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Forensic Genetic Analysis of 11 Rapidly Mutating Y-STRs (RM-YSTRs) in Sindhi Population of Pakistan

Bakhtawar Javed Piracha, Muhammad Shafique, Ahmad Ali Shahid, Amna Sajid,
Faqeeha Javed, Abida Shehzadi, and Muhammad Shehzad

Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan.
Corresponding Author's Email: bakhtawarjavaid333@gmail.com

ABSTRACT:

Forensic Science is robustly associated with DNA that has a complex genetic blue print information intended to resolve the queries related to civil cases, judiciary, investigatory purposes and crime scenes. The study was carried out to contribute the data set of RM-YSTRs (Rapidly Mutating YSTRs) globally. The study focused on the significance of using RM-YSTRs as compared to the set of 9-17 YSTRs (mutation rate 1 × 10-2), as the later one is not being able to attain a high magnitude of male relatives differentiation. In the present work, 100 Sindh DNA male samples were collected, by genotyping the amplified samples, using RM-YSTRs, having a mutation rate of 1 × 10-3. Forensic efficiency parameters such as, PIC (Polymorphism Information Content) was observed in the range of 0.7745 at locus DYS576 to 0.9313 at locus DYF399S1b, maximum gene diversity (0.9350) was found at locus DYF399S1b, minimum gene diversity (0.7978) was seen at locus DYS576. Highest value of allele frequency was found as 0.350 with the allele no 18 at locus DYS576. The results had shown that haplotype frequency and haplotype diversity value were 0.010 and 0.99297, as no haplotype was repeated, which highlighted on the fact that nearly complete male individualization can be obtained using RM YSTRs, thus helpful in avoiding the adventitious matches or exclusion or inclusion of male relatives in forensics cases work. It might be an initiative towards establishing RM YSTR database of Pakistani Sindhi population.

Keywords: *Forensic, Crime scenes, Genotyping, Amplified, Gene diversity, Halotype, Sindhi*

INTRODUCTION

Forensic science is robustly associated with DNA that has a complex genetic blueprint information intended to resolve the queries related to civil cases, judiciary and investigatory purposes (Ruwanpura and Vidanapathirana, 2018). Y chromosome DNA analysis is applied in an ancestry and male lineages identification (Jobling and Tyler-Smith, 2003).

The third generation of DNA analysis or the current method of choice is short tandem repeat or STR analysis (Butler, 2009), as they carry smaller repeat units (2-6bp), used to solve the criminal cases (Willems et al., 2014). There are several uses of Y chromosomes testing in forensic casework on sexual assault evidence, ambiguous paternity testing as it is mostly transferred unchanged through the father's male relatives, and missing person's investigation (Butler, 2011). The patrilineal male relatives may be used in paternity testing or in missing person investigation where the father is not available (Goodwin et al., 2011).

The set of YSTRs, used prolonged in forensics, has low mutation rate in comparison with RM-YSTRs (Rapidly Mutating Y-STRs). These Rapidly Mutating YSTRs have surprisingly high mutation rates Zhou et al. (2018) hence, it was perceived that the set of 13 RM- NYSTR markers was being able to attain a high magnitude of male relatives differentiation as compared to Y filer kit and thus, effectively used to help improving the male lineage differentiation as compared to Y filer set (Olofsson et al., 2015). In forensic science, there are many advantages of using RM-YSTRs, i.e. lower number of markers are required by using RM-YSTRs (13 markers) as compared to Y filer (17 markers), apart from that, RM-YSTRs showed higher discriminating power and maximum haplotype diversity in 7 out of 8 worldwide regions examined, as stated by Ballantyne et al. (2012). In addition to this differentiating ability, RM YSTRs can differentiate father-son in about 50% cases, brother-brother 60% cases and one cousin from other 75% cases Boattini et al. (2016) thus, the homoplasy cases can be resolved by using RM YSTRs.

Keeping in view the negative aspects associated to previously used YSTR markers in forensics, and the compensation provided by RM-YSTRs, this study was designed using multiplex assay incorporating 11 RM-YSTRS. As these markers are highly valuable for the purpose of individual identification, they must be studied in the Pakistani population. We selected Sindhi DNA samples, as no work was done before on this historically important population.

MATERIALS AND METHODS

Blood samples of 100 individuals were collected from unrelated males residing in different regions of Sindh province of Pakistan. The participants accepted to fill the consent form. DNA was extracted from each blood stain of all collected samples in non-amplified laboratory area by phenol chloroform extraction method (Kochl et al., 2005). After the isolation process, the presence, concentration and the amount of DNA was estimated by agarose gel electrophoresis by the method followed in Waye and Fourney (1990).

Concentration and quality of DNA was decided by observing the brightness of DNA band after comparing it with the concentration of standard DNA of 20g and 40g to find the preliminarily quantity of DNA by gel quantification method, shown in Fig. 1.

Real time PCR assay was performed for absolute quantification of the extracted DNA on SDS 7500 Real Time PCR (Applied Biosystems), according to manufacturer's instructions in which DNA Stock solution was diluted in such manner that first dilution was 4x and all from 2-8 dilutions were 3x, the DNA was quantified on 7500 real time PCR in forensic services laboratory. The real-time PCR mix was prepared by using Quantifiler™ Human DNA Quantification kit (Cat. no.4343895) manufactured by Applied Biosystems, in which 6.5µl quantifier reaction mix, 5.5µl quantifier primer mix, 2µl template DNA and total reaction volume was 14µl. Real time PCR was run at three stages of denaturation, annealing and extension. The number of cycles adjusted for the three stages were 1 (95°C for 10 min), 40 (95°C for 15 sec) and 1 (60°C for 1 min). All the extracted DNA samples were diluted to final concentration of 1ng/µl by considering gel electrophoresis and Real time PCR results.

Allele specific Primers for PCR amplification of all the loci were designed using program primer 3 (http://frodo.wi.mit.edu/primer3). Fluorescently labelled primers were synthesized from Applied Biosystems (ABI). Basic Local Alignment Search Tool (BLAST) was used to access the sequences of all 11 RM-YSTR from GeneBank® (www.ncbi.nlm.nih.gov). Single plex PCR was optimized using 10µM working primer solution of each primer in a reaction mix of 12µl.

The multiplex assay and the PCR optimization, used in this work was previously carried out by Javed et al. (2018) in which the selected primer sequences for 11 loci were evaluated at all possible parameters essential for development of multiplex assay. Primer 3 software was used to design the primer sequences. Using this multiplex assay, 100 samples from Sindhi population were genotyped. Amplification of 100 DNA samples was done successfully for all the 11 loci using region specific oligonucleotides. Optimization of region specific amplification was carried out using GeneAmp®PCR system 9700 (ABI) Applied Biosystems. Amplicons were run on agarose gel electrophoresis to confirm the product size range of loci according to Saleem et al. (2020). PCR amplification was checked on agarose gel electrophoresis visually.

In order to perform the Capillary Electrophoresis, for fragment length analysis, genotyping was performed of all the PCR products following the protocol for genotyping using ABI genetic analyzer 3730xl (Applied Biosystem). On completion of electrophoresis each sample information was stored in the form of fsa and imported into Gene Mapper ID Software V3.2 for analysis and interpretation of results. For genetic identification of an individual, statistical parameters such as allele frequency, haplotype frequency, haplotype diversity & gene diversity were evaluated to explicate DNA results.

RESULTS

Extracted DNA was quantified by using agarose gel electrophoresis. DNA of the Extracted samples was compared with the standards of 20ng and 40ng as shown in the Fig. 1. Genotyping of the 100 DNA male Sindhi samples was carried out by using multiplex assay to carry out the genetic analysis of different statistical parameters. Electropherogram of a male DNA sample from Sindhi population of Pakistan has been shown in Fig. 2.

Forensic proficiency statistical parameters such as allele number, gene diversity and PIC (Polymorphism Information Content) calculated for all the markers are shown in Table 1. Maximum gene diversity (0.9350) was found at locus DYF399S1b, minimum gene diversity (0.7978) was seen at locus DYS576. PIC was observed in the range of 0.7745 at locus DYS576 to 0.9313 at locus DYF399S1b. Haplotype frequency and haplotype diversity of all the haplotypes was 0.0100 & 0.99297 (Table 2A, 2B, 2C, 2D, 2E), which concluded that almost complete male individualization could be obtained by making use of RM-YSTRs.

Allele frequencies along with allele numbers have been given in Table 3. Allele numbers` ranged from 9.3 to 55 and most of them were not repeated. Highest value of allele frequency was 0.350 at allele no 18 for DYS576.

DNA Quantification through agarose gel electrophoresis

Extracted DNA was quantified by using agarose gel electrophoresis. DNA of the extracted samples was compared with the standards of 20ng and 40ng, shown in the Fig 1.

