2 Review of Literature

2.1 Phansomba

2.1.1 General Information

Phanasomba is a folk medicine used in the Western Ghats of Maharashtra. It is common bracket mushroom causing heart rot disease of *Artocarpus heterophyllus* Lam. (vernacular name: Phanas). Phansomba is also known as Phanas-alambe, Phanas banda (Khory 1887, Dymok *et al.* 1890, Andalkar 1988, Vaidya & Bhor 1991; Vaidya & Lamrood 2000).

It is believed that Portuguese introduced the medicinal use of mushrooms in India. They used a kind of mushroom as a substitute for *European Tinder Fungus* for wound healing; the local name given to this mushroom lateron was Phansomba (Dymock *et al.* 1980). The description of the mushroom given resembles that of *Phellinus*.

Phanasomba was also named as Phanas-alombe and was considered to belong to *Agaricus* (Khory 1887). There has been an ambiguity regarding the genera to which Pansomba belongs, Phansomba was reported as *Agaricus ostreatus* as well as *Boletus nitus* and *Boletus crocatus* (Dymock *et al.* 1980, Kirtikar 1918, Nadkarni 1954, Shah 1928). Though the genera and the species mentioned in these reports are different, the descriptions of therapeutic uses are similar.

The previous reports note that Phansomba samples were sometimes dry and sometimes moist and when they were moist, showed oozing of reddish juice. Those fungi found on trees like Mango, Babool and Tamarind are also medicinal in addition to those found on Jackfruit. It is further stated that, Phansomba is yellow – orange in colour when fresh and dull brown, when old (Kirtikar 1918, Shah 1928). There are some species of *Innonotus* particularly *I. rickii* which show similar property i.e., oozing of reddish droplets on the basidiocarp surface.

Based on this observation it is proposed by our group that the species of *Innonotus* might be belonging to the folk medicine Phansomba. Further work in this direction is in progress where the pilot work is quite encouraging.

After genuine and critical examination of samples, these mushrooms are sold under the trade name Phansomba resemble *Polyporus* and not *Agaricus*. 

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2.1.2 Uses and preparation methods

Many species of *Phellinus* are being regularly used under the trade name Phansomba by the tribals, local people of Western Maharashtra, learned Ayurvedic doctors (Vaidus) of Western Maharashtra (i.e. Konkan region) not only in several ailments in children but also for throat problems (antitumor), antimicrobial and anti-inflammatory agents in rheumatoid arthritis as well as a general health enhancer along with other ayurvedic drugs (Andhalkar 1988, Chopra *et al.* 1956, Desai 1927, Dymock *et. al.* 1890, Khory 1887, Kirtikar 1918, Nadkarni 1954, Sathe 1982, Sawant 1974, Shah 1928, Vaidya 1995, Vaidya & Bhor 1991, Vaidya & Lamrood 2000).

The whole basidiocarp of *Phellinus* is ground in water to make paste and then applied to affected area, like gums in case of excessive salivation in kids, for wound healing etc. this paste may be administered internally for diarrhoea and dysentery (Desai 1927, Vaidya 1995, Vaidya & Bhor 1991).

The drug preparation procedure suggested by ayurvedic doctors from Maharashtra, is as follows: Phansomba (50g) in 2,000cc of cold water, boiled and this mixture is then concentrated to 1,400cc and kept aside, after adding 2,000cc cold water. This process is repeated three times, all the three solutions are then mixed and refrigerated. The suggested dose of this mixture is 100-150 ml. (about the size of a coffee cup) three times a day. For all the procedures glassware or an herb extractor should be used (Personal communication by local Ayurvedic doctors)
2.1.3 *Phellinus* Quel. as Phansomba

On the basis of knowledge acquired by the extensive market surveys, interviews with local people/ vaidus and a vast sample study, Phansomba was shown to be comprising of many species of *Phellinus*, a medicinal fungus from Hymenochaetaceae, such as *Phellinus adamantinus* (Berk.) Ryv., *P. carteri* (Berk. et Cke.) Ryv., *P. conchatus* (Pers.: Fr.) Quel., *P. fastuosus*, *P. linteus*, *P. merrilli* (Murr.) Ryv., *P. pectinatus* (Klotzsch) Quel., *P. sublinteus* (Murr.) Ryv. and lot many (Vaidya & Bhor 1991).

