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COMPARATIVE STUDY OF PHOSPHATASEENZYME PRODUCED BY PLANT, ANIMAL & BACTERIAL CELL

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ABSTRACT

Phosphatase is an enzyme that removes a phosphate group from its substrate and now widely used in research, diagnosis, Immunoassay, DNA profiling .The present investigation was made to study the expression profile of Phosphatase. For this purpose chicken heart (Gallus gallusdomesticus)was used as the animal sample, Green tomato (Solanumlycopersicum) was used as plant sample, and bacteria sample wasB.Subtilis. Screening and isolation of phosphatase enzyme were carried out from plant, animal and bacteria samples. Lowry,Phosphatase and sulfhydril assays were performed reported to protein concentration, Phosphate and sulfhydrilgroup.After doing the assay, it was found phosphatase enzyme acidicand soluble and localized in cytosol chicken heart& green tomato, whereasalkali inB.Subtilis&localized in periplasmicmembrane. After doing the Phosphatase and sulfhydril assayhighest enzyme activity and highestsulfhydril group localized in mitochondria in green tomato whereas, in chicken heartF5 fraction had highest enzyme activity&highest sulfhydril group localized in nuclei. By Protein Electrophoresis, molecular weight of acid phosphatase in chicken heart was found to be ~29kDa, Green tomato ~28kDa and in B.Subtilis~42 kDa.At last ion exchange chromatography was done which elutes our enzyme of interest to maximum level.

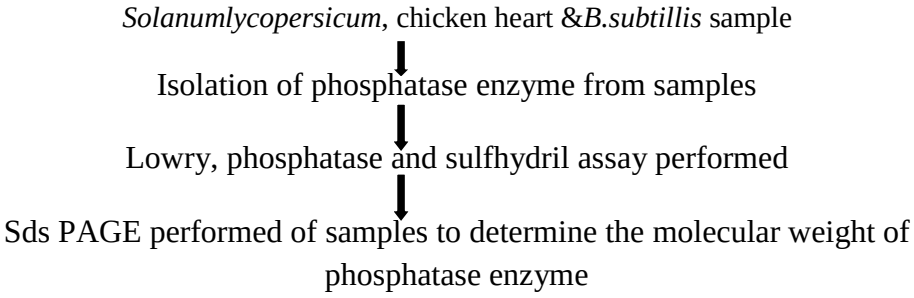
Keyword: Phosphatase, diagnosis, Immunoassay, DNA profiling, Sulfhydril,Fingerprinting

Introduction

Phosphatase is an enzyme that removes a phosphate group from its substrate by hydrolyzing phosphoric acid monoester into a phosphate ion and a molecule with a free hydroxyl group. This action is directly to opposite of phosphorylasesor kinases.Phosphatases enzyme now widely used in research, diagnosis, medical purpose DNA profiling[7, 15]. Phosphatase is an important serum analyte and its elevation in serum is correlated with the pressure of bone, liver, and other diseases. The analysis of the isoenzymes of alkaline phosphatase is an aid in diagnosing liver and/or bone disease, especially the high molecular weight isoenzymes that appear in cholestatic liverdisease. Phosphatase has become a usefultool in molecular biology laboratories [7, 14].

Materials and method

The work was carried out as shown in the proceeding flowchart:



Isolation of phosphatase enzyme from different samples*:*

1. Phosphatase enzyme isolated on the basis of acidic PH, solublization and their cellular location property. 250µl of acidic extraction buffer was added, pH=5.5 in both F1 and F1' and the tissue was grinded in tube-pestle for 10-15mins until homogenous tissue suspension (no clumps) is obtained.

2. Neutral extraction buffer was added, pH=9.0 in both F2 and F2' and the tissue was grinded in tube-pestle for 10-15mins until homogenous tissue suspension (no clumps) is obtained.

3. Alkaline extraction buffer was added, pH=7.0 -7.5 in both F3 and F3' and the tissue was grinded in tube-pestle for 10-15mins until homogenous tissue suspension (no clumps) is obtained.