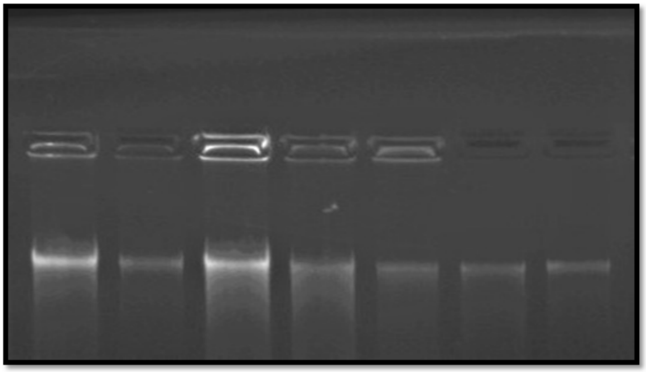

Fig.1: Agarose gel stained with ethidium bromide demonstrating the extracted DNA
Lane 1 = Standard 40ngp; Lane 2 = Standard 20ng;
Lane 3-7 = Extracted DNA samples

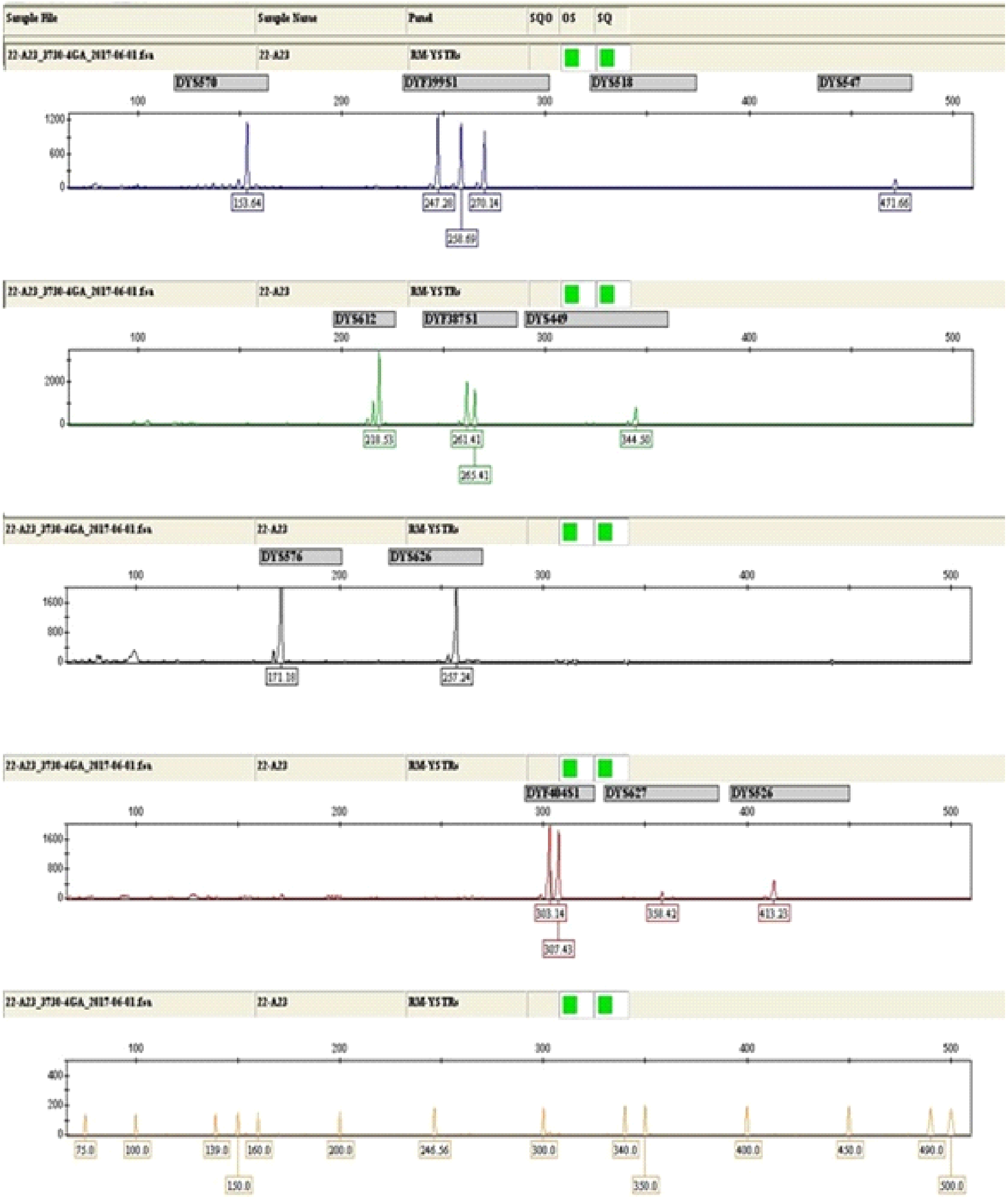

Fig. 2: Electropherogram of a male DNA sample from Sindhi population of Pakistan

Table 1: Forensics proficiency parameters calculated for all the markers under study

Marker	Allele No	Gene Diversity	PIC
DYS570	23.0000	0.9136	0.9077
DYF399S1a	19.0000	0.8920	0.8837
DYF399S1b	26.0000	0.9350	0.9313
DYF399S1c	18.0000	0.8408	0.8307
DYF547	24.0000	0.9142	0.9083
DYS612	21.0000	0.9284	0.9240
DYF387S1a	18.0000	0.8844	0.8744
DYF387S1b	16.0000	0.8714	0.8582
DYS449	28.0000	0.9282	0.9239
DYS576	12.0000	0.7978	0.7745
DYS626	20.0000	0.9168	0.9111
DYF404s1a	20.0000	0.8634	0.8523
DYF404s1b	19.0000	0.9020	0.8942
DYS627	22.0000	0.8972	0.8889
DYS526	25.0000	0.9174	0.9118

Table 1: is showing the forensics proficiency parameters, i.e. allele number, gene diversity and PIC calculated for all the RMYSTR markers under examination.

Table 2A: Haplotypes obtained along with haplotype frequency and diversity from sample ID 1-20

Sample ID	Haplotypes	Frequency	Diversity
S1	19.1-20.1-24-0-47.1-39.1-37-40-33.1-17-31.3-13.1-14.1-17-40	0.01000	0.99297
S2	18-21-22-24.1-46.1-35.1-35.3-38.3-34-18-29.3-15.2-16.2-18-36	0.01000	0.99297
S3	17-20-24-25.1-49.1-37.1-36.3-0-30.1-18-31.3-14.2-0-17.1-39.1	0.01000	0.99297
S4	18.2-20-21.3-22.3-49-38.2-33-35.4-28.2-17.3-31.1-13.2-15.2-18.2-36	0.01000	0.99297
S5	18-19.1-21-23.4-48-37-36.3-38-30.3-18-33.3-13-15-18.1-40	0.01000	0.99297
S6	19.2-20.2-23.2-25.3-48.1-41.2-35.2-38.2-32.3-15-30-14.3-15.3-19.3-37	0.01000	0.99297
S7	18.2-21-23-0-47-38.2-36-39-32.2-18-32.3-10.3-14.2-18-41.1	0.01000	0.99297
S8	18.2-22-23.3-24.3-48-41-35.3-38.1-30.3-17-28.3-13.3-13.2-18-35.3	0.01000	0.99297
S9	18-20-21.1-21.4-49.1-36.1-37.3-40-31.1-18-32.3-14.2-0-17.1-37.1	0.01000	0.99297
S10	15-23.1-23.3-0-45.1-36.1-35.3-39-32.1-16-31.3-13.1-17.2-20-37.1	0.01000	0.99297
S11	19.1-21-24-0-47-37-35.3-38-29.3-16.3-31.3-14-0-17.1-32	0.01000	0.99297
S12	18-21.3-22.3-25.1-46.1-33-35.3-38.3-33.3-18-28.3-15.1-16-18.1-36	0.01000	0.99297
S13	18-20-21.1-21.4-49.1-36.1-37.3-40-31.1-18-32.3-14.2-0-17.1-38.1	0.01000	0.99297
S14	16.3-21-22-24.1-47.2-33.2-35-39.3-33.2-18-28.2-14.2-16.2-20.3-34.3	0.01000	0.99297
S15	19.1-21-23.1-23.4-48.1-36.1-37.3-39-32.1-17-32.3-14.2-16.2-17.1-38.1	0.01000	0.99297
S16	17.2-19.3-21-25.2-47.2-37-36.2-38.3-32.2-18.3-32.1-14.3-15.3-18-39	0.01000	0.99297
S17	18-21-23.1-23.4-48.1-39.1-37-0-31.1-18-30.3-14.2-15.1-17.1-39.1	0.01000	0.99297
S18	18.2-18.3-20.3-21.3-50.2-37-36.2-38.3-32.3-16-31.2-14.3-15.3-18.3-40	0.01000	0.99297
S19	17-21-23-24.1-45.1-36.1-36.3-38.3-31.2-18-31.3-14.2-16.2-17.1-38.1	0.01000	0.99297
S20	18-21-21.1-22.4-47.1-38-36-39-31.1-18-31.3-14.2-15.1-18.1-40	0.01000	0.99297

Table 2B: Haplotypes obtained along with haplotype frequency & diversity from sample ID 21-40

