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Lectin Expression Patterns during *Erythrina abyssinica* and *Abrus precatorius* Seeds Germination: A Prelude towards Understanding the Enigmatic Biological Role of Plant Lectin

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Abstract

Lectins are a divergent group of carbohydrate-binding proteins of non-immune origin that are widely spread in all kingdoms of life. Several postulations have been put forward to describe the physiological obscure functions of plant lectins. Functions such as storage proteins, defensive role against predators or as endogenous enzyme stabilizer were suggested. This work was undertaken to enable further investigation on the possible role of plant lectins. Dry good quality seeds of *Erythrina Abyssinica* (ErA) and *Abrus precatorius* (AbP) were germinated under humid conditions for 21 days. Two-gram seeds were harvested and proteins were extracted every other day. Protein content and lectin activity were monitored. The two seeds under study showed that their protein content was gradually degraded. The three isoforms of lectin, obtained by AmS fractionation, also exhibited a gradual decline in their activities, however, they did not follow similar degradation patterns and some of them prevailed active during the entire germination course. These results support the theories that emphasize a physiological, yet to be disclosed significance for legume lectin other than merely being as a storage protein, and may, therefore, represent a mosaic piece in our race for revealing the exact physiology of plant lectin.

Keywords: Lectin expression; Leguminosae; germination; Erythrina Abyssinica; Abrus precatorius; physiological role.

Introduction

Lectins are defined as proteins or glycoproteins of non-immune origin, which recognize and reversibly bind to carbohydrates and glycoconjugates (Kumar et al., 2012). Lectins are ubiquitous in nature being found from bacteria to humans. Animal lectin is well known to function in cell organization, embryo morphogenesis, cell protection and phagocytosis, and glycoconjugates transport (Santos et al., 2014; Galaly, et al., 2018). They are distributed in all parts of the plant, especially in the seeds while the other parts of the plant contain only little amounts of lectin. It is distributed in various tissues of the plant such as bark (Van Damme et al., 2002), leaf (Konozy et al., 2002; Nielsen et al., 1997), roots (Kalsi and Etzler, 2000), seeds (Jalil and Jebor, 2012; Osman et al., 2016), tubers (Van Damme et al., 1995), rhizomes (Yao et al., 2010), and flowers (Hivrale and Ingale, 2013). Several postulations on the physiological function of plant lectins were suggested. Roles such as reserve proteins, defense, and endogenous enzyme stabilizers have been postulated (Kestwal et al., 2007; Lannoo and Van Damme, 2014). Protein mobilization and degradation have been well documented mostly from the legume plant (Chmielnicka et al., 2017; Kim et al., 2011; Tan-Wilson and Wilson, 2012). Since lectins are generally accumulated in protein bodies derived from the endoplasmic reticulum a role to act as a reserve protein to supply amino acids during early stages of seedlings was earlier postulated (SILVA et al., 2000b). Interactions, common localization as well as N-terminal sequence similarities between lectin and storage proteins from soybean vegetative tissues gave experimental clues to suggest that lectins may function in the packaging or remobilization of storage protein inside protein bodies (Spilatro et al., 1996). *Erythrina abyssinica (ErA)* and *Abrus precatorius* (AbP) are legume plants that are widely distributed in tropical and subtropical areas. The lectins from these plants were already partially characterized, in which both are galactose-specific (Hegde et al., 1991; Mohieddin, 2016.).

In this investigation, we report, for the first time, the lectin expression pattern during various stages of ErA and AbP seeds germination.

Materials and Methods

Materials:

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Good quality, mature, and dry seeds of ErA were collected from the urban area of Western Sudan, whereas AbP seeds were purchased from the local market at Khartoum-Sudan. Human blood cells of type O were taken from healthy donors and used throughout the investigation.

