
Biology and Significance of *Saksenaea vasiformis*

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Abstract

The status of *Saksenea vasiformis* discovered by S.B. Saksena from soil of Sagar forests in 1953, now found distributed all over the world as serious pathogen, is reviewed. Its morphology, clinical aspects, pathogenicity, case reports, antibiotic sensitivity, treatment and molecular phylogeny is also reviewed. Almost all the citations dealing with fungus are included.

Keywords

Saksenaea vasiformis • Sporulation • Sporangia • Rhizoids • Mucormycoses

Introduction

Saksenaea vasiformis S.B. Saksena was first isolated from forest soil of Sagar MP, India, by S.B. Saksena in 1953. This fungus belongs to subphylum Mucomycotina. This is the only species of the genus *Saksenea*. Later Ajello et al. (1976), Chien et al. (1992), Pillai and Ahmed (1993) reported it from wood and grains. The characteristic features of this fungus are typically flask-shaped sporangia, sporangio-phores, oval sporangiospores, and dark rhizoides (Fig. 2.1).

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The flask-shaped sporangia and inability to sporulate on normal media are special features of *Saksenaea vasiformis*. However, Czapeks Dox agar and agar blocks or nutrient deficient media may be favorable for sporulation (Ellis and Ajello 1982; Padhye and Ajello 1988). These workers reported fast growing white colonies without any pigmentation. Aseptate broad hyphae bearing typical flask-shaped with long neck, dividing singly or dichotomously branched hyphae rhizoides are darkly pigmented.

Fig. 2.1 *Saksenea vasiformis* Saksena. X1000



Saksenaea vasiformis can be isolated on several media but grows without sporulation. Only agar blocks and nutrient deficient media favor its sporulation. However, Czapek's dox agar may also favor sporulation but not always. Bearer et al. (1994) and Vega et al. (2006) studied 11 strains from different reference culture collections for DNA extraction, amplification, and sequencing, which were provided by the American Type Culture Collection.

Routine mycological media support mycelia growth of *Saksenia vasiformis* without sporulation. Nutritionally deficient media such as agar blocks stimulate its sporulation and favor development of flask-shaped sporangia. Sterilized yeast extract and/or sterile distilled water is mostly used for development of sporangia (Bearer et al. 1994). A total of 11 strains from different reference culture collections were studied by Vega et al. (2006) for DNA extraction, amplification, and sequencing, which they obtained from the American Type Culture Collection, (ATCC) Manassas, VA; the Centraalbureau voor Schimmelcultures (CBS) Utrecht, Netherlands; the National Reference Center for Mycoses and Antifungal Agents (NRCMA), Institut Pasteur, Paris, France; the Facultad de Medicina de Reus (FMR), Reus, Spain; the Fungus Testing Laboratory at the University of Texas Health Science Center (UTHSC), San Antonio, TX; and the ARS (NRRL) Culture Collection, Peoria.

Reports revealed that these strains were cultured and maintained on PDA, MEA, and CZA at 37 °C. The growth rate on above media temperature ranging 4–50 °C and carbon source assimilation profile were measured by using method described by Schwarz et al. (2007).

Al-Hedaithy (1998) have reported two new species namely *S. oblongispora* and *S. erythrospora* based on molecular phylogeny. Induction of sporulation in the fungus is successful in tap water agar and exposure to sunlight was used.

