

Biochemical Analysis Of Allyl Sulfide Compounds From Pungent Vegetables And Their Gene Expression Analysis Using Bioinformatic Tools.

P.B.RameshBabu¹, Sathik Shajahan²

¹Professor and Head, ²UG Student

Dept. of Genetic Engineering

BIHER, BIST, Bharath University

Chennai- 600073.

rameshbabupb.gen@bharathuniv.ac.in

ABSTRACT

The scientists found that in lab tests, extracts from the tearless onion significantly reduced platelet clumping compared to regular onions or even garlic. Other scientific result showed that the new onion had about the same anti-inflammatory properties as the original. The pungent smell is caused by the enzyme “allinase”, in this paper we have done sequence study of the allinase gene and also the protein study. The location of this particular gene has been found using bioinformatics tools. To design a vector for the restriction enzyme, which is used to restrict that particular gene so that the sulphur content will retain in our body and the lachrymator enzyme will not be released in order to stop the tears completely. The consequences of this dramatic simultaneous down- and up-regulation of secondary sulfur products on the health and flavor attributes of the onion are discussed.

KEY WORDS: Allinase, Restriction enzyme sites, Tearless onions, Lacrimator, lachrymatory-factor synthase

INTRODUCTION

When an onion is sliced or dried, the onion's cells release these compounds into the air. During his process “Allinase enzyme” works to alter the amino acids into lachrymator compounds [4]. This form of sulfuric acid irritates the nerves around the eyes making them tear. Irritations to the tear glands cause it to secrete tears continuously without being influenced by the intent and emotion to cry [10]. The chemical compound released by onion is allyl sulfide and they also exist in garlic and spring onions [2, 3]. The chemical contained in *Allium cepa* are reported to be effective agent against fungal and bacterial growth They protect against stomach, colon and skin cancer and also they have anti-inflammatory [14], antiallergenic, anti asthmatic and antidiabetic action [16] and they treat causes of cardiovascular disorder [17] including hyper tension, hyperglycemia and hyperlipidemia [hepatoprotective

PROCESS INVOLVED IN ONION:

1. Lachrymatory-factor synthase is released into the air when we cut an onion.
2. The synthase enzyme converts the sulfoxides{amino acid} of the onion into sulfenic acid.
3. The unstable sulfenic acid rearranges itself into syn-propanethial-S-oxide.
4. syn-propanethial-S-oxide gets into the air and comes in contact with our eyes. The lachrymal glands become irritated and produces the tears.

Colin C.Eady and colleagues reported that the onion has a unique chemistry that leads to its tear-inducing effects when cut. Its pungency has driven cooks to don goggles, clench wooden spoons in their mouths and try other usually futile techniques to prevent crying at the cutting board. An answer could arrive in the form of a new type of onion that makes less of the protein blamed for making eyes burn and tear up [11]. Eady's team has developed such a version, which instead makes a sulfur compound similar to one found in cut garlic that may be the key to its cardiovascular benefits. Many people eat garlic cloves or take it as a nutritional supplement in pill form to reduce the clumping of platelets in the blood, which can lead to blood clot and clogged arteries [6, 19].

The scientists found that in lab tests, extracts from the tearless onion significantly reduced platelet clumping compared to regular onions or even garlic [17]. Other result showed that the new onion had about the same anti-inflammatory properties as the original. Also, preliminary testing in rats showed that the tearless onion could help control weight gain more so that regular onions are garlic. Japanese food manufacturing company, house food group, has announced the development of a new tearless onion that is less pungent, so no more odour on your hands. Scientists say that have managed to disable the production of a powerful substance an onion releases as the knife slices into it, cutting down on the pungent fumes that bring tears to the eyes. House foods group said in a press release that they bombarded the brown bulb with irradiating ions in a process that drastically reduces the level of a certain enzyme that is the key to this process. This team figured out how to neutralize the onion by halting the production of an enzyme that is released when the onion is cut by zapping it with irradiating ions [9].

