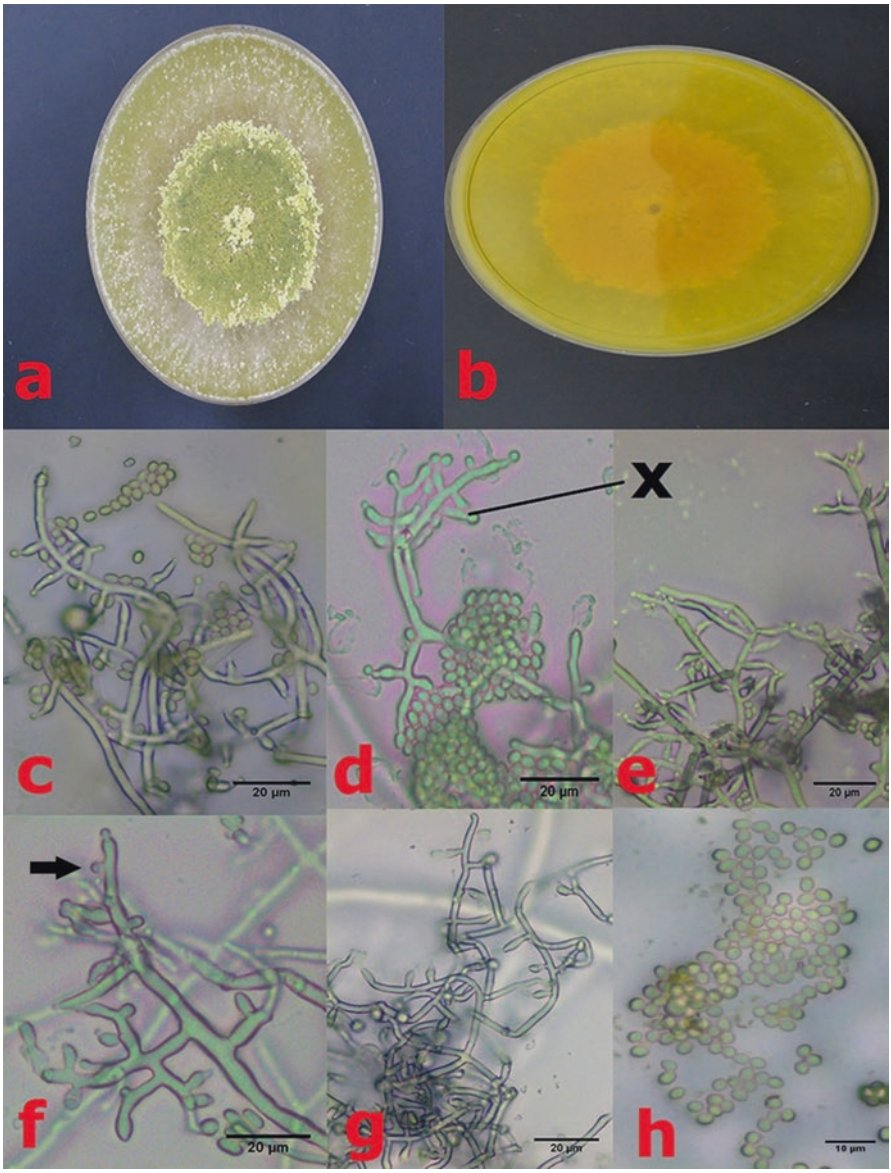

Fig. 4.9 A representative morphological characteristics of *T. reesei*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–g**) Conidiosphores; (**h**) Conidia; *Arrow*: Intercalary phialides; X: Phialide. **c–h** were observed with 400× magnification

4.3.5 *Trichoderma longibrachiatum*

The macroscopic and microscopic characteristics of *T. longibrachiatum* are shown in Fig. 4.10.

Macroscopic characteristics

- The colony is initially smooth, glabrous, translucent and watery white but often to form greenish yellow conidial crusts with dense conidiation (Fig. 4.10(a)) when grown onto the PDA for 5 days at 28 ± 2 °C with a good interval of light and darkness.
- The reverse colony can observe to be pale greenish-yellow in young culture (Fig. 4.10(b)) to brownish chocolates in mature culture.
- No distinct odor appearance.

Microscopic characteristics

- The conidiosphores are typically long and the phialides are bottle shape, mostly solitary and often inflate in the middle.
- The phialides often observe to bent at the apex, and slightly constrict at the base with an average length around 6–9 μm (Fig. 4.10(c–g)).
- They arise singly from the main axis and intercalary phialides commonly produced as shown in Fig. 4.10(c).
- Conidia are smooth walled with ellipsoidal to cylindrical in shape with an average length of 3–6 μm.
- The color of the conidia has observed to be pale green (Fig. 4.10(h)). Sometime a smooth, thick-walled and subglobose to ellipsoidal.
- Chlamydospores can be observed in mature colony.

4.3.6 *Trichoderma spirale*

The morphological characteristics of *T. spirale* are shown in Fig. 4.11.

Macroscopic characteristics

- The colonies grow well onto PDA at 28 ± 2 °C with a less distinctive concentric rings observed. The conidiation of the conidia appear to be compact, cottony, cushion-shaped pustules and the mycelia appear to be white with greenish yellow tint in color (Fig. 4.11(a)).
- A yellow pigment tends to diffuse throughout the agar.
- Reverse colony is colorless; however most of the isolates are developing a dull-yellowish color (Fig. 4.11(b)).
- No distinctive odor produced.

Microscopic characteristics

- The conidiosphores observed to be long and less branches.
- The branches are relatively short and arising singly, alternately or pair from the main axis (Fig. 4.11(c–g)).