2.1.4 International research on medicinal properties of *Phellinus* Quel.

*P. igniarius* is taken internally as a bitter tonic and laxative, externally as a styptic (Nadkarni 1954, Chopra *et al.* 1956).

Much advancement in studies of *Phellinus* spp. has revealed newer dimensions of their medicinal properties, some of which are noted below along with the international senario of the medicinal use of *Phellinus*.

The Khanty people of West Siberia used the ash of *Phellinus nigricans* to cure toothache and tooth decay by mixing in low-grade tobacco (Saar 1991). Same practice of using ash of *Phellinus igniarius* with tobacco was also used in other tribes like Blackfoot, Labrador Eskimo, Micmac, Kwakiut, Inuit, Yup’ik and Inupiaq Eskimos of western Alaska. These people used to either smoke or chew this mixture and it was supposed to be stronger in flavour than just tobacco (Blanchette 2001, Blanchette *et al.* 1992).

The extract of *P. linteus* has anti-oxidant activity, anti-acne effect and it improves immunity (Kim *et al.* 2000). *P. linteus* extracts along with some additives serves as hair tonic and is effective in the regeneration of mother hair cell and the promotion of blood circulation (Kim 2000) Several studies have showed that *Phellinus* species can produce substances with cytotoxic activity (Atsumi *et al.* 1993, Withers & Umezawa 1991, Han *et al.* 1999). Polysaccharides-spachyman and pachymaran extracted from *Phellinus* exhibited strong anti-cancer and immunomodulatory activities (Hobbs 1995).
Phellinus also shows antioxidant activity that highly regarded in traditional medicine and is widely consumed in the belief that it promotes health and longevity lowers the risk of cancer and heart disease and boosts the immune system (Jong & Birmingham 1992, Rajarathnam et al. 1998, Wachtel-Galor et al. 2003).

2.2  

**Artocarpus heterophyllus** Lam.: The Jack-fruit tree

**Family:** Moraceae (Singh et al. 2001)

**Origin and Ecology:** The jackfruit tree is one of the most useful plants of the tropics. While it bears the largest edible fruits known, the tree is also famed for its timber. From the standpoint of quantity and the quality of food contained in its multiple fruit, there is no other fruit that is as popular as this in some of the tropical countries, especially in southern Asia where this plant has been in cultivation since the beginning of Christian era.

The tree is considered to be indigenous to India, a native of the region lying at the foot of the Western Ghats of the Indian Peninsula, whence it is supposed to have spread into other tropical countries. It is now cultivated in several tropical regions such as Sri Lanka, Malaysia, Burma, Assam, Indonesia, Jamaica, Brazil, Mauritius, the Seychelles, Rodriguez Island, East Africa, and in many other countries in the tropical lowlands of both hemispheres.

In Nigeria, this species is of recent introduction and has not become very popular, being represented by very few plants distributed far apart between southern and northern Nigeria (Thomas 1980). It is common avenue tree in western parts of Maharashtra, India. It is planted at roadsides and gardens as well as found in wild. Here it is called by its vernacular name Phanas. Jackfruit is adapted to humid tropical and near-tropical climates (http://www.crfg.org/pubs/ff/jackfruit.html).

**Taxonomy:** Evergreen trees, cauliflorus with massive syncarps. Leaves elliptic or obovate, dark green, 5.25 x 3.5.12.0 cm, base cuneate or subdecurrent, apex obtuse or subacuminate; lateral veins 6.10 pairs, petioles 8.15 mm long. Inflorescence solitary, axillary. Male heads 25.70 x 8.28 mm, narrowly clavate; peduncles 12.50 cm long. Syncarp 30.100 x 25.50 cm,

Medicinal value: Ayurvedic medicinal literature describes many medicinal properties of Jackfruit plant parts. Ripe fruits have laxative properties in addition to be nutritive food where as unripe fruits have strong astringent and carminative properties. Roots can be used in the treatment of skin diseases and the wood of jackfruit tree is an effective medicine in convulsions (Lamrood 2004).

2.3 Taxonomic Studies of Phellinus Quel.

2.3.1 Classification and Evolution in Basidiomycota

It has been noticed that there is an active or driven force towards the evolution of complex forms in homobasidiomycetes. It seems that the driving force is related to selection for efficient spore dispersal, which is the sole function of efficient fruit bodies. Still there is a lot of scope for development of algorithms of efficient models of evolution for multistate characters.

