European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Determination of viability in yeast cell culture for the study of some plant base insulin releasing and insulin like activity therapy plants (*Abelmoschus esculentus L.*, *Musa paradisiaca*, and *Dioscorea dumetorum*)

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doi: https://doi.org/10.37745/ejbmsr.2013/vol12n36692 Published December 15, 2024

Citation: Aloysius M.T., Andreou, E., Felekkis K. , Petrou C. , Egwim C.E. (2024) Determination of viability in yeast cell culture for the study of some plant base insulin releasing and insulin like activity therapy plants (Abelmoschus esculentus L., Musa paradisiaca, and Dioscorea dumetorum), *European Journal of Biology and Medical Science Research*, Vol.12, No.3, pp.,66-92

Abstract: *The determination of viability in yeast cell culture for the study of some insulin – releasing and insulin – like activity therapy plants (*Abelmoschus esculentus L., Musa paradisiaca, *and* Dioscorea dumetorum*) was studied using standard techniques, and data analytical tools respectively. The yeast cell line was used for this study. Yeast cell measurement at OD600 was carried out with the assistance of a spectrophotometer. Yeast cell counting was done first with a hemocytometer to determine the yeast cell number (as a correlation between the OD600). The yeast cell was very viable 95.9%. The trypan blue dye exclusion and OD600 methods both suggest viability of the cells and effectiveness in interacting with the extract fractions as well as the glucose substrate. Single re-suspension, double re-suspension, first and second re-suspension for both re-suspensions, the YPDM with the cells and PBS for the, independent T-test and paired T-test gave similar result in each of the assays, in all significant results were found; suggesting the effectiveness of the trypan blue dye cell viability assay and the OD600 method were compared and verified. Therefore, the baker's yeast (Saccharomyces cerevisiae) could further be recommended as the perfect eukaryotic microorganism for biochemical investigations.*

Keywords: Yeast cell culture, Cell viability, Insulin-Like Therapy, Abelmoschus esculentus L, Musa paradisiaca, and Dioscorea dumetorum

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INTRODUCTION

Viability stages and propagation proportions of cells are decent signs of cell well-being. Physical and chemical mediators can disturb cell well-being and metabolism (Aslantürk, 2017). These mediators may originate harmfulness on cells through diverse devices like the damage of cell membranes, stoppage of protein synthesis, permanent binding to receptors, a reserve of polydeoxynucleotide elongation, and enzymatic responses (Ishiyama *et al*; 1996) to see the death initiated by these devices; there is a need for reasonable, dependable, and repeatable temporary cytotoxicity and cell viability assays. In vitro cell, viability and cytotoxicity assays with cultured cells are extensively used for cytotoxicity experiments of chemicals and various medication screening. The use of these assays has been of skyrocketing attention lately. These assays are also utilized in ontological investigations to measure compound harmfulness and tumor cell growth reserve in the course of drug development. Since they are fast, cheap, and do not involve the use of animals.

A nutrient may be a material used by an organism to live, develop, and replicate. The condition for dietary nutrient intake applies to animals, plants, fungi, and protists. Nutrients are frequently combined into cells for metabolic processes or excreted to get non-cellular structures, like hair, scales, feathers, or exoskeletons. Some nutrients may enter into smaller molecules within the process of freeing energy, like carbohydrates, lipids, proteins, and fermentation products, leading to end-products of water and greenhouse emissions. All organisms need water. Essential nutrients for animals are the energy sources, several amino acids combined to supply proteins, a subgroup of fatty acids, vitamins, and certain minerals. Plants need more varied minerals absorbed through roots, plus greenhouse gas and oxygen absorbed through leaves. Fungi live on dead or living organic matter and meet nutrient requirements from their host.

Diverse sorts of organisms have diverse essential nutrients. For example, vitamin C (ascorbic acid) is crucial, meaning it must be in adequate quantities to humans and a few other animal species, but not to all animals and not plants, which can manufacture it. Nutrients are also organic or inorganic: organic compounds include most compounds comprising of carbon, while all other chemicals are inorganic. Inorganic nutrients include nutrients like iron, selenium, and zinc, while organic nutrients comprise, among numerous others, energy-providing compounds and vitamins.
Usually, *Abelmoschus esculentus L* (okra) may be a high-value crop because it characterizes a source of nutrients that are significant to human health, e.g., vitamins, potassium, calcium, carbohydrates, dietary fiber, besides unsaturated fatty acids similar linolenic also oleic acids, then equally of bioactive compounds (Moyin-Jesu, 2007; Habtamu *et al*; 2014). Okra may be a versatile crop thanks to the various use of its leaves, buds, flowers, pods, stems, and seeds (Mihretu *et al*; 2014). Okra has long been a vegetable and a source of dietary drug (Maganha *et al*; 2010;

Benchasr, 2012; Messing *et al*; 2014; Roy *et al*; 2014). Indeed, apart from its nutritional role, it is appropriate for a few therapeutic and industrialized uses (Benchasr, 2012) The profile of the bioactive constituents in several parts of okra is well accepted: for okra pod polyphenolic mixtures, carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acids (Roy *et al*; 2014; Jain *et al*; 2012; Gemede *et al*; 2015; Petropoulos *et al*; 2018); for okra seed polyphenolic mixtures, mostly oligomeric catechins and flavonol byproducts, protein (i.e., high lysine levels), and oil segment (in specifically, (Durazzo *et al*; 2018) its resultant oil is rich in palmitic, oleic, and linoleic acids) (Arapitsas, 2008; Adelakun *et al*; 2009; Adelakun and Oyelade, 2011; Jarret *et al*; 2011; Dong *et al*; 2014; Hu *et al*; 2014; Steyn *et al*; 2014; Durazzo *et al*; 2018; Wei *et al*; 2016); for root carbohydrates and flavonol glycosides (Sunilson *et al*; 2008), and primarily minerals, tannins, and flavonol glycosides for leaves (Idris *et al*; 2009; Calueta *et al*; 2014). Liao *et al*; (2012) called the occurrence in various proportions of the whole phenolics and total flavonoids and antioxidant properties in an exceedingly diverse part of plants, i.e., flower, fruit, leaf, and seed. Several okra constituents (flavonoids, polysaccharides, and vitamins) own significant biological activities (Durazzo, 2017). The valuation of relations of bioactive constituents during the quantifying of antioxidant properties (Durazzo, 2017) characterizes a first stage for understanding their biological actions and beneficial properties.

Musa paradisiaca (unripe plantain) contains the subsequent proximate composition: Moisture 59g, Crude protein 7.7g%, Crude lipid 1.5g%, Ash 1.4g%, Crude fiber 1.4g%, Carbohydrate 24.4g%, and gross energy 148.6Kcal/100g. At the same time, the mineral composition consists of sodium 200mg, Potassium 370mg, Calcium 126.5g, Magnesium 375mg, Iron 2.53mg, Phosphorus 220mg, Zinc 3.74mg, Manganese 2.99mg, and Copper 1.66mg (Adepoju *et al*;2012).

The proximate mineral and phytochemical composition of aqueous extract of Dioscorea dumetorum (bitter yam) contain the following: Crude protein 6.44g%, Crude fat 0.75g%, Crude fibre 15.00g%, Total ash 3.45g%, Moisture 70.04g, Carbohydrate 19.36%, and energy 109.5kcal/100g. Potassium 17,036.00ppm (part per million), Magnesium 1,630.50ppm, Sodium 521.00ppm, Calcium 484.50ppm, Iron 204.75ppm, Manganese 55.25ppm, Copper 17.40ppm, Zinc 10.70ppm, and phosphorus 11.55ppm. Also, Flavonoids, Alkaloids, Saponins, and Cardiac glycosides are present in small quantities. At the same time, tannins and anthraquinones are absent (Nimenibo and Oriakhi, 2017).

Previously, the invention of insulin in 1922 for the management of diabetes depended largely on dietary processes, which comprised the utilization of plant treatments. Various plant managements for diabetes exist (Bailey and Day, 1989; Swanston-Flatt *et al*; 1991, Gray and Flatt, 1997a). Nevertheless, some through scientific or therapeutic study and the World Health Organization (WHO) have suggested that plant managements for diabetes deserve more assessment (WHO, 1980).

An anti - diabetic agent can exercise a beneficial effect within the diabetic condition by increasing insulin secretion, improving and imitating insulin action (Gray and Flatt 1997a).