4. F4 and F4` were taken with 0.5g of fresh tissue in each. 250µl of protein extraction buffer was added, mild detergent in F-5 and sub cellular buffer in F-6, strong detergent in F-9 are added[3, 4].

Identification of molecular weight by Biochemical tests

Lowry Assay for Protein Quantification

A standard curve is prepared with bovine serum albumin or other pure protein, and the concentration of unknown protein solutions is determined from the graph. Lowry assay was done with the 12 different fractions namely F1, F2, F3, F4-1, F4-2, F4-3, F4-4, F4-5, F5, F6, F7, F8 and F9 [7].

Determination of phosphatase activity

The Phosphatase Assay is designed to measure the activity of phosphatase in biological samples and to screen for agonists and inhibitors of phosphatase. The Phosphatase Assay uses para-nitrophenyl phosphate (pNPP), a chromogenic substrate for most phosphatase, including alkaline phosphatase, acid phosphatase, protein tyrosine phosphatase and serine/threonine phosphatase. The phosphatases remove the phosphate group to generate p-nitrophenol, which is deprotonated under alkaline conditions to produce pnitrophenolate that has strong absorption at 415nm [3, 5].

Sulfhydril Assay for Determination of Free –SH Groups

In this assay DTNB (Ellman's reagent) (5, 5' dithiobis-(2-nitrobenzoic acid) is a chemical used to quantify the number or concentration of thiol groups in a sample. Thiols react with this compound, cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (NTB-) which ionizes to the NTB2- dianion in water at neutral and alkaline ph. This NTB2- ion has a yellow color [7].

Inoculation of Bacillus subtilis cells in phosphatase production broth:

Step-1- Phosphatase production broth was prepared as minimal media with 0.5% glucose (w/v).

Step-2- Bacillus subtilis crude culture was inoculated (1%) in that broth.

Step-3- It was then left for incubation at 37˚C.

Collection of crude extracts of enzyme at regular intervals:

The cells were palette down by low-speed centrifugation (<5000g).

They were then re-suspended in Tris-HCl buffer (pH-7.5) and washed in this buffer twice.

Finally the cells were centrifuged at 10,000g for 5 min to obtain crude extract of

Phosphatase enzyme after 1, 2 and 3 hrs [5, 6].

Protein Electrophoresis (SDS-PAGE)

Under appropriate conditions, one gram of a polypeptide binds 1.4g of SDS resulting in the formation of a rod like particle with length proportional to the molecular weight of the polypeptide.

Following staining, the gel was placed on a glass plate and on top of a light box. The distance travelled by the bromophenol dye front and each polypeptide (standard and unknown) from the top of the resolving gel was measured. For the unknown a couple of protein bands were selected [1, 2].

The Rf (relative mobility) for each polypeptide from the formula was calculated as

Rf = distance of protein migration/distance of dye migration

Rf versus log Mol.Wt. was plotted for the standard using a spreadsheet program.

Protein standards are 250, 120, 100, 55, 37, 30, 21 and 7kDa

Using the RF value of the unknown sample polypeptide to estimate their molecular weight byinterpolation. The Log (Molwt) of unknowns was calculated as:

Log Mol.Wt = (m× Unknown Protein Rf) +c

Molecular weight was calculated as Estimated molecular wt = $10^{\log molwt}$

Results and discussion

1. Plant (green tomato) Sample

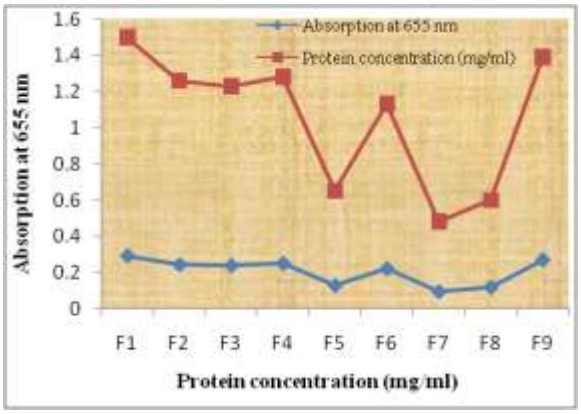


Fig: 1 Protein concentration in *S.lycopersicum* with Lowry assay

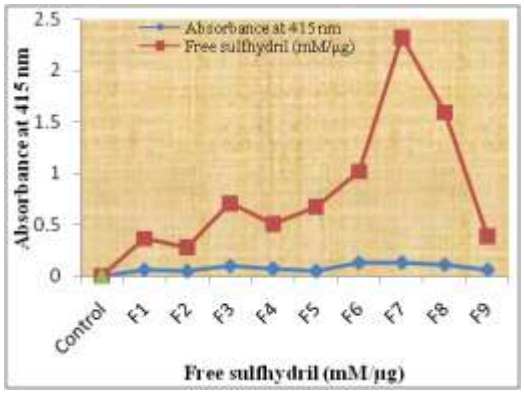


Fig: 2free sulfhydril (mM/µg)*in S. lycopersicum* with sulfhydril assay

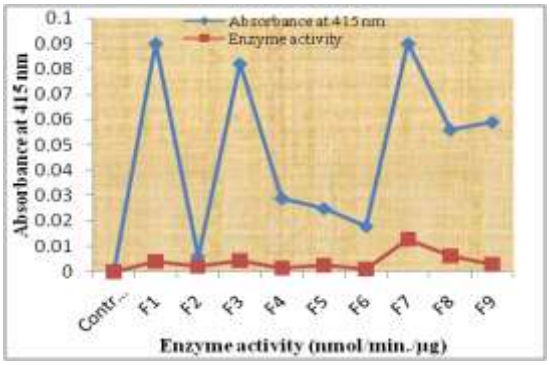


Fig: 3Enzyme activities (nmol/min./µg) in *S. lycopersicum* with ACP assay

Protein Electrophoresis

Relative mobility values of protein marker

Dye front distance = 35mm

Protein marker (kDa):250, 120, 100, 55, 37, 30,21,7

Rf value: 0.198, 0.387, 0.586, 0.697, 0.789, 0.867, 0.967, 1.0

Log molecular weight: 2.39794, 2.079181, 2, 1.740363, 1.568202, 1.477121, 1.322219, 0845098