Pileate-sessile and coralloid clavaroid forms are the most labile forms, Pileate–Stipitate is the most stable form of basidiocarp, Gasteroid forms have evolved through intermediates of pileate stipitate or coralloid clavaroid precursors, it is also suggested that there could be an evolutionary trend towards resupinate forms (Binder & Hibbett 2002).

Corner (1932) introduced a well-known concept of Hyphal systems. The concept of mono, di and trimitic hyphal system was considered as an important conservative classifying character at higher hierarchial level till recent time. His views were strongly supported by many authors lateron (Ryvarden 1991).

There are atleas three types of hyphae constituting the basidiocarp.

- **Generative hyphae**: These are essential component of basidiocarp producing clamp connections and basidia. These are usually freely branched and multiseptate.

- **Skeletal hyphae**: Principle element of basidiocarp originating from generative hyphae. Usually not freely branched although certain kinds may show some branching occasionally.
Binding Hyphae: These originate from generative hyphae usually as side branches. They are profusely branched and relatively slender with a limited potential for elongation.

Pouzar (1966) suggested a notion ‘Vegetative hyphae’ for skeletal and binding hyphae to be morphologically and functionally similar in contrast to generative hyphae.

The latest accepted classification is based on a series of major phylogenetic studies of fungi under the umbrella of ‘Deep hypha’ and AFTOL (‘Assembling the Fungal Tree of Life) projects (Blackwell et.al. 2006, Hibbett et.al. 2007, James et.al. 2006, Kirk et.al. 2008). According to the latest suggested classification system, the presence and absence of macroscopic basidiocarps, the life form, life cycle and the traditional ‘hymenomycetes’ and ‘Gasteromycetes’ meaning the pragmatic subdivisions in the ninth edition can no longer be supported. They can only be considered as informal and not monophyletic categories. The consequence of this is that some of the older literature cited maybe in conflict with the taxon it is cited under (Hibbett et.al. 2007, Kirk et.al 2008).

2.3.2 Hymenochaetales and Hymenochaetaceae

Hymenochaetales was the order of Basidiomycets first coined by Oberwinkler (1977). It was estimated to contain 2 families, 22 genera 382 species in the begining of 21st century (Kirk et.al.2001); whereas according to the latest estimation, the same order contains 2 families, 48 genera and 610 species (Kirk et.al 2008).

Hymenochaetaceae contained 14 Genera and 298 species according to the report in recent past (Kirk et.al.2001); whereas latest knowledge estimated number for genera as 27 and that for species as 487.

Hymenochaetaceae was created by Donk, which Oberwinkler (1977) raised to ordinal level, Hymenochaetales. This group is accepted as a natural entity and a well-defined taxonomic unit in the traditional thought line. The monophyly of this group was supported by characters such as xanthochroic reaction, yellow to deep brown trama, absence of clamp connections and cause of white rot (Donk 1948, Fiasson & Niemela 1984, Oberwinkler 1977, Ryvarden
1991). Hymenochaetaceae members are morphologically distinct and therefore considered as separate monopyletic unit by most of the authors (Larsson et al. 2006).

The monophyly of Hymenochaetaceae cannot be supported by molecular phylogenetic analysis. The monophyly of Hymenochaetaceae is could not be accepted because of inclusion of some corticoid species and two polyporeid genera which were intermixed with other genera of Hymenochaetaceae. The resulting classification of Hymenochaetales based on molecular phylogenetic analysis can be attempted to be justified by morphological, physiological and ecological characters. But micromorphological division between monomitic annual taxa and dimitic perennial ones cannot support it (Wagner & Fischer 2001, 2002a,b). Though many authors reject the monophyly of the group some authors still accept its monophyly stating the exceptions of genera *Coltricia* and *Coltriciella*. It is also worth noting that Hymenochaetaceae can now only be defined only in terms of molecular data (Larsson et al. 2006). The circumscription of Hymenochaetacean genera is rather imprecise even with the traditional systematics (Corner 1991).

Fiasson & Niemela (1984) proposed two new suborders for Hymenochaetales, Phaeolinae and Hymenochaetinae. The former comprising one family, Phaeolaceae and the latter, three families Hymenochaetaceae, Inonotaceae and Phellinaceae (Fiasson & Niemela 1984). Majority of the members of this clade are lignicolous species. All the Hymenochaetaceae members are likely to belong to this clade but it is not fully determined as to how many Corticiaceae and Polyporaceae members are a part of it. The Hymenochaetoid clade is estimated to contain 630 fungal species (McLaughlin et al. 2001).