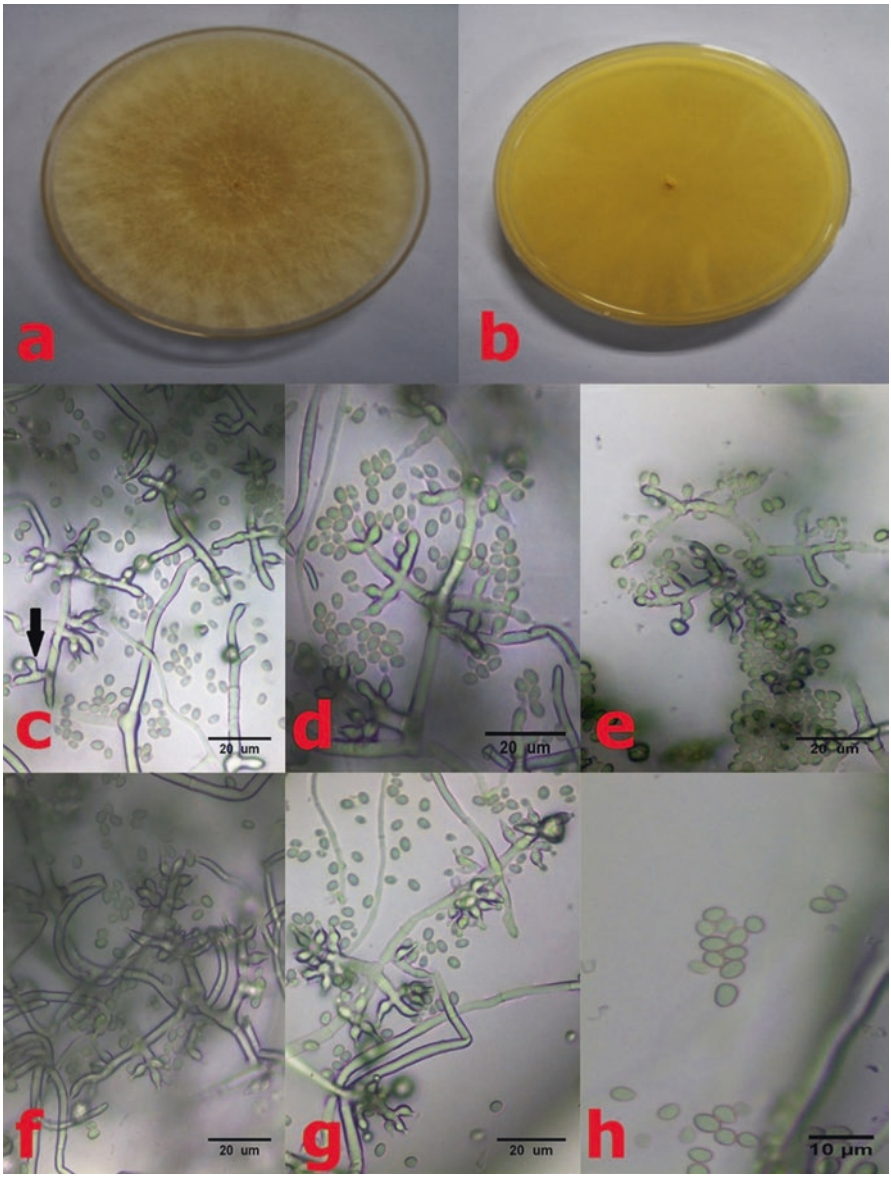

Fig. 4.10 Morphological characteristics of *T. longibrachiatum*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–g**) Conidiosphores; (**h**) Conidia; *Arrow*: Intercalary phialides. **c–h** were observed with 400× magnification