But the Japanese team suspected something else was at play, because garlic also contains alliinase and it's decidedly tear-free. They added alliinase specifically to PRENCSO, so the alliinase alone is not responsible for the breakdown. There's another missing enzyme and it turns out the culprit is Lachrymatory factor (LF) synthase, a powerful enzyme found in onion but not their milder garlic cousins. They reported that they can now disable the enzyme while retaining

the flavor of the onion. They are often compounds that will repel insects or animals that try to bite into it [23-27].

The irritating lachrymatory factor that is released by onions when they are chopped up has been presumed to be produced spontaneously following the action of the enzyme allinase, which operates in the biochemical pathway that produces the compounds responsible for the onion's characteristic flavor [5]. Here we show that this factor is not formed as a by-production of this reaction, but that it is specifically synthesized by a previously[20, 21]. undiscovered enzyme, lachrymatory-factor synthase. It may be possible to develop a non-lachrymatory onion that still retains its characteristic flavor and high nutritional value by down regulating the activity of this synthase enzyme. House foods group researchers identified a previously unknown enzyme responsible for this process and hypothesized that it would be possible to weaken the function of the enzyme, while still preserving nutrition and flavor in the onion [8].

Materials and methods:

EXPASY:

ExPASy is a bio resource portal operated by the Swiss Institute of Bioinformatics (SIB) and in particular the SIB Web Team. It is an extensible and integrative portal accessing many scientific resources, databases and software tools in different areas of life sciences. Scientists can access a wide range of resources in many different domains, such as proteomics, genomics, phylogeny/evolution, systems biology, population genetics, and transcriptomics. The individual resources (databases, web-based and downloadable software tools) are hosted in a decentralised way by different groups of the SIB Swiss Institute of Bioinformatics and partner institutions. Specifically, a single web portal provides a common entry point to a wide range of resources developed and operated by many different SIB groups and external institutions. [12, 13]. The portal features a search function across selected resources. Internally, the availability and usage of resources are monitored. The portal is aimed for both expert users and for people who are not familiar with a specific domain in life sciences: in particular, the new web interface provides visual guidance for newcomers to ExPASy [12-14]

Protein identification and analysis software performs a central role in the investigation of proteins from two-dimensional (2-D) gels and mass spectrometry. For protein identification, the user matches certain empirically acquired information against a protein database to define a protein as already known or as novel. For protein analysis, information in protein databases can be used to predict certain properties about a protein, which can be useful for its empirical investigation. Analysis tools in ExPASy will include Compute pI/Mw, a tool for predicting protein isoelectric point (pI) and molecular weight (Mw); ProtParam, to calculate various physicochemical parameters; PeptideMass, [22,23], a tool for theoretically cleaving proteins and calculating the masses of their peptides and any known cellular or artifactual posttranslational modifications; Peptide Cutter, to predict cleavage sites of proteases or chemicals in protein sequences; ProtScale, for amino acid scale representation, such as hydrophobicity plots. Protein identification tools include TagIdent, a tool that lists proteins within a userspecified pI and Mw

region, and allows proteins to be identified through the use of short “sequence tags” up to six amino acids long [20,21].

Workspace

The SWISS-MODEL Workspace provides a personal web-based area for each user in which protein homology models can be built and the results of completed modelling projects are stored and visualized. In the workspace a list of the current modeling work units and their current status is displayed: *submitted* (the job has been submitted to the pipeline but still queuing), *running* (job is running and programs are calculating), *finished* (job has been completed, final results are available) or *failed/stopped* (if something went wrong during the process). Depending on the type of job the user has submitted a different tag will be associated with a work unit: *Template Identification* for template identification, *Sequence Scanning* for secondary structure and disorder prediction and domain assignment, *Structure Assessment* for structure quality assessment. And *Modelling Automatic*, *Modelling Project*, *Modelling Alignment* respectively for automated, alignment or project mode modeling requests. After completion of the modelling procedure (~ a few minutes up to several hours), the results are stored in the workspace and the user is notified about the completion. The user can access the results output by clicking on the work unit ID number. The results are stored for one week on the server. The remaining time before deletion of a given work unit is also displayed. The user can decide to either delete a work unit or to prolong its life span by clicking on the corresponding link.