### 2.3.3 Phellinus Quel.

The genus *Phellinus* is created by Quelet (1886) to describe brown, poroid, pileate wood inhabiting fungi which were annual or perennial. In the original literature six species of *Phellinus* were described viz *P.igiarius*, *P.rubriporus*, *P.fulvus*, *P. conchatus*, *P. pectinatus* and *P. salicinus*. The author considered *P. igniarius* Quel. as the generic nomenclatural type. Later *P. rubriporus* was also considered as the type species for *Phellinus* (Donk 1960). The generic status of *Phellinus* remained rather stable since it was created (Larsen & Cobb-Poulle 1990).
A large number of species were included in this genus thus making it one of the largest genus of Basidiomycetes. The species concept in the genus *Phellinus* is repeatedly reported as vague, obscure and uncertain at many instances (Larsen & Cobb-Poulle 1990, Parmasto 1985, Gilbertson 1979, Wagner & Fischer 2001, Wagner & Fischer 2002a & b).

A vast contribution by several authors was done over the years regarding inclusion of new species and rearrangements in the existing species. Remarkable work reflected departure from the classical taxonomy. Fiasson (1983) and Fiasson & Niemela (1984) revised the taxonomy and nomenclature of eurpean species of *Phellinus* with additional dimensions of numerical taxonomy, chemotaxonomy, protein profile, nuclear condition and cultural characteristics to a limited extent.

A comparatively recent compilation of *Phellinus* spp. was done by Larsen and Cobb-Poulle (1990) giving type descriptions and dichotomous keys for all the valid species till that time. This could be regarded as landmark work in the taxonomy of genus *Phellinus*.

As per updated knowledge, *Phellinus* is the large genus with 174 valid species (excluding varietis and f.spp) (http://www.cbs.knaw.nl/scripts/Aphyllophorales.dll/Data ). The number of valid species has always changed with the technology advancement. Generally it is difficult to organize a large group of species into one genus.

*Phellinus* s.l is the most diverse genus of basidiomycetes with a high specific richness, mainly in tropical regions (Goes-Neto et.al. 2001).

The generic concepts of *Phellinus* s.l. and *Inonotus* s.l. are traditionally based on mitism. This sytem has been repeatedly shown to be artificial (Fiasson & Niemela 1984, Corner 1991, Dai 1995, Dai 1999, Wagner & Fischer 2001, 2002a & b). Intermediate hyphal systems occur in both *Phellinus* and *Innonotus* eg. *P.* discipes, *P.* erectus, *P.* gilvus, *I.* drydeus. Based on the characters of morphology, anatomy, pigmentation and karyology Fiasson and Niemala (1984) proposed family Phellinaceae in Hymenochaetales. This family comprised seven genera derived from original *Phellinus* Quel. along with two others *Onnia* Karst. and *Inonotopsis* Parm. The seven genera were *Fomitiporia* Murr. (*P.* robustus complex), *Porodaedalia* Murr. (*P.* pini complex), *Ochroprus* J. Schroet (*P.* igniarus complex), *Fulvifomes* Murr. (*P.* rimosus complex), *Phellinidium* (Kotl.) Fiasson & Niemela (*P.* ferrugineofuscus and allies), *Fuscoporia* Murr. (*P.* ferreus and its allies) and *Phellinus* Quel s.str. (*P.* torulosus complex). This system was accepted in a limited scope and rejected.
evolved eventually as it was proposed with the consideration of only European taxa. The subdivision of *Phellinus* and *Inonotus* into smaller groups is strongly supported by phylogenetic studies (Larsson *et al.* 2006).

It is stated that, a clear agreement between the morphological species recognition on one side and phylogenetic as well as biological species recognition on the other was observed only in few cases e.g. *P. lundelli* and, to a lesser extent, *P. laevigatus*, DNA and pairing tests supported the conspecificity of strains originating from different geographic regions throughout the north hemisphere. The majority of the morphological species, however, appeared less uniform particularly *P. igniarius*. In *Phellinus* the sole application of morphological species recognition often does not reflect the actual taxonomic state of the specimens. Synonymy amongst many species of *Phellinus* is noted by many authors (Gilbertson & Ryvarden 1987, Larsen & Cobb-Poulle 1990, Ryvarden & Gilbertson 1994).