Methods

Seed germination: Seeds of ErA and AbP were sterilized with 70% alcohol and then washed with distilled water three times. The ErA seeds were pretreated by scoring with a serrated knife and put in boiled water for 1h. Whereas, AbP seeds were just immersed in boiling water for a short time. Seeds were placed on tissue papers wet with distilled water on a plastic box. The quiescent seeds were taken as day 0, Axes and cotyledons were harvested at 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21 days old seedlings (abbreviated as D1, D3, D5….D21).

Extraction of lectin: Extraction of protein from dry seeds: 2g of dry seeds were taken mixed with a minimal amount of 0.145M NaCl and ground with a coffee grinder; 20mL of 0.145 M NaCl was added (1:10 w/v) and the mixture was stirred for 4hr under cold condition. Clear supernatant, obtained after centrifugation at 16000 *rpm* was collected and used for protein and lectin assay.

Extraction of germinated cotyledons and axes: 2g of imbibed seeds were blended with 0.145 M NaCl (1:10 w/v) by fruit mixer and stirred for 4hrs under cold condition. Insoluble materials were removed by centrifugation at 16000 *rpm* for 20 min (Cavada et al., 1994). The clear supernatants, obtained after centrifugation were used for the determination of the soluble protein content and hemagglutinating activity and were processed for protein fractionation by a salting-out technique using ammonium sulphate (AmS).

Crude extract protein fractionation by ammonium sulphate: Seeds extracts obtained at different stages of germination were fractionated by salting out using AmS at 40, 60, and 80% saturation. Resultant precipitants were dissolved in a minimal amount of 0.145M NaCl, dialyzed exhaustively against an ample amount of 0.145 M NaCl until free of ammonium sulfate, then subjected to hemagglutination assay and protein content estimation. Lectin activity, detected in both ErA and AbP quiescent and subsequent germinated seeds extract obtained after 40, 60, and 80% AmS saturation were termed here-forth as ErA-Lec40, ErA-Lec60, ErA-lec80, AbP-lec40, AbP-lec60, and AbP-lec80, respectively.

Erythrocytes preparation: The hemagglutinating activity was tested with human erythrocytes type O. The blood was centrifuged at 4000 rpm for 10 min, supernatants were decanted and the cell pellet was washed 4-5 times with 0.145 M NaCl until free of plasma. The washed erythrocytes were finally re-suspend in 0.145 M NaCl to reach the final concentration of 2% (Pompeu et al., 2015).

Hemagglutinating activity (HA): This is expressed as Unit, namely, the reciprocal of the highest dilution that gives positive results (Konozy et al., 2002).

Specific activity: Lectin specific activity is defined as Unit per mg protein (Konozy et al., 2002).

Determination of protein content: Protein content was determined according to the Bradford method (Bradford, 1976) using Bovine Serum Albumin (BSA) as the standard.

Results

The germination and early seedlings growth of the mature seeds of two legume plants *A. precatorius* (AbP) *and E. abyssinica* (ErA) were monitored for lectin and protein content every day. Though both the plant seeds exhibited sequential protein mobilization, the expression of lectin isoforms and peaks of activity did not follow the same course.

Protein and lectin behavior during the germination of Abrus precatorius (AbP) seeds

This part started with the extraction of the crude extract of quiescent seeds (denoted as D0) followed by extracting imbibed seeds taken every next two days (Pourahmadi, et al., 2017). The highest protein content (mg/mL) was noticed at D0 seeds (2mg/mL). Upon fractionation of these crude extracts by the classical salting-out technique using AmS at 40, 60, and 80% saturation, major protein precipitant was secured at fraction 40% (3mg/mL) followed by fraction 60% and finally fraction 80%. Interestingly, this fashion of protein dissemination between the three AmS precipitants of quiescent seeds was not observed for the rest of the fractions taken at subsequent days of germination, as protein fluctuations were evident. Upon imbibition of the seeds, a clear decline in the protein content was observed. This decrease in protein content was continued until D9 after which a sudden increase in protein synthesis was also apparent (1.1 mg/mL). After this point, the protein content remained almost the same until the 3rd week of the experiment. Lectin activity was detectable in all protein fractions resulted from AmS saturations indicating the presence of lectin in the isoforms i.e., AbP-lec40, AbP-lec60, and AbP-lec80. AbP-lec40 was the most active form, which maintained its activity throughout the germination course (approx. 200 U/mg to as high as 1000U/mg). In contrary, AbP-lec80 was the isolectin with the least activity, where it was only active till D15 after which no activity was recorded (Figure 1 and Table 1). Figure 2A shows the germination and seedling growth of AbP seeds as well as the expression of isolectins at various stages of seed sprouting.

