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Contents

Identification, Functional Annotation and Analysis of COS Markers in Zingiber and Its Utility in DNA Barcoding

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A B S T R A C T

Species identification is exceedingly difficult in Zingiberaceae due to similarities in morphological characteristics between species, the plants' phenotypic plasticity, and their short and seasonal flowering cycles. DNA barcoding has great potential in Zingiberaceae, both for the confirmation of raw materials in the pharmaceutical sector and as a tool in conservation biology and ecology. A suitable barcode target in the family Zingiberaceae is yet to be discovered. Conserved ortholog set (COS) markers are single-copy evolutionary conserved genes in two or more species that share common ancestry (orthologous). Due to the conserved nature of these markers across genera they may be possible barcode candidates and these conserved set may be used as low-variation universal loci across the monocots. In the present study identification, functional annotation and analysis of COS markers in Zingiberaceae is carried out. About 37 COS markers were identified, validated and tested.The functional annotation revealed house-keeping genes (85.7%), a defense gene, 3 mitochondrial proteins and one chloroplast protein. The utilization of a COS marker (ZE372342) as barcode target for *Zingiber* genera were tested and the results provide a promising target.

Keywords: COS; DNA barcoding; Zingiberaceae; EST; Single-copy gene.

1) Introduction

Zingiberaceae (gingers) is a pantropic family consisting of approximately 53 genera and 1200 species [1], [2]. Most of the gingers are rhizomatous, and the rhizomes (underground stem) of several gingers are used as spices, vegetables, neutraceuticals, drugs or indispensable ingredients in traditional medicines in Southeast Asian countries [3], [4]. Species identification is exceedingly difficult in Zingiberaceae due to similarities in morphological characteristics between species, the plants' phenotypic plasticity, and their short and seasonal flowering cycles. DNA barcoding has great potential in Zingiberaceae, both for the confirmation of raw materials in the pharmaceutical sector [5], [6], [7] and as a tool in conservation biology and ecology. Despite considerable debate over the last 8 years, there is little consensus regarding the choice of loci for barcoding plants. Each plant barcode locus has different strengths and weaknesses, and their resolvability and universality vary considerably between taxa [8], [9]. Shi et al. [4] evaluated the universality and resolvability of five chloroplast loci and nrDNA internal transcribed spacer 2 (ITS2) in Zingiberaceae members in China and found ITS2 be the most promising locus. Contrastingly study by Vinitha et al.[10] suggests that *matK* and *rbcL* loci are suitable for barcoding Zingiberaceae members and they highlight the poor utility of ITS and its intragenomic heterogeneity in the species tested. Another study points out that *Curucuma,* a Zingiberaceae member is a challenging group for DNA barcoding wherein the four barcode regions they used (*matK, rbcL, trnH-psbA* and *trnL-F*) yielded no barcode gaps [11]. Thus this highlights the lack of a suitable barcode target in the family Zingiberaceae.

Conserved ortholog set (COS) markers [12] are single-copy evolutionary conserved genes in two or more species that share common ancestry (orthologous). Due to the conserved nature of these markers across genera they may be possible barcode candidates and these conserved set can be used as low-variation universal loci across the plant kingdom [13]. COS markers have been identified in several flowering plant (angiosperms) families, including the Euasterids-Solanaceae, Rubiaceae, and Asteraceae [14], the legume family (Leguminosae) and grass family, Poaceae [15], the composite family, Asteraceae [16], Rosaceae [17], gymnosperms, Pinaceae [18] and forest trees [19]. A comparative genomic approach is not yet attempted in Zingiberaceae family. In the present study identification, functional annotation and analysis of conserved orthologous sequences (COS) in genus *Zingiber* is carried out. Later on the possibility of a COS marker as putative barcode candidate is tested across *Zingiber* genus.

2 Materials and Methods

2.1 Ginger EST Database

Zingiber officinale EST collection was downloaded from NCBI (National Center for Biological Information) (http://www.ncbi.nlm.nih.gov/dbEST/). The EST collection include 38139 ESTs which were classified into three tissue libraries of leaves 13274 (DV544275-ES560515), rhizomes 12763 (DY350707-DY363469) and roots 12092 (DY363470-DY375561) [20].

2.2 Computational Screening of Conserved Ortholog Set Markers

Conserved orthologs between rice (*Oryza sativa*) and ginger were identified following the method of Fulton et al.[12] with modifications. Rice genome was downloaded from NCBI (http://www.ncbi.nlm.nih.gov/genome). A conservative computational strategy was followed to avoid paralogs. Ginger ESTs were scanned against Rice genome using Spidey, a computer program that aligns spliced sequences to genomic sequences, using local alignment algorithms and heuristics to put together a global spliced alignment (http://www.ncbi.nlm.nih.gov/spidey) [21]. Three major criteria were implied for selecting a conserved ortholog, (i) EST should provide hit against rice genome at an expect value < E^{-20} (ii) Next best rice genome hit must be of lower significance, i. e. expect value should be less than E^{-1} (iii) Match should cover 100% coverage of EST. Those ESTs which obey the above mentioned criteria were validated against the ginger unigene set composed of 38139 contigs to ensure that all COS markers chosen represent unique ginger genes. They were blasted against rice genome also to validate its uniqueness. The selected COS regions were annotated by BLASTX analysis against the Gen Bank protein database maintained at the NCBI (http://www.ncbi.nlm.nih.gov/)

2.3 Primer Designing

After rigorous screening, about 37 regions were selected as putative COS markers. These selected regions were blasted against the available monocot sequences and those which provide hits with an expected value <$E^{-20}$was identified and selected for primer designing. Thus the primers designed were supposed to be universal primers for orthologous genes across monocots. Primers were designed using Primer 3 software [22] and primers were custom synthesized by Metabion, Germany. A total of 51 primer pairs were synthesized and their annealing temperature ranged from 50-66°C.

2.4 Plant Materials

Preliminary screening of the primers was done in one accession each of *Z. officinale* and *Z. zerumbet*. Two accessions each of seven species of *Zingiber* were used for the barcoding studies (Table 1). GenElute Plant Genomic Kit (Sigma) was used to isolate total genomic DNA from the selected samples.

Table 1 List of plant materials used for the study

Species	Accession no.	Source
Z.wightianum	*Z.wightianum_2*	Anamalai, Kerala
	Z.wightianum_1	Anamalai, Kerala
Z. zerumbet	*Z. zerumbet_1*	Poovathode, Kerala
	Z. zerumbet_2	Poovathode, Kerala
Z.parishi	*Z.parishi_1*	Andaman
	Z.parishi_2	Andaman
Z.officinale	*Z.officinale_1*	Valparai, TamilNadu
	Z.officinale_2	Valparai, TamilNadu
Z.oderiferum	*Z.oderiferum_1*	Andaman
	Z.oderiferum_2	Andaman
Z.nimonii	*Z.nimonii_1*	Poovathode, Kerala
	Z.nimonii_2	Palakkad, Kerala
Z.neesanum	*Z.neesanum_2*	Ponmudi, Kerala
	Z.neesanum_1	Idukki,Kerala

* Plants used for initial primer screening

2.5 PCR Amplification

PCR was performed on a GeneAmp PCR System 9700 (Applied Biosystems) in a 10-µL volume containing 10 ng total DNA, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1picomole each of forward and reverse primers, 0.1mg/ml BSA, 4% DMSO, 0.5 U AmpliTaq Gold polymerase (Applied Biosystems) and 1X AmpliTaq Gold PCR buffer. The PCR profile included an initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 30 s, primer annealing at 50 – 66°C for 40 s and DNA strand extension at 72°C for 1 min with a final extension of 7 min at 72°C.The amplified products were visualised in agarose gel electrophoresis. Only the primers which provided single intense band were selected for further sequencing. In the present study only one region (ZE372342) was used for barcoding*Zingiber* genus.