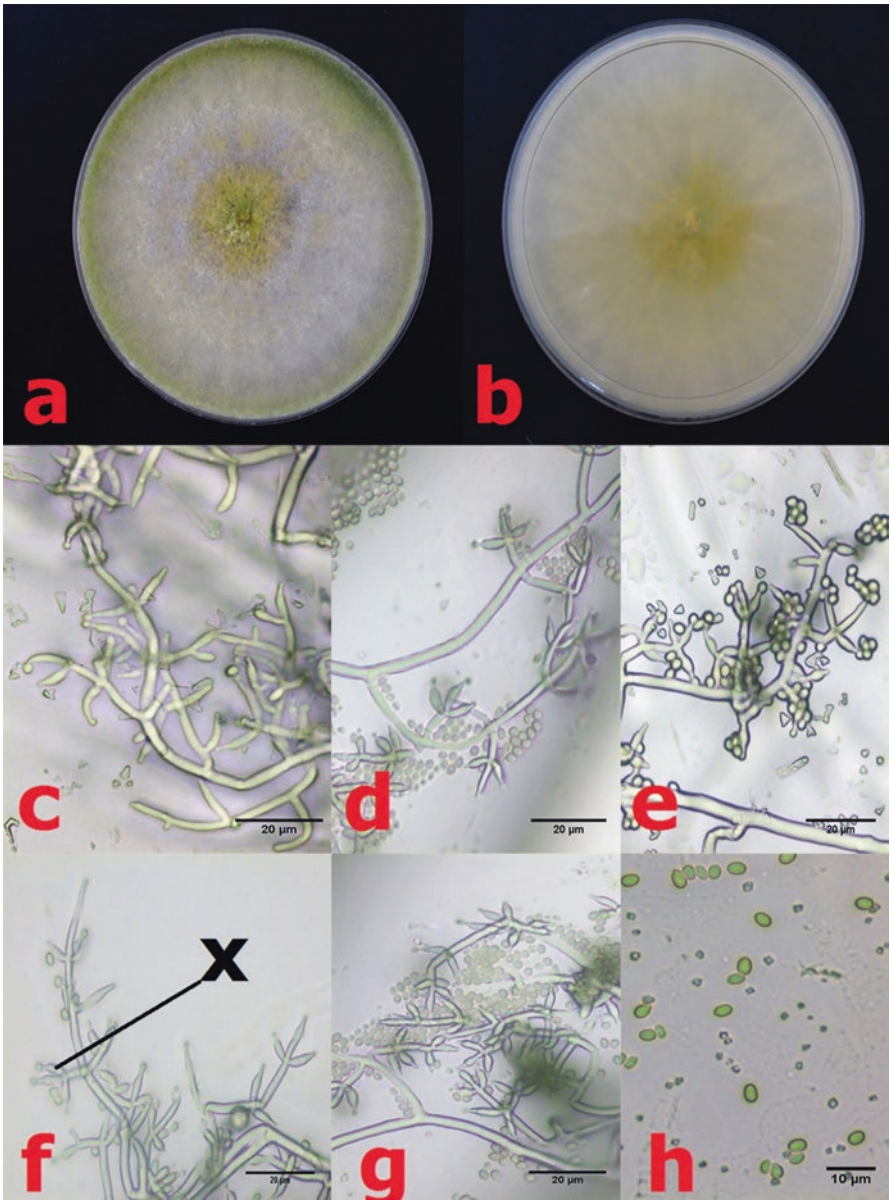

Fig. 4.11 A representative morphological characteristics of *T. spirale*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–g**) Conidiosphores; (**h**) Conidia; X: phialides. **c–h** were observed with 400× magnification

- The primary branches are terminated with fertile elongation (Fig. 4.11(f)).
- Phialides appear to be ampulliform to nearly subglobose which an average length of 4–6 µm. Most of the phialides arising in dense cluster comprising 1–3 whorls.
- Absence of intercalary phialide.
- The conidia observe to be green in color, smooth walled, and oblong to ovoidal with an average length of 3–5 µm.

4.3.7 *Trichoderma brevicompactum*

The morphological characteristics of *T. brevicompactum* are shown in Fig. 4.12.

Macroscopic characteristics

- The colony grows moderately slow at 28 ± 2 °C, getting upto 7 cm in diameter during 72 h of incubation period in PDA plate (Difco, USA) as compared to other isolates.
- After 5 days of incubation under intermittent light, the colony can form conspicuous 1–2 concentric rings with dense yellow-green conidia over the internal concentric ring (Fig. 4.12(a)).
- There is no diffuse pigment occur throughout the agar (Fig. 4.12(b))
- Absence of distinctive odor.

Microscopic characteristics

- The conidia can observe to hyaline and smooth-walled under 400× magnification.
- The branching pattern of the conidiosphores resembles the branching pattern of the *Pachybasium*-type (Fig. 4.12(c–f)).
- Each branches of the conidiosphores terminating in a vertical of 1-3 phialides.
- Phialides appear to be marginally enflamed in the middle and lageniform when arising from crowded branches.
- Absence of intercalary phialide.
- The conidia are smooth, subglobose or short ellipsoidal and yellowish green on color with an average length of 2-3 µm (Fig. 4.12(h)).

4.3.8 *Trichoderma erinaceum*

Trichoderma erinaceum which belongs to the section of *Viride* based on the morphological characteristics are shown in Fig. 4.13.

Macroscopic characteristics

- Conidia can form in dense, flat lawns in concentric rings with some tendency to form flat or infrequently develop pustules at 25–30 °C.
- Diffusing pigments and distinctive odor are absence.