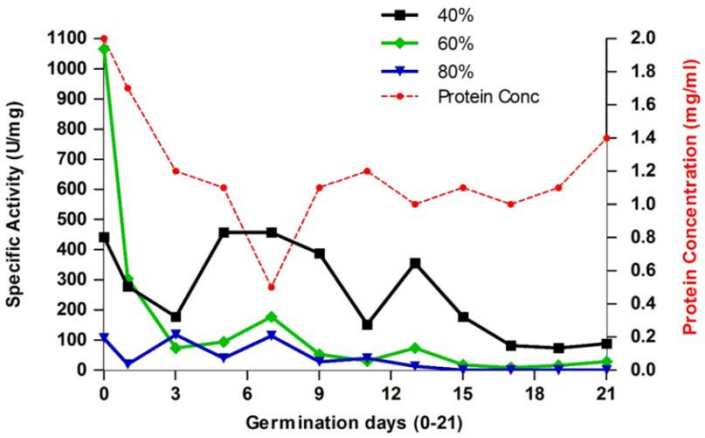

Figure 1: AbP seeds *soluble proteins and isolectins (40, 60, and 80) expression at varying days of germination.*

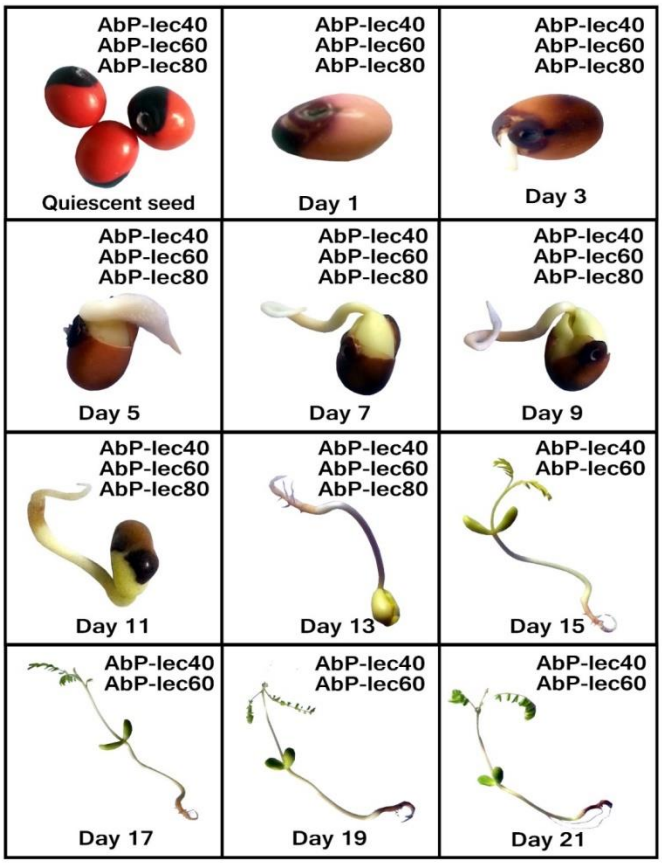

Figure 1B: AbP quiescent seeds and germinated cotyledons and axes with active isolectins at each stage.