The discovery of insulin

In 1889 by German scientists Minkowski and Von Mering acknowledged that total pancreatectomy gave rise to severe diabetes (Bliss, 1993). They postulated that a material secreted by the pancreas was responsible for metabolic regulation. Other scientists advanced this postulation, observed that diabetes with the damage of the islets of Langerhans within the cell of the pancreas. Although Minkowski, as well as Zuelzer in Germany and Scott within the USA, tried, without a successful outcome, to separate and administer the absent pancreatic islet material. Belgian researcher de Meyer in 1909 suggested the name "insulin," as did British scientist Schaefer in 1916 (Wilcox, 2005).

Lastly, in 1921, insulin was eventually separated, purified, and presented in an exceedingly way capable of beneficial management. In May 1921, Toronto surgeon Banting, supported by medical scholar Best, and with the supervision of McLeod, Professor of Carbohydrate metabolism, started experimentations in dogs. They directed ice-cold saline extracts of the pancreas intravenously to dogs induced diabetic by pancreatectomy and detected a decrease in glucose. In December 1921, this effort was made known to the American Physiological Association, and biochemist James Bertram Collip, who became a part of the team, confirmed that this extract also repaired hepatic glycogen utilization and the ability to clear ketones. Within one month, precisely in January 1922, a unique human trial started on a diabetic young boy of 14-years who had clinical indications and biochemical defects that to normal by giving the separated pancreatic substance. May 1922, an active substance was called insulin, and therefore the outcomes of those trials were revealed to the Association of American Physicians. Eli Lilly afterward started producing porcine insulin, improving purification by iso - electric precipitation, producing marketable sizes by 1923. Banting and McLeod were honoured for their efforts in 1923 (Bliss, 1993).

Nutraceuticals

The idea of nutraceuticals as pharma-foods originates way back. The term came from two words "nutrient" and "pharmaceutical," was invented by Stephen DeFelice, and is defined as "a food or a part of a food that supply medicinal or health benefits, likewise because of the inhibition and management of an illness" (DeFelice, 1995). This definition results in a limited reference to the definition of a food supplement. The two promise health benefits; nevertheless, nutraceuticals from food or a part of food; food supplements are only one material used unaided or in combinations with the likelihood of adding micronutrients once the body requires them. The part defined by DeFelice (DeFelice, 1995)— specifically the protective part and therefore the management of an illness—is lacking within the definition and possibility of food supplements, which may be a support for the body but do not seem to be necessary to possess a longtime clinical efficacy on a

health condition. Building on these concerns, it consequently seems to be of paramount significance to coin a special definition for nutraceuticals predicting their usage "outside the diet" as apparatus capable of stopping or delaying the start of some asymptomatic protracted pathological disorders; for example, hypercholesterolemia, and hypertriglyceridemia. The stages in an exceedingly novel nutraceutical preparation ought to start with identifying the most pathologic disorder, akin to what occurs for drug preparations (Santini and Novellino, 2017).

Cell Viability

The present study analyzes the possible presence of natural product(s) in an aqueous extract of mixed food plants, which stimulates insulin secretion.Viability stages and propagation proportions of cells are decent signs of cell well-being. Physical and chemical mediators can disturb cell well-being and metabolism (Aslantürk, 2017). These mediators may originate harmfulness on cells through diverse devices like the damage of cell membranes, stoppage of protein synthesis, permanent binding to receptors, a reserve of polydeoxynucleotide elongation, and enzymatic responses (Ishiyama *et al*; 1996) to see the death initiated by these devices; there is a need for reasonable, dependable, and repeatable temporary cytotoxicity and cell viability assays. In vitro cell, viability and cytotoxicity assays with cultured cells are extensively used for cytotoxicity experiments of chemicals and various medication screening. The use of these assays has been of skyrocketing attention lately. These assays are also utilized in oncological investigations to measure compound harmfulness and tumor cell growth reserve in the course of drug development. Since they are fast, cheap, and do not involve the use of animals.

Besides, they help test an excessive number of samples. Cell viability and cytotoxicity assays are supported several cell functions like cytomembrane penetrability, enzyme action, cell observance, ATP creation, co-enzyme creation, and nucleotide uptake action (Ishiyama *et al*; 1996). In vitro cytotoxicity and cell viability assays have merits, like rapidity, cheap rate, and possibility for computerization, and investigations with human cells could be more applicable than some in vivo animal investigations. Though, they have some demerits since they are not strictly sophisticated enough to switch animal investigations (Chrzanowska *et al*; 1990). it is crucial to understand what percentage of viable cells are left and what number of cells are dead at the close of the experimentation—a wide range of cytotoxicity and cell viability assays have presently been engaged in the fields of toxicology and pharmacology. The selection of assay technique is critical in evaluating the interface nature (Sliwka *et al*; 2016).

Classification of cytotoxicity and cell viability assays

While there are diverse categorizations for cytotoxicity and cell viability assays, these assays exert inversely depending on the analytical endpoints (colour variations, fluorescence, and luminescence).

Dye exclusion: Trypan blue, eosin, Congo red, erythrosine B assays.

Colorimetric assays: MTT assay, MTS assay, TXT assay, WST-1 assay, WST-8 assay, LDH assay, SRB assay, NRU assay, and crystal violet assay.

Fluorometric assays:
AlamarBlue assay and CFDA-AM assay.

Luminometric assays:
ATP assay and real-time viability assay.

Dye exclusion assays

Various methods have experimented with the number of viable cells during a cell population. The only and extensively used approach is the dye exclusion method. Viable cells exclude dyes within the dye exclusion method, but dead cells do not exclude the dye. While the discoloration technique is modest, many samples are challenging and slow (Yip and Auersperg,1972). Nevertheless, the determination of membrane integrity is feasible through the dye exclusion method. A range of such dyes is engaged, including eosin, Congo red, erythrosine B, and trypan blue (Bhuyan *et al*; 1976, Krause *et al*; 1984). Among the dyes listed, trypan blue has been used primary widely (Eisenbrand *et al*; 2002, Aslantürk and Askin, 2013a, Aslantürk and Askin, 2013b, Aslantürk and Askin, 2013c). If dye exclusion assays must be applied, the subsequent factors should be looked at:

1. Lethally impaired cells by cytotoxic mediators may necessitate a number of days to lose their membrane integrity.
2. The living cells may still multiply.
3. Approximately lethally impaired cells are not discolored with dye at the beginning of the culture duration since they will experience an initial fragmentation.

Factors (1) and (3) may bring about miscalculation of necrobiosis when the outcome of the assay is supported by percentage viability observation (Weisenthal *et al*; 1983, Son *et al*; 2003, Lee *et al*; 2005). Dye exclusion assays have distinctive merits for chemosensitivity analysis. They are relatively modest, involve lesser quantities of fast cells, and identify cell dead in non-dividing cell populations. More studies into the likely part of those assays in chemosensitivity analysis are necessary (Weisenthal *et al*; 1983). Conversely, none of those dyes are for use on monolayer cell cultures; but rather, they are for cells in suspension; therefore, monolayer cells must first be trypsinized (Krause *et al*; 1984).

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Trypan blue dye exclusion assay

This dye exclusion assay is employed to see the quantity of viable and dead cells in the same cell suspension. Trypan blue may be a big charged molecule. Trypan blue dye exclusion assay relies on the standard that living cells possess complete cell membranes that exclude this dye, whereas dead cells do not. During this assay, adherent or nonadherent cells with serial dilutions of test compounds for various times. After the compound treatment, cells and suspension. The cell suspension with dye, so visually examined to see whether cells take up or exclude dye. Viable cells will have a transparent cytoplasm, whereas dead cells will have a blue cytoplasm (Johnston, 2010, Strober, 2001). Light microscopy shows the number of viable and dead cells per unit volume as a percentage of untreated control cells (Strober, 2001, Aslantürk and Askin, 2013d).

Merits: This method is easy, cheap, and an authentic sign of membrane integrity (Ruben,1988), and dead cells are coloured blue within seconds of contact with the dye (Stone *et al*; 2009).