2.6 ExoSAP IT clean-up and DNA Sequencing

The amplified PCR product after visualisation in agarose gel electrophoresis, were cleaned up using ExoSAP-IT PCR product clean up reagent, Thermo Fisher Scientific. About 1 µl of ExoSAP-IT is added to 5 µl PCR product and kept for 45 minutes incubation at 37°C. After treatment, ExoSAP-IT is inactivated by incubating at 80°C for 15 minutes. The cleaned up amplicon were sequenced using ABI® prism Big Dye Terminator v3.1 Cycle Sequencing Kit (USA). The PCR profile consisted of an initial denaturation at 96°C for 2 min followed by 25 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for 4 min. in a DNA Thermal cycler (ABI). After post sequencing clean up capillary electrophoresis were carried in ABI 3730 DNA Analyzer

2.7 Sequence Analysis

The sequences obtained from the 14 samples were aligned using the program Geneious Pro 5.0.4 [23] and refined manually as needed. Phylogenetic tree was constructed using distance-based methods, unweighted pair group method with arithmetic mean, UPGMA. In the tree-based approach, a species is considered resolved if it is recovered monophyletically, *i.e.* when all accessions of the species are clustered under one node. Bootstrap analysis (500 replicates) was

3 Results and Discussion

3.1 Selection of COS Markers

The 37 COS markers described here were identified by screening *Zingiber* EST sequences against rice genome (http://www.ncbi.nlm.nih.gov/genome) using the software Spidey. The purpose of this screen was to identify single-copy tomato genes that have a single best match to one region of the rice genome and hence would qualify as potential orthologs.

To obtain the COS markers described here, 38139 *Zingiber* ESTs were screened as described above. To standardize all results, the COS marker set was rescreened against both rice genome and the *Zingiber* EST/unigene set. Only those regions which fulfilled the earlier mentioned three criteria were selected as putative COS markers which were only 37. The number of COS markers were less compared to the earlier studies [12],[18], [19] which may be due to our stringent modifications to obtain single copy genes with 100% mRNA coverage.

3.2 Annotation of COS Markers

Out of 37 selected COS markers 35 were annotated by BLASTX analysis against the GenBank protein database maintained at the NCBI (http://www.ncbi.nlm.nih.gov/). Annotation for the remaining two was not obtained. The identified putative COS genes belonged to diverse functional groups (Table 2). Out of 35 annotated regions, majority were house-keeping genes such as chlorophyll a/b binding protein (ZE377718), transporters (ABC transporters-ZE350073; vesicular transport SNAPs-ZE351074), kinases, proteases,

ubiquitins, enzymes involved in metabolism of purines (phosphoribosylaminoimidazolecarboxamide formyltransferase-ZE352864), chlorophyll (glutamate-1-semialdehyde 2,1-aminomutase-ZE35589; 3,8-divinyl protochlorophyllide a 8-vinyl reductase-ZE350566; Mg-protoporphyrin IX monomethyl ester cyclase-ZE380805), sterols (sterol methyl transferase2-ZE351047) and lipopolysaccharides (bifunctional polymyxin resistance arnA protein-ZE361721), transcription factors, translation factors etc. One gene region identified coded for disease resistance, complement proteins (ZE356530). Mitochondrial proteins were also identified such as carbamoylphosphate synthase large subunit-like gene (ZE355175), formate dehydrogenase 1 (ZE355804) and succinate dehydrogenase flavoprotein subunit (ZE372342). Oxygen evolving enhancing protein of photosystem II (ZE381543) was the chloroplast protein identified. The annotation results are in concordance with previous studies in the presence of majorly house-keeping genes and less number of evolving genes such as disease resistance genes which may be due to their conserved nature across genera and families [18], [19].

Table 2 Functional annotation for the putative conserved ortholog set identified for *Zingiber*

Sr. No.	COS Markers	Sequence Annotation
01	Ze381543	oxygen evolving enhancing protein of photosystem II for *Zea mays*
02	Ze355175	carbamoylphosphate synthase large subunit-like gene for *Triticum monococcum*
03	Ze356530	MAC/Perforin domain containing protein for *Z. mays*
04	Ze361721	bifunctional polymyxin resistance arnA protein for *Z. mays*
05	Ze350073	ATP BINDING CASSETTE PROTEIN 1 for *Arabidopsis thaliana*
06	Ze380805	Mg-protoporphyrin IX monomethyl ester cyclase for *Hordeum vulgare*
07	Ze355842	Protein Kinases *A. thaliana*
08	Ze353441	nucleic acid binding protein for *A. lyrata*
09	Ze349334	chloroplast translational elongation factor Tu (tufA)
10	Ze376518	zinc metalloprotease *Ricinus communis*
11	Ze350166	N-acetylglucosaminyltransferase *Musa acuminata*
12	Ze378062	ATP-dependent peptidase/ ATPase/ metallopeptidase (ftsh9) *A. thaliana*
13	Ze351047	sterol methyl transferase2 (smt2) for *Z. mays*
14	Ze362589	serine hydroxymethyltransferase *Z. mays*
15	Ze366271	lysyl-tRNA synthetase for *Z. mays*
16	Ze350566	3,8-divinyl protochlorophyllide a 8-vinyl reductase *A. thaliana*
17	Ze345717	fructose-1,6-bisphosphatase
18	Ze367620	myb2 for *Z. mays*
19	Ze354692	alpha-amylase for *H. vulgare*
20	Ze382569	AMP-dependent synthetase and ligase family protein for *A. thaliana*
21	Ze353562	heat shock protein 70 (HSP70)-interacting protein *R. communis*
22	Ze345478	eukaryotic translation initiation factor 3 for *Z. mays*
23	Ze355804	formate dehydrogenase 1 for *Z. mays*
24	Ze371351	gcip-interacting family protein-like mRNA
25	Ze377718	chlorophyll a/b binding protein cab-PhE7 for *P. edulis*
26	Ze372342	succinate dehydrogenase flavoprotein subunit for *Z. mays*
27	Ze352864	phosphoribosylaminoimidazolecarboxamide formyltransferase *A. thaliana*
28	Ze351074	alpha-soluble NSF attachment protein for *Elaeis guineensis*
29	Ze355895	glutamate-1-semialdehyde 2,1-aminomutase for *Z. mays*
30	Ze381127	formyltetrahydrofolate synthetase for *Populus*
31	Ze361874	putative cell elongation protein for *E. guineensis*
32	Ze351405	malate dehydrogenase
33	Ze355959	Eukaryotic translation initiation factor 3 subunit for *Ricinus, Arabidopsis*
34	Ze353425	S-adenosyl-L-homocysteine hydrolase (SH6.2) for *T. aestivum*
35	Ze364870	E3 ubiquitin protein ligase upl2 for *R. communis*

3.3 Primer designing and PCR amplification

Primers were designed for the selected 37 regions and their PCR amplification was tested. Altogether 51 primer pairs were designed, custom synthesized and validated. Out of the 51 primer pairs of 37 regions only 17 primer pair belonging to 14 regions yielded single band. All the others yielded multiple non-specific bands and hence were removed from further studies. Out of these 17 primer pair only 11 successfully completed sequencing belonging to 8 regions. Eventually only 8 COS markers were commended for further use belonging to 8 regions (Table 3). These 8 targets can be used across monocots as rate of transferability across species–genera is greater and their probability of orthology is high [18].

In the present study the potential of one COS marker (ZE372342) as barcode target for *Zingiber* species was validated. Two accessions each of seven *Zingiber* species were used for barcoding.