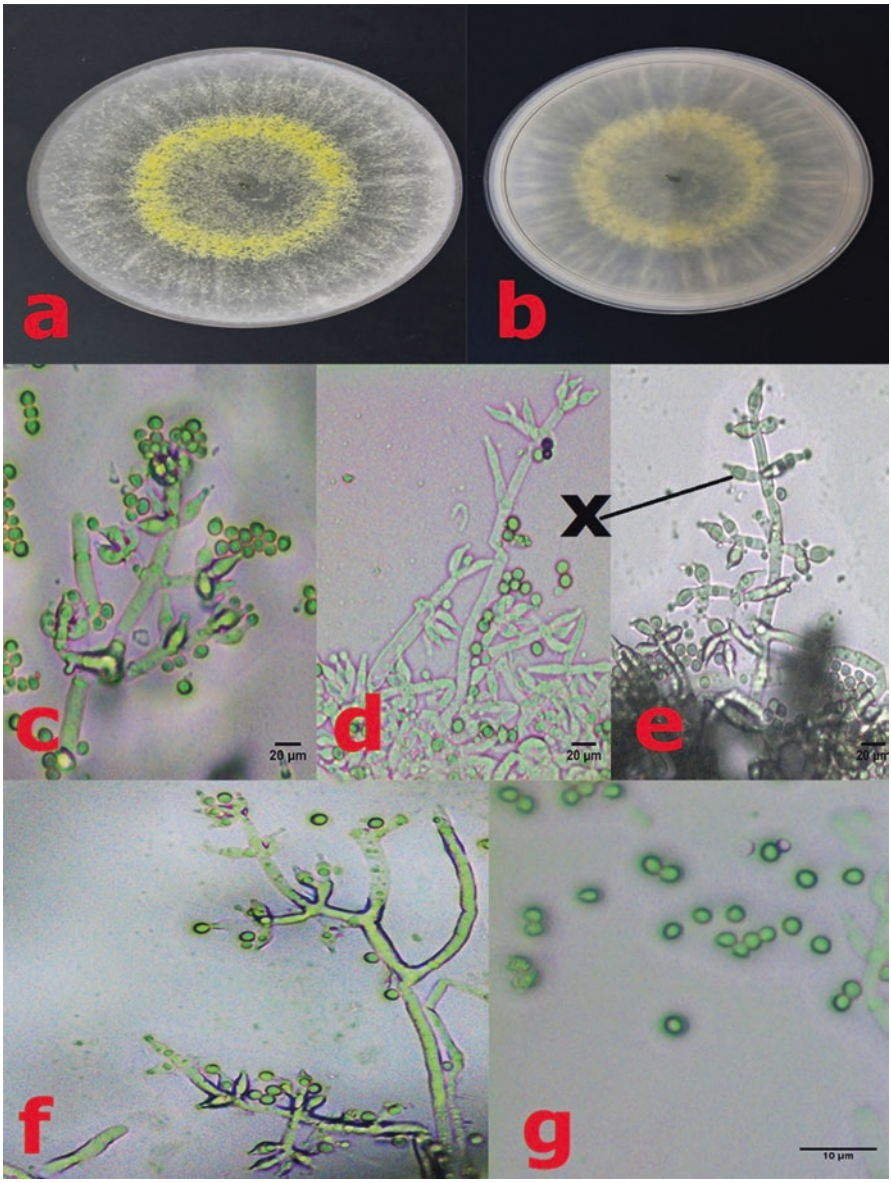

Fig. 4.12 Morphological characteristics of *T. brevicompactum*. (**a**) Front colony which was grown in PDA for 5 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; (**g**) Conidia; X: phialides. **c–g** were observed with 400× magnification

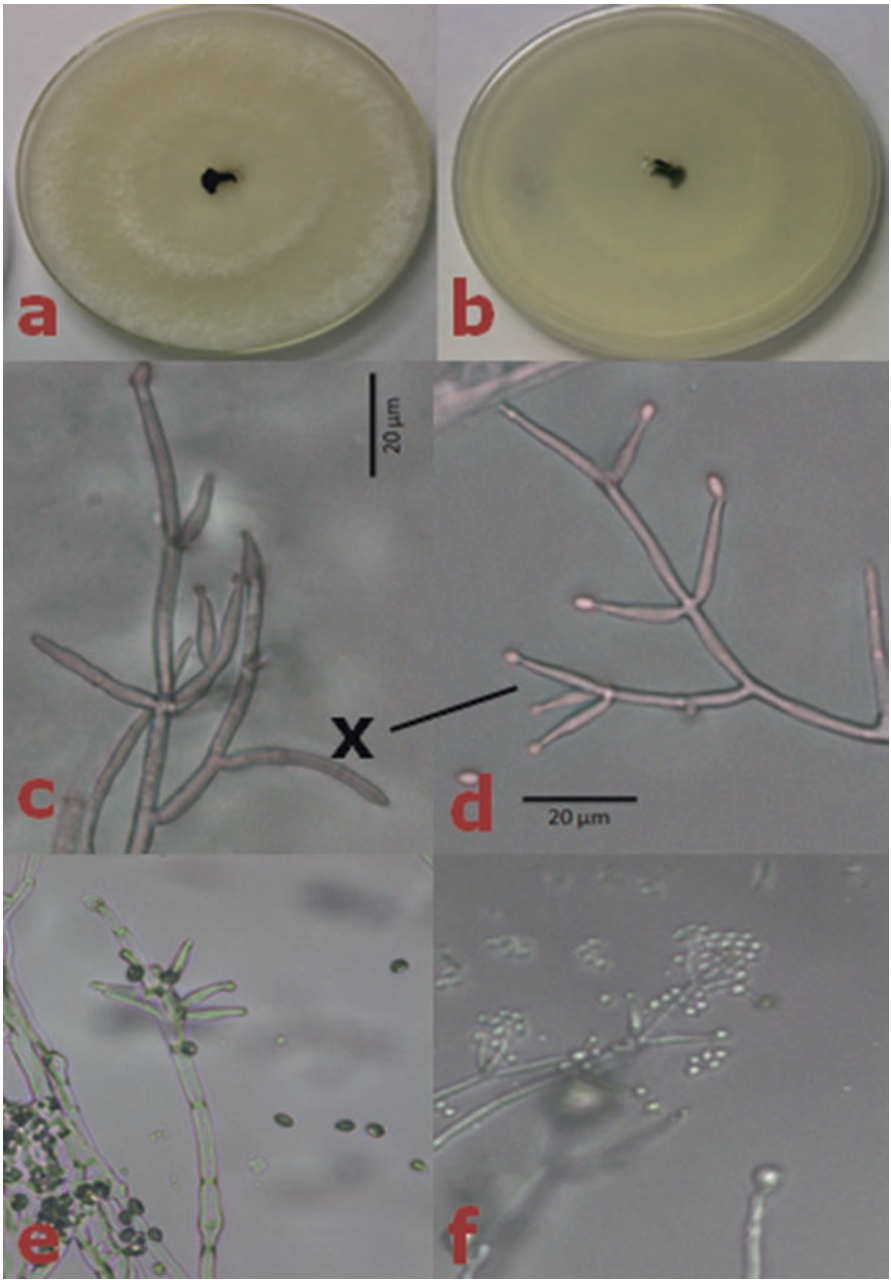

Fig. 4.13 A representative morphological characteristics of *T. erinaceum*. (**a**) Front colony which has grown in PDA for 4 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; X. Phialides. **c–f** were observed with 400× magnification.

Microscopic characteristics

- The conidiosphores branches arising at angles of 90° or less with the main axis.
- The main axis terminates in septate elongation with single phialide at its tip.
- The phialide arising from the branches near the base solitary or in whorls of 2 or 3, straight, nearly cylindrical to swollen in the middle as can be seen in Fig. 4.13. Conidiosphores branches are arising at the angle of 90° directly or not to the conidiosphores with the average of 2.2–3.0 μm wide.
- Chlamydospores are terminally intercalary, globose to subglobose.

4.3.9 Trichoderma koningiopis

The macroscopic and microscopic characteristics of *T. koningiopis* are observed in Figs. 4.14 and 4.15.