Demerits: Cell counting primarily employs a hemacytometer (Absher, 1973). So, counting errors (approximately 10%) can happen. They are counting errors to reduced diffusion of cells, cell loss in the course of cell diffusion, wrong dilution of cells, inappropriate filling of the chamber and incidence of air bubbles within the chamber (Absher, 1973). In addition, although the discoloration technique is modest, it is challenging to process an excessive number of samples simultaneously, mainly where the precise scheduling of advanced cytotoxic effects is necessary (Yip and Auersperg, 1972).

Additionally, trypan blue discoloration cannot differentiate amongst healthy and alive cells but lose cell roles. Consequently, it is not adequately profound to use for in vitro cytotoxicity analysis. Another demerit of trypan blue is the harmful side effect of this dye on mammalian cells (Kim *et al*; 2016).

Erythrosine B dye exclusion assay

Erythrosine B, also called erythrosine or Red No. 3, is mainly used as a colouring means (Kim *et al*; 2016; Marmion, 1979). Erythrosine B has previously been a basic dye for counting viable cells. The standard of this dye exclusion assay is comparable to the trypan blue dye exclusion assay standard. While erythrosine B is another bio-safe dynamic dye for cell counting, it is not extensively accustomed to counting viable or dead cells.

Merits: The merits are low cost, resourcefulness, and bio-safety (Kim *et al*; 2016).

Demerits: Its technique is slow and labour-intensive. Furthermore, possible demerits comprise impurity of reusable cell counting chamber, differences in hemocytometer filling proportions, and inter-user differences (Kim *et al*; 2016).

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Colorimetric assays

The standard of colorimetric assays is that the extent of a biochemical marker to judge the metabolic action of the cells. Chemicals employed in colorimetric assays change colour in reaction to the viability of cells, permitting the colorimetric assaying of cell viability through a spectrophotometer. Colorimetric assays are appropriate for adherent or suspended cell lines, stress-free to execute, and relatively cheap (Präbst *et al*; 2017a, *Präbst et al;* 2017b). Marketable kits of colorimetric assays are obtainable from numerous companies, and routinely investigational techniques of those assays are obtainable in kit packages.

MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide) assay is one among the foremost frequently employed colorimetric assay to evaluate cytotoxicity or cell viability (Mosmann, 1983). This assay controls cell viability mainly by defining cells' mitochondrial role by quantifying the action of mitochondrial enzymes like succinate dehydrogenase (Stone *et al*; 2009). During this assay, MTT is changed to purple formazan by NADH. The outcome may be measured by light absorbance at a selected wavelength.

Merits: This technique is much higher than the earlier stated dye exclusion methods since it is stress-free to apply, harmless, has great repeatability.

Demerits: MTT formazan is difficult to dissolve in water, developing purple needle-shaped cells. Hence, before determining the absorbance, an organic solvent like dimethyl sulfoxide (DMSO) or isopropanol is essential to make the crystals soluble. Moreover, the cytotoxicity of MTT formazan makes it challenging to get rid of cell culture media from the plate wells thanks to freeing the cells with MTT formazan needles, giving important well-to-well inaccuracy (Stone *et al*; 2009, Bopp and Lettieri, 2008). Further regulating experimentations ought to be done to measure back false-positive or false-negative outcomes produced by background nosiness because of the presence of particles. This nosiness can result in overestimating the cell viability. It will often happen by deducting the background absorbance of the cells within the existence of the particles, however lacking the assay chemicals (Stone *et al*; 2009, Bopp and Lettieri, 2008).

MTS assay

The MTS assay (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethyl-thiazolyl)-3-(4-sulfophenyl) tetrazolium, inner salt assay) could be a colorimetric assay. This assay relies on converting a tetrazolium salt into a coloured formazan by the mitochondrial action of living cells. The quantity of formazan formed depends on the viable cell number in culture and might read with a spectrophotometer at 492 nm.

Merits: Prior research advocate that the MTS in vitro cytotoxicity assay syndicates all structures of a good analyzed method in stress-free to engage, accuracy, and a fast sign of toxicity (Berg *et al*; 1994, Tominaga *et al*; 1999). MTS assay could be a fast, profound, cheap, and precise in vitro cytotoxicity. The process of this assay is incredibly modest to other toxicological experiments. This assay offers the best assets for cytotoxicity analysis since it is stress-free to engage, fast, dependable, and cheap. So, it will be for urgent toxicological evaluations (Berg *et al*; 1994, Cory *et al*; 1991, Riss and Moravec, 1992, Promega, 1996).

Demerits: The extent of absorbance read at 492 nm is inclined by the cultivation period, cell type, and cell number. The amount of MTS indicating chemicals to cells in a culture likewise affects the analyzed absorbance level. Earlier research recommended a linear association among cultivation period and absorbance for brief cultivation intervals of about five hours (Cory *et al*; 1991, Rotter *et al*, 1993, Buttice *et al*; 1993). Thus, appropriate cultivation periods for this assay are between one to three hours.

TXT assay

A colorimetric method supported the tetrazolium salt TXT (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-carboxamide-2H-tetrazolium, monosodium salt) was initially defined by Scudiero *et al*. (Scudiero *et al*; 1988). Although MTT formed a formazan compound that is not soluble in water which is essential in liquefying the dye so as to take its absorbance, the TXT yields a water-soluble dye. The technique of TXT is just for determining multiplication and is, consequently, a superb answer for estimating cells and analyzing their viability. TXT is employed to assay cell multiplication as a reaction to diverse growth dynamics. It is likewise required for assaying cytotoxicity. This assay relies on the power to reduce the tetrazolium salt TXT to orange-coloured formazan compounds by lively metabolic cells. Orange-coloured formazan is water-soluble, and its strength could differ with a spectrophotometer. There is a linear association among the strength of the formazan and, therefore, the quantity of viable cells. The employment of multi-well plates and a spectrophotometer (or ELISA reader) permits for analysis of many samples and getting outcomes without difficulty and fast. This assay technique comprises cell culture through a 96-well plate, adding the TXT chemical, and cultivation for a period of two to twenty-four hours. Through the cultivation period, the orange colour and the strength of the colour could differ with a spectrophotometer (Scudiero *et al*; 1988, Promokine manual http://www.promokine.info).

Merits: TXT assay is a rapid, profound, stress-free to engage, and harmless technique. It is high in profoundness and precision (Promokine guide –http://www.promokine.info).

Demerits: TXT assay ability is subject to the decreasing measurements of viable cells with mitochondrial dehydrogenase action. Thus, variations within the decreasing measurements of viable cells subsequent to the enzymatic control, pH, cellular ion concentration (e.g., sodium,

calcium, potassium), cell cycle difference, or further ecological influences may affect the ultimate absorbance analysis (Scudiero *et al*; 1988).

WST-1 assay

WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium monosodium salt) cell multiplication assay could be a modest, colorimetric assay intended to live the comparative production rates of cells in culture. The standard of this assay is predicated on the change of the tetrazolium salt WST-1 into an extremely water-soluble formazan by mitochondrial dehydrogenase enzymes within the existence of an intermediate electron acceptor like mPMS (1-methoxy-5-methyl-phenazinium methyl sulfate) (Ishiyama *et al*; 1993). The water-soluble salt is freed into the cell matter. Within the cultivation time required, the response yields a colour conversion that is equivalent to the number of mitochondrial dehydrogenases in cell culture, and therefore, the assay analyzes the metabolic action of cells. The WST-1 reagent that is prepared to be engaged is added right into the media of cells cultured in multi-well plates to carry out the assay. The cultures are at that point specified thirty minutes to four hours to cut back the chemical into the dye system. The plate is instantly measured at 450 nm with a compared measurement at 630 nm.

Merit: It is stress-free, harmless, has great repeatability, and is extensively accustomed to evaluate both cell viability and cytotoxicity experiments. Moreover, phenol red indicators in cell materials do not inhibit the dye response. Finally, since the coloured dye formed at the top of the investigation is water-soluble, it does not need a solvent and extra cultivation period.

Demerits: The quality cultivation period of WST-1 is two hours. If the one-time adding of WST-1 could reproduce the outcome of the analyzing means at the diverse period on the tendency of comparative cell viability remains uncertain.

