Morphological and physiological biochemical identification biology essay

[Science](), [Biology]()

Yeasts were classified on the basis of their morphology and biochemical characteristics. The workers of the Dutch school were responsible for much of the pioneering work on the classification of yeast species up to year 1950. These workers classified all the yeasts available to them on the basis of cellular morphology, spore shape/number and nature of conjugation process. At species level, they were classified based on the ability to ferment/assimilate sugars, ability to use ethanol and nitrate and to hydrolyze arbutin. As judged by these criteria, the distinction between some species was rather fine. Around the same time, Wickerham and Burton, (1948) and Wickerham, (1951) introduced a number of refinements to the Dutch system, especially the use of a much larger number of carbon compounds. These included additional hexoses, di-, tri-, and tetrasaccharides, 2 polysaccharides and a number of pentoses, polyhydric alcohols and organic acids. They also introduced tests for vitamin requirements. The widely accepted practice is to use approximately 30 carbon compounds and to test for fermentation of at least 11 of these including inulin (Barnett et al., 1990). The ability to use nitrite as well as nitrate at depressed temperature and on media of high sugar or salt content is also noted. The type and number of additional reactions tested vary with the interests and preferences of the individual investigator. Difficulties both major and minor accompany the use of these methods. One is a question of the stability of the biochemical criteria. For e. g. Candida and Torulopsis were separated solely on the ability of the former to produce pseudo hyphae until it was observed that the same species might produce two or more forms simultaneously or at different stages of growth. It has now become evident that different strains of the same species may differ

in their ability to produce pseudo mycelium and the value of this criterion in distinguishing the two genera has approached vanishing point. Another obstacle encountered by an investigator is the instability of physiological characters. Scheda and Yarrow, (1966) observed enough variability in the fermentation and carbon assimilation patterns of a number of Saccharomyces sp. causing difficulties in the assignment of these yeast strains to different species. Yet another problem lies in the relationship of the biochemical tests to metabolism of the organisms. Formerly, it was not sufficiently appreciated that the various carbon compounds are not necessarily assimilated independently but may be metabolized by common pathways. Thus yeasts, which can use a particular compound can also use a structurally related one by the same metabolic pathway, Barnett, (1968) noted that there was a small percentage of yeasts that were exceptions to this rule. In general the conclusions were valid, that the effective number of criteria for the number of substrates reduced distinguishing yeast species metabolized by such linked mechanisms. The metabolism of most or all of the compounds used involves a few distinct central pathways and depends on the ability of the cells to convert the substrates into intermediary metabolites of one of these pathways. Nahvi and Moeini, (2004) isolated and identified the yeast strains with high beta-galactosidase activity from dairy products by using the standard key outlined by Kurtzman and Fell (1998). Savova and Nikolova, 2002 studied taxonomic study of yeast strains from Bulgarian dairy products according to the methods described by Kreger van Rij (1987), Barnett et al., (1990) and Kurtzman and Fell, (1998). As per

Kreger van Rij (1987), Barnett et al., (1990) and Kurtzman and Fell, (1998) the main characteristics used to classify yeasts are as follows.

2. 7. 1. 1 Microscopical appearance

The yeast cells examined microscopically and consider their size and shape, how they reproduce vegetatively (by multipolar/bipolar/unipolar filaments), the form, structure and mode of formation of ascospores and teliospores.

2. 7. 1. 2 Sexual reproduction

Some yeasts reproduce sexually by ascospores, others by teliospores and yet others by basidiospores. For ascosporogenous yeasts, taxonomic significance is given to whether asci are formed from a) vegetative cells b) two conjugating cells or c) a mother cell, which has conjugated with its bud. For yeasts with asci borne on filaments, the arrangement of asci - whether in chains or bunches – may be used to distinguish between genera. The number of ascospores in each ascus, their shape and whether the ascospore walls are smooth or rough are relevant factors used in classification.

2. 7. 1. 3 Biochemical and physiological characteristics

Studies of certain biochemical and physiological characters may influence taxonomic decisions. The factors used for classifying yeasts are chiefly their ability to a) Ferment sugars anaerobically, b) Grow aerobically with various compounds such as a sole source of carbon or nitrogen, c) Grow without an exogenous supply of vitamins, d) Grow at 37ºC, e) Grow in the presence of cycloheximide, f) Split fat, g) Produce starch like substances, h) Hydrolyze urea and i) Formation of citric acid.