Clinical Aspects, Pathogenicity and Case Reports

Kaufman et al. (1988) and Tanphaichitr et al. (1990) further clarified its thermo-tolerant nature growing at 25 and 44 °C and reported to cause infection in immunocompromised and immunocompetent human hosts. Mucormycosis caused by *S. vasiformis* most often occurs after traumatic implantation of the fungus but can also be due to inhalation of spores (García-Martínez et al. 2008), spider bites, insect stings, and the use of indwelling catheters (Chakrabarti et al. 1997; Lechevalier et al. 2008). Clinical cases seem to be more common in tropical and subtropical climates than elsewhere and have been reported from Australia (Ellis and Kaminski 1985; Gonis and Starr 1997; Holland 1997; Stewardson et al. 2009; Wilson 2008), India (Baradkar and Kumar 2009; Baradkar et al. 2008; Chakrabarti et al. 1997; Padhye et al. 1988), the USA (Ajello et al. 1976; Bearer et al. 1994; Oberle and Penn 1983; Pierce et al. 1987; Toreil et al. 1981), Thailand (Tanphaichitr et al. 1990), Tunisia (Lechevalier et al. 2008), the Middle East (Al-Hedaithy 1998), and Central and South America (Blanchet et al. 2008;

Vega et al. 2006). Other workers also reported *S. vasiformis* from several clinical samples (Upton et al. 2002; Wilson 2008; Mogbil Al Wedaithy 1988; Parker et al. 1986; Robeck and Dalton 2002).

The first human infections due to *S. vasiformis* were described by Ajello et al. in 1976. *Saksenaea vasiformis* is most often associated with cutaneous or subcutaneous lesions after trauma. *Apophysomyces elegans* is often being reported (Adam et al. 1949). Up to now, approximately 40 cases of zygomycete infections, mostly cutaneous infections, have been attributed to *Saksenaea* (Vega et al. 2006; Trotter et al. 2008; Baradkar and Kumar 2009), although for the reasons indicated above, it is likely that the actual number of clinical cases has been underestimated. To avoid difficulties in the detection and identification of *Saksenaea* in clinical samples, several authors have emphasized the need for special culture techniques, such as the use of floating agar blocks on water, or the use of Borelli's lactrimel agar (Ellis and Ajello 1982; Lye et al. 1996) to induce sporulation. In contrast, the use of Czapek agar, a culture medium traditionally used for the phenotypic characterization of *Aspergillus* and *Penicillium* species, produced good in vitro sporulation of the *Saksenaea* strains isolated from clinical sources. *Saksenaea vasiformis* was found implicated in human infections of cutaneous and subcutaneous lesions as studied by Ajello et al. (1976). Emerging prominence of cutaneous infections has also been reported by Adam et al. (1949). About 40 cases of cutaneous infections by *Saksenaea vasiformis* reported so far (Vega et al. 2006; Trotter et al. 2008; Baradkar and Kumar 2009). But it appears that clinical cases reported so far are as much lesser than expected. Attempt to induce sporulation of this fungus several techniques such as floating agar blocks on water was suggested (Ellis and Ajello 1982; Lye et al. 1996) in order to correct identification of *Saksenaea vasiformis*. The CZA also proves a good medium for sporulation for *Saksenaea vasiformis*.

Saksenaea vasiformis is an emerging pathogenic fungus of order Mucorales and appeared to be involved in immunocompromised patients such as with leukemia, diabetes, and under treatment and under treatments with corticosteroids. Infection of this fungus is diagnosed by angio-invasion leading to tissue necrosis; also cutaneous and subcutaneous involvement is noted. This is being associated with traumatic inoculations. Alvarez et al. (2010) have reported rhino-orbito-cerebral and disseminated infections. The clinical relevance and incidence of infections are not yet very clear, but *S. erythrospora* was found associated in a wounded person in Iraq.

The association of *Saksenaea vasiformis* always in infection through soil is not very well recognized. The first report of subcutaneous zygomycosis by *Saksenaea vasiformis* was from Vishakhapatnam, India by Padmaja et al. (2006). Ellis and Kaminski (1985), Oberle and Penn (1983) reported 28 cases of *Saksenaea vasiformis* infection of which 11 died. Out of 11 deaths six were observed immuno-competent hosts (Padhye et al. 1988; Patino et al. 1984; Kaufman et al. 1988; Hay et al. 1983; Solano et al. 2000; Oberle and Penn 1983), while three were found with malignancies as described by Toreil et al. (1981), Ellis and Kaminski (1985), Gonis and Starr (1997). And two were in diabetics (Chakrabarti et al. 1997; Campelo et al. 2005). Most of these infections were cutaneous and localized. *Saksenaea vasiformis*

also causes rhinocerebral diseases (Kaufman et al. 1988; Gonis and Starr 1997). Solano et al. (2000) reported some cases of pulmonary sinusitis which were from Spain. These infected patients died in spite of debridement and anti-fungal therapy. Amphotericin B treated patients were recovered. Some cases responded to debridement alone (Padhye et al. 1988; Holland 1997; Chakrabarti et al. 1997; Pritchard et al. 1986).

