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POSSIBLE ENHANCEMENT OF PHOSPHATIDYL INOSITOL (PI) DEPENDS ON ITS METABOLIC PATHWAY COMPONENTS AS SECOND MESSENGERS: EVIDENCE FROM THE GROWTH AND PI LEVELS OF NORMAL, PI GROWN, AND UV EXPOSED *SACCHAROMYCES CEREVISIAE.*

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Abstract

The total phospholipids analyses had been performed in "***Saccharomyces cerevisiae***", which is a baker's yeast. This yeast is very much rich in carbohydrates, proteins, nucleic acids, and lipids, where these basic bio-materials (biological polymers) placed this haploid yeast as "single cell protein" category. Out of all phospholipids (PC, PE, PS, and PI) the world scientists have diverted their concentration towards PI and their metabolic components (e.g., PIP, PIP, PIP3 etc.,) and their applications on further growth of this baker's yeast. Here, in this communication the PI levels were up to the mark on the Silica Gel-G (40 grams mixed in 60 ml of sterile distilled water) TLC plates. Further, the experimentation was extended in the growth levels observation of this haploid yeast in presence of isolated PIs (because of the existed assumption that the PIP, PIP2, & PIP3 are very much nearer in the surrounding of PI spot on Silica Gel-G plates), that may have enhanced the growth of *S. cerevisiae*. In addition, on supplementation of PI with *S. cerevisiae* grown cells stimulated the concentration of other Phospholipids includes PI. These results in total suggested that PI-supplemented cells have shown the stimulated growth and total phospholipids contents in this *S. cerevisiae.* It may be extrapolated that the second messenger PI and its metabolic intermediates may be involved as the remedy for phospholipids metabolic errors that lead to different deficient diseases. In final bit of experimentation the PI was isolated in UV exposed *S cerevisiae* cells which will further clarify metabolic changes and genetic deficiencies of PI metabolism..

Keywords: *Phosphatidyl Choline (PC), Phosphatidyl Ethanolamine (PE), Phosphatidyl Serine (PS), Phosphatidyl Inositol (PI), and Phosphoinositides.*

Introduction

Phospholipids are the major class of lipids in biological plasma membranes and organelle membranes for forming lipid bi-layers of a cell and its organelles. The major phospholipids contain glycerol backbone, a phosphate group and simple organic molecule such as choline or ethanolamine or serine or inositol (Zincer et.al., 1991). Generally, the structure of phospholipids shows that it contains a hydrophobic tail and a hydrophilic head in the formation of cell membrane (Lodish et al., 2008). However, the presence of major four phospholipids (PC, PE, PS, and PI) is there located in the membrane, and there observed also the metabolic intermediates of PI such as PIP (phosphatidyl inositol phosphate), PIP_2 (phosphatidyl inositol 4, 5 bis-phosphate), PIP_3 (phosphatidyl inositol 1, 4, 5 tri-phosphate) are distributed in the cytoplasm of many eukaryotic biological cells (Lodish et al., 2008). Phosphatidylinositol (PI) is a minor constituent of cell membranes that acts as a substrate for several enzymes involved in cell signaling (Irivine, 1992). It can be also phosphorylated by a variety of kinases (Toker & Lewis, 1997) to form phosphatidyl inositol phosphates (PIPs). The PIs are the family of phospholipids as a class of the phosphoglycerides, in such molecules the isomer of the inositol group is assumed to be the myo-conformer unless otherwise stated (Figure. 1). Typically phosphatidyl inositol forms a minor component on the cytosolic side of eukaryotic cell membranes. The phosphate groups give the molecule a negative charge at physiological pH.
The form of phosphatidyl-inositol comprising the isomer muco-inositol act as a sensory receptor in the taste functions of our sensory system (*www.revolvy.com*).