2. 7. 1. 4 Hydrolytic enzyme production

Marine yeasts are reported to be truly versatile agents of biodegradation (Da Costa and D'souza, 1979; Kobatake et al., 1992). They participate in a range of ecologically significant processes in the sea, especially in estuarine and nearshore localities. These activities include decomposition of plant substrates, nutrient-recycling phenomena, and biodegradation of oil and recalcitrant compounds. Biomass data and repeated observations of microhabitat colonization by various marine yeasts support ancillary lab evidence for the contribution of this segment of the marine mycota to productivity and transformation activities in the sea (Meyers et al., 1975). Yeast enzymes were found to be useful in various industrial processes which emphasize their direct contribution to our day to day life. These enzymes are produced mostly extracellular by different metabolic reactions taking place inside the cell and participate in various transformation activities like mineralization of organic compounds. Studies by Paskevicus, (2001) showed that almost all the yeast strains produce lipase enzyme. Lipases catalyze a wide range of reactions like hydrolysis, esterification, alcoholysis, acidolysis, aminolysis etc. (Hasan et al., 2006). Lipases are mainly involved in detergent industry and biodegradation especially oil residues. Wang et al., (2007) isolated 427 strains from different marine substrates and their lipase activity was estimated. They found that nine yeast strains could produce lipase in a medium with olive oil. Some lipases from the yeast strains could actively hydrolyze different oils, indicating that they may have potential applications in industry. A protease producing strain isolated from the sediments of saltern near Qingdao, China, had the highest activity at pH 9 and 45ºC (Chi

et al., 2007). This principal enzyme, protease, has many applications in detergent, leather processing and feed industry besides waste treatment (Ni et al., 2008). Yeast amylases have many applications in bread and baking industry, starch liquefaction and saccharification, paper industry, detergent industry, medical and clinical analysis, food and pharmaceutical industries (Chi et al., 2003; Gupta et al., 2003). Amylolytic yeasts convert starchy biomass to single cell protein and ethanol (Li et al., 2006). Cellulases have application in stone washing, detergent additives, production of SCP, bio fuels and waste treatment (Zhang and Chi, 2007). The enzyme inulinase produce fuel ethanol, high fructose syrup and inulo-oligosaccharides (Pandey et al., 1999). Sheng et al., (2007) isolated a marine yeast strain Cryptococcus aureus G7 from China South Sea sediment which was found to secrete a large amount of inulinase into the medium. The crude inulinase produced by this marine yeast showed the highest activity at pH 5. 0 and 50°C. The enzyme phytase is a component of commercial poultry, swine and fish diets and animal/human nutrition (Haefner et al., 2005). Urease is a nickel containing enzyme that catalyses the hydrolysis of urea. Urease has many industrial applications like in diagnostic kits for determination of urea in blood serum, in alcoholic beverages as a urea reducing agent and in biosensors of haemodialysis systems for determining blood urea (Bakhtiari et al., 2006). Gelatinase have been shown to have many applications in food, chemical and medical industries. The extracellular enzyme production, their properties and cloning of the genes encoding the enzymes from marine yeasts are overviewed by Chi et al., (2009). These enzymes include cellulase, alkaline protease, aspartic protease, amylase, inulinase, lipase,

phytase and killer toxin. It was found that some properties of the enzymes from the marine yeasts are unique than that of the enzymes from terrestrial yeasts.