Sample ID	Haplotypes	Frequency	Diversity
S21	19.3-19.1-21-24.3-47.1-35.2-36.3-39-32.2-20-31.3-14.2-14.1-17-40	0.01000	0.99297
S22	18.2-19.1-22-23.4-45.1-37.2-36.3-38-32.2-17.3-33.3-14.2-15.2-16.1-39	0.01000	0.99297
S23	18-21.2-22.1-23.4-49.1-38.1-36.3-39-27.1-20-28.3-12.3-14.1-24.3-35.1	0.01000	0.99297
S24	16-22-28-0-47.1-37.1-34.3-40-26.1-18-31.3-13.1-16.2-22.3-39	0.01000	0.99297
S25	18.2-21-22.3-0-47.1-36-35.3-38.3-31.3-18.1-30.2-14.3-16.2-18-40	0.01000	0.99297
S26	14-21-22-22.4-47.1-37-34.3-39-25-17-31.3-15.2-0-22-39.1	0.01000	0.99297
S27	17-21-24-0-46.1-36.1-37.3-39-28.2-16-29.3-15-16-20.3-32	0.01000	0.99297
S28	19.1-20-23-27.1-49.1-36.1-36.3-38-31.1-18-30.3-14.2-16.2-19-38.1	0.01000	0.99297
S29	17.2-21-22.2-23.1-48.2-38-35.3-36.3-31.3-17.3-33.1-13.3-15.3-18-55	0.01000	0.99297
S30	18.2-19.3-22-25.2-46.2-40-37.1-39.2-29.3-18-30.2-10.3-14.2-18-36	0.01000	0.99297
S31	15.1-19.3-22.3-24.4-48-40-35.3-38.3-26.2-15-32.2-14.3-16.3-21.3-40	0.01000	0.99297
S32	17.2-21-22.3-23.1-48.1-38-35.3-36.3-30.3-17.3-30-13.3-15.3-22-37.3	0.01000	0.99297
S33	17.2-17.2-20.3-0-48-40-38.2-38.3-30-17-29.2-12.3-11.1-19-35.3	0.01000	0.99297
S34	18.1-20.3-0-0-49.1-36.1-37-39-32.1-17-32.3-14.2-15.2-19-39.1	0.01000	0.99297
S35	15-19-24-24.4-48.1-35.1-38-37.2-30.2-18-30.3-15.2-16.2-20-36.1	0.01000	0.99297
S36	19.3-19.3-22.1-23.3-48.2-40-36.2-39.2-33.2-17-31.1-11-14.2-17-41.3	0.01000	0.99297
S37	19.1-21-24.1-0-49.1-38.1-37-38-32.1-18-33.3-14.2-16.2-17.1-38.1	0.01000	0.99297
S38	18.2-19-19.3-26.2-47.2-37.2-36.2-38.3-32.3-19.3-31.2-12-15.3-17-41.1	0.01000	0.99297
S39	19.2-21-24-0-49.1-38.1-36.3-37.3-32.1-18-33.3-14.2-16.2-17.1-38.1	0.01000	0.99297
S40	16.2-19.3-24.3-25.3-47.1-35.2-37.2-38.3-31.3-16-32.2-14.3-15.2-22.2-37	0.01000	0.99297

Table 2C: Haplotypes obtained along with haplotype frequency & diversity from sample ID 41-60

Sample ID	Haplotypes	Frequency	Diversity
S41	19.3-19.3-22-23.3-53.2-33-36.2-41.2-31.3-18.3-34.1-9.3-13.2-17-37	0.01000	0.99297
S42	15-22-23-24.1-48.1-37.1-35-40-26.1-16-31.3-14.2-16.2-20-38.1	0.01000	0.99297
S43	18.2-21.1-22-0-41.3-38.2-39-39-31.2-18.3-30.3-9.3-13.2-18-40	0.01000	0.99297
S44	19.3-20.3-22-23.3-49-37-37.3-39.3-32.3-17-32.2-14.3-16.3-17-39	0.01000	0.99297
S45	17.2-20.3-26-0-51-38-37-38-32.2-18-34.2-14.3-15.3-17-40	0.01000	0.99297
S46	18.2-21-23.5-23.3-51.2-37-37.2-38.3-32.3-18.3-33.1-14.3-15.3-17-39	0.01000	0.99297
S47	17.2-18-23.3-24.4-48.1-39-37.2-37.3-32.3-17-29.2-14.3-16.3-19-37	0.01000	0.99297
S48	20.1-21-24.1-0-47.1-36.1-37-0-33-17-30.3-14.2-15.1-19-38.1	0.01000	0.99297
S49	18.1-21.1-23-0-47.1-38.1-36-39-31.1-18-31.3-14.2-15.2-18.1-41.1	0.01000	0.99297
S50	18.2-21.1-22-0-41.3-38.2-39-39-32.2-18.3-30.3-10.3-14.2-19-39	0.01000	0.99297
S51	16.2-18.3-23.5-24.4-49.1-36-34.3-39.3-27.3-17-31.1-13.3-15.3-21.2-45	0.01000	0.99297
S52	15.1-20.3-21.3-23.3-48-40-35.3-38-29.2-17-29.2-13.3-13.2-20.3-36.3	0.01000	0.99297
S53	18.2-20.3-23-0-45.1-35.2-35.3-39.3-28.2-18-30.2-14.3-15.2-18-36	0.01000	0.99297
S54	13.1-20.3-22.3-0-51-39-35.3-36.3-29.3-15-31.2-14.3-10.2-21.3-35	0.01000	0.99297
S55	18.2-20-21.5-0-50-36.2-37-40-31.2-17.3-30.3-13.3-14.2-17.1-40	0.01000	0.99297
S56	18-19.1-21-23.4-48.2-34.1-37-38-32.1-18-33.3-14.2-15.1-20-39.1	0.01000	0.99297
S57	17.2-20.2-23.3-0-51.2-39-36.2-38.3-31.3-17-29.2-11-14.3-17-41.1	0.01000	0.99297
S58	20.3-19-22.3-0-47.1-36.2-37.3-38.3-32.2-18-32.3-14.2-16.2-18-39.2	0.01000	0.99297
S59	18.2-18.1-21-21.3-47.1-38.2-35.1-38.3-31.3-18-30.2-11-14.2-18.3-40	0.01000	0.99297
S60	15-20-23-25.1-48.1-39.1-34.3-40-26.1-18-31.3-14.2-16.2-22.3-39	0.01000	0.99297

Table 2D: Haplotypes obtained along with haplotype frequency & diversity from sample ID 61-80

Sample ID	Haplotypes	Frequency	Diversity
S61	19.3-19.3-21.3-26.3-51-36.2-36.3-37.3-32.2-17.3-30.2-14.2-15.2-19-39	0.01000	0.99297
S62	16-22-28-0-47.1-37.1-35-40-26.1-18-31.3-13.1-16.2-22.3-39.1	0.01000	0.99297
S63	21.1-18.1-22.3-0-48-36-37.3-39-31.3-17.3-33.3-14-16-18.1-38.3	0.01000	0.99297
S64	15.1-22-24-0-49.1-39-35-39.3-26.1-17-31.3-10.3-12.2-20.2-42.3	0.01000	0.99297
S65	20.1-21-23.1-23.4-47.1-37.1-36.3-38-33-17-30.3-14.2-15.1-18.1-38.1	0.01000	0.99297
S66	13.1-20.2-22.3-23.3-50.1-39-35.2-36.3-29.3-15-21.1-11-14.3-20.2-35	0.01000	0.99297
S67	18-19.1-20-21.3-49-37-36.3-39-31.3-18.3-30.3-13-17-17.1-39	0.01000	0.99297
S68	19.3-21.2-26.3-0-49.2-37.2-37.2-38.3-32.3-17-31.1-12-15.2-18-40	0.01000	0.99297
S69	17.1-20.3-23.5-0-50-37.2-36.3-37.3-32.2-17.3-34.2-14.3-15.2-17-40	0.01000	0.99297
S70	16.1-19.3-21.3-24.4-50.1-37-36.3-37.3-30.3-18.3-30.2-12.2-15.3-19.3-37	0.01000	0.99297
S71	19.3-19.3-20.3-21.3-49.1-36.2-35.3-37.3-33.3-17-30.2-14.3-15.3-19-36	0.01000	0.99297
S72	19.2-20.1-21-25.3-48.2-35.2-36.3-37.3-32.2-18.3-29.3-11.3-15.2-17.1-39.2	0.01000	0.99297
S73	18.3-21-23.5-22.3-47.1-37-35.3-38.3-31.3-19.3-31.2-15-15.3-18-40	0.01000	0.99297
S74	20.3-20.3-22.3-23.3-47.1-39.2-36.2-37.3-33.3-17-30.2-11.3-15.2-18-39	0.01000	0.99297
S75	17.1-18-24-0-47.1-38-36.3-38-32.1-17-29.3-13.1-16.2-19-36	0.01000	0.99297
S76	18.2-20-22.1-0-46-41.2-37.3-39.3-29.1-19-30.2-14.2-15.1-18-36.3	0.01000	0.99297
S77	19.3-20.3-23-23.3-49-37-37.2-38.3-32.3-17-32.1-14.3-16.3-17-39	0.01000	0.99297
S78	17.2-19.3-23.2-24.3-47.3-37-36.2-39.2-34.3-18.3-31.1-10.3-14.2-16-41.3	0.01000	0.99297
S79	19.1-21-23.3-25.1-47-40-35.3-39-31.3-17-28.3-14-15-21-36	0.01000	0.99297
S80	18.2-21.3-24.3-25.4-48.1-36.2-34.3-37.3-32.2-18-29.2-14.3-16.2-18-39	0.01000	0.99297

Table 2E: Haplotypes obtained along with haplotype frequency & diversity from sample ID 80-100