As far the applications have considered (i) The PI has been widely used in food biotechnology, to improve the quality, as well as to make with delicious and tasty for better general metabolic activities of the body (Martini & Martini, 1989). (ii) It is one of the key phospholipids of cell membranes in all eukaryotic cells. Phosphorylated forms of phosphatidylinositol (PI) are called phosphoinositides and play important roles in lipid signaling, cell signaling and membrane trafficking. The inositol ring can be phosphorylated by a variety of kinases on the three, four and five hydroxyl groups in seven different combinations. (Muller & Pical, 2002). (iii) It is a second

messenger for the control and enhancement of many metabolic activities of a cell (Michel et. al., 1981). (iv). It also helps in calcium metabolism, especially more prominent in muscle tissue (Braunger et. al., 2014).

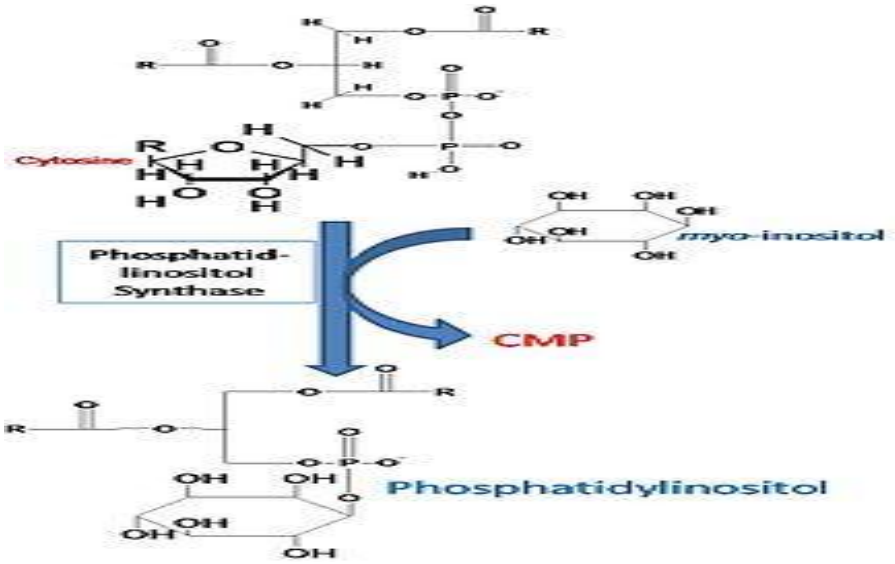

Figure. 1. The synthesis Phosphatidyl inositol in the laboratory is catalysed by phosphatidylinositiol synthase and involves CDP-diacylglycerol and L-myoinositol.

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Materials and Methods

Saccharomyces cerevisiae (MTCC 180) has been obtained from, IMTECH, Chandigarh, India. The broth, slants & plates were made from readymade YEPD broth and YEPD agar obtained from Hi-media, Mumbai, India. The rest chemicals and organic solvents are from Glaxo and/or Ranbaxy, India. All the glass wares used were of Corning, India. All the polymer containers, centrifuge tubes, and other laboratory wares etc., are of polypropylene and polystyrene made of India. The major instruments used in this communication are laminar air flow, rotary shaker, and BOD incubator, TLC applicator, and autoclave. The micropipettes and tips are of Gilson made.

Preparation of culture:

Culture medium preparation was form readymade YEPD agar or YEPD broth proportionally by adding to the range of 100 ml to 1 liter distilled water conical flasks. The YEPD agar was poured into sterile Petri-plates in a laminar air-flow. Mother culture was maintained by streaking the stock (lyophilized powder of *S. cerevisiae*) on the YEPD agar plates. These streaked plates were placed inside the BOD incubator in inverted position for 48 to 72 hours at temperature of 37 degrees centigrade (Trivedi et.al., 1982). A loop- full of thus grown culture of *S. cerevisiae* from the Petri-plate was inoculated in to the YEPD broth and placed in the rotary shaker for 24 hours for stationary cell culture at 37 degrees centigrade. This stationary culture was used for sub-culture in to a fresh YEPD broth by transferring at the rate of 1ml inoculums per 100ml broth, and is grown to active mid-log phase culture in the rotary shaker (Rao et. al., 1985). Thus, the mid-log grown cells were centrifuged at 5000 rpm for five minutes and washed twice with sterile distilled water and the pellet was taken for lipid extraction.