2. 7. 2 Commercial yeast identification systems

Two commercially available yeast identification systems are API 20C Yeast Identification System (API Analytab Products, Plainview, NY) and the Biomerieux Vitek System (Hazelwood, MO). These two yeast identification systems are easy to use. The API 20C requires less preparation of reagents. The Vitek system is an automated system. The two systems are based on modifications of the classic auxanographic technique of carbohydrate assimilation. When an organism is able to assimilate a particular carbohydrate, in the cupules of reconstituted substrates (API) or accompanied by a color change, in the wells containing the substrate of Vitek, the systems must be supplemented with morphological studies, and both systems should have germ tube tests done in conjunction with them. The performance of four miniaturized biochemical test systems (API 20C Auxanogram, Mycotube, Oricult and Uni-Yeast-Tek) for the identification of yeasts was evaluated using standard biochemical tests as reference by Bergan et al., (1982). The VITEK 2 Compact is a fully automated microbiological system that performs microbial identification utilizing growth-based technology. The system uses the colorimetric reagent cards that are incubated and interpreted automatically. This system delivers fast, accurate microbial identification and antibiotic susceptibility testing for a wide range of organisms from environmental sources or final products. The newly

redesigned colorimetric VITEK-2 Compact system with updated Advanced Expert System (AES) (bioMerieux, Marcy l'Etoile, France) was evaluated for its accuracy and rapidity to identify clinical isolates and to detect several antimicrobial resistances by Isamu Nakasone et al., (2006). Overall, the VITEK-2 gave 95. 8% of compatibility with the reference API strips (bioMerieux) in the identifications (IDs) of Gram-positive cocci (GPC), Gram-negative rods (GNR) and yeasts.

2. 7. 3 Molecular identification

Yeasts occurring in marine and other aquatic environments in high concentrations often comprised basidiomycetes yeasts. This made identification troublesome, owing to the unstable reactions in the assimilation tests and the lack of morphological information on the sexual reproduction of many basidiomycetes. But such problems have been overcome with the progress of molecular taxonomy in the last two decades. Instability of the biochemical criteria and physiological characters and obscurity in the interrelationship of biochemical tests to the metabolism of the organisms has led to development of new technique such as rDNA sequencing for taxonomic identification of yeasts rather than classical methods based on morphology, physiological and biochemical characteristics. The molecular identification is a key step for most protocols in molecular biology studies (Sambrook et al., 1989). DNA extraction methods are widely used to isolate DNA from yeast including phenol extraction but they often involve multiple, time consuming steps including the handling of toxic chemicals (Ausbel et al., 1995). PCR based detection of

fungal DNA sequences can be rapid, sensitive and specific (Makimura et al., 1994). The nucleotide sequence of the D1/D2 domain of the 26 S rRNA is sufficiently variable to allow reliable identification of yeast species (Kurtzman and Robnett, 1997). An efficient, inexpensive method for obtaining yeast genomic DNA from liquid cultures or directly from colonies was developed by Harju et al., (2004). PCR based detection of fungal DNA sequences can be rapid, sensitive and specific (Makimura et al., 1994). The nucleotide sequence of the D1/D2 domain of the 26 S rRNA is sufficiently variable to allow reliable identification of yeast species (Kurtzman and Robnett, 1997). Fell and Kurtzman, (1990) reported the nucleotide sequence analysis of a variable region of the large subunit rRNA for identification of marine occurring yeasts. More recently, the differences in the rRNA internal transcribed spacer have been used to identify yeast species. Coding regions of the 18 S, 5. 8 S and 28 S rRNA genes evolve slowly are relatively conserved among fungi, and provide a molecular basis of establishing phylogenetic relationships. Between coding regions are the internal transcribed spacer 1 and 2 regions (ITS1 and ITS2 respectively) which evolve more rapidly and therefore vary among different species within a genus. From the conserved sequences of 18 S and 28 S rRNA genes at the ends of the ITS region two universal primers ITS1 (F) and ITS4 (R) were designed by White et al., (1990). This amplify a fragment of approximately 580bp containing the ITS 1, 5. 8S and ITS 2 regions and are widely used for identification purpose. Differentiation of closely related species requires analysis of both D1/D2 and ITS regions (Fell, 2001). A phylogenetic tree or evolutionary tree is a branching diagram or " tree" showing the inferred