Sample ID	Haplotypes	Frequency	Diversity
S80	18.2-21.3-24.3-25.4-48.1-36.2-34.3-37.3-32.2-18-29.2-14.3-16.2-18-39	0.01000	0.99297
S81	19.3-20-20.3-0-52.2-38.2-36.3-37.3-32.2-17.3-29.2-14.3-16.3-16-38.3	0.01000	0.99297
S82	18.1-21.1-25-0-50.1-37.1-33-39-32.1-18-29.3-14.2-15.2-17.1-41.1	0.01000	0.99297
S83	17-21-26-0-49.1-37.1-37-38-32.1-18-34.3-14.2-15.1-17.1-39.1	0.01000	0.99297
S84	15-23.2-24-0-45.1-36.1-36-39-32.1-16-31.3-13.1-17.2-20-37	0.01000	0.99297
S85	18.1-22-24.1-0-46.1-39.1-36.3-39.3-25.1-19-31.3-14.2-16.2-20.3-34.1	0.01000	0.99297
S86	18-20.1-21-23.4-48-36-36.3-39.3-30-18.3-33.3-14.1-15-17.1-39	0.01000	0.99297
S87	18.2-22-23.3-24.3-48-41-36-36-30.2-17-28.3-9.3-13.2-18-36	0.01000	0.99297
S88	19.3-19.3-23.5-22.4-51-37.2-36.3-38.3-24-17.3-31.2-14.3-16.3-18-41.3	0.01000	0.99297
S89	19-21.2-22.1-24.4-48-38-37.3-40-26.3-21.3-28.3-12.2-13.3-25-34.3	0.01000	0.99297
S90	15-21-22-27.1-51.2-34.1-37-39-31.2-16-29.3-13.1-14.1-19-34.1	0.01000	0.99297
S91	15-22-27-0-47.1-37.1-34.3-40-26.1-18-31.3-13.1-15.1-24.2-39.1	0.01000	0.99297
S92	18-20.1-21-23.4-48.1-36.1-36.3-40-31.1-19-32.3-14.1-15.1-17.1-38.1	0.01000	0.99297
S93	15.1-18.3-19.3-23.3-49-36-34.2-37.3-30.3-18-30.2-15.3-16.3-19.3-37	0.01000	0.99297
S94	19.3-19.3-22.3-24.4-48.1-37-34.1-37.3-31.3-18.3-32.1-11.3-15.3-17-40	0.01000	0.99297
S95	21.2-21-24-27.1-45.1-36-36.3-38-32.1-18-32.3-14.2-15.1-17.1-41.1	0.01000	0.99297
S96	19.1-19.1-21-24.4-49.1-36.1-36-39-32.1-19-30.3-14.2-17.2-18-41.1	0.01000	0.99297
S97	17.1-20-21-25.4-49.3-37.2-37-38-32.2-18-34.2-14.3-15.3-17-40	0.01000	0.99297
S98	20.1-18.2-20.1-23.4-49.1-38-37.3-38.3-30.1-18-30.3-13.3-16.2-20-33.1	0.01000	0.99297
S99	18.2-19.3-24.3-0-48.2-34-36.3-37.3-34.3-18-31.1-15.3-16.3-20.3-35	0.01000	0.99297
S100	19.1-22-23-25.1-52.1-36.1-35.3-37-32.1-18-25-11.1-13-21-32.3	0.01000	0.99297

Table 2A, 2B, 2C, 2D & 2E are indicating that among 100 male samples of Sindhi population, all unique haplotypes were obtained in which no profile was repeated. Haplotype frequencies and diversity values calculated for all samples at each locus have been given, in which the haplotype frequency is 0.0100 and haplotype diversity is 0.99297.

Table 3: Indicating allele numbers and allele frequencies of Sindhi samples, at all the 11 RM-YSTR markers under study

Allele	9.3	10.2	10.3	11	11.1	11.3	12	12.2	12.3	13	13.1	13.2	13.3
DYS570											0.020		
DYF399S1 (1)													
DYF399S1 (2)													
DYF399S1 (3)													
DYS547													
DYS612													
DYF387S1 (1)													
DYF387S1 (2)													
DYS449													
DYS576													
DYS626													
DYF404S1(1)	0.030		0.050	0.040	0.010	0.030	0.020	0.020	0.020	0.020	0.080	0.010	0.070
DYF404S1 (2)		0.010			0.010			0.010		0.010		0.050	0.010
DYS627													
DYS526													

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
14	0.010											0.030			
14.1												0.020	0.040		
14.2												0.280	0.070		
14.3												0.190			
14.3													0.020		
15	0.070									0.040		0.020	0.030		
15.1	0.040											0.010	0.100		
15.2												0.030	0.120		
15.3												0.020	0.140		
16	0.020									0.070			0.030	0.020	
16.1	0.010													0.010	
16.2	0.020												0.180		
16.3	0.010									0.010			0.080		

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
17	0.040									0.220			0.010	0.130	
17.1	0.030													0.170	
17.2	0.080	0.010											0.030		
17.3										0.100					
18	0.120	0.020								0.350				0.180	
18.1	0.040	0.020								0.010				0.060	
18.2	0.180	0.010												0.010	
18.3	0.010	0.030								0.110				0.020	
19	0.010	0.030								0.040				0.100	
19.1	0.080	0.060													
19.2	0.030														
19.3	0.110	0.130	0.020							0.020				0.030	
20		0.100	0.010							0.020				0.060	

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
20.1	0.030	0.040	0.010												
20.2		0.030												0.020	
20.3	0.020	0.090	0.040											0.050	
21		0.230	0.100											0.020	
21.1	0.010	0.040	0.030								0.010				
21.2	0.010	0.030												0.010	
21.3		0.020	0.040	0.040						0.010				0.020	
21.4				0.020											
21.5			0.010												
22		0.090	0.100											0.020	
22.1			0.04												
22.2			0.010											0.010	
22.3			0.100	0.020										0.030	

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
22.4				0.030											
23			0.090												
23.1		0.010	0.030	0.020											
23.2		0.010	0.020												
23.3			0.060	0.090											
23.4				0.100											
23.5			0.050												
24			0.100						0.010						
24.1			0.030	0.040											
24.2														0.010	
24.3			0.030	0.040										0.010	
24.4				0.080											

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
25			0.010						0.010		0.010			0.010	
25.1				0.050					0.010						
25.2				0.020											
25.3				0.030											
25.4				0.020											
26			0.020												
26.1									0.060						
26.2				0.010					0.010						
26.3			0.010	0.010					0.010						
27			0.010												
27.1									0.010						
27.3									0.010						

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
28			0.020												
28.2									0.030		0.010				
28.3											0.060				
29.1									0.010						
29.2									0.010		0.060				
29.3									0.040		0.060				
30									0.020		0.020				
30.1									0.020						
30.2									0.020		0.100				
30.3									0.050		0.110				
31.1									0.070		0.060				
31.2									0.040		0.050				
31.3									0.110		0.170		31.3		

Allele	32	32.1	32.2	32.3	33	33.1	33.2	33.3	34	34.1	34.2	34.3	35
DYS570													
DYF399S1 (1)													
DYF399S1 (2)													
DYF399S1 (3)													
DYS547													
DYS612					0.020		0.010		0.010	0.020			
DYF387S1 (1)					0.020					0.010	0.010	0.060	0.040
DYF387S1 (2)													
DYS449		0.130	0.130	0.080	0.020	0.010	0.020	0.030	0.010			0.020	
DYS576													
DYS626		0.030	0.030	0.080		0.020		0.070		0.010	0.030	0.010	
DYF404S1(1)													
DYF404S1 (2)													
DYS627													
DYS526	0.020			0.010		0.010				0.020		0.020	0.030

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
35.1						0.020	0.010								0.010
35.2						0.040	0.020								
35.3							0.160								0.020
35.4								0.010							
36						0.060	0.060	0.010							0.090
36.1						0.130								0.010	
36.2						0.050	0.080								
36.3							0.220	0.040						0.020	
37						0.130	0.110	0.010						0.070	
37.1						0.080	0.010							0.020	0.020
37.2						0.060	0.050	0.010							
37.3							0.100	0.130							0.010

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
38						0.070	0.010	0.130							
38.1						0.040		0.010							0.100
38.2						0.060	0.010	0.010							
38.3								0.190							0.020
39						0.050	0.020	0.190							0.130
39.1						0.040									0.080
39.2						0.010		0.030							0.020
39.3								0.080							
40						0.060		0.110							0.160
41						0.020									
41.1															0.070
41.2								0.010							
41.3					0.020										0.030

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
42.3															0.010
45															0.010
45.1					0.060										
46					0.010										
46.1					0.040										
46.2					0.010										
47					0.030										
47.1					0.170										
47.2					0.030										
47.3					0.010										
48					0.090										
48.1					0.110										
48.2					0.050										

Allele	DYS570	DYF399S1 (1)	DYF399S1 (2)	DYF399S1 (3)	DYS547	DYS612	DYF387S1 (1)	DYF387S1 (2)	DYS449	DYS576	DYS626	DYF404S1(1)	DYF404S1 (2)	DYS627	DYS526
49					0.050										
49.1					0.140										
49.2					0.010										
49.3					0.010										
50					0.020										
50.1					0.030										
50.2					0.010										
51					0.040										
51.2					0.030										
52.1					0.010										
52.2					0.010										
53.2					0.010										
55															0.010

Allele frequencies along with allele numbers have been shown in Table 3. Allele numbers ranging from 9.3 to 55 and most of them are not repeated. Maximum allele frequency value is 0.350 with the allele no 18 at locus DYS576.