DNA Extraction from Onion:

An onion is used because it has a low starch content, which allows the DNA to be more clearly seen. The salt shields the negative phosphate ends of DNA, which allows these ends to come closer so the DNA can precipitate out of a cold alcohol solution. The detergent causes the cell membrane to break down by dissolving the lipids and proteins of the cell disturbing the bonds that hold the cell membrane together. The detergent then forms complexes with these lipids and proteins causing them to precipitate out of solution. You will be altering the onion solution so that you can extract DNA. For a variety of reasons, the DNA extracted for cells may not spool. Temperature controls and the purity of the extraction are the most common factors.

Results and Discussion:

The comparison of nucleotide or protein sequences from the same or different organisms is a very powerful tool in molecular biology. By finding similarities between sequences, scientists can infer the function of newly sequenced genes, predict new members of gene families, and explore evolutionary relationships. Now that whole genomes are being sequenced, sequence similarity searching can be used to predict the location and function of protein-coding and transcription regulation regions in genomic DNA. Basic Local Alignment Search Tool (BLAST) is the tool most frequently used for calculating sequence similarity. BLAST comes in variations

for use with different query sequences against different databases. All BLAST applications, as well as information on which BLAST program to use and other help documentation, are listed on the BLAST homepage.

Protein Sequence Of Alliinase:

Alliinase *Alliumsativum*

GenBank AAB32477.1 GenPept Identical Proteins Graphics [gigb](#) AAB.

```
MICLVILTCIIMSNSFVNNNNMVQAKMTWTMKAEEAEAVANINCSEHGRAFLDGIISE
GSPKCECNTCYTGPDCEKIQGCSADVASGDGLFLEEWKQHQKEASAVLVSPWHRMSY
FFNPVSNFISFELEKTIKELHEVVGNAAAKDRYIVFGVGVVTQLIHGLVISLSPNMTATPDA
PESKVVAHAPFYPVFREQTKYFDKKGYVWAGNAANYVNVSNPEQYIEMVTSPNNPEGL
LRHAVIKGCKSIYDMVYYWPHYTPIKYKADEDILLFTMSKFTGHSGSRFGWALIKDES
VYNNLLNYMTKNTGTPRETQLRSLKVLKEIVAMVKTQKGTMRDLNTFGFKLRERWV
NITALLDQSDRFSYQELPQSEYCNVFRMRPPSPSYAWVNCEWEEDKDCYQTFQNGRIN
TQSGVGFEASSRYVRLSLIKTQDDFDQLMYYLKDMVKAKRKTPLIKQLFTDETETASRR
PF
```

Table 1 : Rank ID DIST LEN2 POS1 POS2 pI DE

1	alln_allsa	2.45	486	1	486	8.08	Alliin lyase precursor (EC 4.4.1.4) (Alliinase) (Cysteine sulphoxide)
2	alln_allce	3.50	479	1	479	8.08	Alliin lyase precursor (EC 4.4.1.4) (Alliinase) (Cysteine sulphoxide)
3	alln_allas	4.39	447	1	447	8.32	Alliin lyase precursor (EC 4.4.1.4) (Alliinase) (Cysteine sulphoxide)
4	cpk3_oncmy	5.11	491	1	491	6.47	Cytochrome P450 2K3 (EC 1.14.14.1) (CYPIIK3) (P450 LMC2).
5	tyr1_yeast	5.32	452	1	452	6.58	Prephenate dehydrogenase [NADP+] (EC 1.3.1.13) (PRDH).

Restriction Mapper result:

Name: Untitled

Conformation: linear

Overhang: five_prime, three_prime, blunt

Minimum Site Length: 5 bases

Maximum Number of Cuts: all

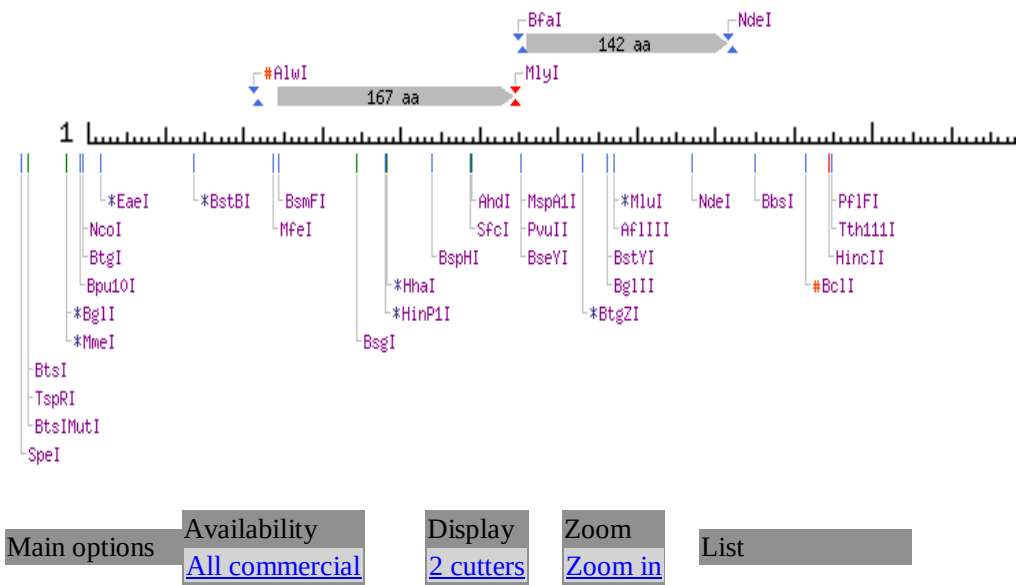
Included: all commercial, prototypes only

Noncutters: AarI, AatII, AbsI, AccI, AclI, AcyI, AflII, AgeI, AjuI, AlfI, AloI, ApaI, ApaLI, AscI, AvaI, AvrII, BaeI, BalI, BamHI, BarI, BbvCI, BcgI, BciVI, BfiI, BpII, BsaAI, BsaBI, BsaXI, BsePI, BseSI, BsmI, Bsp1407I, BspMI, BstEII, BstXI, BtrI, Cfr10I, ClaI, CspCI, DraII, DraIII, DrdI, EciI, Eco47III, Eco57I, EcoNI, EcoP15I, EcoRI, EcoRII, EcoRV, Esp3I, FauI, FseI, FspAI, HaeII, HpaI, Hpy99I, KpnI, MauBI, NaeI, NarI, NheI, NmeAIII, NotI, NruI, NspI, OliI, PacI, PaeI, PflMI, PfoI, PmaCI, PmeI, PpiI, PpuMI, PshAI, PI-PspI, PspXI, PsrI, PstI, PvuI, RsrII, SacI, SacII, SalI, SanDI, SapI, PI-SceI, SduI, SexAI, SfiI, Sgfi, SgrAI, SgrDI, SmaI, SnaBI, SphI, SrfI, Sse8387I, StuI, SwaI, VspI, XbaI, XcmI, XhoI, XmnI

Table 2 : Allinase gene Restriction Enzyme sites with cut position details

Name	Sequence	Site Length	Overhang	Frequency	Cut Positions
HindII	GTYRAC	6	blunt	1	1835
PvuII	CAGCTG	6	blunt	1	1143
AflIII	ACRYGT	6	five_prime	1	1352

Figure 1 : Diagrammatic representation of positions of Restriction Enzyme cutting sites in gene



New DNA	All	3 cutters	More...	0 cutters
Custom digest				1 cutters
View sequence	Minimum ORF length to display: 100 aa.			All sites
ORF summary				Save all sites
Save project				Flanking enzymes
Print				

In this paper bioinformatic tools were used to find where the particular gene are present in the onion, which will throw more light in identifying DNA sequences where restriction enzyme will be used to digest them into smaller fragments. Using this information it will be possible to design a vector for the restriction enzyme digestion for making tearless onion by manipulating the Allinase gene in onion gene families.[37-40] The enzyme used to restrict that particular Allinase gene so that the sulphur content will retain in our body and the lachrymator enzyme will not be released, thereby preventing or stopping the tears completely while onion is cut.[41-45]

REFERENCE:

1. Nimal, R.J.G.R., Hussain, J.H., Effect of deep cryogenic treatment on EN24 steel, International Journal of Pure and Applied Mathematics, V-116, I-17 Special Issue, PP-113-116, 2017
2. Parameswari, D., Khanaa, V., Deploying lampert clocks and linked lists, International Journal of Pharmacy and Technology, V-8, I-3, PP-17039-17044, 2016
3. Parameswari, D., Khanaa, V., Case for massive multiplayer online role-playing games, International Journal of Pharmacy and Technology, V-8, I-3, PP-17404-17409, 2016
4. Parameswari, D., Khanaa, V., Deconstructing model checking with hueddot, International Journal of Pharmacy and Technology, V-8, I-3, PP-17370-17375, 2016
5. Parameswari, D., Khanaa, V., The effect of self-learning epistemologies on theory, International Journal of Pharmacy and Technology, V-8, I-3, PP-17314-17320, 2016
6. Pavithra, J., Peter, M., GowthamAashirwad, K., A study on business process in IT and systems through extranet, International Journal of Pure and Applied Mathematics, V-116, I-19 Special Issue, PP-571-576, 2017
7. Pavithra, J., Ramamoorthy, R., Satyapira Das, S., A report on evaluating the effectiveness of working capital management in googolsoft technologies, Chennai, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-129-132, 2017
8. Pavithra, J., Thooyamani, K.P., A cram on consumer behaviour on Mahindra two wheelers in Chennai, International Journal of Pure and Applied Mathematics, V-116, I-18 Special Issue, PP-55-57, 2017
9. Pavithra, J., Thooyamani, K.P., Dkhar, K., A study on the air freight customer satisfaction, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-179-184, 2017

10. Pavithra, J., Thooyamani, K.P., Dkhar, K., A study on the working capital management of TVS credit services limited, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-185-187, 2017
11. Pavithra, J., Thooyamani, K.P., Dkhar, K., A study on the analysis of financial performance with reference to Jeppiaar Cements Pvt Ltd, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-189-194, 2017
12. Peter, M., Dayakar, P., Gupta, C., A study on employee motivation at Banalari World Cars Pvt Ltd Shillong, International Journal of Pure and Applied Mathematics, V-116, I-18 Special Issue, PP-291-294, 2017
13. Peter, M., Kausalya, R., A study on capital budgeting with reference to signware technologies, International Journal of Pure and Applied Mathematics, V-116, I-18 Special Issue, PP-71-74, 2017
14. Peter, M., Kausalya, R., Akash, R., A study on career development with reference to premheerasurgicals, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-415-420, 2017
15. Peter, M., Kausalya, R., Mohanta, S., A study on awareness about the cost reduction and elimination of waste among employees in life line multispeciality hospital, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-287-293, 2017
16. Peter, M., Srinivasan, V., Vignesh, A., A study on working capital management at deccan Finance Pvt Limited Chennai, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-255-260, 2017
17. Peter, M., Thooyamani, K.P., Srinivasan, V., A study on performance of the commodity market based on technical analysis, International Journal of Pure and Applied Mathematics, V-116, I-18 Special Issue, PP-99-103, 2017
18. Philomina, S., Karthik, B., Wi-Fi energy meter implementation using embedded linux in ARM 9, Middle - East Journal of Scientific Research, V-20, I-12, PP-2434-2438, 2014
19. Philomina, S., Subbulakshmi, K., Efficient wireless message transfer system, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-289-293, 2017
20. Philomina, S., Subbulakshmi, K., Ignition system for vehicles on the basis of GSM, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-283-286, 2017
21. Philomina, S., Subbulakshmi, K., Avoidance of fire accident by wireless sensor network, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-295-299, 2017
22. Pothumani, S., Anuradha, C., Monitoring android mobiles in an industry, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-537-540, 2017
23. Pothumani, S., Anuradha, C., Decoy method on various environments - A survey, International Journal of Pure and Applied Mathematics, V-116, I-10 Special Issue, PP-197-199, 2017
24. Pothumani, S., Anuradha, C., Priya, N., Study on apple iCloud, International Journal of Pure and Applied Mathematics, V-116, I-8 Special Issue, PP-389-391, 2017