WST-8 assay

WST-8 assay could be a colorimetric assay for the analysis of viable cell statistics and may be accustomed to cell propagation assays in addition to cytotoxicity assays. WST-8 (2-(2-methoxy4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt), an extremely steady and water-soluble WST, is employed in Cell Counting Kit-8 (CCK-8). It is additionally profound than WST-1, mainly at neutral pH. Due to the electron mediator, 1-methoxy PMS during this kit is extremely steady, and CCK-8 is steady for a minimum of six months at a temperature of 0–5^{o}C and for one year. Subsequently, WST-8, WST-8 formazan, and 1-methoxy PMS do not have any cytotoxicity on cells within the culture media, the identical cells from the earlier assay could be accustomed for different experimentations.

Merits: WST-8 is not cell-permeable, which ends in very little cytotoxicity effect. Hence, it is likely to carry on additional experimentations using identical cells after the assay. Also, it yields the water-soluble formazan once the cells are reduced, which might offer further merit to the strategy by permitting a more straightforward assay technique and not demanding an additional stage to liquefy the formazan (Tominaga *et al*; 1999).

Demerits: A critical concern is the decrease of assay substrates affected by variations in intracellular metabolic actions that has no real influence on the general cell viability (Strober, 2001).

LDH (lactate dehydrogenase) assay

LDH (lactate dehydrogenase) cytotoxicity assay may be a colorimetric technique of analyzing cellular cytotoxicity. LDH Cytotoxicity Assay Kit with diverse cell types not only for analyzing cell-mediated cytotoxicity but also for measuring cytotoxicity mediated by lethal chemicals and additional experimental compounds. The assay quantitatively analyzes the steady, cytosolic, lactate dehydrogenase (LDH) enzyme. This enzyme is emitted from impaired cells. LDH is an enzyme within the cell cytoplasm. Once cell viability has decreased the leakiness of the cell walls increases, so the LDH enzyme is emitted into the cell substance. The emitted LDH with a coupled enzymatic response ends up changing a tetrazolium salt (iodonitrotetrazolium (INT)) into a red colour formazan by diaphorase. Within the start, LDH catalyzes the changing of lactate to pyruvate, and therefore, NAD to NADH/H+. In an exceedingly succeeding step, catalytic agent (diaphorase) handovers H/H^+ from $NADH/H^+$ to the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT), which to red formazan (Decker and Lohmann – Matthes, 1988, Lappalanien *et al*; 1994). The LDH action as NADH oxidation or INT decrease over an outlined fundamental measure. The subsequent red formazan absorbs excellently at 492 nm and may be analyzed for the quantity of the substance at 490 nm. The detergent Triton X-100 is often used as an anion standard within the LDH assay to see the utmost LDH emission from the cells. Furthermore, prominent membranolytic particles like crystalline silica could be accustomed as an anion standard in LDH assay (Schins *et al*; 2002).

Merits: Dependability, rapid, and straightforward assessment are a number of the features of this assay. Since the loss of intracellular LDH and its emission into the medium; is a sign of permanent death thanks to semipermeable membrane impairment (Decker and Lohmann – Matthes, 1988, Fotakis and Timbrell, 2006).

Demerits: The critical constraint of this assay is that serum and a few other compounds have characteristic LDH action. For instance, the fetal calf serum has tremendously great background analysis. Thus, this assay is proscribed to serum-free or low-serum settings, restraining the assay culture timing (subject to the cells' capability to low serum) and decreasing the possibility of the

assay because it cannot permit the measurement of necrobiosis initiated as a result of natural growth circumstances (i.e., in 10% fetal calf serum). Therefore, at least, one ought to always initially experiment the assay with a new aliquot of the media one proposes to accustom and equate the analysis from media requiring enhancements (e.g., straight DMEM) (Kumarasuriyar, 2007).

SRB (Sulforhodamine B) assay

SRB (Sulforhodamine B) assay could be a fast and profound colorimetric technique for determining the therapeutic-induced cytotoxicity in either attached or suspension cell cultures. As initially defined by Skehan and associates, this assay is within the disease-orientated, extensive anticancer medication detection program of the National Cancer Institute (NCI) that was propelled in 1985. SRB could be a bright pink aminoxanthene dye through two sulfonic groups. In slightly acidic environments, SRB attaches to protein primary aminoalkanoic acid residues in TCA-fixed (trichloroacetic acid) cells to supply a profound guide of cellular protein. In addition, SRB assay assesses colony development and elimination (Skehan *et al*; 1990).

Merits: The SRB assay is easy, rapid, and profound. It gives a better straightforward process with cell quantity, allowing the utilization of flooding dye applications, is a smaller amount profound to ecological variabilities, is free of intermediary metabolism, and gives a set endpoint that does not need a period-profound quantification of first response velocity (Skehan *et al*; 1990). The repeatability of this assay is great.

Demerits: It is essential to get and retain an identical cell suspension. Cellular clumps/aggregates have to be eluded for excellent assay outcomes.

NRU (neutral red uptake) assay

The neutral red uptake (NRU) assay is one of the foremost accustomed colorimetric cytotoxicity and cell viability assays. This assay was established by Borenfreund and Puerner (Borenfreund and Puerner, 1984). This assay has supported the flexibility of viable cells to require up the supravital dye neutral red. This faintly positive dye enters cell membranes by non-ionic inert dispersal and stays within the lysosomes. The dye is then removed from the viable cells accustoming an acidified ethanol solution, and therefore the absorbance of the dye is read accustoming a spectrophotometer. Neutral red uptake relies on the ability of cells to maintain pH slopes with ATP formation. At physiological pH, the overall charge of the dye is zero. This charge enables the dye to enter the cell membranes. A proton gradient keeps up a pH under the cytoplasm inside the lysosomes. Therefore, the dye develops charges and within the lysosomes. Once the cell dies or the pH slope is decreased, the dye cannot be reserved. Furthermore, the uptake of neutral red by viable cells alters cell exterior or lysosomal membranes. Therefore, it is likely to differentiate amongst viable, impaired, or dead cells (Borenfreund and Puerner, 1984). Lysosomal uptake of neutral red dye may be an excellent sign of cell viability. The assay can quantify cell

viability and evaluate cell duplication, cytostatic effects, or cytotoxic effects on the cell's density (Repetto *et al*; 2008). Absorbance is read at 540 nm in a multi-well plate reader spectrophotometer.

Merits: NRU assay could be a better marker of lysosomal impairment. Similarly, rapidity and ease of assessment are some merits of this assay.

Demerits: It is known that the NRU assay is either slightly or not in the most petite full of usual influences, like temperature and salinity, but by contaminants (Ringwood *et al*; 1998).

CVS assay (crystal violet assay)

Supporter cells separate from cell culture plates through necrobiosis. This parameter is often accustomed for the indirect evaluations of cell death and to analyzed changes in propagation rate after inducement with cytotoxic substances. A straightforward technique to identify sustained supporter cells is crystal violet assay. During this assay, crystal violet dye attaches to the proteins and DNA of viable cells and therefore binds cells with this dye. Cells lose their support through necrobiosis and are afterward lost from the cell group, decreasing the number of anthelminthic discolorations through a culture. Antimycotic substance assay may be a fast and dependable analyzing technique appropriate for investigating the effect of therapeutic substances or added compounds on cell existence and growth hindrances.

Merits: Bactericide staining may be a fast and multipurpose assay for determining cell viability in different inducement settings (Geserich *et al*; 2009). Though, it by proliferative responses that coins with necrobiosis reactions. Consequently, chemical hindrances of caspases and necroptosis into the assay (Degterev *et al*; 2008, Sun *et al*; 2012). On the other hand, molecular investigations (e.g., higher estimations or reduction) are executed to more precisely cater to the issues of cell death (Feoktistova *et al*; 2011).

Demerits: Antimycotic substance assay is not versatile to variations in cell metabolic action. So, this assay is unsuitable for investigations involving cell metabolism-affected substances. In addition, while helminthic assay is appropriate for investigating the effect of therapeutic substances or added compounds on cell existence and growth hindrance, it is not capable of living cell propagation rate (Feoktistova *et al*; 2011).

Fluorometric assays

Fluorometric assays of cell viability and cytotoxicity are simple to carry out with the employment of a fluorescence microscope, fluorometer, fluorescence microplate reader, or flow cytometer, and that they provide numerous merits over customary dye exclusion colorimetric assays. Fluorometric assays similarly are appropriate for supporter or suspended cells and are simple to accustomed. These assays are more versatile than colorimetric assays (Page *et al*; 1993, O' Brien *et al*; 2000,

Riss *et al*; 2013). Marketable kits of fluorometric assays are accessible from numerous corporations, and customarily investigational techniques of those assays are accessible in kit packages.