Subcutaneous zygomycosis is often found due to *Saksenaea vasiformis* starting with mild to fatal infections. Padmaja et al. (2006) reported that early diagnoses and treatment may give better results. Since this species normally do not sporulate on common media and it takes time on nutritionally deficient media to sporulate and diagnose.

The infections caused by *Saksenia vasiformis* are subcutaneous, disseminated, rhinocerebral, and fatal disseminated (Toreil et al. 1981; Hay et al. 1983). Subcutaneous infections reported in 3-month- and 11-year-old children. This fungus was also able to invade tissue in traumatic injury. Padhye et al. (1988) reported subcutaneous zygomycosis by this fungus in India for the first time in a foot infection rice mill worker revealing multiple sinuses. However, this infection was cured by split thickness graft and potassium iodide treatment. Cranial wound, subcutaneous tissue necrotizing cellulites, and disseminated infections were also reported but are not common in clinical laboratories. (Ajello et al. 1976; Ellis and Kaminski 1985; Oberle and Penn 1983; Toreil et al. 1981).

Al-Hedaithy (1998) reported rhinocerebral infection by *Saksenea vasiformis*, while cutaneous infection suggested to be primary or secondary infection was suspected from some other sources. Hay et al. (1983) reported fatal disseminated infection in a woman. A three-month-old infant and 11-year-old child were reported to have subcutaneous infection (Al-Hedaithy 1998). *S. vasiformis* is filamentous fungus belonging to Mucorales causing human infection. Ajello et al. (1976) reported to cause human infection for the first time.

Alvarez et al. (2010) reported a cutaneous lesion of the abdominal wall in a woman in French Guinea which was due to zygomycosis and was treated with liposomal amphotericin B and itraconazol. Vega et al. (2006) found a child in Paris who developed lesion due to scorpion sting with many areas of necrosis and non-specific inflammation from where later on zygomycetous branched, non-septate fungal hyphae were observed. These were failed to sporulate. Fluorescent microscopy after proper staining with calcofluor white also revealed zygomycete hyphae.

Some animal cases of *S. vasiformis* in Texas, Australia, were reported including marine mammals which were found to develop various symptoms as a result of fungus infection.

In 2010 *Saksenaea oblongispora* Alvarez, Stchigel, Cano, Garcia-Hermoso, et Guarro sp. nov. MycoBank MB 518626; *Saksenaea erythrospora* Alvarez, Cano, Stchigel, Garcia-Hermoso, et Guarro sp. nov. MycoBank MB 518627 were discovered. *Saksenaea oblongispora*, characterized by oblong sporangiospores and unable to grow at 42 °C, and *Saksenaea erythrospora*, characterized by large