Protein and lectin behavior during germination of Erythrina Abyssinica (ErA) seeds

Unlike AbP quiescent seeds, ErA contains comparably high protein content (6mg/mL). By the end of the 3rd week of germination, this high protein content was degraded very rapidly to reach as minimum as approximately 2mg/mL. Upon fractionation of protein by AmS at varying saturations as shown in AbP seeds crude extract, no systematic or fixed protein content was segregated among different precipitants. The lectin activity started at as high as 11 U/mg, which dropped within 4 days of germination by almost 70%. After that, the activity was again

increased to the same activity of the quiescent seeds and remained throughout the course of germination in almost the same level of activity. Whilst AbP exhibited apparent protein content fluctuations, ErA showed progressive protein degradation till the end of the 3rd week of the experiment. Fractionation of the germinated seeds crude protein extract (D1 to D21) with different concentrations of AmS (40, 60 and 80%) led not only to protein dissemination but also the lectin activities. Reasonably high lectin activity was detected in all AmS fractions. These lectin activities were named ErA-lec40, ErA-lec60, and ErA-lec80 according to AmS saturation at which lectin was detected. During the course of germination, the activity of three lectin isoforms exhibited apparent activity fluctuations as shown in Figure 2. ErA-lec60 had the highest activity, with three peaks at D3, D7, and D17, followed by ErA-lec80 which had two maxima at D6 and D15, and finally, ErA-lec40 which kept the least activity among the three isolectins (Table 2 and Figure 2). Figure 2B shows ErA seeds germination and seedling growth as well as the expression of isolectins at various stages of seed sprouting.

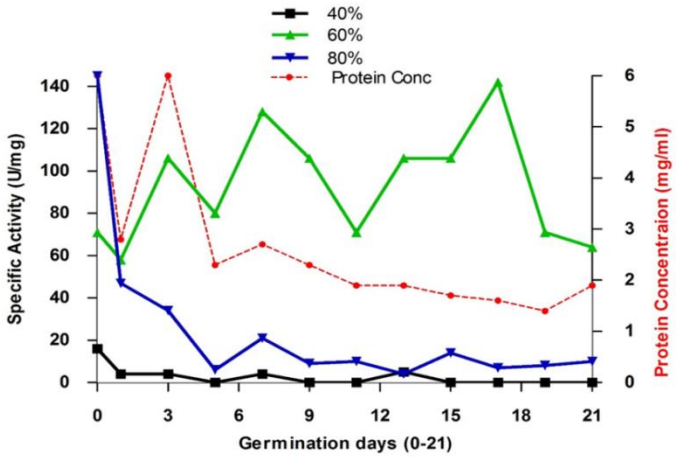

Figure 2A: ErA seeds' soluble proteins and isolectins (40, 60 and 80) expression at varying days of germination.

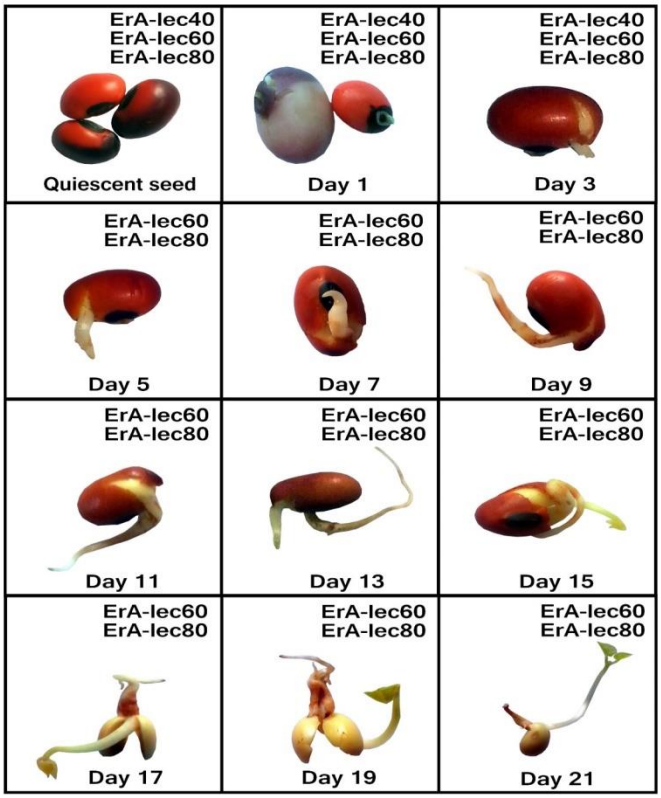

Figure 2B: ErA quiescent seeds and germinated cotyledons and axes with active isolectins at each stage