Alamar-Blue (AB) assay

The Alamar-Blue assay is additionally called resazurin decreasing assay. The Alamar-Blue assay relies on changing the blue non-fluorescent dye resazurin, which is changed to the pink fluorescent resorufin by mitochondrial and other enzymes like diaphorase (O' Brien *et al*; 2000). Resazurin could be a phenoxazin-3-one dye and cell-permeable reduction-oxidation sign, which will screen viable cell quantities with procedures the same as those applying the tetrazolium compounds (Ahmed *et al*; 1994). It is recognized to perform as an intermediary electron acceptor within the electron transport chain amongst the ultimate losing of oxygen ions and cytochrome oxidase by replacing molecular oxygen as an electron acceptor (Page *et al*; 1993). It is a harmless and cell-permeable substance. The colour of this substance is blue, and it is non-fluorescent. Subsequently, when applied to cells, resazurin changes to resorufin. Resorufin is red and an extremely fluorescent substance. Viable cells change resazurin constantly to resofurin, accumulating the general fluorescence and colour of the cell substance. The number of resofurin formed is the number of viable cells. The proportion of viable cells will be measured employing a microplate reader fluorometer fitted out with a 560 nm excitation per 590 nm emitting filter fixed. Absorbance changes can even measure Resofurin, but identifying absorbance is a lowly versatile than fluorescence evaluation. The cultivation time essential to get enough fluorescent indicator beyond the background is sometimes an interval of one to four hours, relying on the metabolic action of the cells, the cell bulk per well, and added circumstances like the culture medium nature (Riss *et al*; 2013).

Merits: AlamarBlue (resazurin reducing ion) assay is comparatively cheap and more versatile than tetrazolium assays. Likewise, it with other techniques like determining caspase action to collect added data regarding the cytotoxicity process.

Demerits: Fluorescent nosiness from experimental substances and the frequently ignored direct lethal impacts on the cells are likely (Riss *et al*; 2013).

CFDA-AM assay

CFDA-AM (5-carboxyfluorescein diacetate, acetoxymethyl ester) is an alternative fluorogenic dye accustomed for cytotoxicity measurement. It is a sign of semipermeable membrane reliability. The dye CFDA-AM may be a harmless esterase substrate that may be changed by basic esterases of viable cells may as well change from a membrane-permeable, nonpolar, non-fluorescent compound to polar, dyestuff, carboxyfluorescein (CF). The change of CFDA-AM to CF by the

cells point toward the reliability of the cell membrane because just a complete membrane could sustain the cytoplasmic environment required.

Merits: CFDA-AM and alamarBlue assays were revealed to be relevant in equivalent on the identical fixed cells, meanwhile both are harmless to cells, needs equal cultivation periods, and may detect at diverse wavelengths devoid of nosiness (Ganassi *et al*; 2000, Scheer *et al*; 2005, Bopp *et al*; 2006).

Demerits: Fluorescent nosiness from experimental substances is feasible.

Protease viability marker assay (GF-AFC assay)

The evaluation of viable cells preserved and constitutive protease enzyme action is an authentic sign of cell viability. A cell-permeable fluorogenic protease substrate (glycylphenylalanyl-aminofluorocoumarin; GF-AFC) has been lately established to identify protease action that is limited to viable cells (Niles *et al;* 2009). The GF-AFC substrate will enter viable cells. In these cells, cytoplasmic aminopeptidase action eliminates the gly and the phe amino acids to discharge aminofluorocoumarin (AFC) and yields a fluorescent indicator comparative to the number of viable cells (Riss *et al*; 2013). After cells die, this protease action is quickly missing. Consequently, this protease action could be a careful marker of the viable cell population. The sign produced from this assay method connects well with other conventional techniques of measuring cell viability, like an ATP assay (Riss *et al*; 2013).

Merits: It is comparatively harmless to cells in culture. Similarly, instead of exposing the cells to tetrazolium, continual exposing of the GF-AFC substrate cells affects the modification within the viability of the cells. This assay is appropriate for substituting with further assays since the cell population does not lose its viability at the top of the assay and may be for successive assays. Likewise, the cultivation period is far smaller (thirty minutes to one hour) than the one to four hours necessary for the tetrazolium assays (Riss *et al*; 2013).

Demerits: Fluorescent nosiness from experimental substances is feasible.

Luminometric assays

Luminometric assays offer a quick and straightforward measurement of cell propagation and cytotoxicity in mammalian cells. These assays may occur during an appropriate 96-well and 384-well microtiter plate design and identification by a luminometric microplate reader (Riss *et al*; 2013, Duellman *et al*; 2015, Mueller *et al*; 2004). A motivating characteristic of the luminometric assays is the continuous and steady light sign formed once the chemical is added. This quality supplies viability and cytotoxicity values from the identical well (Niles *et al*; 2009). Marketable kits of luminometric assays are accessible from numerous corporations, and usually, investigational protocols of those assays are accessible in kit packages.

European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

ATP assay

ATP (adenosine tri-phosphate) characterizes the primary energy reservoir in cells and for biological production, indication, carrying, and translocation processes. Consequently, cellular ATP is one of the foremost versatile outcomes in determining cell viability (Maehara *et al*; 1987). Once cells are impaired severely and devoid of membrane reliability, they are also devoid of the power to produce ATP, and likewise, the ATP measure of cells reduces vividly (Riss *et al*; 2013, Garcia and Massieu, 2003). The principle of ATP assay depends on the reaction of changing luciferin to oxyluciferin. The enzyme luciferase converts luciferin to oxyluciferin in this reaction within the presence of magnesium ions and ATP, producing a luminescent indication. Thus, there seems to be a direct relationship between the strength of the luminescent indication and ATP concentration (Mueller *et al*; 2004) or cell quantity (Andreotti *et al*; 1995). The ATP assay interaction can identify less than ten cells per well, so it has been extensively accustomed in the 1536-well this related design.

Merits: ATP assay is the rapidly known cell viability assay to accustomed, the foremost versatile, and only a little amount is at risk of objects being involved than similar viability assays. The luminescent indication gets to a balanced steady state in the interval of ten minutes when the required chemical is added. In addition, it does remove a cultivation stage for changing the substrate into coloured substances. It also excludes a plate regulating stage (Riss *et al*; 2013).

Demerits: The ATP assay versatility is reduced by the repeatability of measuring duplicate samples instead of the outcomes of the assay interaction (Riss *et al*; 2013).

Real-time viability assay

Lately, there has been a novel techniquein advancing the determining the number of viable cell statistics in real-time (Duellman *et al*; 2015). Now, this technique describes a contrived luciferase from an aquatic shrimp and a lesser low molecule pro-substrate is accustomed through this assay. The pro-substrate and luciferase are mixed with the cell matter straightaway as a chemical. The pro-substrate does not belong as a substrate of luciferase. Dynamic breakdown of viable cells decreases the pro-substrate into a substrate, made possible by enzyme luciferase, and produces a luminescent indication. This assay can be carried out in two ways; i.e. continuous read and endpoint measurement. Within the continuous read method, the luminescent indication may be constantly being recorded from the sample wells over a prolonged time to determine the number of cells in "real-time" (Riss *et al*; 2013, Duellman *et al*; 2015).

Merits: This assay is a single known assay that permits real-time cell viability and cytotoxicity evaluations. The quick way in which luminescent indication reduces once cells are dead allowed combining this assay with other luminescent assays comprising a lysis stage that will destroy cells.

In addition, the decline in luminescence following necrobiosis is significant to remove nosiness with later luminescent assays (Riss *et al*; 2013, Duellman *et al*; 2015).

Demerits: The constraint involved in the real-time assay emanates from the ultimate reduction of pro-substrate by the breakdown of active cells. Largely, the luminescent indication produced is associated with the number of active cells broken down. Conversely, the time interval required for the luminescent indication to be equal to cell number will rely on the number of cells per well and the breakdown action. Consequently, the utmost cultivation period to keep up equality should be for every cell type and the measured density (Riss *et al*; 2013, Duellman *et al*; 2015).