DISCUSSION

This study was designed to investigate the forensics efficiency parameters of 11 RM- YSTRs loci in Sindhi Population of Pakistan. Because of the high mutation rate of RM-YSTRs, and less number of markers required as compared to Y-STRs, they can be used to resolve the identity crisis of males. It is reported that RM-YSTRs successfully discriminated 70% out of 20 investigated father son pairs and only 3 haplotypes were shared in 8 individuals out of 604 males (Ballantyne et al., 2010). In another study, 250 haplotypes were observed in which only 2 haplotypes were shared by 2 males respectively. According to Javed et al. (2018) haplotypes of RM Y-STRs were unique in 97 unrelated Araein ethnic group. By using RM-YSTRs, the haplotype diversity reached 0.999937 and the discrimination capacity was 99.21%, showing a great discrimination power in Chinese Han population (Zhang et al., 2016). All haplotypes obtained from RM Y-STRs were the most diverse and showed strong discriminatory power in Korean population (Lee et al., 2017). Adnan et al. (2016) separated 45% of 1568 relative male Pakistani samples, by using RM-YSTRs and 15% of 1484 with commercial AmpFlSTR Y-filer kit, thus concluding higher discriminating power of RM-YSTRs as compared to the YSTRs. A recent research on the significance of RMYSTRs, carried out by Almohammed and Hadi (2019) on the Qatari population concluded the 100% resolution power of haplotype with maximum gene diversity. Saleem et al. (2020) examined a 250 residential Christian population of Lahore, Pakistan and 17 Y-chromosomal STRs loci was evaluated by AmpF/STR®Yfiler PCR amplification kit. Ancestral lineages and parameters of forensic importance showed recognition of 135 unique out of 175 total haplotypes with diversity value of 0.991 which was noticed similar to our result with 0.9927 halotype diversity.

Similar results were found in our study, in which no profile was repeated among 100 Sindhi DNA samples. This information and the previously mentioned results suggested that haplotype frequency and haplotype diversity of all markers, included in this study, was equal to 0.0100 and 0.9927. Nearly complete male individualization can be obtained using RM YSTRs, thus helpful in avoiding the adventitious matches or exclusion or inclusion of male relatives in forensics case work (Ballantyne et al., 2012). These results were in agreement with previous studies which reflected that RM-YSTRs set could significantly increase the discrimination capacity as compared to set of markers used in commercially available kits. Maximum allele frequency was found as 0.350 with the allele no 18 at locus DYS576.

However, further studies might be conducted in this population in comparison to commercially available kits to expand these results on larger scale.

CONCLUSION

In the vantage point of the findings of this research work we can conclude that the set of 11 RM-YSTRs, used in this experimentation, can be manipulated in order to resolve the forensics cases, having unrelated male samples, due to the maximum discrimination capacity as the results have shown that there was no repetition of haplotypes, thus almost absolute male individualization can be obtained by making use of RM-YSRs as compared to the other forensics tools. However, further studies might be conducted in this population in comparison to commercially available kits to expand these results on larger scale. Nevertheless, the main purpose of the research was to contribute the data set of RM-YSTR for the initiation of RM-YSTR database of Pakistani population over the succeeding years.

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Isolation and Characterization of Mesophilic Rhizobacteria from Gilgit-Baltistan and their Impact on the Growth of *Triticum aestivum L.*

Saboor-ul-Hassan, Ambreen Ahmed*

Department of Botany, University of the Punjab, Lahore 54590, Pakistan
***Corresponding Author's E-mail: ambreenahmed1@hotmail.com**

ABSTRACT:

The basic aim of this work is the isolation of mesophilic rhizobacteria from Gilgit-Baltistan region of Pakistan to study their impact on growth promotion of plants. Plant growth promoting rhizobacteria (PGPR) play their part in enhancing overall plant growth and can serve as potential bio-fertilizers. These isolated bacterial strains were screened by checking out their capability to produce auxin. Morphological and physiological characterization was also done. Plant growth-stimulating activities of these isolates were assessed through biological assay. Triticum aestivum seeds were inoculated with these isolates and the effect of these isolates was studied on the growth of wheat. Auxin producing ten bacterial strains were selected and used for the current study. Bacterially treated plants have shown significant enhancement in growth parameters as compared to non-treated plants. All bacterial strains have shown variable behavior. However, bacterial strain Mi5 performed most efficiently. In this case increment of 12.09, 68.82 and 39.89 % have been recorded in plant shoot, root and fresh weight as compared to control. Improvement in various plant growth parameters have shown that these indigenous bacterial strains can be used as biofertilizers in areas like with low temperature.

Key Words: *Mesophiles, PGPR, IAA, Triticum aestivum*

INTRODUCTION

Bacteria are classified into three categories on the basis of temperature tolerance i.e., psychrophiles, mesophiles and thermophiles. Psychrophiles are the bacteria which can grow at low temperatures. Those bacteria which can grow at slightly mild temperature which is neither low nor high i.e. between 20°C and 45°C, are termed as mesophiles while those bacteria which can grow at high temperature are termed as thermophiles (Ramle, 2018). There is a symbiotic association between plants and soil microorganisms, notably bacteria and fungi. These microbes through different mechanisms help plants in improving their overall growth and development (Raza et al., 2016). These rhizobacteria which have the ability of plant growth promotion are specified as PGPRs (Di Benedetto et al., 2019). Thus plant growth enhancing rhizobacteria have inherent property of phytostimulation (Akhtar et al., 2012).

Rhizosphere which is also known as microbe repository. Basically it is an area beneath the earth surface that is surrounding the plant roots. Here, plant roots are in direct contact with microbes specially bacteria and fungi. Biological activities of microbes determine the characteristics of soil of this area (Kundan et al., 2015). Association of plants and PGPRs is widely used at different levels and has great potential of scientific applications for the betterment of crops (Gonzalez et al., 2015). Applications of bacterial symbiotic association with plants have been studied in a wide variety of cereal crops, vegetables and fruits (Gray and Smith, 2005). PGPRs are involved in a wide range of biotic and abiotic processes. Biotic activities of PGPRs are of great benefit as they make the soil ecosystem more dynamic and versatile in a good manner. These activities are beneficial for sustainable crop production and for increasing turnover of different crops (Gupta et al., 2015). Rhizobacteria simply inhabit beneath the soil and they create a sort of competition in rhizosphere and improve plant growth through different mechanisms such as bacteria solubilize phosphates present in soil and thus make phosphate available to plants (Ahemad and Khan, 2012). Bacteria get protection by inhabiting plants and in return they fix nitrogen and make it available for plants as nutrient (Glick, 2012). Auxin production by bacteria also helps a lot in improving plant growth and development (Jahanian et al., 2012). Rhizobacteria have variety of enzymes that help plants in improving their development. ACC enzyme helps in lowering ethylene level in plants which improves root development (Liu et al., 2016). Rhizobacteria also degrade various environmental pollutants and produce different hormones and metabolic enzymes. In addition to all the traits mentioned above, some PGPRs possess the ability to degrade heavy metals that retard plant growth. Similarly some of these PGPRs have the ability to tolerate salt which in return helps plants to grow in saline environment. Moreover, some rhizobacteria act as biological control agent against various plant pathogens (Egamberdieva and Lugtenberg, 2014). The current study is about the plant growth promoting impact of mesophilic bacteria on wheat and their possible potential to act as biofertilizers and can replace harmful chemical fertilizers to improve plant growth. For the current study rhizospheric samples were collected from Khaplu and Skardu. These are the cities in Gilgit-

Baltistan region of Pakistan with average lower temperature range i.e., 27 to -10 ℃

MATERIALS AND METHODS

Isolation

For bacterial isolation, different plants were collected from various regions of Gilgit Baltistan such as Khaplu (35°10′N 76°20′E) and Skardu (35°17′25″N 75°38′40″E). Juglans regia (Walnut) and Morus nigra (Mulberry) samples were collected from Khaplu and Prunus armeniaca (Apricot) samples were collected from Skardu. Rhizosphere of the collected plants was used to isolate different bacterial strains. Isolation of bacteria was done through serial dilution procedure. From rhizosphere of these plants, total sixty seven strains of bacteria were obtained and these strains were regularly grown on L-Agar medium to obtain pure culture for further use.

Auxin Production

Bacterial isolates were selected on account of their ability to produce auxin. Auxin production estimation was done following Ahmed and Hasnain (2010). IAA standard curve was used to find out the concentration of IAA (Sarwar and Kremer, 1995).

Characterization

To characterize bacterial isolates, morphology of their colony and cells was examined. The bacterial isolates were streaked on media plates and incubated overnight. Different aspects of bacterial colonies were observed under stereomicroscope. Fresh cultures of bacterial isolates were taken in order to examine their cell morphology after gram-staining. Shape, colour and arrangement of bacterial cells were observed under oil immersion lens of microscope.

For physiological characterization of bacterial isolates, two parameters were recorded i.e. temperature range and growth curve. For the determination of optimum temperature for bacterial growth, bacterial cultures were grown at 25, 37 and 45°C. After 24 hours, optical density was taken for each culture at 600nm. Growth curve indicates the growth of bacteria over different time intervals.

Plant Microbe Interaction

Certified Triticum aestivum seeds var. FSD 2008 were obtained from Punjab Seed Corporation, Lahore. These seeds were sterilized using 0.1% $HgCl_2$ solution followed by numerous washings with distilled water. Optical density of bacterial cultures was set to 106 to 107CFU ml-1 so that there is equal number of bacteria in each culture. Surface sterilized wheat seeds were then infused with bacterial cultures following Ahmed & Hasnain 2010. For experimental control plants, seeds were treated with distilled water. For germination, wheat seeds inoculated with bacterial cultures were placed in autoclaved petri-plates having moistened Whatman filter papers. Germinated seedlings were then moved from petri-plates to properly tagged pots, each containing about 150g sieved soil. Each treatment had three replicates. Experimental temperature was adjusted to 20ºC. Garden soil was used in this experiment. Experiment was conducted in laboratory. After 20-25 days of sowing, plants were harvested and their different growth parameters like shoot and root length and fresh weight were noted down.