Macroscopic characteristics

The representative's morphological characteristics of *T. koningiopsis* are shown in Figs. 4.14 and 4.15.

- The conidia start to grow after 2 days incubation at 28 ± 2 °C.
- It has dense lawn or the centre remaining sterile, forming 2–3 concentric rings, abundant in light-grown colonies, and less abundant.
- No discoloration seen on the agar.
- Distinctive odor not detected.

Microscopic characteristics

- The conidiosphores abundant branches arising along the length of the main axis, pair with longer or shorter internodes between branches with *pachybasium*-like pustules, short and crowded phialides.
- Branches arise at the angle less than 90°.
- Phialides are straight, hooked or sinuous, narrowly lageniform and sometime obviously swollen in the middle.
- Several phialides are arising from the same appoint and crowded.
- The conidia are deep to dark green, seldom with yellow coloration, ellipsoidal, lacking visible basal abscission scar and smooth.
- Chlamydospores are fertile to sparse, terminal to intercalary, globose to subglobose with the range of 9.0–9.5 μm.

4.3.10 Trichoderma melanomagna

Trichoderma melanomagna which belongs to the section of *Lutea* based on the morphological characteristics as shown in Fig. 4.16.

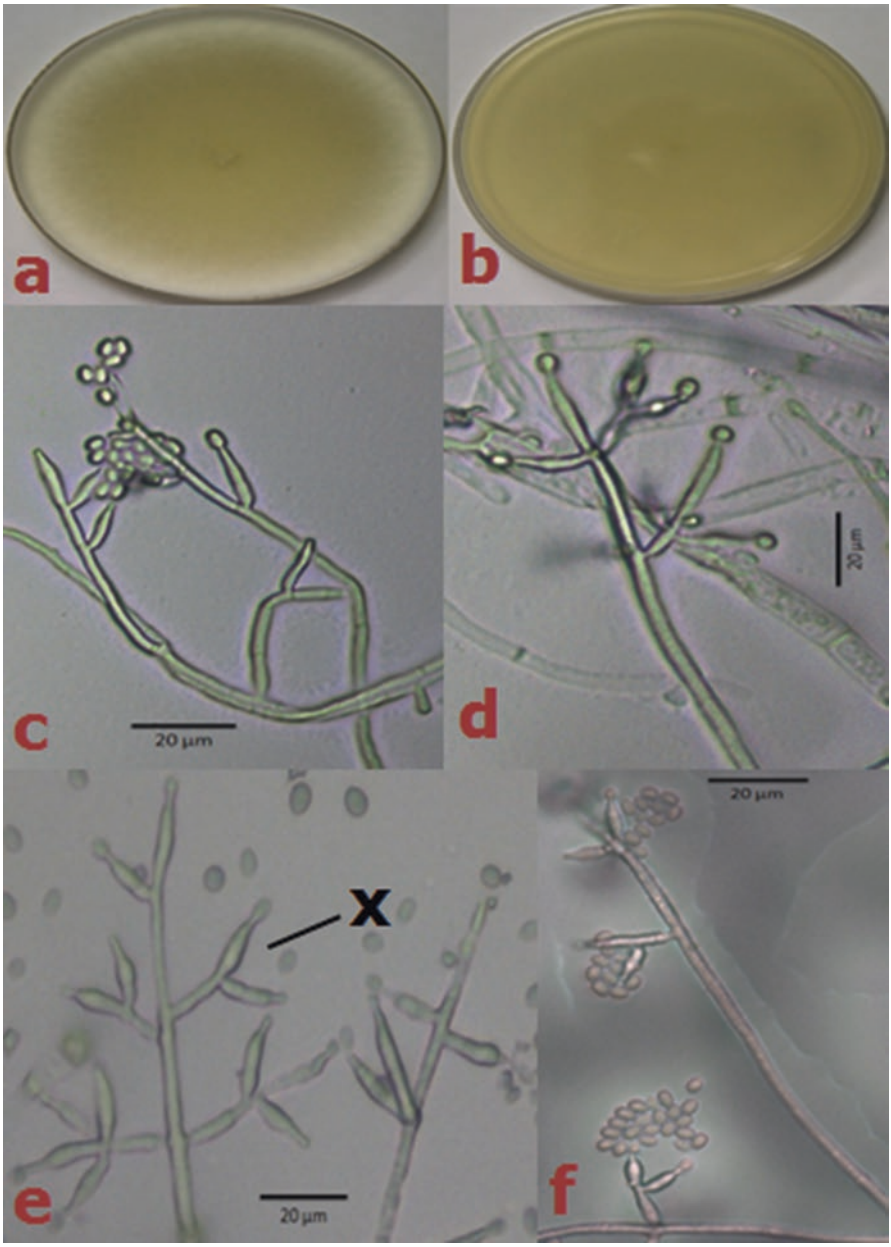

Fig. 4.14 A representative morphological characteristics of *T. koningiopsis*. (**a**) Front colony which has grown in PDA for 4 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; X. Phialides. **c–f** were observed with 400× magnification