Discussion

Most legumes often contain lectin, which might contribute up to 15% of the total soluble seed protein (Cummings et al., 2015). Though animal lectins are assigned to perform several important cellular roles (Gabius, 1997), the definitive role of plant lectins remains debatable, though several publications have greatly contributed towards understanding this elusive biological assignment (De Hoff et al., 2009; Osman and Konozy, 2017; Rüdiger and Gabius, 2001). In the current investigation, we chose two legume plants, AbP and ErA to study the expression pattern of lectin during their germination. The selection of these two plants, in particular, was because of their being characterized proteins with respect to sugar specificity and some physicochemical parameters and in addition to a high level of lectin activity (Mohieddin, 2016.; Wu et al., 2001). The use of ammonium sulphate (AmS) for protein fractionation is known since a long time ago, being routinely applicable to isolate specific protein with the least contaminants (King, 1972). The bringing of the crude seeds' extracts of quiescent and subsequent germinated seeds to different saturations of AmS, allowed us to fractionate the crude extracts into three precipitant fractions. Upon analysis of the precipitants, lectin activity was detected in all of them, which might suggest either the presence of lectin in different isoforms or different proteins with variable molecular weights, however, since no data on the purification of iso/different lectins from these two legume plants are currently available, it becomes arduous to judge. When seeds commenced absorbing water, a significant decrease in protein content was evident in both plant seeds, most likely attributed to the mobilization of the softened cotyledon storage proteins to provide nutrients to the growing axes for the growth during the germination (Silva et al., 2000a). Bakskin *et al*. reported that soluble proteins from the seeds of six grass species were significantly degraded in their course of germination to meet their energy needs (Zhao et al., 2018). In spite of the fact that we used two genetically correlated plants belonging to the same family (Leguminosae) (Lagarda-Diaz et al., 2017) their lectins activity varies, this may be due to the different lectin composition in these seeds, amount of the third structure, and hydrophobic and hydroxyl groups, which are the parameters that may determine agglutination (Bhagyawant et al., 2015). The mature dry seed lectin of *Dolishose biflorus* found at a high level, the authors suggested that this is attributed to the conversion of precursors but not due to the de-novo synthesis of lectin (Talbot and Etzler, 1978). In germinated castor bean, after the storage proteins were consumed, the lectins in the endosperm were rapidly degraded (Harley and Beevers, 1986). In young seedlings of 3-9 days old of the two peanut varieties, more than 90% of the total amount of lectins, detected in the plants was in the cotyledons (Pueppke, 1979; Hagab, et al., 2018). The total soluble protein content found at ErA seed extracts was relatively higher than that of AbP seeds extracts. However, on the contrary, lectin activity was very much higher in the later. We observed that during the germination and early seedlings growth of the two plants under examination, there was a decrease in the cotyledonary lectin specific activity accompanied by a slow rate of total soluble protein degradation. Though there was a noticeable stepwise drop in the isolectins activities from the quiescent to germinated seeds, the three isolectins did not degrade equally. These results might strongly highlight role variations, though yet to be proven, between these isolectins. Several researchers have shown the localization of lectin along with other storage proteins in protein bodies vacuoles derived from the endoplasmic reticulum (Huang, 1985; Moreira Rde et al., 1991; Ramos et al., 2002). This finding led other researchers to suggest a function for plant lectins as storage proteins, however, the presence of the carbohydrate binding sites as well as the ability of some lectins to interact with hydrophobic–molecules through a hydrophobic binding pocket, in some lectins (Konozy et al., 2006; Shetty et al., 2013), might strongly argue against this allegation. Oliveira *et al.* in their work on *Erythrina velutina* seed germination showed different inconsistent route for degradation of storage proteins and lectin and thus, suggested a role for lectin beyond being merely a storage protein (Oliveira et al., 1998). The finding of Cavada *et al*. was in agreement with Oliveira *et al*. results, investigating on *Dioclea guianenis var. lasiophylla* seeds where an evident delay in lectin degradation was observed in comparison to the storage proteins, and therefore a role for plant lectin as a defense wall was put forwards (Cavada et al., 1994). Da Silva *et al.* published a report on lectin expression from germinated *Pisum arvense* seeds in which lectin was found to remain active in the entire course of germination resistive to protease action (SILVA et al., 2000b).