RESULTS

Screening and Characterization

From the screening of the isolated sixty seven bacteria, 10 bacterial isolates were preferred because of their auxin production capability. Morphological and physiological characterization of selected bacterial isolates i.e., Wi2, Wi3, APi1, APi2, Mi3, Mi5, APr2, APr3, APr9 and APr10 was done. All the isolated bacteria produced cream coloured round colonies with entire margins except Mi5 which were yellow in colour. All bacterial isolates were gram-positive except APi1. The isolates Wi2, Wi3, APi2, APr9 and APr10 were rods while APi1, Mi3, Mi5, APr2 and APr3 were cocci. The optimum temperature for the growth of bacterial isolates was found to be 25°C. The growth of majority of the isolates was optimum after 24 hours of incubation.

Auxin Production

All bacterial isolates showed auxin production. The isolate Wi2 showed maximum auxin production i.e., 110µg/ml while minimum auxin production was shown by bacterial isolate APi2 i.e.,19µg/ml (Fig. 1).

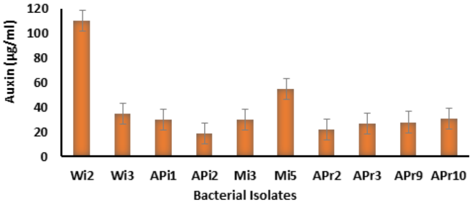

Fig. 1. Auxin production of bacterial isolates. Data represent mean of three replicates

Plant-Microbe Interaction

The plant growth promoting effect of these bacterial isolates was checked through biological assay. Plants that were inoculated with bacteria showed great enhancement in growth over control treatment. Prominent enhancement in shoot length of plants was observed. Plants treated with bacterial isolates APr3, Mi3 and Wi3 showed maximum increase in shoot length i.e., 13.69, 12.09 and 11.21% respectively over control plants (Fig. 2).

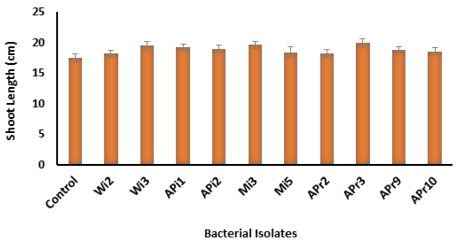

Fig. 2: Effect of bacterial inoculations on the shoot length of Triticum aestivum. Data represent mean of fifteen replicates

Maximum increment in root length was noted in plants inoculated with bacterial isolates Mi5, APr2 and Mi3 showing upto 75.41, 70.87 and 68.82% increase respectively, as compared to control plants. APi1 treated plants showed upto 3% decrease in root length in comparison with control plants (Fig. 3).

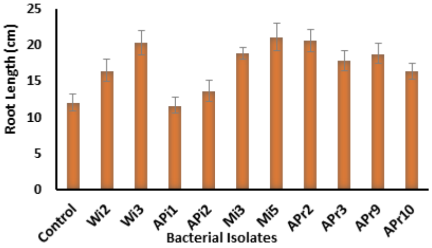

Fig. 3: Effect of bacterial inoculations on the root length of Triticum aestivum. Data represent mean of fifteen replicates

Bacterial isolates Mi3, Wi3 and APr10 enhanced plant fresh weight upto 39.89, 15.93 and 6.47% respectively as compared to control. Reduction in weight of freshly harvested plants was also noted which were inoculated with APr3, APr2, APi2 and Mi5. They showed upto 3.62, 5.5, 2.7 and 9.06% decrease respectively, in comparison with control (Fig. 4).

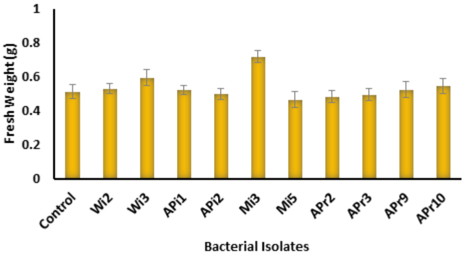

Fig. 4: Effect of bacterial inoculations on the fresh weight of Triticum aestivum. Data represent mean of fifteen replicates

DISCUSSION

Pakistan is an agricultural country and large part of its economy depends on agriculture sector. In recent times, there is an excessive utilization of synthetic fertilizers to increase the yield of major crops. In agriculture, excess use of chemicals is deteriorating soil and is hazardous for the environment and ecosystem. Owing to the adverse effects of chemical fertilizers, scientists are now exploring biofertilizers to improve plant growth organically. Among the biofertilizers, rhizobacteria are very important as they increase yield of the plants (Vejan et al., 2016). Moreover, utlization of biofertilizers containg mesophilic PGPR would enable farmers to use low temperature areas for agricultural purpose as well (Glick, 2012). In the current study, the main focus was on mesophilic rhizobacteria regarding their impact on wheat for growth improvement. In the present study, the impact of the isolated bacteria Wi2, Wi3, APi1, APi2, Mi3, Mi5, APr2, APr3, APr9 and APr10 was very obvious. These mesophilic bacterial isolates enhanced the shoot length. The isolate APr3 increased length of shoot upto 13.69% as compared to shoot length of non-inoculated plants. Bacteria that produce auxin have the potential to improve the expansion of plant roots by modifying different mechanisms of plants that maintain and control root growth. Nutrient uptake increases with increase in root development which ultimately enhances plant size and girth.

Variability was observed

regarding root length in the inoculated treatments. Some bacterial isolates like Mi5 and APr2 showed maximum enhancement in roots upto 75.41 and 70.87% respectively, in comparison with control plants where as, bacterial isolate Api1 caused decrease in the root length upto 3% when compared with control. Auxin is master hormone controlling various plant activities. Exogenous bacterial auxin interact with plant metabolic activities and maintain the plant hormonal status in homeostatic conditions and also regulate the expression of various genes. Auxin under sub-optimal concentrations cause increment in root length but under higher auxin levels initiate development of secondary roots and root hair. This development of extensive root system increase thr absorption area of root that ultimately enhace nutrient and water uptake from soil (Ahmed and Hasnain, 2014).

Similarly enhancement in fresh weight was observed quite evidently with maximum increment of 39.89% in plants inoculated with Mi3 in comparison with control and 9% decrease in fresh weight was noticed in plants which are inoculated with Mi5.

CONCLUSION

Improvements in various growth parameters in treated wheat plants showed the potential of these mesophilic isolates for plant growth improvement. Thus, these bacteria can be used as indigenous biofertilizers in the areas of Gilgit-Baltistan.

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Synthesis, Characterization, and Photocatalytic Degradation of Nickel Doped Copper Oxide Nanoparticles

Iqra Karim[1], Khurram Shahzad Munawar[1]*, Syed Mustansar Abbas[2], Hira Karim[1], Shabbir Hussain[3], Muhammad Ashfaq[4], Mehrine Rehman[5]

[1]Department of Chemistry, University of Mianwali, Pakistan
[2]Nanoscience and Technology Department, National Centre for Physics, Islamabad, Pakistan
[3]Department of Natural and Social Sciences, Sharif College of Engineering and Technology, Raiwind Road Lahore, Pakistan
[4]Department of Physics, University of Sargodha, Pakistan
[5]Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan
*Corresponding Author: Khurramchemist@gmail.com

ABSTRACT:

In the present study, the degradation of organic dyes was performed in the presence of pure and nickel doped copper oxide nanoparticles. In this co-precipitation method was used for the synthesis of pure and nickel doped copper oxide nanoparticles. Sodium hydroxide was used as a precipitating agent. These nanoparticles were characterized by scanning electron microscopy (SEM), diffuse reflectance microscopy (DRS), and Ultraviolet-Visible spectroscopy. SEM results showed that the prepared particles were uniform, regular, pure, and in nano-range. DRS result showed that the copper oxide nanoparticles had a bandgap of 3.37 eV while nickel oxide had a bandgap of 2.29 eV. After doping of Ni the bandgap of copper oxide decreases from 3.37 eV to 2.39 eV. UV-Vis spectroscopic analysis showed that the photocatalytic degradation of organic dyes increases with time. Nickel doped copper oxide nanoparticles have excellent photocatalytic degradation ability.

Keywords: *Nanoparticles, Doping, Co-precipitation, photocatalytic degradation of dyes.*

INTRODUCTION

Nanotechnology is the branch of science that produces materials of various types at the nanoscale level (Salata, 2004). Nanomaterials have different properties i.e electronic, magnetic and optical properties which are different than their bulk material (Nikam et al., 2018).

Nanoparticles are those

materials which have dimensions of 100^{th} nm at least (Khan et al., 2019). Depending on shape and size, the properties of nanoparticles can be varied.

In recent years, nanotechnology has gained importance in the industrial sector. It has applications in the field of electronic storage systems (Kang et al., 1996), biotechnology (Pankhurst et al., 2003), magnetic separation and pre-concentration of target analytes, embattled drug delivery (Rudge et al., 2001; Dobson, 2006), and vehicles for gene and drug delivery. To improve the properties of pure nanoparticles, different dopants are used. These dopants are metals, metalloids, or non-metals. Doped nanoparticles have improved properties than pure (Bryan and Gamelin, 2005; Mahmed et al., 2013; Laguna et al., 2015). Dopants creates the intermediate energy level by decreasing the bandgap of materials.