To overcome the difficulties in identifying *Phellinus* spp. up to some extent, it was then eventually distributed into species complexes. *P. rimosus* complex including *P. rimosus*, *P. badius* and *P. robinae* was described (Kotlaba & Pouzar 1978). Likewise *P. pini* complex, *P. igniarius* complex i.e. *Phellinus* s.str. were also described by various authors. Remarkable attempt was made to split the genus into subgenera viz. *Fulvifomes*, *Fuscoporia*, *Phellinidium*, *Porodaedalia* (Dai 1999).

Even after the split up, the members if species complexes are morphologically similar and very difficult to differentiate (Larsen & Cobb-Poulle 1990, Ryvarden 1991).

Basionyms of *Phellinus* include genera like *Fomes*, *Poria*, *Ochroporus*, *Polyporus*, *Fuscoporia*, *Fuscoporella*, *Scindalma*, *Scalaria*, *Pseudofomes*, etc. (<http://www.cbs.knaw.nl/scripts/Aphyllophorales.dll/Data>, <http://www.indexfungorum.org/Names/Names.asp>, <http://www.mycobank.org/mycotaxo.aspx>). To trace back the history of the basionyms of *Phellinus*, all these genera previously were included in the family Polyporaceae. Initially Polyporaceae was not formed on the basis of collection of homologous characters but worked as an aggregation of those genera and species which could not be included in any other more natural families (Donk 1964, Ryvarden 1991). As a result, diverse character states are seen to be occurring for every selected character in each group. The same situation seem to be occurring at generic level as been specified at family level (Kim & Jung 2002). To consider
the case of *Phellinus* in this respect, this genus also shows diverse character states like two types of hyphal systems, resupinate to pileate forms of basidiocarps, diverse basidiocarp morphology, different types of sexuality etc. This has resulted in chaotic condition over the years, which in turn resulted in many species having their taxonomic position uncertain.

The polypores were segregated into lamellate and poroid fungi initially (Persoon 1801, Fries 1821). Patouillard (1900) was the first author to delimit the taxa based on microscopic characters. Corner (1932) distinguished hyphal system amongst the polypores, this system was widely accepted later in mid 20th century (Cunningham 1946). Much work was done on different aspects of polypores by many scientists like Overholts, Lowe, Donk, Gilbertson, Ryvarden, Johansen, Niemela, Rajchenberg, Larson, Cobb-Poulle and many more who were major contributors for building todays knowledge of Polypores.

Eventually Polyporaceae was split up into more natural families, separating more homogenous groups and make them their own families. Hymenochaetaceae Imaz. & Toki is an ideal example of such a situation.

### 2.3.3.1 Classification of *Phellinus* Quel. (Kirk et.al. 2008)

- **Domain**: Eukaryota
- **Kingdom**: Fungi
- **Phylum**: Basidiomycota
- **Subphylum**: Agaricomycotina
- **Class**: Agaricomycetes (17 Orders, 100 Families, 1147 Genera, 20951 species)
- **Order**: Hymenochaetales (2 Families, 48 Genera, 610 species)
- **Family**: Hymenochaetaceae (27 Genera (+47 Synonyms), 487 species)
- **Genus**: *Phellinus* (174 Species)
2.3.4 Phylogenetic relationships of Phellinus spp.

Interspecific shifting of Phellinus spp. has been occurring in the taxonomy of this genus because of personal judgement of many authors regarding micro and macro morphology of the species. In conclusion it is happened so that, species have been renamed or merged. The process is continuously been occurring till date. The same condition occurred for years in transferring many genera of Polyporaceae and other families to Phellinus (Gilbertson & Burdsall 1972, http://www.cbs.knaw.nl/scripts/Aphyllophorales.dll/Data, http://www.indexfungorum.org/Names/Names.asp, http://www.mycobank.org/mycotaxo.aspx). In taxonomy this situation seems to be an ongoing process.

The switching amongst the species of Phellinus as well as shifting of Phellinus spp to allied genera has always been taking place till date even after using modern techniques (Wagner & Fischer 2001, Wagner & Fischer 2002 a & b).

In delimiting the species environmental influence seem to play a role, eg. *P. linteus* and *P. baumii*, both these species show gross morphological similarities and when critically studied showed many microscopic differences to denote them as two different species. These two species are related, *P. linteus* is a tropical to sub-tropical species whereas *P. baumii* is its temperate kin (Lim et.al. 2003). *P. chinensis* and *P. inermis* show striking micromorphological similaries but their occurence is different. Where *P. chinensis* occurs in northern Asia, *P. inermis* occurs in south and North-America (Parmasto et.al. 1980). Another example of it is *P. torulosus*, which is a temperate counterpart of *P. gilvus* (Goes-Neto et.al. 2001).