Table 3 List of primer combinations successfully sequenced and validated in *Zingiber* genus

Sr. No.	OligoName	5'<-----Sequence----->3'	Amplicon size	Annealing temp
01	ZE356530F	TGGGCCCAAAGCTCTTCATTAGCAC	1kb	55°C
	ZE356530R	GTGCACCCAGGAACCCTGGARAA		
02	ZE376518F	AAGCTGTGGAGGCATCCATGAACAC	500bp	55°C
	ZE376518R	ACGTGCCAACTCTGCAAGATCYTCT		
03	ZE367620F	AACCTGCACGTCCTGATCCTGTTGA	900bp	58°C
	ZE367620R	AATTCTTGGAGTAGCACCAGGSCCA		
04	ZE345478F	GGCTTCGCTGGATCTTTCACAAGCA	1kb	55°C
	ZE345478R	CCCGCAACTTCTTCAAGGTCACCAC		
05	ZE372342F	CCACTGTGCACTATAATATGGGTGGAA	500bp	60°C
	ZE372342R	ACACGTTGCATGTTGAGACGTATTT		
06	ZE381127F	TTAAAATGCATGGAGGGGGCCCTGA	1kb	55°C
	ZE381127R	CAACGCCACTAGCACCATAGAACTTRGC		
07	ZE355959F	AGATCATTGCCTTTGCATGGGAGCC	700bp	55°C
	ZE355959R	CCTGGTCCTCTTGTTCGTACTTCTTRC		
08	ZE353425F	CTCCCTCCACATGACCATCCAGACC	500bp	55°C
	ZE353425R	TCTTGACACCGGTGGTGGTCTCCTC		

* Primer pair used for DNA barcoding studies

3.4 Sequencing and Analysis

For all the 14 samples studied bidirectional sequencing was performed. The sequences were multiple aligned and sequence characteristics were studied. Out of the 467 sites, 439 were conserved sites (94%) and 28 were variable sites (6%). The variable sites included 24 parsimony informative sites (5.13%) and 4 singleton sites (0.87%). Phylogenetic tree was constructed using distance-based methods and the resultant UPGMA dendrogram resolved all the seven *Zingiber* species studied proving its potential in DNA barcoding (Fig. 1). All the nodes showed a high bootstrap value (> 50) highlighting the robustness of the dendrogram. Sequence databases are comparatively poor in the case of Zingiberaceae compared to other angiospermic plant families with high economic importance. A comparative genomic approach is preferable because of the lack of genetic resources for primer designing. The success of COS markers as a tool for phylogenetic analyses have been promising in crop species [14], [16].

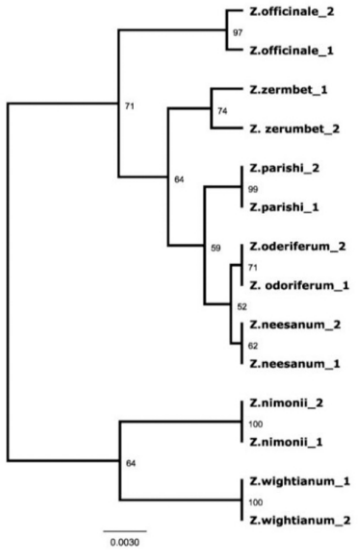

Figure 1 UPGMA dendrogram of 14 individuals belonging to sevenspecies of *Zingiber* obtained using distance based methods computed from the data obtained from sequencing

4 Conclusions

In the present study we have identified COS markers in *Zingiber* which can be utilized across the monocots. These conserved orthologous markers were annotated and majority of the genes were house keeping. All the 37 regions were used for primer designing and all of them were tested in one accession each of *Z. officinale* and *Z. zerumbet*. The utility of one of the COS markers in DNA barcoding was analysed and it proved to be a putative barcode target in *Zingiber* genera. As plant barcoding is still not a complete success, identification of new putative targets and their utility across species are essential. Our study paves a new path in exploiting the utility of comparative genomic approach in less sequenced non-model plant genera.

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"Convolution of an Integral Equation With the H-Function as its Kernel"

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A B S T R A C T

The object of this paper is to solve an integral equation of convolution form having H-function of two variable as it's kernel. Some known results are obtained as special cases

Keywords - integral equation, convolution, generalized type geometric function of two variables

AMS Subject classification No. 45E10

1.Definition and Introduction

The following definition and results will be required in this paper

(i) The Laplace Transform if

$$F(P)=L[f(t);p]=\int_0^{\infty}e^{-pt}f(t)dt\ ,\quad \mathrm{Re}(p)>0 \qquad ...(1.1)$$

Then F(p) is called the Laplace transform of f(t) with parameter p and is represented by F(p) = f (t) Erdelyi [(3) pp.129-131]

(ii) $L[f(t);p]=F(P)$ then $L[e^{-at}f(t)]=F(p+a)$ ...(1.2)

And if $f(0)=f'(0)=f''(0)=.....=f^{m-1}(0)=0\quad ,f^{n}(t)$ Is continuous and differential, then

$$L[f^{n}(t);p]=P^{n}F(p) \qquad ...(1.3)$$

(iii) If $L[f_1(t)]=F_1(p)$ then $L[f_2(t)]=F_2(p)$

Then convolution theorem for Laplace transform is

$$L\{\int_0^1 f_1(t)f_2(t-u)du\}=L\{f_1(t)\}L\{f_2(t)\}=F_1(p).F_2(p) \qquad ...(1.4)$$

(iv) The H-Function Defined by Saxena and kumbhat [1] is an extension of Fox's H-Function on specializing the parameters , H-Function can be reduced to almost all the known special function as well as unknown

The Fox's H-Function of one variable is defined and represented in this Paper as follows [see Srivastava et al [2] ,pp 11-13]

$$H[x]=H_{P,Q}^{M,N}\left[x/\begin{matrix}(a_j,\alpha_j)_{1,P}\\(b_j,\beta_j)_{1,Q}\end{matrix}\right]=\frac{1}{2\pi\omega}\int_{\theta=N-1}\theta(\xi)x^{\xi}d\xi \qquad ...(1.5)$$

$$\theta(\xi)=\frac{\prod_{i=1}^{n}\Gamma b_j-\beta_j\xi\prod_{j=1}^{N}\Gamma 1-a_j-\alpha_j\xi}{\prod_{i=M+1}^{Q}\Gamma 1-b_j+\beta_j\xi\prod_{j=N+1}^{P}\Gamma a_j-\alpha_j\xi} \qquad ...(1.6)$$

For condition of the H-Function of one variable (1.5) and on the contour L we refer to srivastava et al [2]

(V) The H-Function of two variable occurring in this paper is defined and represented as follows [see Srivastava et al [2] ,pp 83-85]

$$H[x,y]=H^{0,n_1,m_2,n_2,m_3,n_3}_{p_1,q_1,p_2,q_2,p_3,q_3}\left[x_y/\begin{matrix}(a_j,\alpha_j,A_j)_{1,p_1}&(c_j,z_j)_{1,p_2}&(e_j,E_j)_{1,p_3}\\(b_j,\beta_j,B_j)_{1,q_1}&(d_j,\delta_j)_{1,q_2}&(f_j,F_j)_{1,q_3}\end{matrix}\right] \qquad ...(1.7)$$

$$=-\frac{1}{4\pi^2}\int_{L_1}\int_{L_2}\phi_1(\xi,\eta)\psi_2(\xi)\psi_3(\eta)x(\xi)y(\eta)d\xi\,d\eta \quad \text{Where}$$

$$\phi_1(\xi,\eta)=\prod_{j=1}^{n_1}\Gamma(1-a_j+\alpha_j\xi+A_j\eta)\times\left[\prod_{j=n_1+1}^{p_1}\Gamma a_j-\alpha_j\xi-A_j\eta\prod_{j=1}^{q_1}\Gamma 1-b_j-\beta_j\xi+B_j\eta\right] \qquad ...(1.8)$$

Where the $\psi_2(\xi)$ and $\psi_3(\eta)$ are defined as (1.6) and for conditions of existence etc. of the $H(x,y)$ we refer to srivastava et al [2]

2. Main Result

Result I $L\left\{t^{\alpha}H_{P,Q}^{M,N}\left[at^{\alpha}/\begin{matrix}(a_j,\alpha_j)_{1,P}\\(b_j,\beta_j)_{1,Q}\end{matrix}\right],P\right\}$

$$=P^{-1-\alpha}H_{P+1,Q}^{M,N+1}\left[at^{-\lambda}/\begin{matrix}(-\alpha,\lambda)(a_j,\alpha_j)_{1,P}\\(b_j,\beta_j)_{1,Q}\end{matrix}\right]$$