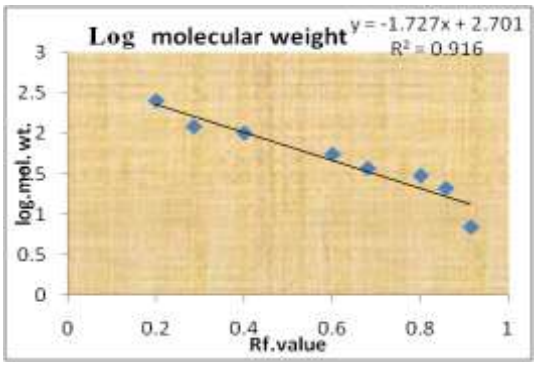


Fig: 4 Log. MolecularWt.of marker

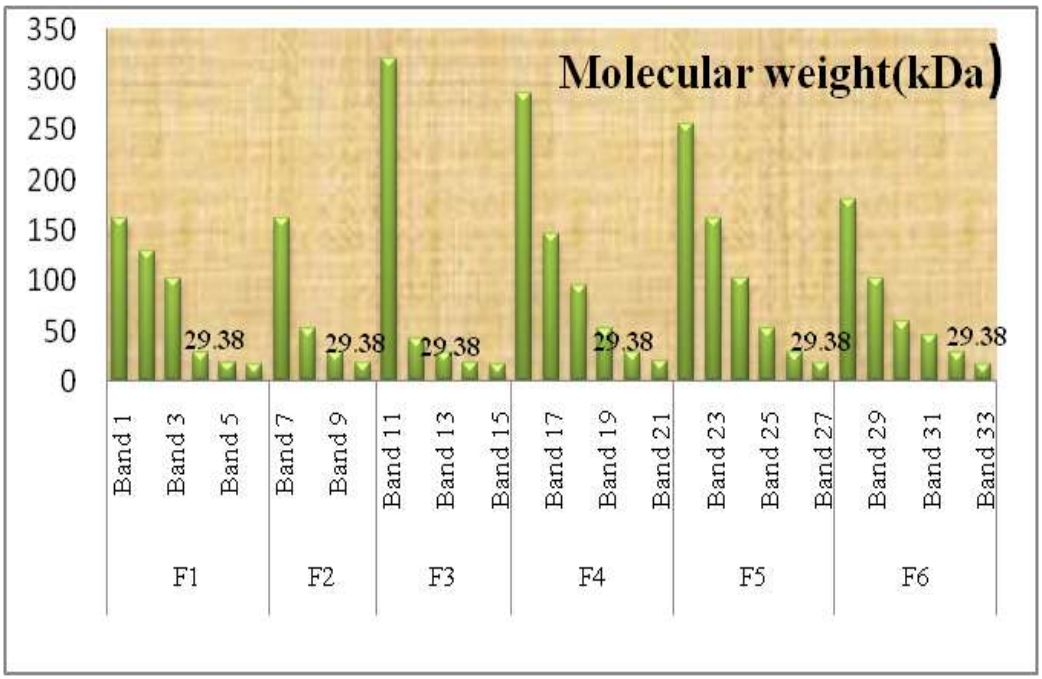


Fig: 5 Molecular weightof*S. lycopersicum*samples

2. Animal (Chicken Heart) Sample

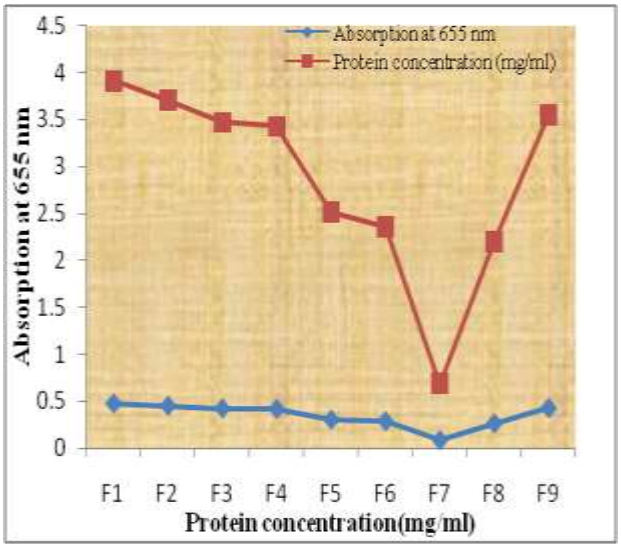


Fig: 6 Protein concentrations in*G.domesticus*with Lowry assay

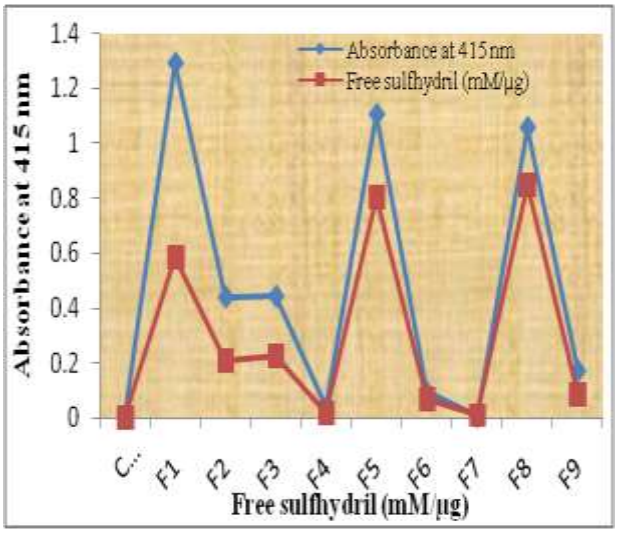


Fig: 7 free sulfhydril (mM/µg)*inG.domesticuswith*sulfhydril assay