With the latest studies, it is evident that Phellinus s.l. is polyphyletic and has to be divided into discrete groups based on evolutionary account. Five clusters of Phellinus s.l were resolved by Wagner & Fischer (2001) viz. Phellinus s.str. (*P. igniarius* group), Phylloporia (*P.ribis* group), Porodaedalia (*P.pini* group), Phellinidium (*P. ferrugineofuscus* and relatives) and Fuscoporia (*Phellinus ferruginosus* and relatives including *P. torulosus, P.wahlbergii*). *P. nigrolimitatus* was not resolved into any clade in the same analysis which was later proposed as a new genus Phellopilus with type species Phellopilus nigrolimitatus (Niemela et.al. 2001).
Two more clusters of *Phellinus* spp. were identified, i.e., *Fomitiporia* (*Phellinus hartigii, P. robustus* & *P. hippophaecola*) and *Fomitiporella* (*Phellinus cavicola*) in addition to the above stated clusters. In the same article, *P. baumii, P. linteus, P. pachypholeus, P. tropicalis, P. vaninni* and *P. weirianus* were suggested as part of *Inonotus* s.l., *Phellinus kawakamii* and *P. nilgheriensis* as a part of *Fulvifomes* cluster, *P. gilvus* as a species of *Fuscopria* and *P. pini* group as a part of genus *Porodaedalea* (Wagner & Fischer 2002b).

The changes of species suggested by these studies are now accepted widely with some exceptions discussed later in the thesis (Kirk et al. 2008).

*P. rimosus* was found to be closely related to *Aurificaria luteoumbrina* than other species of *Phellinus rimosus* complex, the justification probably lies in the mitic system (Goes-Neto et al. 2001). Previously it was denoted as a part of *P. rimosus* complex which was then classified as a part of *Fulvifomes* subgenus of *Phellinus* (Kotlaba & Pouzar 1978, Dai 1999).

In spite of an extended period of molecular and pairing studies, traditional Macro and micromorphological characters (explained below) still define the vast majority of fungal taxa on species level.

**Basidiocarp:** Mainly three broad categories of basidiocarps are found in *Phellinus* Quel., Stipitate, Pileate and Resupinate. But the transitions between these classes are found to occur because of which sharp distinctions in these categories cannot be made. In spite of that the type of basidiocarp is considered as an important character in defining generic notions (Ryvarden 1991). Two life strategies were noted in *Phellinus* i.e. annual and perennial.

**Basidiospores:** Basidiospore morphology is noted as an important taxonomic character for *Phellinus* spp. The basidiospores could be globose oval or cylindrical. If complexity is assumed to be an advanced stage, cylindrical spores can be considered to have a strong adaptive force (Ryvarden 1991). Also a variation in basidiospore pigmentation and spore wall thickness is noted (Larsen & Cobb-Poule 1990).

**Pores:** Hymenial pore shape and the number of pores per millimeter are considered as important characters for *Phellinus* taxonomy (Larsen & Cobb-Poule 1990).

**Tube layer and Context:** The colour, texture and arrangement of tubes are considered to be taxonomically important characters for *Phellinus*. The colour, texture and arrangement of
context with respect to that of tube layer are also noted while taxonomically describing *Phellinus* spp. (Larsen & Cobb-Pouille 1990).

**Hyphal system:** Type of hyphal system was considered as important generic distinguishing character for Hymenochaetaceae till recent time (Kirk *et al.* 2001).

**Setae:** Larsen & Cobb-Pouille in their “*Phellinus* (Hymenochaetaceae) A survey of the World Taxa” considered the presence or absence of setae at the topmost hierarchy for classifying *Phellinus* spp.

**Anamorphic stages:** Imperfect stages are generally rarely found in nature among *Phellinus* spp. Nevertheless they are reported in the form of chlamydospores in some species eg. *P. kawakamii*. Presence of anamorphic stage is considered as species delimiting character for *Phellinus* (Larsen & Cobb-Pouille 1990).