evolutionary relationships among various biological species or other entities based upon similarities and differences in their physical and/or genetic characteristics. The taxa joined together in the tree are implied to have descended from a common ancestor. Phylogenetic analyses have become essential in researching the evolutionary tree of life. A phylogenetic tree is a construction which describes the ancestor-descendants relations of a set of entities. Most phylogenetic trees have an implicit notion of evolution from acestors to current-day entities. Phylogenetic tree construction is an important area of research in biology. Today most phylogenetic trees are built from molecular data: DNA or protein sequences. Originally, the purpose of most molecular phylogenetic trees was to estimate the relationships among the species represented by those sequences, but today the purposes have expanded to include understanding the relationships among the sequences themselves without regard to the host species, inferring the functions of genes that have not been studied experimentally (Hall et al., 2009), and elucidating mechanisms that lead to microbial outbreaks (Hall and Barlow, 2006) among many others. Building a phylogenetic tree requires four distinct steps: (Step 1) identify and acquire a set of homologous DNA or protein sequences, (Step 2) align those sequences, (Step 3) estimate a tree from the aligned sequences, and (Step 4) present that tree in such a way as to clearly convey the relevant information to others. MEGA5 (Tamura et al., 2011) is an integrated program that carries out all four steps in a single environment, with a single user interface eliminating the need for interconverting file formats. At the same time, MEGA5 is sufficiently flexible to permit using other programs for particular steps if that is desired. MEGA5

is, thus, particularly well suited for those who are less familiar with estimating phylogenetic trees. The most basic assumption of phylogenetic analysis is that all the sequences on a tree are homologous, that is, descended from a common ancestor. Alignment programs will align sequences, homologous or not. All tree-building programs will make a tree from that alignment. However, if the sequences are not actually descended from a common ancestor, the tree will be meaningless and may quite well be misleading. The most reliable way to identify sequences that are homologous to the sequence of interest is to do a Basic Local Alignment Search Tool (BLAST) search (Altschul et al., 1997) using the sequence of interest as a query. The BLAST programs are widely used tools for searching protein and DNA databases for sequence similarities. Many investigators constructed phylogenetic tree and established their relationships to ancestors (Hirimuthugoda et al., 2006; Burgaud et al., 2009). Hirimuthugoda et al., (2006) analyzed the 10 strains by its partial sequences of the 18S rDNA were found to be closely related to Hanseniaspora uvarum (strain WZ1), Yarrowia lipolytica (strain W2B), Kodamaea ohmeri (strain BG3), Candida carpophila (strain N12C), Issatchenkia orientalis (strain YF04C), Candida tropicalis (strain MA6), Yarrowia lipolytica (strain YF08), Candida carpophila (strain NY4E), Candida tropicalis (strain YF12C) and Candida tropicalis (strain MB2). Kurtzman and Robnett, (1998) analyzed the phylogeny of approximately 500 species of ascomycetous yeasts including members of Candida and other anamorphic genera from variable D1/D2 domain of large subunit (26S) ribosomal DNA. Species of Saccharomyces, Arxiozyma, Eremothecium, Hanseniaspora (anamorph Kloeckera), Kazachstania, Kluyveromyces,

Pachytichospora, Saccharomycodes, Tetrapisispora, Torulaspora and Zygosaccharomyces, as well as three related anamorphic species assigned to Candida (C. castellii, C. glabrata, C. humilis) were phylogenetically analyzed from divergence in genes of the rDNA repeat (18S, 26S, ITS), single copy nuclear genes (translation elongation factor 1alpha, actin-1, RNA polymerase II) and mitochondrially encoded genes (small-subunit rDNA, cytochrome oxidase II) (Kurtzman and Robnett, 2003). Species assigned to the genera Debaryomyces, Lodderomyces, Spathaspora, and Yamadazyma, as well as selected species of Pichia and Candida that also form coenzyme Q-9, were phylogenetically analyzed from the combined sequences of the D1/D2 domains of the large subunit and the nearly complete small subunit rRNA genes by Kurtzman and Suzuki, (2011). They assigned species to Debaryomyces partitioned into three clades and species assigned to Pichia were distributed among six clades. These well-supported clades were interpreted as genera, and from this analysis, the following new genera are proposed: Babjeviella, Meyerozyma, Millerozyma, Priceomyces and Scheffersomyces. The genus Schwanniomyces was reinstated and emended, and the genus Yamadazyma was phylogenetically defined.