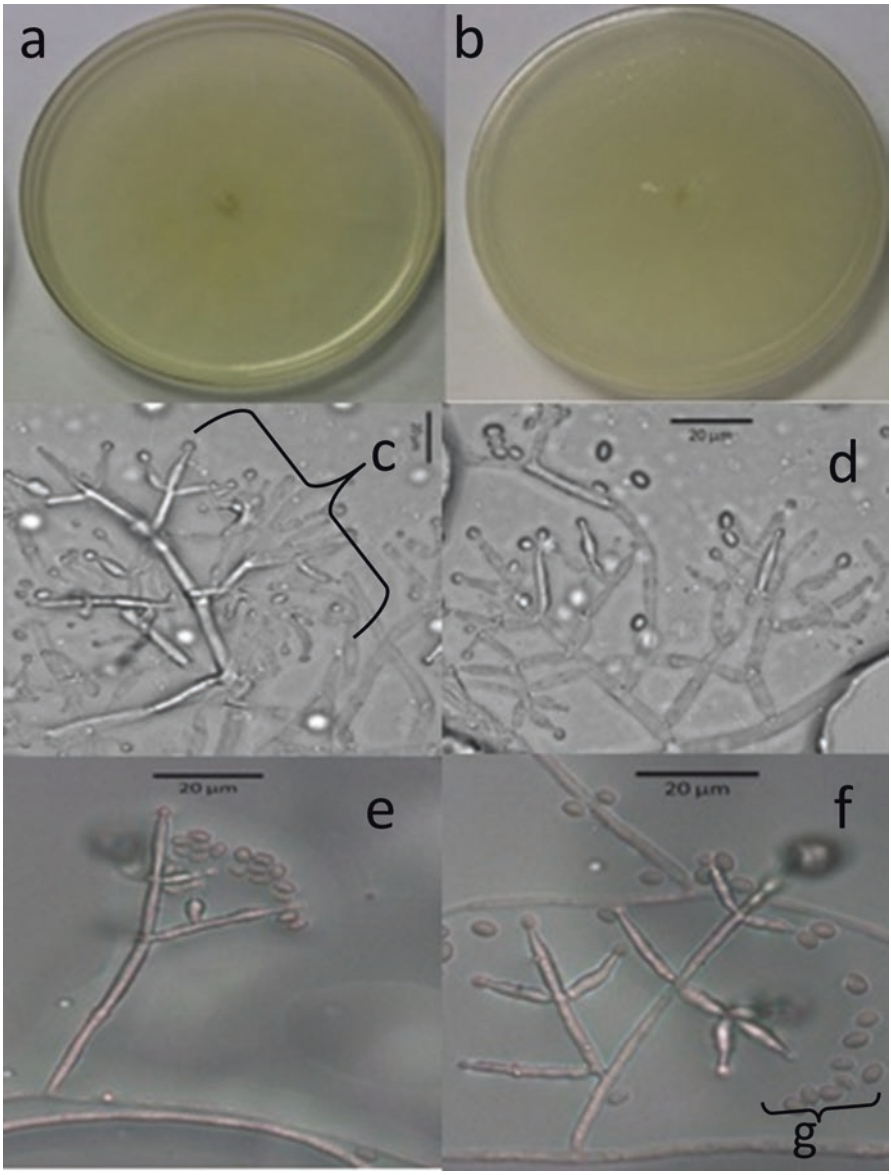

Fig. 4.15 A representative morphological characteristics of *T. koningiopsis*. (**a**) Front colony which has grown in PDA for 4 days; (**b**) Reverse colony; (**d–f**) Conidiosphores; (**c**) Phialides and (**g**) Conidia (observed with 400× magnification)

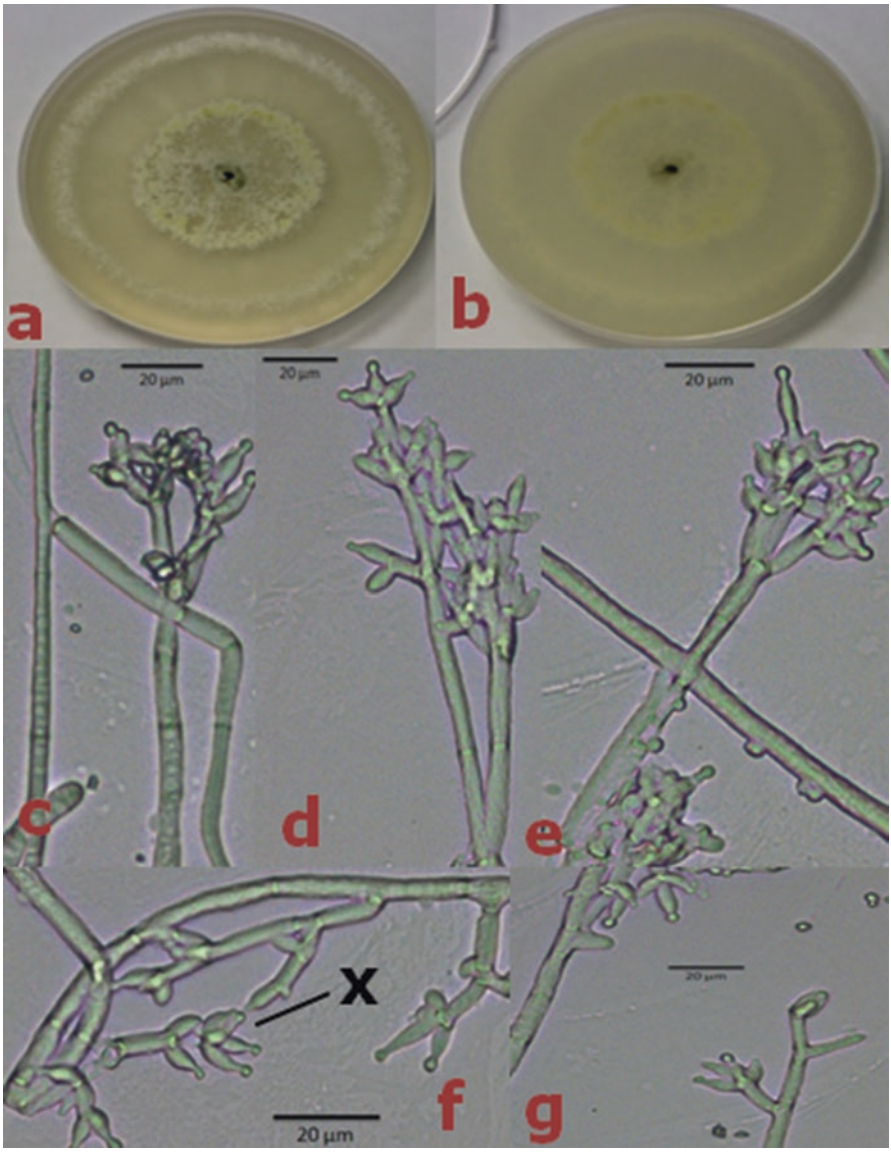

Fig. 4.16 A representative morphological characteristics of *T. melanomagna* (**a**) Front colony which has grown in PDA for 4 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; X. Phialides. **c–f** were observed with 400× magnification