Provided Re(p) >0 1≥λ>a and Re(1+∝)>0

Result II

$$\int_0^1\left\{x^{\alpha-1}(1-x)^{\beta-1}H_{P_1,Q_1}^{M_1,N_1}\left[z_1x^{\lambda}/\begin{matrix}(a_j,\alpha_j)_{1,P_1}\\(b_j,\beta_j)_{1,Q_1}\end{matrix}\right]H_{P_2,Q_2}^{M_2,N_2}\left[z_2(1-x)^{\mu}/\begin{matrix}(c_j,\gamma_j)_{1,P_2}\\(d_j,\delta_j)_{1,Q_2}\end{matrix}\right]\right\}dx$$

$$=H_{P+2,Q+1}^{0,N+2,M_1,N_1,M_2,N_2}\left[\begin{matrix}z_1\\z_2\end{matrix}/\begin{matrix}(1-\alpha,\lambda)(1-\beta,\mu)(a_j,\alpha_j)_{P_1}(c_j,\gamma_j)_{P_2}\\(1-b-\beta,\lambda;\mu)(b_j,\beta_j)_{Q_1}(d_j,\delta_j)_{Q_2}\end{matrix}\right]$$

Provided Re (α) >0 Re (β) >0 λ, μ >0

$$\mathrm{Re}\left(\alpha+\lambda\frac{b_j}{\beta_j}\right)>0\ \mathrm{Re}\left(\beta+\mu\frac{d_j}{\delta_j}\right)>0 \quad j=1,2....m\ \ k=1,2....g$$

$$|\arg z_1|<\tfrac{1}{2}\pi\Delta_1 \qquad |\arg z_2|<\tfrac{1}{2}\pi\Delta_2 \quad \Delta_1\ \Delta_2>0$$

$$\Delta_1=\sum_1^{M_1}b_j-\sum_{M_1+1}^{Q_1}b_j+\sum_1^{N_1}a_j-\sum_{N_1+1}^{P_1}a_j$$

$$\Delta_2=\sum_1^{M_2}d_j-\sum_{M_2+1}^{Q_2}d_j+\sum_1^{N_2}c_j-\sum_{N_2+1}^{P_2}c_j$$

Result III

$$L\left\{e^{-nt}t^{h}H_{P,Q}^{M,N}\left[zt^{k}/\begin{matrix}(a_j,\alpha_j)_{1,P}\\(b_j,\beta_j)_{1,Q}\end{matrix}\right],P\right\}$$

$$=(P+a)^{-1-h}H_{P+1,Q}^{M,N+1}\left[z(p+a)^{-k}/\begin{matrix}(-h,k)(a_j,\alpha_j)_{1,P}\\(b_j,\beta_j)_{1,Q}\end{matrix}\right]$$

Provided Re(p) >0 1≥λ>a and Re(1+∝)>0

Proof I First Taking by mellin barnes type contour integral for H-function for one variable and then convolution of laplace transform for H-function and get required result.

Proof II Taking by mellin barnes type contour integral for H-function for two variables and then using beta function we get required result.

Proof III same as proof I

3. Conclusion

From this Paper we get some many solution of integral equation of convolution from having H – Function of one or more veriables

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Kursa Short Message Encryption Technique

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ABSTRACT

In the present paper we made an effort to propose a short message encryption technique named KURSA. The present encryption technique can be used successfully for the encryption of very short messages as well as secret keys. We believe the present encryption technique will be very useful for secure secret key distribution because the technique adapted is very strange. Data is protected against known cipher text, plain text and brute force attacks. Data is in safe hands till the secret key taken is unknown.

Keywords: Plaintext, Cipher text, Encryption, Decryption, Secret Key.

1.Introduction

Providing security to the information is enforced in now-a-days, so every care should be taken to protect the information. Here we are proposing a **block cipher technique** called KURSA. The word kursa is a Telugu language word, kursa means very short. The present encryption technique can be used for success full encryption of the very short and sensitive information or secret keys. The encryption technique is robust against known plain text, cipher text and brute force attacks because length of the plain text is very short, so there will be less scope for experimenting on it. The secret key which is used in the encryption and decryption process should be exchanged via a secure communication channel or it should be send to the receiver after encrypting it with public key encryption technique. The encryption technique strength is based on the secrecy of the secret key

2. Encryption Algorithm

Step 1: Divide the plain text into blocks of 5 letters.
Step 2: Convert the plain text block into cipher text block as follows.

$$Ci = (Pi + Ki) \bmod 26 \quad \text{for all } 0 <= i <= 4 \quad \text{---- (1)}$$

Where

Cii th cipher text letter
Pii th plain text letter
Kii th keyword letter

Step 3: Perform a left circular shift on the key to get the key for the next block.
Step 4: Repeat step 2 and 3 till entire blocks of the plaintext is converted into cipher text.

3. Decryption Algorithm

Step 1: Divide the cipher text into blocks of 5 letters.
Step 2: Convert the cipher text block into plain text block as follows.

$$Pi = (Ci - Ki) \bmod 26 \quad \textit{for all } 0<=i<=4 \text{ --- (2)}$$

where

Ci i th cipher text letter
Pi i th plain text letter
Ki i th keyword letter

Step 3: Perform a left circular shift on the key to get the key for the next block.
Step 4: Repeat step 2 and 3 till entire blocks of the plaintext is converted into cipher text.

4. Encryption Process

The encryption algorithm is a block cipher technique in which each time it converts 5 successive letters of the plaintext into cipher text using a keyword. The plain text and cipher text's are strings or phrases in English. The letters of the English alphabet *a to z* are used for preparing the keyword. Assume, each letter in the alphabet is assigned a numerical value starting from zero such that a=0, b=1, c=2 ... z=25 and the letters are arranged in a circular passion. In the first step the original message will be divided into blocks of 5 letters then in the second step the individual blocks will be converted into cipher text in a sequential order. At the recipient end the receiver should use the decryption algorithm which takes same key for re conversion of cipher text into plain text.

For example consider the plaintext, prepar eforthewar and key aeiou . The original text now will be divided into four blocks, prepa-refor-thewa-r and this blocks will be converted into cipher text blocks, pvmdu-vmtir-bvywe-f. The process involved in the encryption of the message prepareforthewar is illustrated below.

First Block prepa
Keyword aeiou

C 0 = (P0 + K0) mod 26 = (15+0)mod 26 =15 mod 26 = 15 =p
C 1 = (P1 + K1) mod 26 = (17+4)mod 26 =21 mod 26 = 21 =v
C 2 = (P2 + K2) mod 26 = (4+8)mod 26 =12 mod 26 = 12 =m
C 3 = (P3 + K3) mod 26 = (15+14)mod 26 =29 mod 26 = 3 =d
C 4 = (P4 + K4) mod 26 = (0+20)mod 26 =20 mod 26 = 20 =u

Second Block refor
Keyword eioua

C 0 = (P0 + K0) mod 26 = (17+4)mod 26 =21 mod 26 =21 =v
C 1 = (P1 + K1) mod 26 = (4+8)mod 26 =12 mod 26 = 12 =m
C 2 = (P2 + K2) mod 26 = (5+14)mod 26 =19 mod 26 = 19 =t
C 3 = (P3 + K3) mod 26 = (14+20)mod 26 =34 mod 26 = 8 =i
C 4 = (P4 + K4) mod 26 = (17+0)mod 26 =17 mod 26 = 17 =r

Third Block thewa
Keyword iouae

C 0 = (P0 + K0) mod 26 = (19+8)mod 26 =27 mod 26 = 1 =b
C 1 = (P1 + K1) mod 26 = (7+14)mod 26 =21 mod 26 = 21 =v
C 2 = (P2 + K2) mod 26 = (4+20)mod 26 =24 mod 26 = 24 =y
C 3 = (P3 + K3) mod 26 = (22+0)mod 26 =22 mod 26 = 22 =w
C 4 = (P4 + K4) mod 26 = (0+4)mod 26 =4 mod 26 = 4 =e

Forth Block r
Keyword ouaei

C0 = (P0 + K0) mod 26 = (17+14)mod 26 =31 mod 26 = 5 =f

5. Decryption Process

Decryption algorithm takes the cipher text as input and converts it into plaintext using a keyword. Decryption process is opposite to the encryption process where plaintext blocks were converted into cipher text here the cipher text blocks will be converted into plaintext using the same 5 letter keyword. The decryption logic is very simple; the cipher text letter's numeric value needs to be subtracted from the keyword letter's numeric value. And over the result mod 26 should be performed in a sequence one after another using the following linear equation 2.
For example consider the cipher text in the above example pvmduvmtirbvywef which will be divided into four blocks as, pvmdu- vmtir-bvywe-f and these four blocks will be converted into plaintext prepareforthewar using the same key aeiou. The entire process involved in the decryption of the cipher text is illustrated as follows.