The trypan blue dye exclusion assay is employed to establish the number of viable cells present during a cell suspension. It is based on the principle that living cells have intact cell membranes that exclude some dyes, like trypan blue, Eosin, or propidium iodide (PI), while dead cells do not. Cell suspension with the dye is then visually observed to establish whether cells like yeast take up or exclude dye. The viable cell will have a transparent cytoplasm, while the non-viable cell will be blue. Cell viability valuation offers the chance of a timely sign of the standard of recent cells before freezing. Viabilities above or up to 95% are excellent.

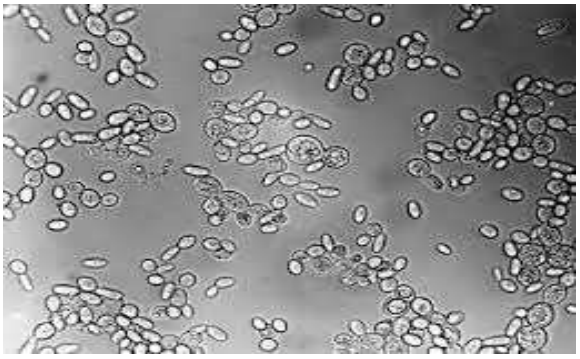
What are yeasts?

Yeast is a single cell organism called fungi. The exact organization employs the physiognomies of the cell, a formed spore, as well as a group of discrete organisms; physiognomies likewise are classified species. Moreover, the most recognizable physiognomies have the capacity to ferment sugars to produce ethanol. Yeast that buds are considered as true fungi of the phylum *Ascomycetes* of the class *Hemiascomycetes.* The true recognizable yeasts are divided into one central order referred to as *Saccharomycetales*.

Yeast is considered through an extensive distribution into the non-human-made settings and circumstances that makes every living and non-living thing that are present in the universe. Yeast is very often on the leaves of plant, flowers, on the soil, as well as the saltwater. In addition, yeasts could likewise be located on the outsides of skin and within the intestinal tracts of animal species that could sustain a body temperature greater than their surroundings, so that they could co-exist in close contact among two or more diverse species as well as in an association among species with one profiting at the detriment of the other. Furthermore, the only infection that is caused by a yeast is called Candidiasis which is initiated through the yeast-like fungus referred to as *Candida albicans*. Besides, *Candida albicans* causes vaginal yeast infections, Candida is also a reason for dermatitis and oral Candidiasis where *Candida albicans* is stored in the mouth enough to bring about symptoms.

European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Furthermore, yeasts increase as single cells that split by budding (example is, *Saccharomyces*) or direct splitting up (splitting up into parts, for instance, *Schizosaccharomyces*), or they could propagate as modest asymmetrical threads (mycelium). In sexual reproduction, the number of yeasts form asci, which incorporates eight haploid formed spore in the ascospores. These formed spore in the ascospore could blend with touching nuclei and proliferate by asexual splitting up or, like specific yeasts, blend with other ascospores.



Yeast cell line *(Saccharomyces cerevisiae)*

The tremendous influence of yeast genetics is partly because of the capacity to rapidly express the comparative sites of the visible physical properties of an entity splitting the chromosomes into lesser fragments that could be proliferated as a part of the *S. cerevisiae* genome. Additionally, in the last twenty years, *S. cerevisiae* has been the ideal organism for most of molecular genetic investigations since the fundamental method of double-stranded DNA molecule being copied to form two duplicate DNA molecules, fragments of DNA being fragmented as well as recombined to form fresh blends of alleles, procedures that are essential for an entity to exist, as well as form its abilities for relating with its surroundings, and biochemical responses in the physical cells that convert food into energy are mostly well-maintained among yeast and higher eukaryotes, as well as mammals.

The well-recognized, as well as profitable and important yeasts, are yeast's associated genetic variant of *Saccharomyces cerevisiae*. These organisms have for a very long time been employed to fermenting the sugars of rice, wheat, barley, and corn to yield drinks of ethanol origin as well as in baking to increase or enlarge dough. *Saccharomyces cerevisiae* is usually used as baker's yeast and for a few kinds of fermentation. Also, yeast is frequently recommended as a vitamin complement since it is fifty percent protein and could be rich in the B vitamins, like niacin, as well as vitamin B complex.

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Print ISSN: ISSN 2053-406X,
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History of yeast as well as yeast in history

Several anthropologists contemplate humans to ascertain agricultural, wheat-growing sophistication in 10,000 years Before Christ Existence (B.C.E). Within the rich hemispherical milieu of Sumeria, contemporary Iraq. Alcoholic beverage production started during a similar region, some six thousand years ago, that is; before the physical existence of Abraham.

Fermentation of crushed grains was perhaps well-thought-out as supernatural stuff of a suitably cared-for container. Today people have been moving around with these containers. Subsequently, yeast cultivation has constantly been meticulously related to human culture. It had been the work of a renowned chemist as well as microbiologist Louis Pasteur that a colony-purified was yeast. Also, baker's yeast (*Saccharomyces cerevisiae*) was gotten from European alcoholic beverages through purification. *Schizosaccharomyces pombe*was similarly discovered from the purification of African millet alcoholic beverages.

Yeast in respect to the tree of life

The classification system of living things into five kingdoms (plants, animals, fungi, protists, and monera) was developed before the existence of macromolecule as well as their contrasts among them. Plants, animals, and fungi are completely and meticulously connected to eukarya.

Yeast could be a model eukaryote

The baker’s yeast (Saccharomyces cerevisiae) is the perfect eukaryotic microorganism for biochemical investigations. The "tremendous influence of yeast genetics" has been developed into famous organism and is the desire of researchers working with advanced eukaryotes. The excellent sequencing of its genome has shown to be exceptionally valuable as an allusion in the direction of the sequencing of human as well as similar advanced eukaryotic genetic factor. Also, the benefit of the genetic operation of yeast permits it to be employed for suitably evaluating as well as the role of separating the fundamental component of inheritance that inhabits precise site on a chromosome product from similar eukaryotes.

Yeast growth phase

Once yeast is budded through a liquid substratum on which cells explanted can be made to bud again, the cultivation of microorganisms is programmed that in a secured method or in a batch cultivation in which no food is added to it and no waste is removed from it; yeast will bud in an expectable form, bring about a budded curve which consist of five separate phases of budding: the lag (initial) phase, the exponential growth (log) phase, deceleration (decline) phase, stationary (stop) phase as well as the death phase. (Bacteria also adopts similar overall form, though they split much more quickly). Cultivations through adding a definite substance to a different substance with a little number of cells. The initial (lag) phase also adopts the adding of a definite substance to a different substance, through which cells are developed to be familiar with the fresh

surroundings and start to specifying the temperature as well as concentration of the substratum on which cells are explanted through their particular biochemical procedures that ensue inside a biological entity so as to sustain life. The lag (initial) phase is accompanied through an exponential(log) phase once the number of cells proliferates rapidly (exponential or log phase). Furthermore, the log phase of yeast could be defined through the mathematical equation: $N = N0\ e^{kt}$

Where N denotes the number of cells at any time (t), as well as N0 denotes the number of cells at the start of the break. Experts frequently discover, it is more suitable to consider the constant expansion k in expressions of the expanding time of the cultivation. During this interpretation, $k = \ln 2/T$ (T = the expanding time of the cultivation). The expansion rate of yeast differs through temperature. Yeast expands well at normal environmental temperature, but they expand faster at 30^{o}C. Furthermore, cultivations expanding in a very airily environment, do so very rapidly than those expanding under a different condition, therefore liquid cultivation are typically expanded on a rotating control. Moreover, at 30^{o}C, gene of yeast that prevails in a normal population of yeast strains have an expanding time of roughly ninety minutes in yeast peptone-dextrose media (YPDM).

Subsequently, depending on the number of expanding times, cells start to reduce the glucose nutrients within the cultivation, their expanding rate decelerates, (deceleration or decline phase) as the cells go into the stationary (stop) phase. Yeast going into the stationary (stop) phase regulate their biochemical reactions in the physical cells that convert food into energy by changing the data in the component of DNA which is duplicated into a fresh molecule of mRNA of many genes, resulting in several physical as well as chemical variations, together with the buildup of carbohydrate assets and, therefore, the association of a high resilient plasma membrane. Within the stationary (stop) phase, the speed of cellular splitting up is analogous to the speed of death. Therefore, the total number of cells does not vary substantially. Cells could live within the stationary (stop) phase for a prolonged time, recommencing expansion once situations are auspicious. Finally, cells go into the death phase when situations remain unchanged.