Macroscopic characteristics

- Colonies grow onto PDA rapidly at 25 °C with radius of 35 mm after 3 days.
- No distinctive odor detected.
- The production of diffusing pigment was not commonly observed.
- However, some pigments appeared, it often inconsistently formed within individual.

Microscopic characteristics

- The conidiosphores have *pachybasium*-like morphology with irregular branching pattern.
- The main axis terminates in a sterile or terminally fertile extension.
- The branches tend to be paired or in whorls, however, unilateral branching commonly found. Branches often not uniformly spaced and "pyramidal" shape in the central to the traditional form concept of *Trichoderma* has not apparent.
- Phialides are longer and slender ranging between 0.8–1.5 × 3–4 μm, in whorls with 3–4 of each phialides axis at angle of 90° to its nearest neighbour.
- Stromatal size ranges from 0.5 to 15.0 mm diameter with shades in white, mostly yellowish, orange, brown, green or black in color.
- Most stromata are pulvinate with a broad or narrow base, peltate or almost cylindrical.

4.3.11 Trichoderma viride

The morphological characteristics of *T. viride* are shown in Fig. 4.17.

Macroscopic characteristics

- The mycelium and conidia formed concentric rings (Fig. 4.17).
- The colony grown rapidly at 25 °C.
- No discoloration found.
- Occasionally the coconut odor found or sometime no distinctive odor detected.

Microscopic characteristics

- The conidiosphores typically comprised abundant central axis or the central axis 100–150 μm long and flexuous.
- The branches can pair or some not, arising at the angle at or near 90° with respect to its supporting branch.
- Sometime lateral branch can widely spaced intervals.
- Phialides are singly arising from the main axis or in whorls at 2–3 at the tip of the lateral braches or at the tip of the conidiosphores.
- It can cylindrical and sometime swollen in the middle with elongated neck, straight, sinuous. The central axis is the average of 2.2–3.2 μm wide.
- The pustules diameter of 0.5–1.0 mm, hemispherical, uniformly cottony and terminally projected fertile conidiosphores can observe.
- Chlamydospores are globose to subglobose with 8.5–1–0.5 μm diameter.

4.3.12 Trichoderma theobromicola

The macroscopic and microscopic characteristics of *T. theobromicola* are shown in Fig. 4.18. *T. theobromicola* which belongs to the section of *Pachybasium* based on the morphological characteristics.