First Block pvmdu
Keyword aeiou

P 0 = (C0 - K0) mod 26 = (15 - 0) mod 26 = 15=p
P 1 = (C1 - K1) mod 26 = (21 - 4) mod 26 = 17=r
P 2 = (C2 - K2) mod 26 = (12 - 8) mod 26 = 4 =e
P 3 = (C3 - K3) mod 26 = (3 - 14) mod 26 = -11 mod 26=15=p
P 4 = (C4 - K4) mod 26 = (20 -20) mod 26 = 0=a

Perform left circular shift for one character on the keyword aeiou then it becomes eioua, use it as keyword for second block processing.

Second Block vmtir
Keyword eioua

P 0 = (C0 - K0) mod 26 = (21 - 4) mod 26 = 17=r P 1 = (C1 - K1) mod 26 = (12 - 8) mod 26 = 4=e
P 2 = (C2 - K2) mod 26 = (19 - 14) mod 26 = 5 mod 26 =5=f
P 3 = (C3 - K3) mod 26 = (8 - 20) mod 26 = -12 mod 26=14=o
P 4 = (C4 - K4) mod 26 = (17 -0) mod 26 = 17=r

Perform left circular shift for one character on the keyword eioua then it becomes iouae use it as keyword for third block processing.

Third Block bvywe
Keyword iouae

P 0 = (C0 - K0) mod 26 = (1 - 8) mod 26 = -7mod26=19=t
P 1 = (C1 - K1) mod 26 = (21 -14) mod 26 = 7=h
P 2 = (C2 - K2) mod 26 = (24 - 20) mod 26 =4 mod 26 =4=e
P 3 = (C3 - K3) mod 26 = (22 - 0) mod 26 =22 mod 26=22=w
P 4 = (C4 - K4) mod 26 = (4 -4) mod 26 = 0=a

Perform left circular shift for one character on the keyword iouae then it becomes ouaei use it as key for forth block processing.

Fourth Block f
keyword ouaei

P 0 = (C0 - K0) mod 26 = (5 -14) mod 26 = -9mod26=17=r

Plaintext: prepareforthewar
Ciphertext: pvmduvmtirbvywef

6. Implementation

The Kursa block cipher technique is implemented in C language. The method **void encrypt(char p[], char k[] , char c[])** is used for encryption of the key and **void decrypt(char c[] , char k[] , char p[])** is used for the decryption of the cipher text into plain text. The character arrays p,c and k are used for storing plain text, cipher text and secret key. To the encryption function we have to pass 3 character array parameters, p, k and c, in which first two p and k are input parameters used to pass plain text and secret key, and third one is output parameter used to store cipher text.

The method **void getblock(char s[] , char block[] , int i)** is used to get a fresh block from the plain text or cipher text. The character array **s** and **block** are used to store plain text and present block. The integer variable i represents the block number. A **void rotate (char k[])** is used to perform left rotation on the key to get the key for the next block. The functions **void encrypt_b(char p[] , char k[] , char c[])** and **void decrypt_b(char c[] , char k[] , char p[])** are used to encrypt and decrypt the individual blocks of the plain text and cipher text.

```
void encrypt(char p[], char k[] , char c[])
{
char key[5] ,b[5] ;
int i ;
int len = strlen(p) ;

strcpy(key,k) ;
for(i=0 ; i< len/5 + 1 ; i++)
{
getblock( p , b , I) ;
encrypt_b(b,key,c+i*5) ;
rotate(key) ;
}
c[len] = '\0' ;
}

void decrypt(char c[] , char k[] , char p[])
{
char key[5] , b[5] ;
int i ;
int len = strlen(c) ;
strcpy(key,k) ;
for(i=0 ; i< len/5 + 1 ; i++)
{
getblock( c , b , i) ;
decrypt_b(b,key,p+i*5) ;
rotate(key) ;
}
p[len] = '\0' ;
}

void getblock(char s[] , char block[] , int i)
{

int j = i*5 , k ;

for(k=j ; k<j+5 && s[k] != '\0'; k++)
block[k-j] = s[k] ;
block[k-j] = '\0' ;
}

void encrypt_b(char p[] , char k[] , char c[])
{
int I ;

for(i=0 ; i<5 && p[i] != '\0' ; i++)
c[i] = ((p[i]+k[i]-194)%26) + 97 ;
}
```

```
void decrypt_b(char c[] , char k[] , char p[])
{
int l ;

for(i=0 ; i<5 && c[i] != '\0' ; i++)
p[i] = ((c[i]-k[i]+26)%26) + 97 ;
}

void rotate(char k[])
{
char first = k[0] ;
int i;
for(i=0 ; i<4 ; i++)
k[i] = k[i+1] ;
k[i] = first ;
}
```

7. Strength and Weakness

The data encrypted with the kursa encryption algorithm is highly confidential because it is safe against known cipher text, plain text and brute force attacks. The procedure we have adapted for the encryption and decryption of the messages is unique and for each letter of the plaintext and cipher text we are using a different key letter. If there are no repeated letters in the plain text the encryption algorithm will be extremely stronger. If the letters in the plain text are repeated there may be a bit of chance to identify the secret key.

8. Conclusion

Data is secure till the secret key is confidential. So, every precaution should be taken to protect the secret key. The secret key should be given to the receiver through secure communication channel and in the cipher text form so that unauthorized persons do not get the secret key. The encryption technique is suitable for exchanging the secret keys and very short messages.

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Promising Updates in Biodiversity as Anti-Cancer Drugs: A Short Review

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ABSTRACT

Owing to the modern life style cancer is a major disease of the modern society. Though lot therapies are there to control cancer, drug based control is considered as a major control measure. The biochemicals isolated from the natural organisms are of immense value as these products have fewer side effects. The action of the biochemical is majorly by intercalation or the groove binding to DNA and regulating the uncontrolled proliferation of tumor by chromosomal breakage or cytolysis. The phyto-chemicals are promising candidates a have diverse type of secondary metabolites, some of which are proved to be efficient drugs in cancer therapy. Another source of cancer drugs are the marine biodiversity, ranging from bacteria to Mollusca. These organisms also develop secondary metabolites for self defense and predation, which isaining wide attention presently. The current review seeks the advancements of cancer therapy in this context.

Keywords: Anticancer Drugs; Biodiversity; Phytochemicals; Marine Products; Secondary metabolites.

1. Introduction

In human body billions of cells are present which perform different functions .The functioning of cells are complicated process and are under incredible phenomenal control. In cancer, cells lose the ability to follow the normal control that the body exerts on it [1, 2]. Slight deviation in a particular cell function that escapes the normal control mechanism continues to grow abruptly and cause tumor [3,4]. Significant advances in science and technology has changed the scenario of understanding and treatment of cancer more effective in this century, even though cancer has been known since antiquity [5, 6]. Theses advancements led to the major landmark in timely and accurate diagnosis, surgery and radiation therapy [6,7]. The discovery of radiation therapy has become a remarkable method in cancer treatment. Through advancement in cell biology, genetics and biotechnology researchers gained knowledge regarding the changes within cells that paved way for converting normal cells to malignant cells. The actual gains delivered from conceptual gains in the practice of cancer diagnosis and treatment, led to the most promising area of tailored cancer therapy in which biological anomalies unique to the diseases.