Conclusions

Taken the results of the present investigation along with other reports on legume lectin, we may conclude that these lectins apparently play a role other than merely being storage proteins. Presence of the lectin, in these plants, in isoforms with distinct variability in their activities during seeds germination stages may emphasize on different and possibly unrelated biological roles.

Conflict of Interest: Authors declare no conflict of interest.

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Abbreviations Used:

AbP: *Abrus precatorius;* **ErA:** *Erythrina Abyssinica;* **ErA-lec40:** *Erythrina Abyssinica* seed lectin activity detected at 40% ammonium sulphate saturation; **ErA-lec60:** *Erythrina Abyssinica* seed lectin activity detected at 60% ammonium sulphate saturation; **ErA-lec80:** *Erythrina Abyssinica* seed lectin activity detected at 80% ammonium sulphate saturation; **AbP-lec40:** *Abrus precatorius* seed lectin activity detected at 40% Ammonium sulphate saturation; **AbP-lec60:** *Abrus precatorius* seed lectin activity detected at 60% Ammonium sulphate saturation; **AbP-lec80:** *Abrus precatorius* seed lectin activity detected at 80% Ammonium sulphate saturation; **AmS:** Ammonium sulphate; **D:** day of germination; **HA:**

Hemagglutination; **BSA**: Bovine Serum Albumin; **U:** Unit; **NA:** No activity detected.

Table 1: purification and activities of *AbP* seed isolectins at different days of germination.

DAYS	fraction	Protein (mg/ml)	Unit	Specific activity (U/mg)	Total Units	Yield %	Fold Purification
D0	Crude	2	256	128	25600	100	1
	40%	0.29	128	441	1920	7.5	3.4
	60%	0.24	256	1066	3840	15	8.3
	80%	0.15	16	106	240	0.93	0.82
D1	Crude	1.7	128	75	12800	100	1
	40%	0.46	128	278	1920	15	3.7
	60%	0.39	64	304	960	7.5	4
	80%	0.39	8	20	120	0.93	0.26
D3	Crude	1.2	256	213	25600	100	1
	40%	0.36	64	177	960	3.75	0.8
	60%	0.43	32	74	480	1.8	0.34
	80%	0.27	32	118	480	1.8	0.55
D5	Crude	1.1	128	116	12800	100	1
	40%	0.28	128	457	1920	15	3.9
	60%	0.34	32	94	480	3.7	0.8
	80%	0.1	4	40	60	0.46	0.34
D7	Crude	0.5	128	256	12800	100	1
	40%	0.28	128	457	1920	15	1.7
	60%	0.36	64	177	960	7.5	0.69
	80%	0.07	8	114	120	0.93	0.44
D9	Crude	1.1	128	116	12800	100	1
	40%	0.33	128	387	1920	15	3.3
	60%	0.3	16	53	240	1.8	0.45
	80%	0.07	2	28	30	0.23	0.24