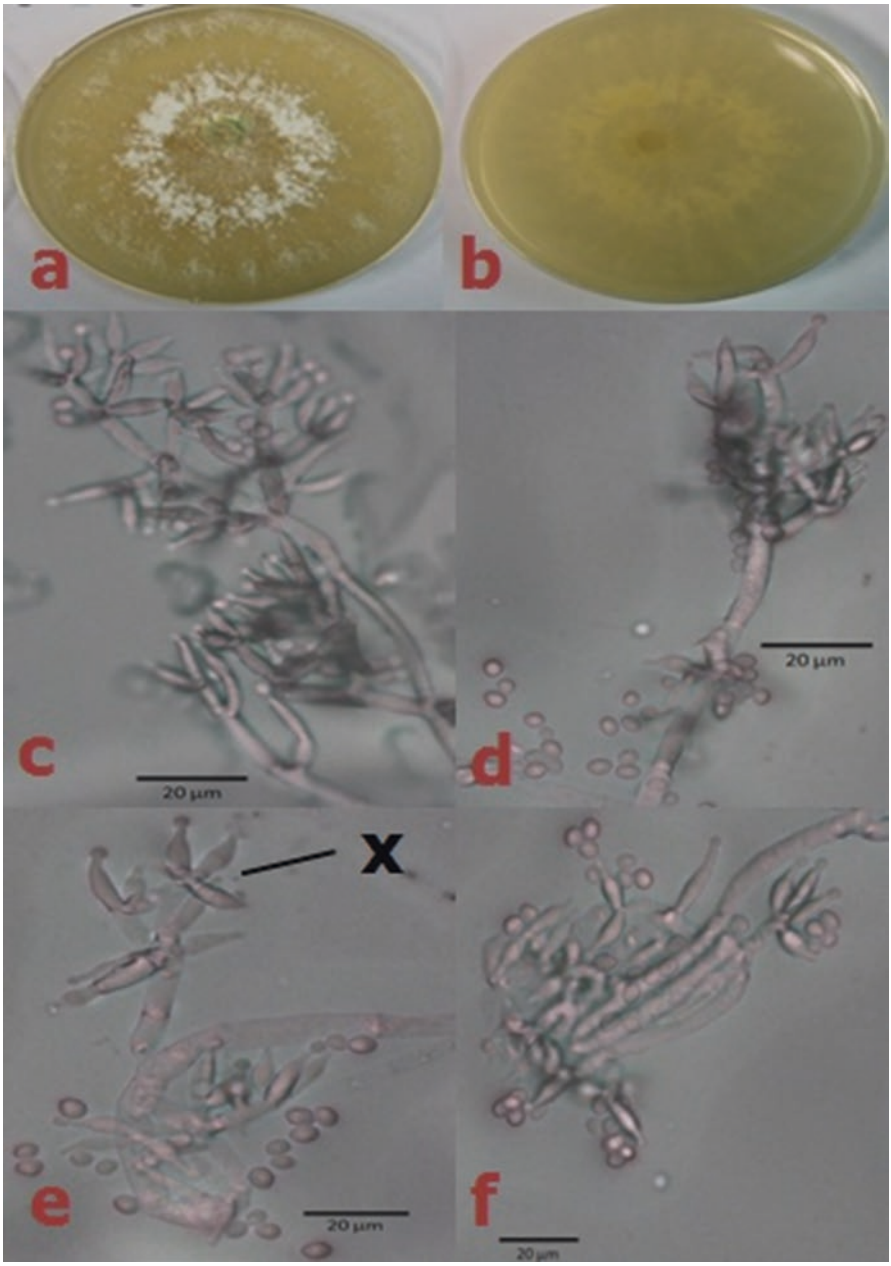

Fig. 4.17 A representative morphological characteristics of *T. viride*. (**a**) Front colony which has grown in PDA for 4 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; X. Phialides. **c–f** were observed with 400× magnification

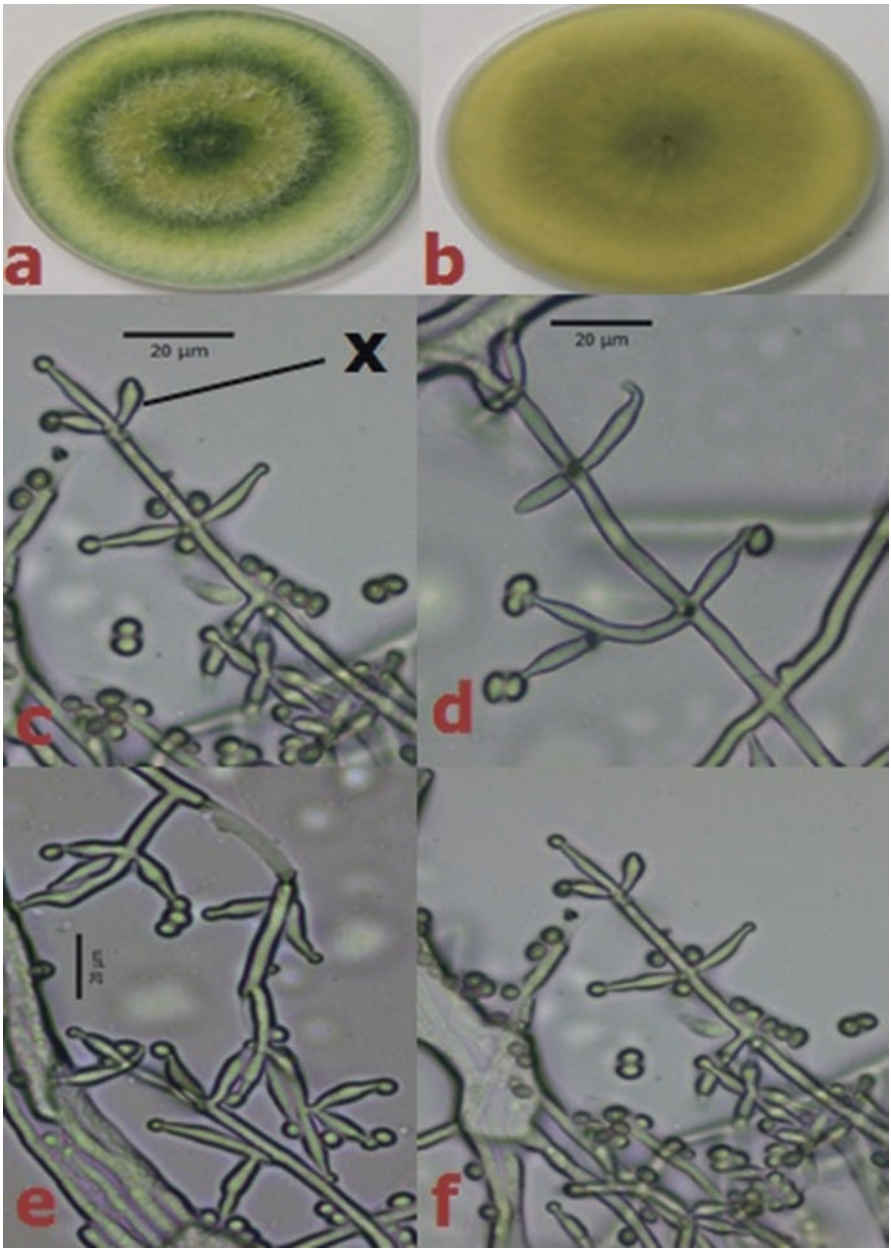

Fig. 4.18 A representative morphological characteristics of *T. theobromicola*. (**a**) Front colony which has grown in PDA for 3 days; (**b**) Reverse colony; (**c–f**) Conidiosphores; X. Phialides. **c–f** were observed with 400× magnification

Macroscopic characteristics

- Its grow rapidly onto PDA at 25 °C after 4 days of incubation, the growth of isolates colony found in the range of 50–65 mm on plates.
- It produced strong aromatic odor of coconut.
- No distinctive yellow pigment appeared.

Microscopic characteristics

- *T. theobromicola* is easily recognized based on its abundantly produced green conidia which formed in dense and thick lawns.
- It is mostly ellipsoidal to subglobose and unicellular.
- They can regularly be branched with two branches tending to arise at each node; with short internodal distances.
- The pustules are densely aggregated and papillate.
- Phialides are long and slender with the range of 1.8–3.4 μm, *gliocladium*-like at the tips of branches of the conidiosphores (Samuels et al. 2006).

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