Photocatalysis is the green and clean method of water purification from dyes (Karimi-Maleh et al., 2020). In this method materials absorb radiations equal or greater than their bandgap (Bedia et al., 2019). This absorption causes the excitation of electrons from the valence band to the conduction band. This creates an electron-hole pair that generates radical for the redox system of substrates. The resulting free radicals degrade the pollutants (Soltani et al., 2012). The decrease in bandgap helps the materials in photocatalytic process. Doped nanoparticles have great importance in photocatalysis (Hamal and Klabunde, 2007; Habibi and Mokhtari, 2011), solar cell (Kruefu et al., 2010; Iwashina and Kudo, 2011; Regan et al., 2012; Kharel et al., 2018), fuel cell (Yang et al., 2009; Hensel et al., 2010; Yang et al., 2011), light-emitting diodes laser and rechargeable batteries (Alivisatos, 1996; Demir-Cakan et al., 2011; Chen et al., 2019).

In the present paper nickel doped copper oxide nanoparticles are prepared by the co-precipitation method. These doped nanoparticles have excellent photocatalytic degradation of organic dyes. These nanoparticles were characterized by scanning electron microscopy (SEM), diffuse reflectance microscopy (DRS), and Ultraviolet-Visible spectroscopy.

MATERIALS AND METHOD

Materials

The chemicals including copper sulfate ($CuSO_4.5H_2O$), nickel sulfate ($NiSO_4.7H_2O$), and sodium hydroxide (NaOH) were obtained from Sigma Aldrich. Eriochrome Black T (EBT), methylene blue, and sodium dodecyl sulfate (SDS) were obtained from Across Organics. All the chemicals used in experimental work were of reagent grade and used without further purification.

Preparation of Pure Copper Oxide Nanoparticles

One gram of sodium dodecyl sulfate was taken into the 100 mL volumetric flask and distilled water was added up to the mark to make a 0.03 M solution. This solution was stirred for 20 minutes. 6g $CuSO_4$ was added in that solution on stirring and kept on ultrasonicator for 35 minutes at 60°C. The pH of the mixture was maintained at 8-9 by using 5M NaOH solution. The respective mixture was kept on stirring for 24 hours at room temperature. After that, it was filtered and the precipitates were washed with distilled water (Equation 1 & 2). The product was dried in an oven at 80°C for 2 hours. The precipitates were annealed at 225°C and calcined at 800 °C for 2 hours .

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + Na_2SO_4 \text{ ------ Equation 1}$$

$$Cu(OH)_2 \longrightarrow CuO + H_2O \text{ ------ Equation 2}$$

Preparation of Nickel doped Copper Oxide Nanoparticles

For the synthesis of Ni-doped nanoparticles calculated amounts of nickel sulfate and copper sulfate were added in 0.03 M sodium dodecyl sulfate solution to make 5 %, 10 %, 15 %, 20 %, and 25 % solutions as represented in Table 1. The doped nanoparticles were obtained by adopting the procedure described above.

Table 1: Quantity of $NiSO_4.7H_2O$ and $CuSO_4.5H_2O$ to make 5 %, 10 %, 15 %, 20 % and 25 % solutions.

Sr. No	Solution % age	$CuSO_4.5H_2O$ (g)	$NiSO_4.7H_2O$ (g)	Water (mL)
1	5	5.7	0.3	100
2	10	5.4	0.6	100
3	15	5.1	0.9	100
4	20	4.8	1.2	100
5	25	4.5	1.5	100

Method for Degradation of Organic Dyes

0.05 g and 0.1 g of pure and doped copper oxide nanoparticles were taken in 100 mL volumetric flask and filled with the 10 ppm Methylene blue respectively. The solution was stirred for 5 minutes and a small portion of it was taken in the cuvette and its degradation was observed under UV spectrophotometer.

RESULTS AND DISCUSSION

Diffuse Reflectance Spectroscopic Analyses

Diffuse reflectance spectroscopy (DRS) was employed for the analyses of reflectance, absorbance, and transmittance of the compound. UV-Visible/NIR spectrometer (Perkin Elmer, Lambda 950/UK) with wavelength up to 3300 nm was used. Fig. 1(a) and (b) showed the bandgap of 3.37 eV for pure CuO and 2.29 eV for NiO. The bandgap is of CuO is shifted to a lesser value of 2.39 eV after doping with Ni (Fig. 1 (c)) (Siddique et al., 2018).

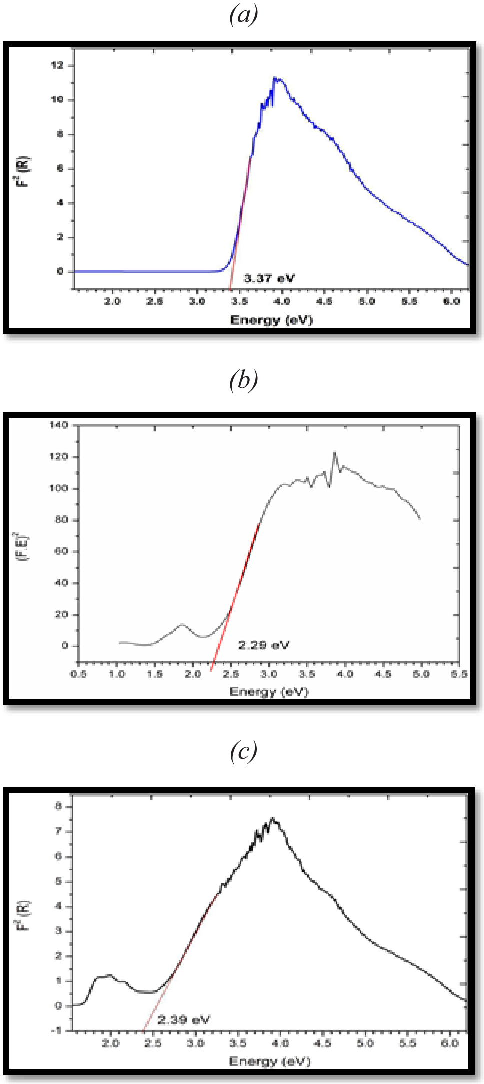

Fig. 1: (a) DRS of pure CuO nanoparticles (b) DRS of NiO nanoparticles (c) DRS of 25 % Ni-doped CuO nanoparticles

A graph was plotted which showed the relationship between reflectance and factor energy (F.E). The lower bandgap indicates that electrons become free and will be responsible for the degradation. So Ni-doped CuO nanoparticles are more effective for increasing the conduction of copper.

Scanning Electron Microscopic analysis

Scanning electron microscopic analyses tell us about crystalline nature, external morphology, and orientation of material making the sample. Fig. 2 shows the SEM of copper oxide nanoparticles which are pure, regular, uniform, and are within nano-range (Lange et al., 2010). After doping with Ni the copper oxide nanoparticles are more uniform and have a crystalline appearance as shown in Fig. 3 (Luna and Commission, 2015).

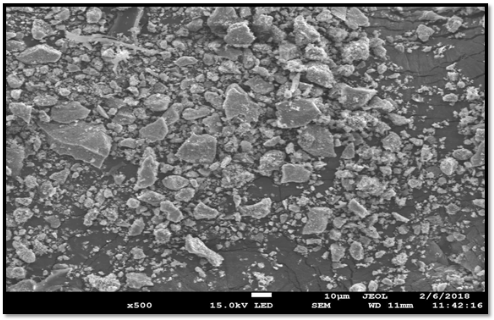

Fig. 2: SEM images of pure CuO nanoparticles

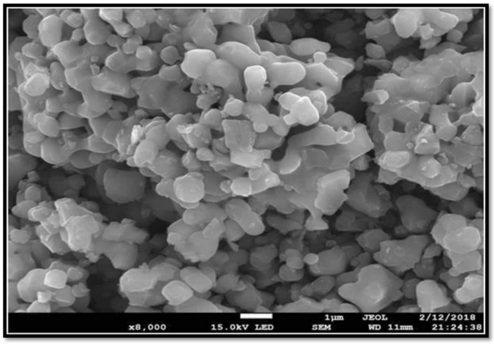

Fig. 3: SEM image of 25 % Ni-doped CuO nanoparticles

Degradation of Methylene Blue with Pure Copper Oxide

Copper oxide nanoparticles were synthesized by the co-precipitation method displayed a good degradation in the presence of light. 10 ppm MB solution was subjected to photocatalytic reaction with copper oxide. The degradation process was recorded throughout the range of UV-Visible and λ_{max} of absorbance was observed at 665 nm. Fig. 4 and Fig. 5 show the MB dye degradation with pure copper oxide nanoparticles. The top line in Fig. 4 shows the blank Methylene blue absorbance which shows that it is not degraded. After some time it starts degrading and the values of absorbance decreases. Two peaks were obtained in the UV-Visible spectra of methylene blue. The first peak (665 nm) represents maximum absorbance because of the n-π* transition by the lone pair present on nitrogen and sulfur. The second peak (~ 300 nm) was because of the π-π* transition of the aromatic rings (Sonia et al., 2015).