Total lipid extraction:

The washed pellet in a polypropylene centrifuge tube was taken, and the chloroform: phenol in the ratio of 2:1 was added, mixed thoroughly with the help of vortex. The same tube was taken and added 2 volumes of 0.9% NaCl, mixed, and left for overnight (minimum for 6 hours) and allowed to separate 'lipids dissolved chloroform layer' in the bottom of the tube. The lower visible chloroform layer was carefully separated with the help of pasture pipette and collected in to fresh eppendorf tubes (Rao et. al., 1985). The concentration was done by taking 100 micro liters of lipid containing chloroform 'slow evaporation at 35 to 40 degrees' centigrade instead of flushing the nitrogen gas. However, the minimum oxidation of the chloroform dissolved lipid samples was observed in a series of experiments of TLC (Thin Layer Chromatography) where we have considered confidently for the 'qualitative' comparisons of separated phospholipids (Gober et. al., 1993).

TLC plate preparation:

Silica gel-G and sterile distilled water were mixed in the ratio of 4:6 w/v and poured and prepared the layer manually with hand to spread on the dried glass plates (which were washed with common detergent followed by wiping with acetone). Then the plates were placed on a plane surface of work bench which facilitated the poured silica gel G solution on glass plates to form a thin gel layer of 0.75 mm to 1 mm (Figure.2. A and B). The glass plates were left in that condition for 1 hour at room temperature for proper drying. Further, it was placed inside the hot air oven for 1 hour around 90 degrees for binder activation. The plates were removed from hot air oven and left

it for cooling and spotted 10 micro liters of lipid samples on above 2 centimeters of bottom edge of the glass plate. Each plate was containing the duplicate samples with identical volumes.

Preparation of Irrigation Mixture for TLC:

The irrigation mixture for developing chromatogram of lipids was by mixing chloroform: methanol: ammonia solution: water (was in the ml ratio of 66: 27: 3:0.8). This mixture was poured in a TLC jar and allowed for 3 hours for saturation. Slowly the total lipid spotted silica plate is placed in a slight tilted way in the chamber and covered with lid and allowed to run the sample (Figure 2.C) in between 1 hour and 2 hours till it reaches just above the 1/3 of the plate. The TLC plate was removed and dried at room temperature and put in the desiccators in presence of iodine fumigation and left it in such condition for two hours. The visible identified yellow spots are phospholipids and were compared with the standards according to Prasad (Prasad, R., 1996).

Figure.2A

Figure.2B

Figure.2C

Figure.2. Different methods were followed for making Silica Gel G Plates. These methods have given the idea of standardization and the results observed on TLC plates only of manually made with hands were worth promising for repetitions.

Figure. (2A**): TLC** plate made by using applicator. Figure. (2B): TLC manual hand made plate Figure. (2C). TLC runs in irrigation mixture in a jar.

Results and Discussion

The results (Figure. 3. A, 3. B, and 3.C) and discussions in this communication are based on 'qualitative comparisons' of different phospholipids, and with special reference to one of the prominent phospholipids that is phosphatidyl inositol (PI). Since 35 years it has been considered PI-