In chemotherapy the cancer cells are destroyed using bystander mediated killing [8]. In a tumor, the cell population will be of heterogeneous nature [9]. They possess malignant as well as non-malignant cells [10]. The nonmalignant cells contain immune cells, endothelial cells and fibroblast that interact with themselves as well as malignant cells [11]. Tumor associated macrophages are the most abundant immune cells present in tumors [12]. Tumor associated macrophages can be targeted with the help of protein [13] and approximately more than 60% of tumor mass can be reduced thereby increasing therapeutic index. In this report, the characterization and the biological properties of a group of bioactive potential compounds which are used for anticancer treatment have been furnished.

Anticancer Drugs

Anticancer drugs or the antineoplastic drugs are used in association with surgery, radiotherapy and immunotherapy for the treatment of cancer. These drugs have enough potential to target the metastatic fast dividing oncogenic cells [14]. Based on the mode of action or origin, they are classified as alkylating agents, antimetabolites, plant or microbe derived biodiversity products and hormones [15]. Alkyl sulfonates, triazene, nitrosoureas, platinum Coordination complexes (cisplatin, carboplatin, oxaliplatin), Methyl hydrazines, Nitrogen mustards, melphalan, cyclophosphamide and ifosfamid and some examples of the alkylating agents. While, purine antagonists, folate antagonists (methotrexate) pyrimidine antagonists (5-fluorouracil, cytarabibe) are classified as antimetabolites. There are also compounds which do not be included in any of the above category but are potential anticancerous drugs.

DNA Targeting

Molecular pathways of compound drug effects and specific compound–substrate interaction is very crucial in the development of new drugs and also for existing drugs. Molecular studies had led to the identification of promising and efficient antitumor strategies [16]. Versatile therapeutic results were achieved by the gene expression alternations through the small DNA binding particles. The product targeting the DNA can modify gene expression leading to alterations in the regulation of cell growth. Systems biology approach suites to capture the complexity of drug activities in cells [17,18, 19]. Prediction of mode of drug action has been attempted by using gene expression profiles. The steps involved in this are the treatment using appropriate drug, identifying and comparing the side effects, literature text mining and applying chemo-informatics tools

Gene Signature

Gene signature is the most promising approach for discovering the connections among drug pathways [20, 21], disease or compounds [22,23]. Gene signature is based on the subset of genes whose differential expression can be utilized as a marker of the given pathway/disease/compound using a large collection of transcriptional responses. These are followed by compound treatment like connectivity map.

Drug-DNA Interaction

DNA is the molecular target for many drugs in cancer treatment and is considered as a nonspecific target to many cytotoxic agents [24]. Anti-neoplastic agents inhibit DNA synthesis and makes irreversible or reversible damage to DNA. Anti-cancer treatment basically depends on small molecules [24,25] which are protein in nature that can target receptors. DNA drugs generally works by two methods, neither by intercalation nor by groove binding [25].

Intercalating drugs enter and intercalate with DNA molecules of the cancerous cells and interfere in the late S or G2 phase [26,27]. This can cause block in the DNA replication and cell cycle, hindering the cell from replication. Intercalating agents decrease the DNA helical twist and lengthening of the DNA. Main intercalaters are compounds with bi or tri cyclic ring structure [28]. Significant amount of free energy is needed for the intercalation of drugs in DNA (approximately 4 kilocal Mol) [26,27,28,29]. The Intercalated compound interfere with the topo-isomers, bind to nuclear DNA leading to DNA breakage, chromosome damage and sister chromatid exchange in the cells. DNA intercalation induces structural changes in DNA leading to intrusion in the recognition and function of DNA associated proteins such as transcription factors, DNA repair systems, polymerases and topoisomerases. This can lead to cytotoxicity and the damage to cancer cell.

DNA groove binding molecules are characterized by several aromatic rings that are connected and allowing freedom of movement and torsion [26, 29]. Groove binders are crescent shaped molecules that bind to the minor groove of DNA. It includes standard lock and key models for ligand macromolecular binding. They are stabilized by intermolecular interaction. Their association constants are higher as they do not require free energy for binding site [30]. Groove binders have been clinically used as anticancer and antibacterial agents. The DNA groove binders will not alter the structure as well as there is no change in free energy structure [27,28,29]. The most promising characteristics of DNA binding compound is that they bind to AT rich area due to the powerful hydrophobic interaction between aromatic ring the compound and second C atom of adenine. Groove Binding drugs are very promising because of their possible effect on pronounced affinity, gene expression and selective binding.

Plant Derived Anticancer Compounds

It is well known that the treatment of cancer is highly complicated, as some of the medicines target not only cancer cells but normal cells also. This leads to the side effects associated with the drug usage [31]. Anticancer agents are generally toxic and cause various side effects that include hair loss, mouth sores, bone marrow toxicity, anemia, cardiac anomalies, severe nausea, vomiting and in rare cases permanent infertility. In this context, derivatives from medicinal plants have gained immense significance in the treatment of cancer (www.cancer-concerns.com/chemsi deffects .htm). According to WHO, 80% of world's population rely on plant derived medicines for healthcare. About 60% of the drugs approved for cancer treatment are of natural origin. Fruits and vegetables contribute main sources of β carotene, α-tocopherol, fibers, vitamins C, B, E that have cancer healing capacity. Plant derived agents are successfully utilized in cancer treatment [32]. Herbal drug formulations for cancer treatment and the natural sources of potential therapeutic effects of these products increase the life expectancy.

Antioxidants play an important role in reducing the development of tumor by regression of premalignant lesions and inhibit their development into cancer [33]. Studies have indicated that some antioxidants like β carotene benefit the treatment conditions of oral leukoplakia. The major antioxidant phyto-compounds are isoflavones, flavones, anthocyanins, flavonoids, lignans, coumarins, isocatechins and catechins [32].

Some examples include vinblastine and vincristine (Catharanthus roseus), epipodophyllotoxin, an isomer of podophyllotoxin (Podophyllum peltatum roots), paclitaxel (Taxus baccata, T. brevifolia, T. canadensis), camptothecin (Camptotheca acuminata), homoharringtonine (Cephalotaxus harringtonia var. drupacea), elliptinium (Bleekeria vitensis), flavopiridol (Dysoxylum binectariferum), and ipomeanol (Ipomoea batatas). The two plant derived natural products, paclitaxel and camptothecin were estimated to account for nearly one third of the global anticancer market, respectively [34]. Numerous types of bioactive compounds have been isolated from plant sources. Several of them are currently in clinical trials or preclinical trials or undergoing further investigation.

Marine Anticancer Compounds

Oceans constitute 70% of the world's surface and majority of species diversity is in the ocean fringe. Total marine species may approach about 1 to 2 million. Intense efforts have been conducted over the past decade to explore the bio-chemical diversity offered by marine life for anti-cancer treatment (http://shodhganga.inflibnet.ac.in/bitstream /10603/28190/11/11_chapter%207.pdf). The intense

concentration of species co-existing in the marine habitat makes them highly complex and competitive. Motile and non-motile marine species are in constant battle for sufficient nutrient, light, temperature, water current etc. This intense competition led a high percentage of species to evolve chemical means to defend against predation, overgrowth by competing species or to subdue motile prey species for ingestion. The major biochemical used in defense are terpenoids, polypeptides, peptides, alkaloids, shikimic acid derivatives, sugar, steroids and a multitude of mixed biogenesis metabolites. Clinical trials for anti-cancer compounds derived from diverse marine life is increased today. It is considered that the evolution in the field of marine natural product drug discovery will be successful in identifying future anticancer drugs [35].

Several types of unique toxins have been identified by marine biologicals and naturalists. Early investigations gave rise to broad surveys of marine life for novel natural products with useful biological properties. They prioritize description of structural chemistry rather than drug discovery. Some of the microbial processes are also utilized in the generation of bioactive and potentially useful marine products. Marine microalgae, cyanobacteria and heterophobic bacteria which are seen in association with invertebrates produce bioactive and useful constituents. Sea weeds are also used as dietary fibers function as antimutagenic, anticoagulant, antioxidant and antitumorous agents [36]. Sarcodictin, bryostatins, discodermolide and elutherobin are some of the agents isolated from marine bacteria against cancer. Streptomycet species isolated from the sediments showed the presence of Guntingimycin (a trioxacarcin derivative) which is a potent anti-cancerous drug [37].