### 2.3.4.1 Indian approach to studies in *Phellinus* Quel.

Pioneering work on taxonomy of polyporaceous fungi from India was done by Bose (1919-1946). He gave a comprehensive account of polypores collected by him from Bengal region. Scanty work in the genus *Phellinus* has been carried out from India. Himalayan and central peninsular region is studied up to some extent (Baghchee 1961, Baghchee & Bakshi 1950, Bakshi 1958, 1971, Roy 1979, Sharma 1993, Singh 1966, Tiwari *et al.* 1989). Some Indian Polypores were described by Berkley, in his ‘Decades of fungi’

Some *Phellinus* flora from southern India has also been studied (Ganesh & Leelavathy 1986, Natarajan & Kolanduvelu 1985). Studies of *Phellinus* spp from Western Ghats of Maharashtra with respect to some regions are carried out (Rabba *et al.*1994, Lamrood & Goes-Neto 2006). Besides these scanty reports no other studies pertaining to genus *Phellinus* from India could be found. The present work contributes significantly to the taxonomic knowledge of *Phellinus* Quel. from Indian subcontinent.
2.4 Database construction and species identification

Diagnostic key is a device for identification of taxa whose properties can be identified in terms of tests each of which has only a finite number of possible results (Payne 1978). There could be several possible tools for species identification.

- **Field guides**: these are books with photographs and descriptions. Routinely used but may be crude. Identification using field guides may be useful for genus identification.

- **Dichotomous Keys**: It is a traditionally used and most popular tool for species identification for field biologists (Kendrick 1990). In spite of its popularity, at times it could be confusing to use such dichotomous keys for a beginner (Neville & Kendrick 1999).

- **Synoptic keys**: this is probably the best traditional method used till now for the identification purpose. The advantage of this method is that the user can enter the search at any point and the characters can be entered at any order.

- **Numerical Identification**: This is an identification method based on numerical taxonomy matrix. An unknown species can be compared with each standard species dataset to identify the taxon. With this system, likelihood can also be attached to identification (Brenner et al. 2004).

There are quite a few software programs available that could be used for species identification. One of which is askSam. It is a database management program. Other one is ActKey, which is a web-based interactive identification key program (Brach & Song 2005). Besides database management, there are softwares, which can be used to construct and print diagnostic keys. Genkey is one of such system exclusively developed to serve this purpose. Many upgraded versions of the software are also developed to achieve performance efficiency (Payne 1975, 1977, 1978, 1981, 1984).
2.5 Statistical methods and tools

2.5.1 Numerical Taxonomy

Numerical taxonomy (sometimes called as taxometrics) developed as a part of multivariate or cladistic analysis, aiming to classification of organism. In numerical taxonomy, groups of organisms showing similar characteristics together form Operational Taxonomic Unit (OTU). Every OUT shows its own set of constant and variable characters. A character is defined as any property that can vary between OTUs and the values it can assume are called character states (Brenner et.al 2004). The dataset build using taxonomic description can then be subjected to cluster analysis.

2.5.2 Cluster Analysis and Parsimony

Cluster analysis is an exploratory data analysis tool which aims at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise. Given the above, cluster analysis can be used to discover structures in data without providing an explanation/interpretation. In other words, cluster analysis simply discovers structures in data without explaining why they exist. Biologists to hypothesize evolutionary linkages between organisms use this tool. Cluster analysis has been used widely till now to derive phylogenetic inferences. The most important task in carrying out cladistic analysis is identifying the variables to be studied (http://www.statsoft.com/TEXTBOOK/stcluan.html ).

Parsimony is one of the methods followed for carrying out cluster analysis. It is a rule to choose amongst the number of cladogram produced and it refers to minimum number of steps taken towards to the last step of the cladogram.

Many software packages are available today viz. PHYLIP, PAUP, Gene Tree, Binumerics, “Biodiversity” Pro and many others (http://evolution.genetics.washington.edu/phylip/software.html). Amongst the widely used packages PAUP is used most widely today it is the most recommended pacage for drawing phylogenetic inferences (Blackwell et.al. 2006, Goes-Neto et.al.2001, Hibbett et.al.2007,

The principle of Maximum parsimony is also successfully applied to the qualitative multivariate analysis using morphological parameters (Goes-Neto et al. 2001, Kim & Jung 2002, Lamrood & Goes-Neto 2006).
2.6 Culture Studies

2.6.1 Cultures as a taxonomic tool

For many years the fungal taxonomy was purely based on micro and macro morphological characters until Nobles stated the significance of culture parameters for fungal taxonomy and phylogenetic studies (Nobles 1948, 1958a, 1965). Nobles (1958b) has shown that a considerable number of polyporaceous species may be arranged in groups separately homogeneous for cultural characteristics, but some of these groups may not be entirely natural. Some groups are easily referable to recognize genera, *Phellinus* Quel. is one of those.