Figure.3A. TLC runs in normal *S.cerevisiae*

PI

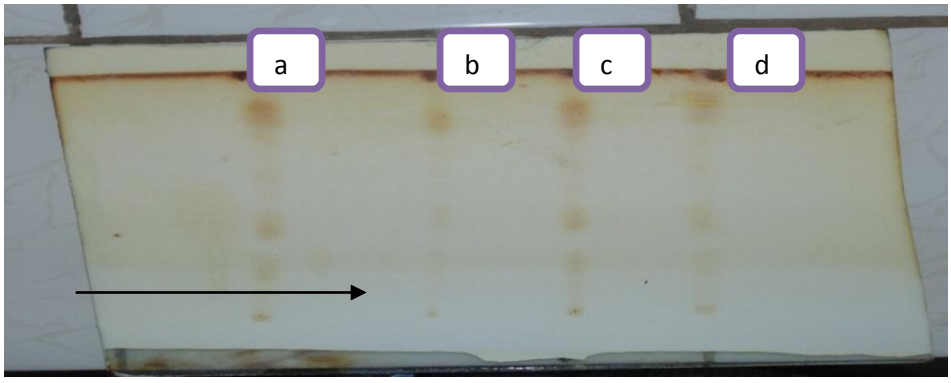

Figure.3B. TLC runs of PI grown S.cerevisiae

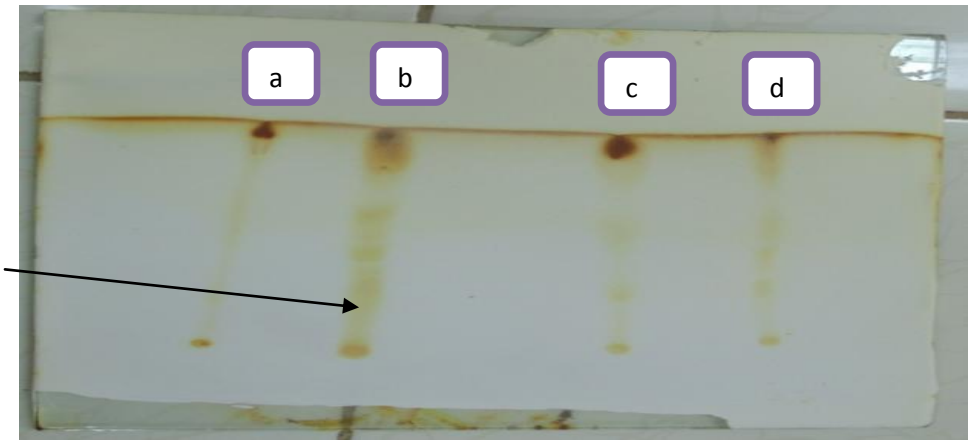

Figure.3C. TLC runs of UV exposed *S.cerevisiae*

Figure.3. 10 micro liters of total lipids dissolved chloroform placed as spots at the bottom above 2cms of TLC plate developed in irrigation mixture in a jar, and was removed after reaching the defined Rf, which is clear by developing yellow spots on iodine fumigation in the desiccators.

as one of the major important phospholipids that is playing an important role in cell signal based metabolic changes for the consequent metabolic biochemical reactions in the cytoplasm, as per the responses made by cell for the external signals (Irvine, 1992). The prominent among the PI metabolic reactions are the formation of PIP (phosphatidyl insitol phosphate), PIP_2 (phosphatidyl inositol 4, 5 bis-phosphate), and PIP_3 (phosphatidyl insitol 1, 4, 5 tri-phosphate) which are categorized by a cumulative name as 'Phospho-inositides' (James et. al., 1990).

The three main experiments followed in this communication were based on the treatment of baker's yeast *S. cerevisiae* among the applications of PI in different contexts. They are in (i) normal cells (cells that normally used for the baking with stable concentrations of phospholipids and other bio-chemicals), in (ii) PI grown cells (cells that are used for baking with altered phospho-inositides), and in (iii) UV click exposed cells that is cells are used for baking with altered phospholipids and also with altered phospho-inositides acquiring with the changes by the formation of switched haploid yeast cell morphologies, and/or also with tolerable genetic alterations (Jani & Lopes, 2009; Flick & Thorner, 1993).

The normal *S. cerevisiae* yeast cells are prominently contains all the major phospholipids (PC, PE, PS, and PI) and also neutral lipids (Trivedi et. al., 1982; Trived et al., 1983). The qualitative comparison of all the four major phospholipids gives an immense idea that all the phospholipids, neutral lipids and sterol precursors comparatively important in the cells and for their normal metabolic activity (Figure. 3 A). As such the wild type baker's yeast has been extensively used for baking the bread, biscuits, cakes etc., food items which are made to think about useful ideas of adding these applications in 'food biotechnology' (Lai & Lin, 2006).