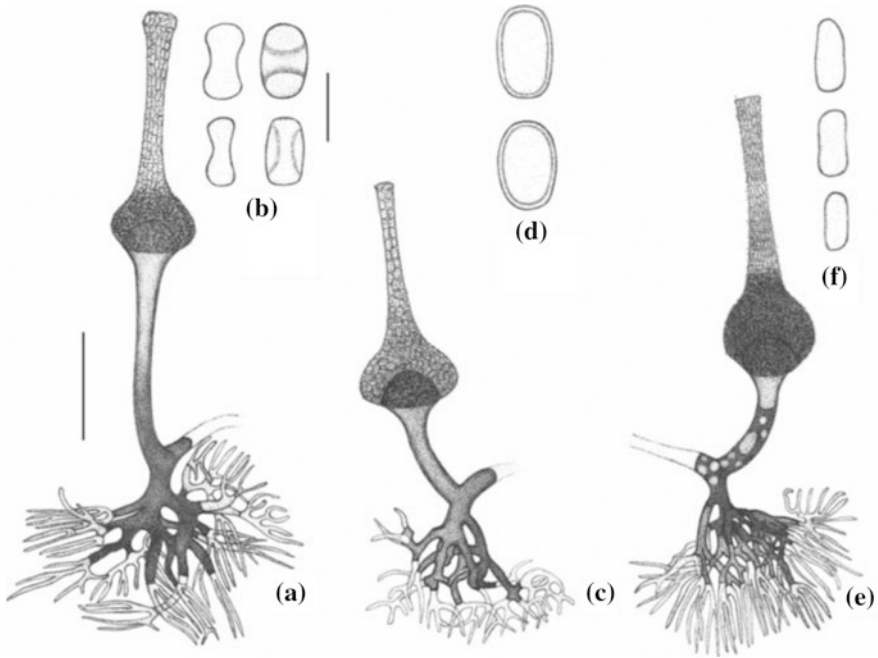


Fig. 2.2 *Saksenaea erythrospora* (a, b). *Saksenaea oblongispora* (c, d). (*Saksenaea vasiformis* (e, f). Bars, 50 µm for (a, c, and e); 5 µm for (b, d, and f) (Alvarez et al. 2010)

sporangiophores and sporangia and by ellipsoid sporangiospores, biconcave in the lateral view (Alvarez et al. 2010) (Fig. 2.2).

deHoog et al. (2000) and Ribes et al. (2000) reported often fatal infections caused by species of *Rhizopus*, *Mucor*, *Absidia*, *Rhizomucor*, *Apophysomyces* including *Saksenaea*. These infections may be localized or disseminated and most commonly rhinocerebral form of diseases. Ribes et al. (2000) discussed predisposing factors. The published first report of *Saksenaea vasiformis* infection is of Ajello et al. (1976) along with 26 more cases. This is rare fungus worldwide in distribution causing mild to chronic infection leading to fatal acute infection (Dean et al. 1977).

Phylogenetic Studies

Alvarez et al. (2010) mentioned small number of isolates availability hinders correction on the basis of phenotypic characters. In the case of *S. vasiformis*, molecular studies were based on internal transcribed spacer (ITS) sequences which revealed relatively high specific intraspecific diversity (Blanchet et al. 2008; Lechevalier et al. 2008). This study disclosed more than one phylogenetic

species of *S. vasiformis*. DNA sequences generated should be useful for further characterization of clinical isolates and/or identification of causative species from tissue biopsy specimens. They may also allow the identification of other species within the genus or the *S. vasiformis* complex. *S. vasiformis* was redefined as a complex, and two new cryptic species were identified, *S. erythrospora* and *S. oblongispora* (Alvarez et al. 2010). These workers showed that possibility exists as a complex species (Fig. 2.3).

Al-Hedaithy (1968), Blanchet et al. (2008), Padmaja et al. (2006) reported 35 cases of *S. vasiformis* infection. Vega et al. (2006) reported 22 cutaneous infections, three sinusitis and three disseminated infections. Infection also occurred after insect and spider bites (Padhye et al. 1988). Study of Padmaja et al. (2006) showed that most of the infections are from USA, Australia, New Zealand, Colombia, Ecuador, French Guiana, Israel, Thailand, Spain, India, and Iraq which are from tropical subtropical regions. In addition to above 35 cases of infection, one more case of *S. vasiformis* infection was reported from Tunisia. In case of non-sporulation of the fungus identification relies on molecular analysis directly on the infected tissue. Frozen- or paraffin-embedded tissues are generally used for these analysis (Vega et al. 2006) of *S. vasiformis* infected tissue.

