Assessment of sugar binding lectins from *Eudrilus eugeniae* on invertebrate and vertebrate cells

Anusha Krishnamurthy, Megha Reddy, Deepika Raj,
Sinku Rana, Preetha Nair, Shweta Murthy

Department of Biotechnology, Mount Carmel College, Bangalore, Karnataka, 560052, India,
Email: shwetanori@gmail.com, preetram@gmail.com

ABSTRACT

The aim and objective of this study is to isolate the sugar binding lectin like proteins from Coelomocytes (CC), muscle cells (MC), gut/whole body (WE) and vermicompost (VC) of earthworms and assess them for their mitogenic property on invertebrate and vertebrate cells. Partially purified lectins were estimated for their protein content by Biuret method and it was observed that lectins isolated from CC have more protein content of 0.178 mg/ml as compared to any other source. Their sugar binding specificity was checked by DNS method and it was observed that CC lectins and VC lectins have more affinity for glucose (CC glu and VC glu) while MC lectin and WE lectins have more affinity for galactose (MC gal and WE gal). The different lectins were then assessed for their mitogenic activity on vertebrate (chicken liver cells) and invertebrate cells (earthworm cells). The cells were cultured in HBSS media and three different concentrations, i.e., 0.1mg/ml. 0.01mg/ml and 0.001mg/ml of lectins from different sources were added to the cells along with control. It was observed that invertebrates showed mitogenecity or proliferation of 83.6% at 0.1mg/ml of lectin from WE that is with more concentration of lectin more proliferation was observed while vertebrates showed inhibitory effect with lectin that is with more concentration of lectin less proliferation was observed.

Keywords: lectin, DNS, HBSS, mitogenecity, CC, MC, VC, WE

Lectins are proteins that occur widely in animal and plant kingdom [1], but their function is not fully understood. It may either have a role in the developmental stages or in the defense mechanism by providing immunity [2]. In earthworm species like *Eudrilus eugeniae* presence of sugar binding lectin like proteins, in various locations were reported [3]. The objective of the research work is isolation, characterization and assessment of partially purified proteins from coelomic fluid, muscles, gut and vermicompost of earthworms for their specificity for binding to monosaccharides like glucose and galactose. They will be assessed for their mitogenic effect on invertebrate and vertebrate cells. This will enable to understand their potential use in future pharmaceuticals. *Eudrilus eugeniae* cultured in kitchen waste and leaves in 3:1 ratio for around 1-2 months. Adult earthworms were used for lectin isolation from different parts like coelomocytes (CC) by cold shock method, and were cultured in CO_2 incubator for one week in HBSS medium. Muscle cells (MC) were cut in fine pieces and was followed by the same procedure, after one week Coelomocytes and muscle cells were trypsined and pelleted and then lysed with 1% SDS and 0.1mM EDTA to release lectin. They were incubated separately with 20 mM glucose and 20 mM galactose in EDTA-MEPBS buffer overnight, Similarly whole body (WE) of earthworms fed on filter paper for 48 hrs were homogenized and cells were pelleted to release lectin with same procedure as above.

pISSN 2319-1244, eISSN 2279-0160

Vermicompost (VC) was added with ME-PBS buffer to collect supernatant and lectin was isolated. Later all the different lectins were purified by dialysis method using appropriate membrane.

The protein concentration of all different proteins was achieved by Biuret method taking BSA as positive control [4]. Sugar binding specificity was determined by DNS method where the colour intensity will give the appropriate result [5]. Mitogenic activity or proliferation activity of all lectins on vertebrate (chicken liver cells in HBSS media) and invertebrate cells (earthworm muscle cells) was checked to find whether the lectins added can act as growth factor for the developing cell [6,7]. The cells were cultured in HBSS media and three different concentrations, i.e., 0.1mg/ml. 0.01mg/ml and 0.001mg/ml of lectins from different sources were added to the cells along with control for one week in CO_2 incubator. Protein estimation assay showed that all the lectins isolated from different parts that is from coelomocytes, muscle cells, whole earthworm body and vermicompost of the earthworm *Eudrilus eugeniae* showed protein content in it which was estimated by Biuret method taking BSA as control at 540 nm. The lectins isolated from Coelomocytes with affinity for glucose (CC glu) showed maximum protein content of 1.78mg/ml. Sugar binding assay with DNS was done to find out the specificity of the lectins for a particular sugar. All the lectins showed affinity for sugars glucose and galactose but Coelomocytes and Vermicompost showed greater affinity for glucose while muscle cell and whole earthworm body showed more affinity for galactose. When sugar and lectin bind together, DNS will be left free and hence show less absorbance reading and less colour intensity (Table 1, 2 and 3).