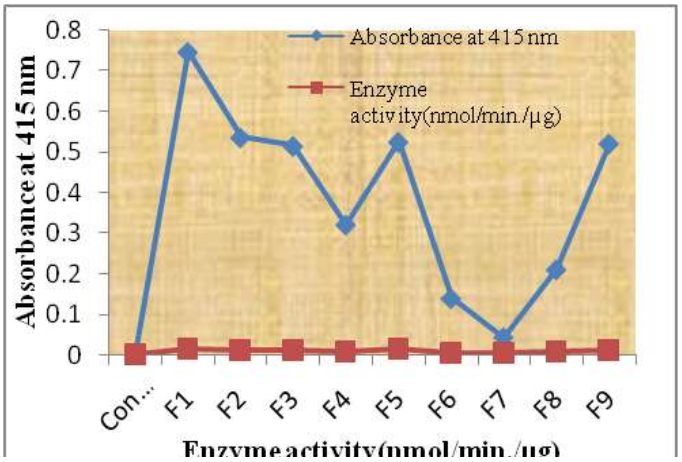


Fig: 8Enzyme activities (nmol/min./µg)in*G.domesticus*with ACP assay

Protein Electrophoresis

Relative mobility values of protein marker

Dye front distance = 47mm

Protein marker (kDa):250, 120, 100, 55, 37, 30,21,7

Rf value: 0.212, 0.297, 0.425, 0.574, 0.702, 0.808, 0.893, 1.0

Log molecular weight: 2.39794, 2.079181, 2, 1.740363, 1.568202, 1.477121, 1.322219, 0.845098