In recent years, the use of animals for biochemical investigations is being resisted – A near ban situation (Sunstein,2002). Cell line culture is the current approach globally; but the cost of these cells has been an issue, hence; the need to acquire the skill of suspending yeast cell for use as cell lines.
The sole aim of this paper was to present the versatility of yeast cell and how it has become the interest of researchers for several biochemical investigations recently with a focus on determining its viability before proceeding with any form of assay.

European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

MATERIALS AND METHODS

Abelmoschus esculentus L. (Okra seed), *Musa paradisiaca* (unripe plantain fruit), and *Dioscorea dumetorum* (Bitter yam tubers) were obtained from a commercial source in Benue and the Nasarawa States of Nigeria; The plants were peeled, washed, cut into small pieces, and air-dried at 37^{o}C for three days to scale down the moisture content. The air-dried plant materials were pulverized, extracted with 80% methanol, sonicated, filtered, concentrated, freeze-dried and partitioned according to the solvent polarity (n-hexane < chloroform < ethyl acetate < distilled water) using separating funnel.
A glucose-responsive insulin-secreting yeast cell line (Saccharomyces cerevisiae) established glucose uptake that retains glucose-inducible insulin secretion with respect to the expression of glucose transporter iso forms. Cells at a level of $0{\cdot}2x10^4$ cells per tubing were used.

Cell viability assay using trypan blue dye exclusion method

The National Institute of Environmental Health Sciences (NIH) method of trypan blue dye exclusion assay was employed. 50 µL of cell suspension in cryo-vial and equal parts of 0.4% trypan blue dye was added to the cell suspension to get a 1 to 2 dilution (that is., 50 µL of cells to 50 µL of trypan blue) and was mixed by pipetting the mixture up and down for two minutes at 37^{o}C. Cell counting was done within five minutes to avoid inaccuracy of cell viability as a result of necrobiosis. Then, with the help of a cover slip, both sides of a hemocytometer counting chamber were filled with 20µL of cell suspension by placing the pipette's tip at the notch. The hemocytometer was placed on two tiny wooden sticks on top of a moistened filter paper in a culture plate for two minutes; this was to permit the loaded cell suspension to settle down properly before mounting it onto the stage of a light-microscope and was focused onto the grid lines of the hemocytometer with a x10 objective lens. Both sides of the hemocytometer contain multiple squares; the hemocytometer contains nine (9) big squares. Each of the squares contains 10^4 mL of cell suspension. Each of the large squares contained sixteen (16) small squares. For counting purposes, only the four big squares at the corners as well as the centre (top left, top right, bottom left, bottom right, and centre during the counting; cells at right and the bottom boundary were ignored). Both the clear as well as blue cells (live and dead cells) in each large square in the four corners and centre of the hemocytometer) were counted by employing a hand tally counter; one to count clear cells (live cells) and the other to count blue cells (dead cells). Record of the number of clear as well as blue cells, and the total number of cells were kept separately. The following cell parameters were calculated:

1. Percentage viable cells = Number of viable cells divided by the number of total cells multiplied by 100
2. Average Number of cells per square = Number of viable cells divide by the number of squares counted.

3. The dilution factor = Final volume divide by the volume of cells.
4. Concentration of viable cells per ml = Average number of cells per square multiplied by the dilution factor and multiplied again by the number of cell suspension per square.

Direct yeast cell counts at OD600

Yeast cell measurement at OD600 was carried out with the assistance of a spectrophotometer. The method was easily achieved within the cell culture hood. Yeast cell was measured at 600nm **(**Mira *et al*; 2022, Beal et al;2020, Haase *et al*; 2017, Mira *et al*; 2022, Domańska *et al*; 2019).

Initial calibration

Yeast cell counting was done first with a hemocytometer to determine the yeast cell number (as a correlation between the OD600).

Yeast cultivation: 1g of yeast was suspended in 250mL "Yeast Extract-Peptone-Dextrose" (YPD) media and incubated at 37°C through the night in an Erlenmeyer flask. Serial dilutions of the stock solution were carried out for each measurement and calibration.

The sample stuck within the cuvette cavity was taken out by touching a clean paper. Three measurements in every serial dilution of the yeast cells and calibration were used in plotting the OD600 and correlate the result with the hemocytometer count. The cells in the YPD media and calibration concluded the first stage. In the second stage, the yeast cells were centrifuged and washed with phosphate buffer saline (PBS) and re-suspended in PBS, and calibration was repeated with PBS. While in the third stage, two selected fractions from the partitioned plant extracts with the best glucose uptake were measured together with YPD media and the yeast cells and calibration were done with distilled water.

Statistical analysis

Data were collected using a one-way, and two-way analysis of variance (ANOVA) as well as independent T-test, and paired T-test analysis. Groups were considered significant if $P<0{\cdot}05$ and, a F-value was significant for ANOVA; the differences between all pairs were carried out using Ducan Post Hoc Test; SPSS version 26, and Microsoft excel windows 10 were used for statistical analysis, and data figure generation.

European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

RESULTS AND DISCUSSION

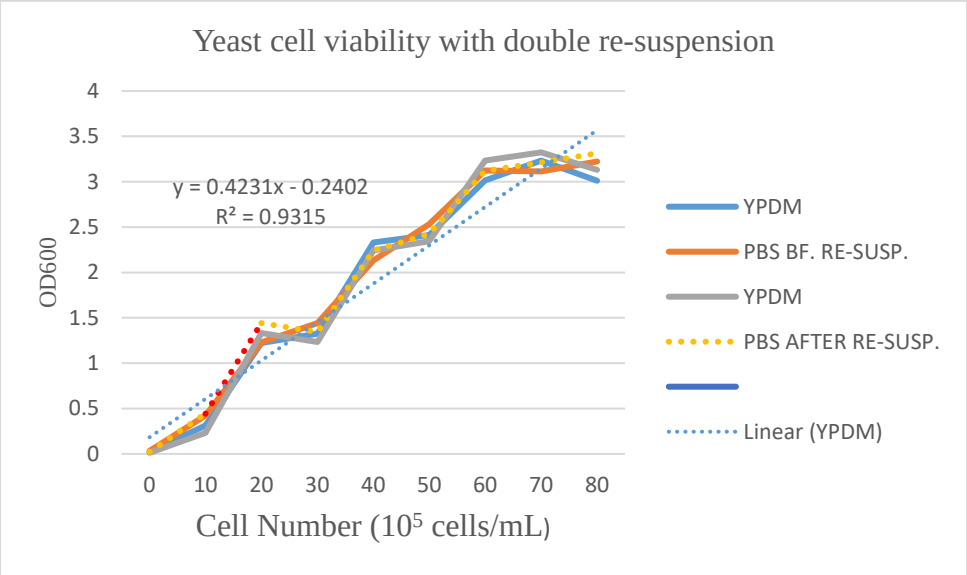
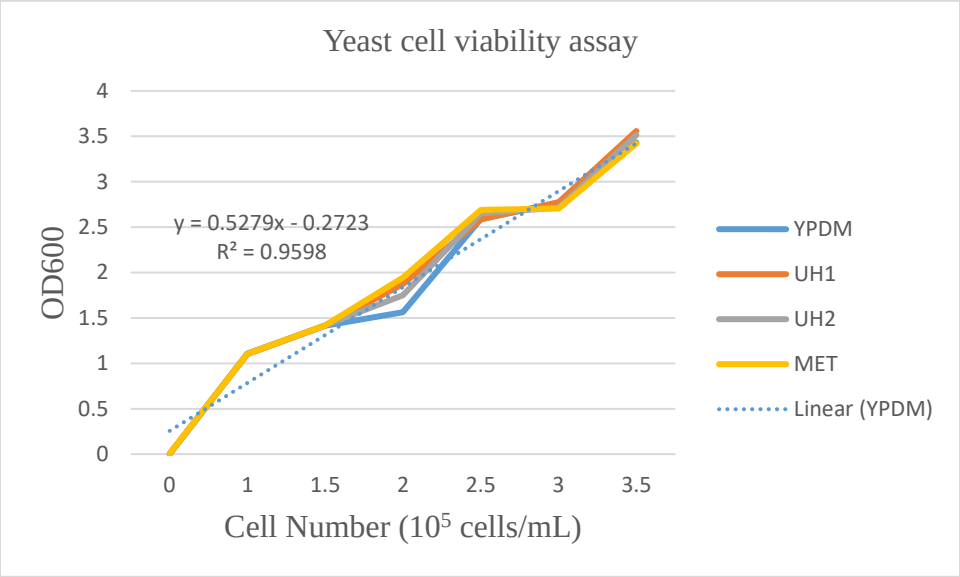


Figure 1: OD600 showing calibration curve for yeast cells up to OD 3.2 with double resuspension in phosphate buffer saline (PBS). Values are presented as mean ± standard deviation of triplicates. Values with high activities concentration are significantly different at $P< 0.05$.