Alignment of the sequences of other described species with *S. vasiformis* sequences allowed us to identify the fungus with confidence as the species *S. vasiformis*. It is interesting that there are relatively large genetic distances between isolates of *S. vasiformis*, as previously noted (Padmaja et al. 2006). A more comprehensive phylogenetic study of this rare genus would be of interest (Alvarez et al. 2010; Castresana 2000). *S. vasiformis* ITS sequence available with the gene bank database is suggestive of more and more phylogenetic study of the genus.

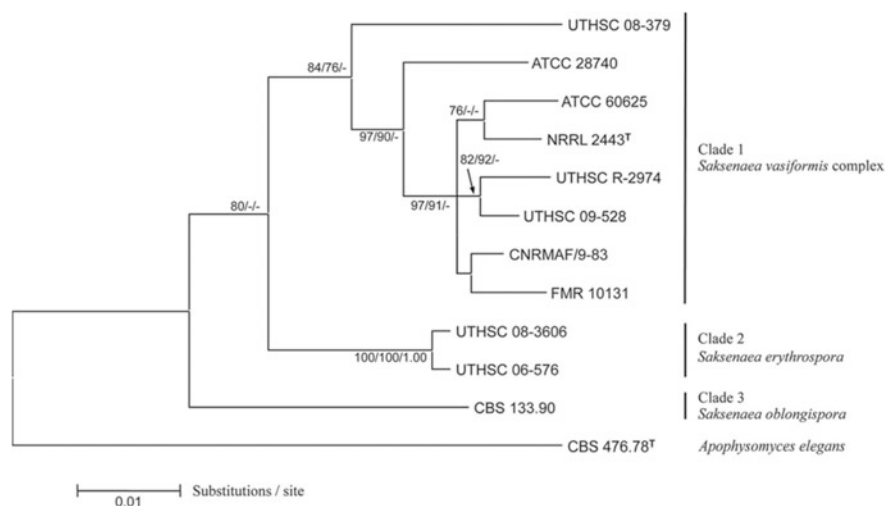


Fig. 2.3 Maximum-likelihood (ML) tree obtained from the combined DNA sequence data from three loci (ITS, D1/D2, EF-1-1 α) (Alvarez et al. 2010)

Vega et al. (2006) studied genetic and phenotypic diversity among 11 strains of and showed useful markers for species label identification of genus *Saksenea*. The genus proved to be genetically heterogenous having more than one species. The most important characteristic features were shape, size, thermotolerance, carbon assimilation for the species differentiation.

Antibiotic Sensitivity and Treatment

Sporangiospore suspension was counted microscopically and adjusted to the required density. Rodriguez-Tudela et al. (2008) screened *S. vasiformis* against amphotericin B, itraconazole, voriconazole, posaconazole, and caspofungin and found the MIC of 0.5 µg/mL for posaconazole effective. Alvarez et al. (2010) found AMB and PSC effective against *S. vasiformis*.

Antifungal susceptibility testing of above strains was carried out on CZA for 7–20 days at 30 °C or 37 °C. Sporangiospores were then collected in water, and the suspension was adjusted to 2×10^4 CFU⁵/ml per well. Pure active powders, of known potency, of amphotericin B (Sigma-Aldrich, Saint Quentin Fallavier, France), voriconazole (Pfizer Central Research, Sandwich, United Kingdom), itraconazole (Janssen-Cilag, Issy-les-Moulineaux, France), posaconazole (Schering-Plough Research Institute, Kenilworth, NJ), terbinafine (Novartis Pharma AG, Basel, Switzerland), caspofungin (Merck & Co., Inc., Rahway, NJ), micafungin (Astellas Pharma, Osaka, Japan), and anidulafungin (Pfizer) were used by Alvarez et al. (2010). And antifungal susceptibility testing was performed by a broth microdilution technique according to the guidelines of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for the testing of conidium-forming molds, with some modifications (Alvarez et al. 2010).

More aggressive approach is required to induce sporulation of zygomycetes that are initially sterile. *S. vasiformis* infections are now found globally, but still the genus is very poorly studied and a very few numbers are preserved in culture collections. Unfortunately no any culture is available in India.

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