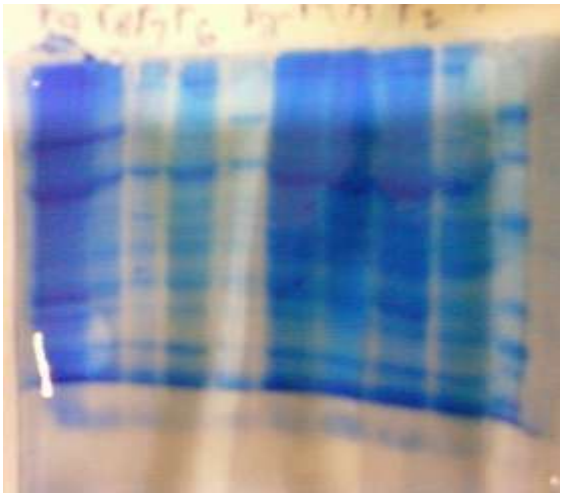


Fig: 9 Image of SDS-PAGEG.domesticus

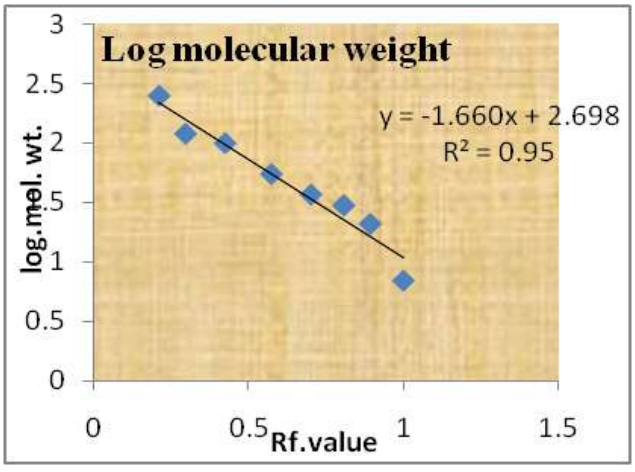


Fig: 10 Log. Molecular Wt.of marker

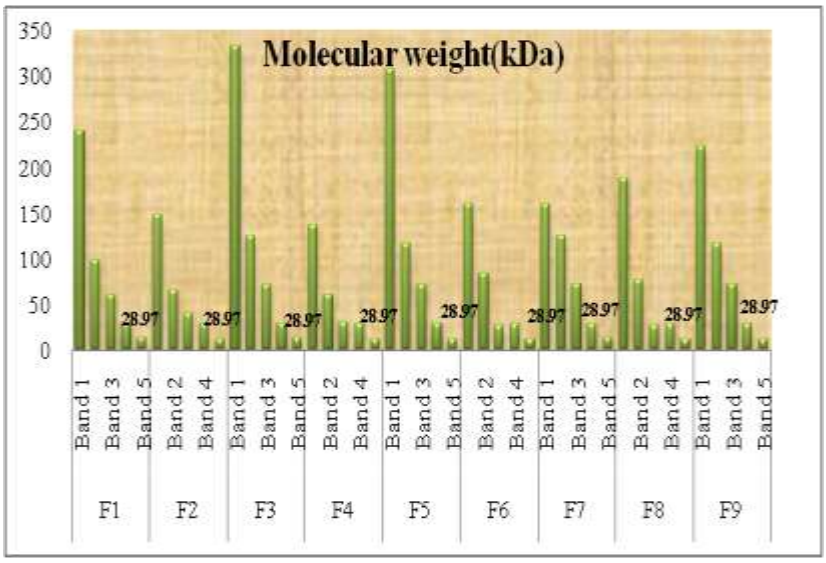


Fig: 11Molecular weight of*G.domesticus*sample

3. Bacteria (*B. Subtilis)* Sample

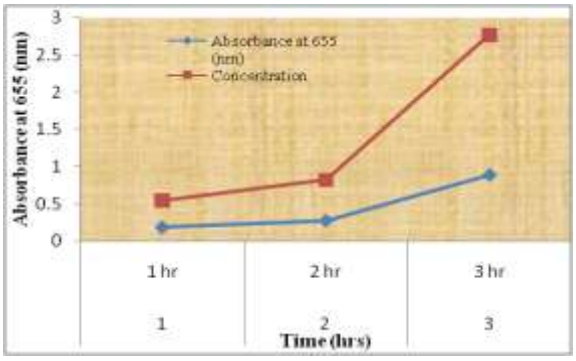


Fig: 12 Proteinconcentrations in *B.subtillis*with Lowry assay

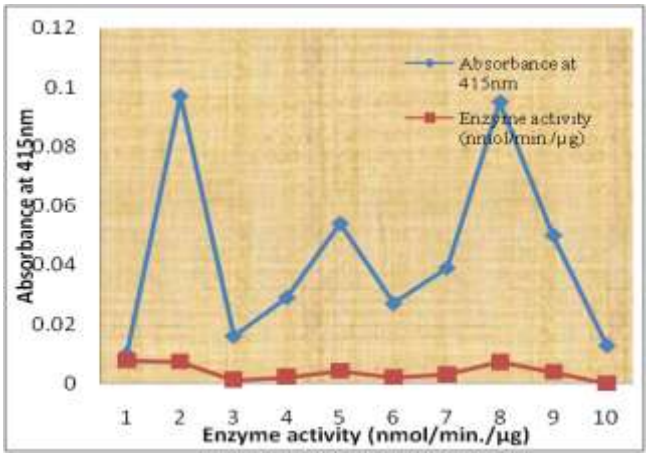


Fig: 13Enzyme activities (nmol/min./µg)in*B.Subtilis*with ALP assay

Relative mobility values and Mr.Wt.of Protein marker (kDa):

250, 120, 100, 55, 37, 30,21,7

Relativemobility: 0.198, 0.387, 0.586, 0.697, 0.789, 0.867, 0.967, 1.0

Log molecular weight: 2.39794, 2.079891, 2, 1.740363, 1.568202, 1.477221, 1.322219, 0.845098