Molluscan Dipeptide

From the molluscan species, Elysia rubefescens a dipeptide called Kahalalide F (KF) is isolated. This peptide is synthesized by microbes associated with the animals. KF blocks cell cycle in G1 phase in a P 53 independent manner and induces cytotoxicity [38]. The activity of this compound is demonstrated in breast, colon and prostrate cancer lines. It is also reported that a metabolite macrolactin A present in the Noctiluca scintillans, inhibits B16-F10 murine melanoma cancercells, protects T lymphocytes against human immun odeficiency virus replication as well as mammalian herpes simplex virus [36,37,38]. Marine actinomycetes (Family Micromono sporacea) are very promising as it is found to be a potent source of anticancer agents which target proteasome function. Thiocoraline is a bioactive anti-cancerous actinomycet derived dipeptide inhibits RNA synthesis [39].

Bacterial Anticancer Compounds

It is well known that the probiotic bacteria such as Lactobacillus and Bifidobacteria control the pathogenic microbes. They produce antibacterial protein called bacteriocin and anticancer substances [40]. The effect of this help in reducing colon cancer by stimulating and modulating the mucosal immune system .The effect of pro inflammatory cytokines block NFKB pathways increasing production of cytokines such as IL-10 and host defense peptide such as Betadefensin enhancing IgA defenses. This defenses dendritic cell maturation as well as modulation of cell proliferation and apoptosis through cell responses to short chain fatty acids. They are gram-positive, spore-forming soil organisms that form a true mycelium. Thiocoraline is cytotoxic against melanoma, colon and lung cancer. It exerts preferential anti-proliferative effects on colon cancer cells with defective P 53 system [41].

Cancer and Challenges

One of the most fundamental advantages in the pathway of research on the treatment of cancer is the outcome from patient affected by the disease. Development of more effective and less toxic treatment has to be researched. Compared to surgery, chemotherapy and radiation therapy, the implementation of targeted therapy, immunotherapy and cancer vaccines need to be developed further [42]. Further studies has to be conducted to concentrate on the better management of toxic effects of the treatment wherein the patients ability to receive effective treatment is prioritized.

Research on cancer therapy has revealed that even within a given cancer, there are differences in the behavioral aspects as well as how it responds to treatment. Scientists have identified various epigenetic, genetic and molecular changes that promote the development of tumor. Tumors have the ability to manipulate the immune system and turn down the immune responses and reprogram the normal cells and promote them to grow and spread. Researchers also worked on the ways how the tumor thrive and survive in the body. This understanding created opportunities to develop target therapies. Growth and development of cancer is reduced by targeting specific changes in protein. The advantage over the existing cancer therapies like surgery, chemotherapy and radiotherapy is the emergence of target therapy and immunotherapy that harness the power of immune system to fight cancer.

Researchers identify genomic similarities between endometric and other type of cancers (breast,ovarian and colorectal) in which similar molecular changes are shared. Target therapies that target specific molecular changes may act against the existing cancer but also against tumors from other sites that specifies same alterations. In conclusion many research advancements in the field of cancer treatment has been developed. Numerous challenges remain which affect the goal of providing required outcome for patients.

1. Major challenge is to eradicate the lethal effects of radiation and chemotherapy drugs on the surrounding normal tissues. Need to develop drugs which are more effective in treatment as well as alleviate the side effects of all form of cancer treatment.

2. Research is required to overcome the drug resistance acquired over a period of time with respect to traditional chemotherapy drugs.

3. In target therapy, some of the drugs responses that lead to autoimmune damage to normal tissue.

4. Genomic characterization of tumors paved way for cancer treatment identifying various molecular aspects of the tumor as it may vary in a single tumor that is present in an individual. This leads to a condition wherein the drug effective in one part of the tumor may not be effective in other part.

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Wear Rate of (Aluminium) AL-6061 on Different Loads

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A B S T R A C T

Wear is the important factor for various applications in automobile and aeronautical industries. Various researches are going on to improve the wear by either alloying the material or using the composite material. Wear is related to interactions between surfaces and more specifically the removal and deformation of material on a surface as a result of mechanical action of the opposite surface. The research work summarized in this thesis present an experimental investigation on the effect of abrasive wear rate of the material Al-6061 alloy on various load. The experiment was carried out in the laboratory using an experimental set up for the analyzing the wear rate of selected material. The experiment for analyzing the wear rate conducted on various load as well as various orientation against grinding disc. Here we study the working life of an engineering component is expired when dimensional losses exceed the specified tolerance limits. The abrasive wear behavior of selected material is analyzing at different loads and four different orientations. In the above paper I have analyzed the abrasive wear of selected material, which shows the estimated life of said material.

OBJECTIVE OF THE STUDY:

To analyzing the wear rate for different load and different orientation for ma selected material during abrasive wear rate. This shows the material life against the selected Load.

INTRODUCTION-

In materials science, wear is erosion or sideways displacement of material from its "derivative" and original position on a solid surface performed by the action of another surface. Wear is related to interactions between surfaces and more specifically the removal and deformation of material on a surface as a result of mechanical action of the opposite surface Rabinowicz, E 1995 [1]. The need for relative motion between two surfaces and initial mechanical contact between asperities is an important distinction between mechanical wear compared to other processes with similar outcomes. Williams, J.A.2005[2].

The definition of wear may include loss at the interface between two sliding surfaces. However, plastic deformation such as yield stress is excluded from the wear definition if it doesn't incorporates a relative sliding motion and contact against another surface despite the possibility for material removal, because it then lacks the relative sliding action of another surface. Impact wear is in reality a short sliding motion where two solid bodies interact at an exceptional short time interval. Previously due to the fast execution, the contact found in impact wear was referred to as an impulse contact by the nomenclature. Impulse can be described as a mathematical model of a synthesized average on the energy solids in opposite converging contact. Cavitations wear is a form of wear where the erosive medium or counter-body is a fluid. Corrosion may be included in wear phenomenon, but the damage is amplified and performed by chemical reactions rather than mechanical action.

Wear can also be defined as a process where interaction between two surfaces or bounding faces of solids within the working environment results in dimensional loss of one solid, with or without any actual decoupling and loss of material. Aspects of the working environment which affect wear include loads and features such as unidirectional sliding, reciprocating, rolling, and impact loads, speed, temperature, but also different types of counter-bodies such as solid, liquid or gas and type of contact ranging between single phase or multiphase, in which the last multiphase may combine liquid with solid particles and gas bubbles.

When surfaces in contact move relative to each other, the friction between the two surfaces converts kinetic energy into heat. This property can have dramatic consequences, as illustrated by the use of friction created by rubbing pieces of wood together to start a fire. Kinetic energy is converted to heat whenever motion with friction occurs, for example when a viscous fluid is stirred. Another important consequence of many types of friction can be wear, which may lead to performance degradation and/or damage to components. Friction is the component of science of tribolgy.

Friction is not a fundamental force but occurs because of the electromagnetic forces between charged particles which constitute the surfaces in contact. Because of the complexity of these interactions, friction cannot be calculated from first principles, but instead must be found empirically. Some factors affecting wear rate such as Coefficient of friction, Specific energy of material, Material removal rate. This includes the width of the grinding wheel, Depth of cut, Feed rate of the specimen, power unit- torque & speed. In recent decades, aluminum alloy based metal matrix are gaining important role in several engineering applications. Al6061 alloy has been used as the matrix material because of its good formability, excellent mechanical properties and manufacturing properties. Wide spectrum of the applications in the commercial and industrial sectors.

Chemical composition of the Al6061 alloy are as

Elements	Cu	Mn	Mg	Si	Fe	Cr	Ti	Zn
Wt %	0.15-0.40	0.15	0.8-1.2	0.4-0.8	0.7	0.04-0.35	0.15	0.25

Aluminum alloy(Al6061)- By the adopting the suitable treatment, mechanical properties of wear resistance of Al composites can be improved. Studying wear is characterized by many different aspect and it is mostly influenced by the complexity of materials interaction on a functional surface as well as by operation conditions. In machine element, there are gradual wear in the result of friction. Therefore, we have to search for the possibilities to prevent it thus extending the technical life of a component.