Table 1. Estimation of protein concentration in various lectin samples.

Lectin sample	Protein concentration (mg/ml)
CC glu	1.78
CC gal	1.29
MC glu	1.52
MC gal	1.36
WE glu	1.23
WE gal	1.17
VC glu	0.87
VC gal	0.84

Table 2. Sugar binding specificity (mg/ml protein bound to sugar) of extracted lectins.

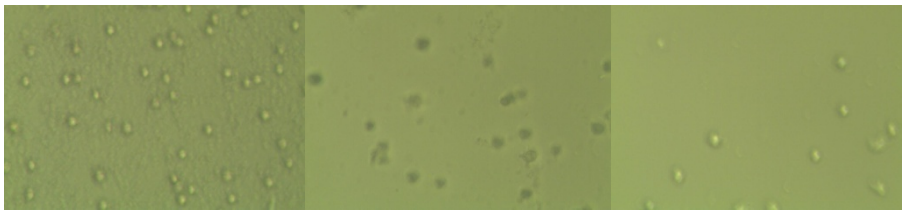
Lectin	Glucose	Galactose
CC	0.952	0.801
MC	0.841	0.950
WE	0.730	0.860
VC	0.954	0.855

The different lectins were then assessed for their mitogenic activity on vertebrate (chicken liver cells) and invertebrate cells (earthworm cells). The cells were cultured in HBSS media and three different concentrations, i.e., 0.1mg/ml. 0.01mg/ml and 0.001mg/ml of lectins from different sources were added to the cells along with control (Figure 1). It was observed that the invertebrates showed mitogenecity and proliferation of 83.63% at 0.1mg/ml of lectin from Whole earthworm

body having affinity with galactose that is with more concentration of lectin more proliferation was observed while vertebrates showed 85.7% in 0.001 mg/ml with lectin from MC that is with more concentration of lectin less proliferation was observed hence on vertebrates it has inhibitory effect.

Table 3. Percentage mitogenic activity of extracted lectins.

Sample	Control	0.1mg/ml	0.01 mg/ml	0.001mg/ml
For earthworm muscle cells (invertebrates)				
CC glu	92.30	83.30	77.77	60.00
CC gal	88.88	76.66	68.82	55.50
MC glu	58.14	66.66	62.50	60.00
MC gal	66.60	66.60	65.21	57.14
WE glu	60.00	64.70	62.5	60.55
WE gal	68.29	83.63	72.33	69.20
VC glu	61.70	68.66	62.92	60.58
VC gal	60.60	67.85	64.70	60.68
For earthworm muscle cells (vertebrates)				
CC glu	67.00	54.40	58.00	63.50
CC gal	80.00	68.18	75.00	78.12
MC glu	66.66	60.00	63.00	65.20
MC gal	94.60	77.27	81.25	85.71
WE glu	75.00	52.63	60.00	70.00
WE gal	81.25	73.07	77.77	79.16
VC glu	63.63	45.45	40.00	66.66
VC gal	79.50	74.00	75.00	78.50



Live cells Dead cells Control

Figure 1. Assessment of mitogenic activity of different lectins on live and dead cells.

REFERENCES

[1] Dodd RB, Drickamer K. J. Glycobiology 2001, 11:71-79.
[2] Kilpatrick DC. J. Biochim Biophys Acta 2002, 1572:187-197.
[3] Licata A, Mauceri L. Eur J Histochem 2002, 46:173-178.
[4] Ferdinand R. J. Mathematical-Natural Sciences 1833, 24:335-337.
[5] Miller GL. J. Anal. Chem 1959, 31:426.
[6] Hrzenjak MD, Kobrehel S, Levanat M, et al. J. Comp. Biochem. Physiol. 1993, 104:723-729.
[7] Imanishi K, Ishiguro T, Saito H, et al. J. Cell. Mol. Life Sci. 1981, 37(11):1186-1187.