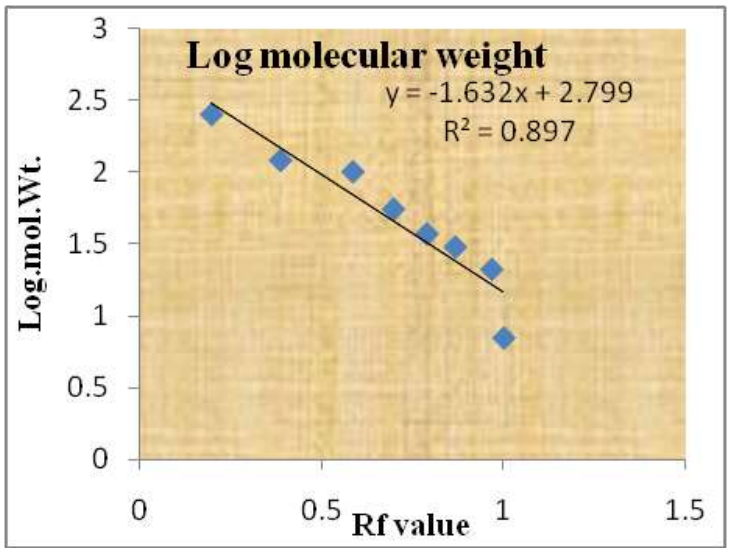


Fig: 14 Log. Molecular Wt.of marker

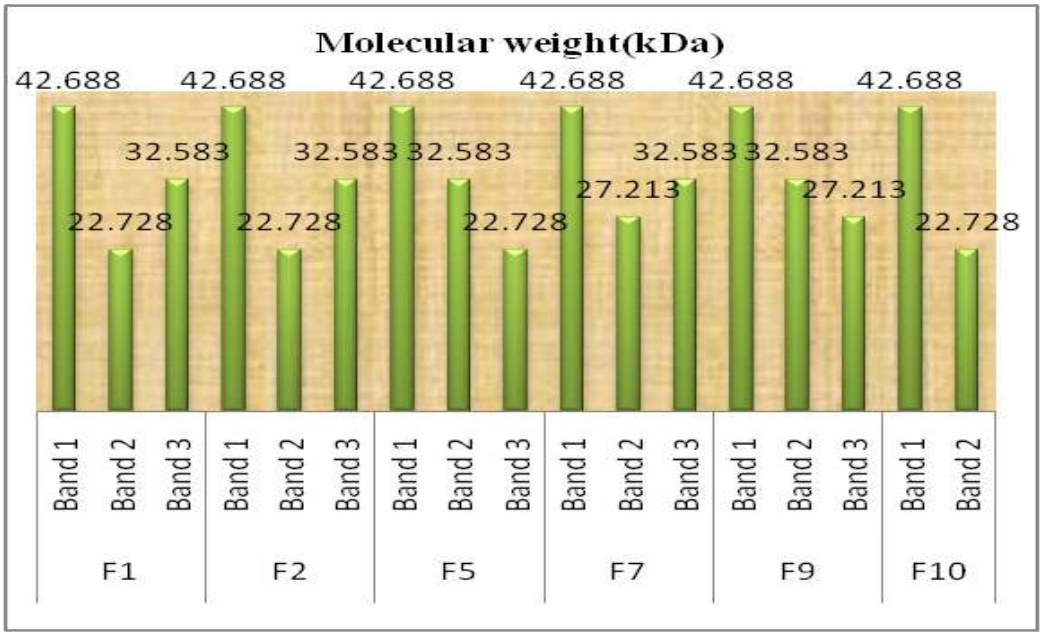


Fig: 15 Molecular weight of*B.Subtilis*sample

Conclusion

The present research was done using three samples green tomato, chicken heart and bacterial sample was *B.Subtilis*. After doing theLOWRY ASSAY, it was found that phosphatase enzyme acidic and soluble in green tomato and localized in cytosol. After doing ACP ASSAY for determining Acid Phosphatase activityit was noticed that highest enzyme activity in mitochondria in green Tomato. After that SULFHYDRYL ASSAY was done it was found that highest sulfhydril group localized in mitochondria in green Tomato.After that SDS-PAGE used for molecular weight determination. Molecular weight of acid phosphatase in green Tomato was found ~28kDa.

Then all the tests were done with chicken heart... From LOWRY ASSAY, it was found that phosphatase enzyme acidic and soluble in nature and localized in cytosol. By ACP ASSAY, it was known cytosol had highest enzyme activity. From SULFHYDRYL ASSAY, it was found that highest sulfhydril group was localized in nuclei. Then SDS PAGE was done. It was shows that acid phosphatase present in chicken heart sampleof 29kDaMol.Wt.

In *B.Subtilis* it was found that phosphatase enzyme alkali in nature. Molecular weight of alkali phosphatase in *B.Subtilis*was found ~42kDa.At last ION EXCHANGE CHROMATOGRAPHY was donewhich elutes our enzyme of interest to maximum level.

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