In the second or another bit of experiment is planned to grow the *S .cerevisiae* cells in presence of PI. PI was from the silica gel spot of *S. cerevisiae* (normal) that is the identified PI from the developed chromatogram plate was scratched and dissolved in 100 micro liters of chloroform. Thus dissolved PI in chloroform was further concentrated and mixed with 100 micro liters of fresh broth (i.e., mixed thoroughly by vortex until the formation of micelles' that were added with inoculums, and allowed the wild type cells to grow up to mid-log stage in a fresh liquid medium which is nothing but a kind of PI-supplemented growth of cells). The duplicate experiments of PI-supplemented *S. cerevisiae* cells have shown prominent increase in the concentrations in almost all phospholipids (PC, PE, and PS) and also increase in the concentration of PI [Figure 3. B, lines from left (a) and (c)]. The basic idea behind this is PI may accumulate in cell membrane and also may be helpful in the formation of phosphoinositides in the cytoplasm that are helping in the enhanced metabolic rates of all kinds of substrates. The main application in this experiment is with a thought that it may give a direction that the cell response to the external signals by enhanced rate of metabolic reactions, there by cell may find a better remedy for external physical signals (e.g., light, temperature, and radiation from the sun) and also for receptor mediated biochemical signals through the extra cellular matrix (Mignery & Sudhof, 1990).

The third parameter was on the radiation exposure to normal *S. cerevisiae* cells. The cells after the centrifugation and wash were poured into sterile Petri plate and gave a click of UV radiation in the laminar air flow. However, the maximum exposure to UV radiation of the cells was between 2 and 5 seconds, also did not exceed 2 to 5 seconds time period. The UV exposed cells of 1 ml quantity was inoculated in fresh broth and grown up to mid log phase for phospholipids isolation. Figure.3 C shows the different phospholipids composition of UV exposed *S. cerevisiae* cells. In Figure.3 C lines (b) and (d) are the phospholipids contents of UV exposed cells. In the equal amount of inoculums and equal volumes of broths for mid log growth clearly explained that the phospholipids (PC, PE, PS, and PI) contents concentrations have been increased when compared to normal cells. This increase indicated that the click of UV have the capacity of enhancing the total lipids that are containing PC, PE, PS, PI, neutral lipids, and sterol precursors etc which are creating a solid cell physiology in the growth and development

eukaryotic cell. In addition, one can expect the enhancement of phosphoinositides for antagonistic effects of UV radiation stress like other stress factors for the better survival of the cells (DeWald et. al., 2001). One can also easily expect biochemical applications of the metabolism by producing lot of important and key bio-chemicals in the cells (Yang & Liu, 2014).

Conclusions

The main conclusions based on these experimental set ups of enhancement of phospholipids that are detected with the help of Thin Layer Chromatograms (TLC) give the total picture of qualitative phospholipids contents in different experimental parameters. Our main concentration is on PI and its metabolic conversions to other phosphoinositides. The increasing concentrations of second messengers and their role on correcting lipid disorders is another branch of lipid biochemistry and may also be applied to lipid molecular biology. We are now in the isolation and supplementation of phosphoinositides (PI, PIP, PIP_2, PIP_3 etc.,) from baker's yeast used in the baking process of different food items preparations in restaurants, baking industries for healthy, active, to get resistance too many diseases, and tasty preparations. Of course the rDNA technology may solve the problems in the productions larger quantities phospholipids and other prominent essential lipids for the supplementations in food biotechnology. The lots of companies are there for baking with the help of manipulating simple growth techniques of *S.cerevisiae*. These growth technologies are by applying different techniques such as (a) manipulating the cells at cell cycle stages, (b) at the stages of different cell phenotypic morphologies, (c) exposure of bio-systems to different environmental physical agents, (d) supplementation of different bio-chemicals for required growth of normal and auxotroph cells that are for required end products, (e) mutagenesis of cells for specific products etc., by utilization of the useful microbial organisms like yeasts and bacteria (Zhong & Blount, 2013).

Now, these are the days of computers and soft wares, and also the satellite utility by the population of world. Another important needed and necessary of these TLC phospholipids applications are for the 'generation of computer soft wares' for a glance 'qualitative observations' for concluding the effects of food items that are baked with *S. cerevisiae* and also for the medical applications including GM foods in lower and higher organisms (Lee, 1996).

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