2. 9 Biodiversity of marine yeasts

It has been determined that there is an enormous biological diversity the world’s oceans. Biological diversity means the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

Any shift in diversity, positive or negative, as a result of fishing disturbance also is cause for concern. Diversity index provides a good measure of the community composition along with its survival strategy. There are many measures of species diversity ranging from the original Simpson's diversity and the Shannon-Wiener indices to new techniques at the species level incorporating factors such as the taxonomic relatedness of species. Incorporating evenness into analysis has wide implications, as the removal of target species could increase or decrease evenness and thus diversity, through predator-prey or competitive interactions. Once the genus was identified, their respective numbers can analyze for diversity indices such as, Shannon-wiener diversity (H' (log2)) (Shannon and Wiener, 1949; Peilou, 1966), Peilou's evenness (J') (Peilou, 1966), Species richness (d) (Gleason, 1922) and Species dominance (λ)) (Peilou, 1966) using the PRIMER V5 (Plymouth Routine in Multivariate Ecological Research) tool pack (Clarke and Gorley, 2001). Species diversity is one of the components of biodiversity (Wilson 1988) and although the inventorying of global species is far from complete, species diversity is often the most convenient ' proxy' for other components of biodiversity, such as genetic diversity and landscape diversity. The situation for fungi and other groups is likely to be even worse. Recently, growing concern about the loss of biodiversity caused by human activities led to new challenges for the development of powerful and affordable methods for quantifying species diversity, in order to monitor and predict the erosion of biodiversity caused directly or indirectly by human activities. The need to assess such changes in biodiversity has given a sense of urgency for basic and applied research in species diversity. Since it is not

possible to monitor all the components of species diversity at broad spatial scales, its estimation by indirect methods is receiving much current attention. However, these methods have not yet been adequately evaluated (Colwell and Coddington, 1994). A combination of the number of species and their relative abundance defines species diversity. Ecologists define species diversity on the basis of two factors: (l) the number of species in the community which ecologists usually call species richness and (2) the relative abundance of species, or species evenness. Species diversity can be partitioned into two components: richness and evenness. The concept of species richness is one of the oldest and most fundamental in community ecology (Hutchinson 1959, Whittaker 1965, Peet, 1974; Magurran, 1988; Gotelli and Colwell, 2001). Many authors have reported the significant effects of species richness on important ecological processes such as ecosystem productivity and stability (Tilman and Downing, 1994; Naeem et al., 1994; Loreau, 2000); this work remains controversial (Aarsen, 1997). The influence of species richness on community diversity is clear. A community with 20 species is obviously less diverse than one with 80 species. Species richness is simply the total number of species recorded and is highly dependent on sampling effort; thus comparisons can only be made if sampling procedures are the same. Species diversity indicates both richness and the distribution of the number of individuals among species of the assemblage (evenness). Biodiversity measures vary from counts of the number of entities present (species richness) to indices of the abundance distribution among those entities (species diversity). In measures of species diversity, high evenness and low dominance (the converse of one another) generally indicate a more

diverse assemblage than one of low evenness and high dominance. Species evenness refers to how close in numbers each species in an environment are. Evenness is an important feature of all ecological communities. The evenness was calculated based on Peilou, 1966. Mathematically it is defined as a diversity index, a measure of biodiversity which quantifies how equal the community is numerically.

Biodiversity in the marine environment tends to be in a state of dynamic equilibrium with variable environmental productivity and disturbance causing continuous shifts in community composition, which obviously complicates attributing changes to specific events or activities. Not only is there temporal variability, species richness also varies spatially among habitats; those with greater structural complexity hosting assemblages of greater diversity. As species composition can vary greatly between different depths and habitat types, studies obviously need to standardize the substrata and depths they survey if comparison between different intensities of fishing is the goal. Studies of species richness and diversity are reliant on good taxonomic skills to avoid problems of misidentification or inconsistent identification that could have significant effects on the results. Carrasco et al., (2012) studied the diversity of yeast species isolated from King George Island, the sub-Antarctic region stated that a high diversity of yeasts was isolated in this work including undescribed species and species not previously isolated from the Antarctic region, including Wickerhamomyces anomalus, which has not been isolated from cold regions in general.