DAYS	fraction	Protein (mg/ml)	Unit	Specific activity (U/mg)	Total Units	Yield %	Fold Purification
D11	Crude	1.2	64	53	6400	100	1
	40%	0.42	64	152	960	15	2.8
	60%	0.27	16	31	240	3.75	0.58
	80%	0.1	4	40	60	0.93	0.75
D13	crude	1	64	64	6400	100	1
	40%	0.36	128	355	1920	30	5.5
	60%	0.43	32	74	480	7.5	1.1
	80%	0.15	2	13	30	0.46	0.20
D15	crude	1.1	64	58	6400	100	1
	40%	0.36	64	177	960	15	3
	60%	0.43	8	18	120	1.8	0.31
	80%	0.1	NA	-	-	-	-
D17	crude	1	32	32	3200	100	1
	40%	0.39	32	82	480	15	2.5
	60%	0.42	4	9	60	1.8	0.28
	80%	0.09	NA	-	-	-	-

D19	crude	1.1	16	14	1600	100	1
	40%	0.43	32	74	480	30	5.2
	60%	0.3	8	16	120	7.5	1.1
	80%	0.09	NA	-	-	-	-
D21	crude	1.4	16	11	1600	100	1
	40%	.0.36	32	88	480	30	8
	60%	0.27	8	29	120	7.5	2.6
	80%	0.1	NA	-	-	-	-

NA: No activity detected

Table 2: purification and activities of *Erythrina abyssinica* seed isolectins at different days of germination.

DAYS	fraction	Protein (mg/ml)	Unit	Specific activity (U/mg)	Total Units	Yield %	Fold Purification
D0	crude	6	64	11	6400	100	1
	40%	1.08	16	16	240	3.75	1.4
	60%	1.8	128	71	1920	30	6.4
	80%	0.44	64	145	960	15	13
D1	crude	2.8	8	2	800	100	1
	40%	0.47	2	4	30	3.75	2
	60%	1.1	64	58	960	120	29
	80%	0.34	16	47	240	30	23
D3	crude	6	16	3	1600	100	1
	40%	0.44	2	4	30	1.87	1.3
	60%	1.2	128	106	1920	120	35
	80%	0.47	16	34	240	15	11
D5	crude	2.3	32	13	3200	100	1
	40%	0.32	NA	-	-	-	-
	60%	0.8	64	80	960	30	6.1
	80%	0.72	4	6	60	1.8	0.46
D7	crude	2.7	32	11	3200	100	1
	40%	0.42	NA	-	-	-	-
	60%	1	128	128	1920	60	11
	80%	0.37	8	21	120	3.75	1.90
D9	crude	2.3	32	13	3200	100	1
	40%	0.32	NA	-	-	-	-
	60%	1.2	128	106	1920	60	8
	80%	0.42	4	9	60	1.87	0.69

DAYS	fraction	Protein (mg/ml)	Unit	Specific activity (U/mg)	Total Units	Yield %	Fold Purification
D11	crude	1.9	16	8	1600	100	1
	40%	0.37	NA	-	-	-	-
	60%	0.9	64	71	960	60	8.87
	80%	0.40	4	10	60	3.75	1.25
D13	crude	1.9	8	4	800	100	1
	40%	0.39	NA	-	-	-	-
	60%	1.2	128	106	1920	240	26.5
	80%	0.96	4	4	60	7.5	1
D15	crude	1.7	16	9	1600	100	1

	40%	0.34	NA	-	-	-	-
	60%	1.2	128	106	1920	120	11.7
	80%	0.56	8	14	120	7.5	1.5
D17	crude	1.6	16	10	1600	100	1
	40%	0.41	NA	-	-	-	-
	60%	0.9	128	142	1920	120	14.2
	80%	0.56	4	7	60	3.75	0.7
D19	crude	1.4	16	11	1600	100	1
	40%	0.38	NA	-	-	-	-
	60%	0.9	64	71	960	60	6.45
	80%	0.45	4	8	60	3.75	0.7
D21	crude	1.9	16	8	1600	100	1
	40%	0.37	NA	-	-	-	-
	60%	1	64	64	960	60	8
	80%	0.38		10	60	3.75	1.25

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