European Journal of Biology and Medical Science Research
Vol.12, No.3, pp.,66-92, 2024
Print ISSN: ISSN 2053-406X,
Online ISSN: ISSN 2053-4078
Website: https://www.eajournals.org/
Publication of the European Centre for Research Training and Development -UK

Figure 2: OD600 showing linear part of the calibration curve up to OD ~1.4 with YPDM, UH1, UH2, and MET. Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P< 0.05$.

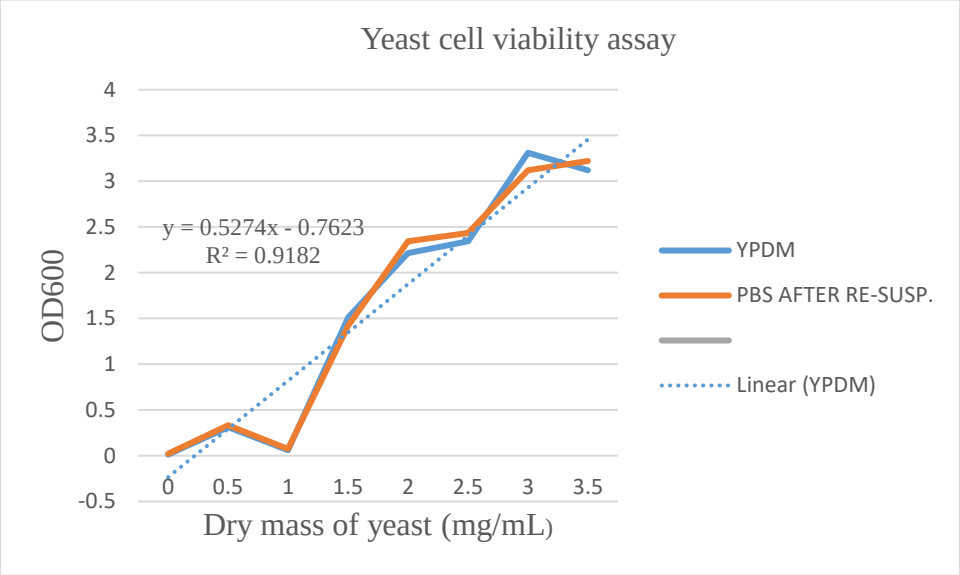


Figure 3: OD600 showing yeast cells as a function of the dry cell mass with second resuspension in phosphate buffer saline (PBS). Values are presented as mean ± standard deviation of triplicates. Values with high activity concentration are significantly different at $P< 0.05$.

Calculation of parameters to determine the quantity of viable (live cells) cells after counting the cell suspension loaded within the hemocytometer and mounted onto the stage of a light–binocular microscope shows that:
1. Percentage of viable cells was equal:
Number of clear cell (live cells) equal to 94.
Number of blue cells (dead cells) equal to 04.
Total number of counted cells equal to 98.
Therefore, percentage viable cells equal to 94 divided by 98 multiplied by 100 equal to 95.918367347.
2. Average number of cells per square equal to
94 divided by 5 (number of squares counted) equal to 18.8
3. Dilution factor equal to: final volume divided by volume of cells equal to 100µL divided by 50 µL equal to 2.
4. Concentration of viable cells per ml equal: 18.8 multiplied by 2 and multiplied by 10^4equal to 376,000 cells per ml.
In scientific notation, this was written as 3.76 x 10^5 cells per ml; therefore, the quantity of viable

cells was approximately 95.9%. Values are presented as mean ± variance of triplicates.The calibration curve of figure 1 shows a linear range up to OD 1.4 with a high R^2; this corresponds to 20 x 10^5 cells per ml.

Figure 4.1 showed calibration curves for plateau absorbance values above OD 3.2. This can be always observed for samples containing particles (yeast cells), because the OD600 measurement is largely a turbidimetric measurement. By using polynomial regression, cell counts of up to 80 x 10^5 cells are possible at a high R^2 of 0.99. We found a yeast cell number of 70 x 10^5 cells per 1 OD600 unit; this can be within the information found within the literature.The linear part of the calibration curves was up to OD ~1.4 was observed in figure 1 (replicated in figure 2). A yeast cell number of 1.5 x 10^5 cells was found from the calculation formula; 1.5 multiplied by 2 multiplied by 10^4 equal to 3.0 x 10^5; therefore, this correlates with cell viability of 95.9% from the R^2.

We also determined the yeast biomass as "dry mass" as a function of the OD600. From figure 3, it is seen that a dry mass of 1 mg/mL roughly correlates to an OD of 3.2 leading to 3.2 x10^5 cells. From this, the dry mass of one yeast cell is calculated as 6.4 x 10^7mg. Recalibration was not necessary because the temperature of the environment remained at 37^{o}C throughout the assay (e.g. different temperatures). The best accuracy was achieved by direct calibration before the measurement.

The viability of the cells was not effectively different in concentrations. This was seen in the similarity of the whole mean plot of the cell viability with the actual plots being similar with figures 1, 2, 3 respectively. Furthermore, the comparison between extracts and cells was only effectively different at higher concentrations and not at lower concentrations. However, single re-suspension of the cell was effectively different at lower concentration; while at higher concentration, there was no effective difference on the cells. In the double re-suspension (first re-suspension) of the YPDM, there was an effective difference in the concentration on the cells and the mean plot of cell viability was similar to the OD600 plot and mean plot to OD600 respectively. Similarly, the double re-suspension (second re-suspension) of YPDM, there was also an effective difference in concentration on the cells and the mean plot of cell viability was also similar to the OD600 plot. Furthermore, the first re-suspension with YPDM shows that there was an effective difference in the concentration of the extract on the cells and PBS.

The independent T-test of concentration between samples at first resuspension was not effectively different on the cells at low concentrations. However, at 30 to 50mg/L ceased effectiveness at a high concentration of 60 to 70mg/L and suddenly restored effectiveness at higher concentration of 80mg/L. This suggests that the extract fractions could be used both at high and low concentrations effectively. In the same way, independent T-test of concentration between samples at second re-suspension surprisingly was effective on the cells at a very low concentration of 0 mg/L; While at

high concentrations of 10 to 80mg/L, there was no effect of the extract on the cells. This again further suggests that the extract could be very effective at a very low concentration.

In the same vein, paired T-test of the comparison between the concentration of first and second re-suspension with cells was not effectively different. This suggests that there were no differences in the two suspensions (first and second suspension) of the yeast cells in phosphate-buffer saline (PBS). The viability of the yeast cell was high enough (95.9%) to warrant their use with the extract fractions in the assays. In each of the assays, significant results were found; suggesting the effectiveness of the trypan blue dye cell viability assay and the OD600 method compared and verified.

CONCLUSION

The yeast cell line used in this study was very viable. The trypan blue and OD600 both suggest viability and effectiveness in interfacing with the extracts as well as the glucose substrate. The effectiveness of the viability of the cells with the first and second suspensions of yeast cells with phosphate buffered saline (PBS) was not different suggesting that the yeast cells were indeed viable as both suspensions were the same.

Contributors

"Conceptualization, MA. and EA.; methodology, MA and EA. EE; software, MA and EA.; validation, KF., CP. and EE.; formal analysis, MA.; investigation, MA, EA, KF, CP and EE.; resources, MA and EA.; data curation, MA and EA.; writing - original draft preparation, MA.; writing - review and editing, MA, EA; visualization, MA, EA, KF, CP, and EE; supervision, EA, KF, CP, and EE; project administration, EA; funding acquisition, MA, EA, KF, CP, and EE. All authors have read and agreed to the published version of the manuscript."

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of University of Nicosia; approval date is 15 January 2020.

Funding

This research received no external funding

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