25. Pothumani, S., Hameed Hussain, J., A novel economic framework for cloud and grid computing, International Journal of Pure and Applied Mathematics, V-116, I-13 Special Issue, PP-5-8, 2017
26. Pothumani, S., Hameed Hussain, J., A novel method to manage network requirements, International Journal of Pure and Applied Mathematics, V-116, I-13 Special Issue, PP-9-15, 2017
27. Pradeep, R., Vikram, C.J., Naveenchandra, P., Experimental evaluation and finite element analysis of composite leaf spring for automotive vehicle, Middle - East Journal of Scientific Research, V-12, I-12, PP-1750-1753, 2012
28. Pradeep, R., Vikram, C.J., Naveenchandran, P., Experimental evaluation and finite element analysis of composite leaf spring for automotive vehicle, Middle - East Journal of Scientific Research, V-17, I-12, PP-1760-1763, 2013
29. Prakash, S., Jayalakshmi, V., Power quality improvement using matrix converter, International Journal of Pure and Applied Mathematics, V-116, I-19 Special Issue, PP-95-98, 2017
30. Prakash, S., Jayalakshmi, V., Power quality analysis & power system study in high voltage systems, International Journal of Pure and Applied Mathematics, V-116, I-19 Special Issue, PP-47-52, 2017
31. Prakash, S., Sherine, S., Control of BLDC motor powered electric vehicle using indirect vector control and sliding mode observer, International Journal of Pure and Applied Mathematics, V-116, I-19 Special Issue, PP-295-299, 2017
32. Prakesh, S., Sherine, S., Forecasting methodologies of solar resource and PV power for smart grid energy management, International Journal of Pure and Applied Mathematics, V-116, I-18 Special Issue, PP-313-317, 2017
33. Prasanna, D., Arulselvi, S., Decoupling smalltalk from rpcs in access points, International Journal of Pure and Applied Mathematics, V-116, I-16 Special Issue, PP-1-4, 2017
34. Prasanna, D., Arulselvi, S., Exploring gigabit switches and journaling file systems, International Journal of Pure and Applied Mathematics, V-116, I-16 Special Issue, PP-13-17, 2017
35. Prasanna, D., Arulselvi, S., Collaborative configurations for wireless sensor networks systems, International Journal of Pure and Applied Mathematics, V-116, I-15 Special Issue, PP-577-581, 2017
36. Priya, N., Anuradha, C., Kavitha, R., Li-Fi science transmission of knowledge by way of light, International Journal of Pure and Applied Mathematics, V-116, I-9 Special Issue, PP-285-290, 2017
37. Priya, N., Pothumani, S., Kavitha, R., Merging of e-commerce and e-market-a novel approach, International Journal of Pure and Applied Mathematics, V-116, I-9 Special Issue, PP-313-316, 2017
38. Raj, R.M., Karthik, B., Effective demining based on statistical modeling for detecting thermal infrared, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-273-276, 2017
39. Raj, R.M., Karthik, B., Energy sag mitigation for chopper, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-267-270, 2017

40. Raj, R.M., Karthik, B., Efficient survey in CDMA system on the basis of error revealing, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-279-281, 2017
41. Rajasulochana, P., Krishnamoorthy, P., Ramesh Babu, P., Datta, R., Innovative business modeling towards sustainable E-Health applications, International Journal of Pharmacy and Technology, V-4, I-4, PP-4898-4904, 2012
42. Rama, A., Nalini, C., Shanthi, E., An iris based authentication system by eye localization, International Journal of Pharmacy and Technology, V-8, I-4, PP-23973-23980, 2016
43. Rama, A., Nalini, C., Shanthi, E., Effective collaborative target tracking in wireless sensor networks, International Journal of Pharmacy and Technology, V-8, I-4, PP-23981-23986, 2016
44. Ramamoorthy, R., Kanagasabai, V., Irshad Khan, S., Budget and budgetary control, International Journal of Pure and Applied Mathematics, V-116, I-20 Special Issue, PP-189-191, 2017
45. Ramamoorthy, R., Kanagasabai, V., Jivandan, S., A study on training and development process at Vantec Logistics India Pvt Ltd, International Journal of Pure and Applied Mathematics, V-116, I-14 Special Issue, PP-201-207, 2017