LITERATURE REVIEW-

- **Chang Chongyi Wang Chenggu and Jin Ying 2010**[14] They conducted their study on numerical method to predict wheel/rail profile evolution due to wear. A wheel/rail profile wear prediction methodology was developed and applied to the wheel/rail disc test about the wear of flange and gauge. Three-dimensional nonlinear finite element dynamic analysis code ABAQUS was also used in the simulation of wheel/rail disc rolling contact process. The simulation results are compared with measurements of laboratory wear test and the effectiveness of the wear prediction ethodology was verified.

- **Dharma R. Maddala, Arif Mubarok and Rainer J. Hebert 2010** [15] conducted study on Sliding wear behavior of Cu50Hf41.5Al8 .5 bulk metallic glass. Sliding wear behavior of a copperbased bulk metallic glass (Cu50Hf41.5Al8.5) was investigated for both as-cast and annealed samples. The wear resistance increased during isothermal annealing near the glass transition temperature. Nano crystals developed during the annealing for annealing times up to 300 min. A linear relation between hardness and wear resistance was observed during the early stages of devitrification, but at longer annealing times the wear resistance increased less than the hardness.

- **H.C. How and T.N. Baker 1997**[18] In their investigation of wear behavior of Al6061-saffil fiber, concluded that saffil are significant in improving wear resistance of the composite.

- **Liang Y. N. et. al. 1995** [19] Reported that the MMCs containin g SiC particles exhibit improved wear resistance.

- **Basavarajappa S., et. al. 2005** [20] Stated that the micro struct - ural characteristics, applied load, sliding speed and sliding distance affect the dry sliding wear and friction of MMCs. However, they conclude that, at higher normal loads (60N), severe wear and silicon carbide particles cracking and seizure of the composites occurs during dry sliding. Liang Y. N. et. al. Reported that the MMCs containing SiC particles exhibit improved wear resistance.

- **Chandramohan G., et. al**[22]. Reported that the sliding distance has the highest effect onthe dry sliding wear behavior of MMCs than that of the load and sliding speed.

- **Y. Reda et.al**[23] Vol.9, No.1 Studies on Al6061-SiC and Al7075 - Al2O3 Metal Matrix Composites 45 and **R. Clark et. al**.[24] In their studies on Al7075 reported that, pre-aging at various retrogression temperatures improves the hardness, tensile properties and electrical resistivity.

- **Surappaet al 1982** [25]**.** Noted that aluminum reinforced with 5 % alumina possessed an adhesive wear rate comparable to that of Al-11.8Si or AI--S1 hypereutectic alloys. Other work published by the same workerss4 involving Al, Al-11.8Si, Al-16Si alloys and Al reinforced with A1203p (5%) indicated that increased silicon content reduced the wear rate.

- **Hoskings et al.1982** [26]reported a decrease in adhesive wear rate with increasing particle content (at constant particle size) and dimension (at constant volume fraction) for Al 2014 and 2024 alloys reinforced with Al,O,p and SiCp (1-142 pm) of various weight fractions (2-30 %). SIC was shown to be more effective than alumina in resisting wear, when tested in a ball-on-disc rig. These findings were in disagreement with those reported by Surappa.54 However, it should be noted that the former work involved only a small reinforcement content (5 %).

ABRASIVE WEAR:

Abrasive wear occurs when a hard rough surface slides across a softer surface.[1] ASTM International (formerly American Society for Testing and Materials) defines it as the loss of material due to hard particles or hard protuberances that are forced against and move along a solid surface. Standard Terminology Relating to Wear 1987 [9].

Abrasive wear is commonly classified according to the type of contact and the contact environment. The type of contact determine s the mode of abrasive wear. The two modes of abrasive wear are known as two-body and three-body abrasive wear. Two-body wear occurs when the grits or hard particles remove material from the opposite surface. The common analogy is that of material being removed or displaced by a cutting or plowing operation. Three-body wear occurs when the particles are not constrained, and are free to roll and slide down a surface. The contact environment determines whether the wear is classified as open or closed. An open contact environment occurs when the surfaces are sufficiently displaced to be independent of one another ASM Handbook Committee 2002 [10].

There are a number of factors which influence abrasive wear and hence the manner of material removal. Several different mechanis-ms have been proposed to describe the manner in which the materi-al is removed. Three commonly identified mechanisms of abrasive wear are:

1. Plowing
2. Cutting
3. Fragmentation

Plowing occurs when material is displaced to the side, away from the wear particles, resulting in the formation of grooves that do not involve direct material removal. The displaced material forms ridges adjacent to grooves, which may be removed by subsequent passage of abrasive particles. Cutting occurs when material is separated from the surface in the form of primary debris, or microchips, with little or no material displaced to the sides of the grooves. This mechanism closely resembles conventional machining. Fragmentation occurs when material is separated from a surface by a cutting process and the indenting abrasive causes localized fracture of the wear material. These cracks then freely propagate locally around the wear groove, resulting in additional material removal by spalling. Abrasive wear can be measured as loss of mass by the Taber Abrasion Test according to ISO 9352 or ASTM D 1044.

RESULT AND DISCUSSION:

Effect of Orientation on Wear

- It is observed that as the orientation of the specimen changes from 0^{o} to 60^{o}, the wear mass (Wt. Loss) decreases from 0.798 gm to 0.626 gm when applied load is 5N.
- It is observed that the wear mass follows the same pattern as in graph 6.1.The wear mass decreases from 0.914gm to 0.737gm as the orientation changes from 0^{o} to 60^{o} when applied load is 10N.
- Similarly it is observed that wear mass decreases from 0.937 gm to 0.778 gm the orientation of the specimen changes from 0^{o} to $60^{o}$when applied loads is15N

Effect of Load on Wear

- It is observed that the wear mass increases from 0.798 gm to 0.937 gm as the applied load on the specimen increases from 5N to 15N when orientation of the specimen is 0^{o}.
- It is observed that the curve follows the same pattern as in graph 6.5.The wear mass increases from 0.750 gm to 0.886 gm as the applied load on the specimen increases from 5N to 15N when orientation of the specimen is 30^{o}.

- It is observed that the wear mass increases from 0.707 gm to 0.853 gm as the applied load on the specimen increases from 5N to 15N when orientation of the specimen is 45°.

It is observed that the wear mass increases from 0.626 gm to 0.778 gm as the applied load on the specimen increases from 5N to 15N when orientation of the specimen is 60°.

CONCLUSION: The study of this experimental analysis is an overview of research work on abrasive wear of selected material at different load. It will give you full information about the abrasive wear, its important factors, and techniques used to minimize the wear of aluminum alloy. On the basis of experimental work the following conclusion can be drawn:

- Maximum wear occur when the test specimen is held at 0o angle for given applied load.
- Minimum wear occur when the specimen is held at 60o angle for given applied load.
- As the orientation of the specimen changes from 0o to 60o, the wear mass decreases from 0.798 gm to 0.626 gm when applied load is 5N.
- The wear mass decreases from 0.914gm to 0.737gm as the orientation changes from 0o to 60o when applied load is 10N.
- Wear mass decreases from 0.937 gm to 0.778 gm as the orientation of the specimen changes from 0o to 90o when applied loads is15N.
- Minimum wear occur when load applied on the specimen is 5N for given orientation load.
- Maximum wear occur when applied load is 15N for the given orientation of the specimen.
- When applied load is varied from 5 N to 15N the wear mass increases from 0.798 gm to 0.937 gm for 0o orientation.
- When applied load is varied from 5N to 15N the wear mass increases from 0.750 gm to 0.886 gm when orientation of the specimen is 30o.
- When applied load is varied from 5N to 15N the wear mass increases from 0.707 gm to 0.853 gm when orientation of the specimen is 45o.
- When applied load is varied from 5N to 15N the wear mass increases from 0.626 gm to 0.778 gm when orientation of the specimen is 60o.

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