Nobles (1965) established a system for studying culture characteristics of wood rotting fungi which was then followed by many researchers lateron (Nakasone & Glibetzon 1978, Stalpers 1978).

A vast culture study for wood inhabiting Aphylophorales was carried out by Stalpers (1978). Herein each species was characterized by a numerical code of 96 characters and a system for identification of such a way that it could be used as a synoptic key.

The importance of cultural characteristics as a part of traditional fungal taxonomy is noted repeatedly (Nobles 1965, Stalpers 1978).

The aim of culture studies in the scope of this thesis mainly remained rather restricted to exploring general culture characteristics of genus *Phellinus* and alignment of the same with the traditional taxonomic investigations.

Culture studies of basidiomycetes, mainly polypores from tropical regions has been done way back in 1970s to investigate its relevance with the classical taxonomic approach that time (Bakshi et al. 1969, 1970, Sen 1973). Unfortunately this work does not establish any comparison with previously worked out temperate basidiomycetes (Stalpers 1978).

Besides studying different culture parameters and microscopic observations, drop tests for various enzymes also serve as differentiating characters in culture studies (Stalpers 1978, Biodin 1951, Nobles 1958a, Taylor 1974).

A more recent work was done by Rajchenberg and Greslebin (1995), which included culture studies of seven Aphylophoralaceous fungi. Also culture characteristics of seven polypores
from Brazil were documented according to Nobles’ system (1965) (Neves & Loguercio-Leite 1999). All these studies confirm the usefulness of culture characteristics for species delimitation.

## 2.6.2 Exopolysaccharide production

Polysaccharides are the best known and most potent bioactive compounds in mushrooms with antitumor and immune modulating properties (Wasser, 2002; Mizuno, 1999; Wasser & Weis, 1999). The historic evidence for the use of mushroom polysaccharide could be as old as the tradition of mushroom hot water extract in the eastern countries. Large number of basidiomycetes have been screened for polysaccharides, 651 species and 7 intraspecific taxa from 182 genera of higher hetero and homo basidiomycetes have been studied (Wasser, 2002). An exhaustive list of basidiomycetes studied for the antitumor activity against, Sarcoma 180 solid cancer and Ehrlich solid cancer have also been listed by Wasser (2002). A number of promising ones are still to be explored for the biological activities. Further, there is an urgent need to study the down stream mechanism of action at molecular level that occur in specific immune modulation such as receptors and the various events triggered by the binding of these polysaccharides to the target cells (Daba & Ezeronye, 2003).

A great deal of recent interest has been shown in the ability of fungi to synthesize exopolysaccharides. Fungi produce exopolysaccharides that have chemical and physical properties of considerable commercial potential. Surprisingly little is known about how and why fungi overproduce these metabolites and how yields are affected by both the physical and chemical environments (Seviour et.al. 1992).

Three polysaccharide based carcinostatic (immuno therapeutic) agents viz., Lentinan, Kerstin and Schizophyllan have already been commercialized from mushrooms namely, *Lentinus edodes*, *Coriolus versicolor* and *Schizophyllum commune* respectively. These are used currently in the treatment of cancer of the digestive organs, lungs, breast, stomach and cervical cancers (Daba & Ezeronye, 2003). Exopolysachharides are the purest form of polysaccharides produced by fungi, which could be isolated in liquid cultures.
Results showed that most of the Basidiomycetes strains screened are potential exopolysaccharide producers. The possibility of using these biopolymers for medical application promises a large opportunity to improve the study of such group of fungi. Besides the Brazilian mycobiota has been scarcely investigated although its great potentiality (Maziero et.al. 1999).

### 2.6.3 Optimization of submerged cultivation

Since biomass and exopolysaccharides produced from submerged cultures were responsible for healthy properties of medicinal mushrooms, both these should be considered as criteria for optimization process (Kim et.al. 2002).


Building statistical models could serve as a useful tool in optimization of culture conditions for maximum productivity. Most of the above reviewed publications make use of “One factor at a time” model for optimizing submerged cultivation. Besides this, there are other statistical models which could be applied for extrapolating optimal values in such experiments like, Plakett- Burman Design and Central composite Design (Xu et. al. 2002).