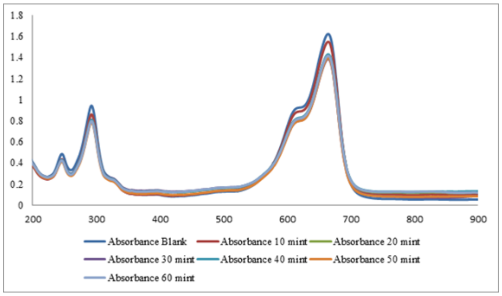

Fig. 4: Degradation methylene blue with 0.05 g of pure CuO NPs

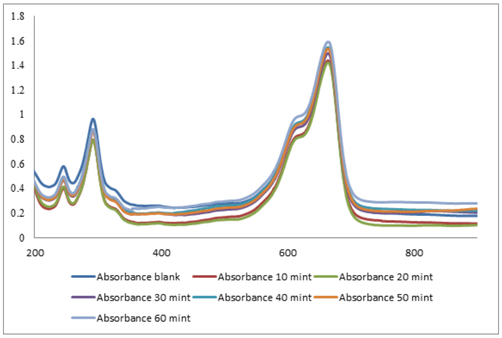

Fig. 5: Degradation of methylene blue with 0.1 g of pure CuO NPs

Degradation of Methylene Blue with Doped Copper Oxide

The degradation of the 10 ppm solution of methylene blue was observed with doped copper oxide nanoparticles. The amount of nanoparticles was 0.05 g and 0.1 g. The results are shown in Fig. 6 and Fig. 7.

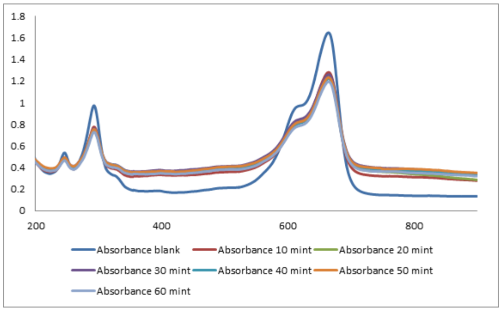

Figure 6: Degradation of 10 ppm Methylene Blue with 0.05 g of Ni-doped CuO NPs

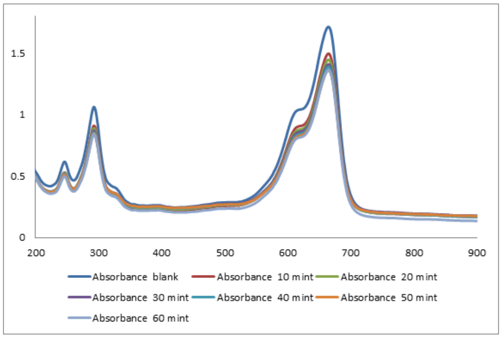

Fig. 7: Degradation of methylene blue with 0.1g of Ni-doped CuO NPs

Photocatalytic Degradation of Methylene Blue

The degradation shows surprisingly good results in the presence of light. The effect of light was carried out for 7 hours. More than 80 % of the dye is degraded as shown in Fig. 8.

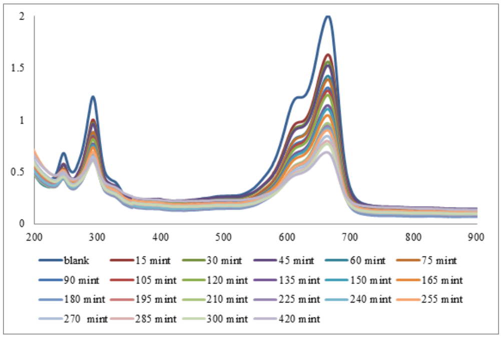

Fig. 8: Degradation of methylene blue in the presence of light

CONCLUSION

The co-precipitation method is used for the preparation of pure and nickel doped copper oxide nanoparticles. SEM results showed that the particles were pure, regular, uniform, and in nano-range. DRS spectra showed that after Ni doping the band gap decreases which enhances the conductivity of Copper. Degradation of methylene blue showed that the doped nanoparticles have a greater capacity to degrade the organic dye than pure copper oxide. It was observed that with increasing time degradation will be increased. Light enhanced the photocatalytic activity of doped CuO nanoparticles.

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Impact of Natural Antioxidants on Biological Systems

Malka Samra Malik, Muhammad Azam, Muhammad Asim Raza Basra*

Institute of Chemistry, University of the Punjab, New Campus, Lahore, Pakistan.
***Corresponding Author's E-mail: asimbasra@gmail.com**
Tel +924299230463 Ext. 139, +92-3320388300

ABSTRACT:

Free radicals are the unstable electron-deficient species that reacts with different molecules to gain stability and to eliminate their unpaired condition. Antioxidant molecules neutralizes the free radicals by donating their electrons and inhibits the unwanted oxidative reactions in biological system. The imbalance between antioxidants and free radicals generated oxidative stress which leads to severe impairment of the biological systems. The purpose of the present review is to highlight the beneficial role of naturally occurring antioxidant systems in minimizing the damage and maintaining the homeostasis in the biological system. Enzymatic and non-enzymatic antioxidants are the major classes of natural antioxidants which executes diverse functions in the biological system to provide defense against the destructive accumulating effects of ROS/RNS. Superoxide dismutase (SOD) are responsible for providing first line of defense to the biological system, by converting the superoxide anion radical (O_2^{o-}) into hydrogen peroxide (H_2O_2) which eventually converted into water and oxygen. Non-enzymatic antioxidants either endogenous or exogenous provides numerous crucial mechanisms to quench the ROS/RNS in the biological system. Endogenous antioxidants inhibits lipid peroxidation in the cellular membranes while the exogenous antioxidants acts as chain breakers and terminates the oxidative chain reaction. It is significant to sustain the level of exogenous antioxidants in the body through diet so that the normal biological processes can be maintained at optimal levels.

Keywords: *Antioxidants; free radicals; oxidative stress; homeostasis; protection*

INTRODUCTION

In the biological system, normal metabolic activities and ATP (energy) production relies on oxygen because it acts as the final electron acceptor in cellular respiration (Stanley and Chandler, 2002). Difficulties occur when the energy production and electron flow become disengaged which leads to generation of free radicals (FR) in the biological system (Nohl et al., 2005). FR are unstable and highly reactive molecules, produced during oxidative/cellular metabolic processes and due to the presence of unpaired electrons, FR can easily react with other molecules to acquire stability (Nasri, 2016). FR have a half-life of about 10^{-10} seconds and their reactivity depends upon their extent of stability in a paticular system (Suma, 2014). FR are the derivative of oxygen and nitrogen, termed as reactive oxygen and reactive nitrogen specie, ROS and RNS respectively. ROS/RNS have significant roles in cellular apoptosis (programmed cell death), cellular signaling pathways, ion transportation and gene expression (Franco et al., 2009). FR formed due to the exogenous sources includes the contaminants, chemicals, ionizing radiation, toxins while those which are produced from the endogenous sources includes the proteins, enzymes (Xanthine Oxidase and Monoamine Oxidase) and other organic molecules (Valko et al., 2006; Dhiman et al., 2017). Excessive level of ROS attacks and damages the proteins, nucleic acids, side chains of different amino acids, unsaturated fatty acids, DNA and RNA, thereby generating oxidative stress in the body (Baeza et al., 2010; Holmgren and Lu, 2010; Lu et al., 2010). RNS produced in insignificant amounts during usual cellular processes; ATP production for cellular energy, neurotransmission and cell signaling, blood pressure modulation, phagocytosis and regulation of cell growth (Santos-Sanchez et al., 2019).

Oxidative stress is a complex yet harmful response evoked by ROS/RNS in the body of living organism (Colin-Gonzalez et al., 2015), which is generated when ROS/RNS production go beyond the defense capacity of the cellular antioxidants (Limon-Pacheco and Gonsebatt, 2009). Oxidative stress existed at tissue, cellular, molecular and even at genetic levels of all living organisms and characterized by the accumulation of severe deleterious impacts on the cells and tissues which increases the risk of diseases and death (Rahal et al., 2014). In response to various stimuli, the body immunity is suppressed due to the presence of oxidative stress, inflammatory reactions, incidence of apoptosis (programmed cell death) and various devastating reactions (Cooksey et al., 2004, Galanis et al., 2009). Oxidative stress ultimately intensifies the risk for various deadly diseases which includes cancer, autism, cardiovascular disease, rheumatoid arthritis, diabetes and many other (Table 1) (Valko et al., 2007; Piwkowska et al., 2011).

Table 1: Positive Correlation of Oxidative Stress with Different Diseases

Sr. No.	**Etiology**	**Organs**	**Disease**	**Reference**
1.	Reactive oxygen intermediates	Eyes	Macular degeneration	(Beatty et al., 2000)
2.	ROS	Multi-organ	Diabetes	(Maritim et al., 2003)
3.	Reactive C-protein	Multi-organ	Chronic fatigue	(Fulle et al., 2007)
4.	NADPH (Reduced) oxidase system	Blood vessels	Atherosclerosis	(Singh and Jialal, 2006)
5.	ROS	Brain	Neurodegenerative diseases	(Uttara et al., 2009)
6.	ROS, H_2O_2	Lungs	Asthma	(Dozor, 2010)
7.	ROS	Joints	Rheumatoid and osteoarthritis	(Blackburn et al., 2011)
8.	Glutathione transferase kappa	Kidney	Nephritis	(Ziskoven et al., 2010)
9.	Lipid peroxidation (LPO) and DNA damage	Skin	Melanoma	(Sander et al., 2003)
10.	ROS	Heart	Myocardial infarction	(Filippo et al., 2006)

In the biological system, oxidative stress irreplaceably activates the phagocytic system and causes destruction of various proteins and enzymes by reducing, carboxylating, peroxidating or hydroxylating the side chains of amino acids (Grimsrud et al., 2008; Forman et al., 2014).

Antioxidant molecules are capable of neutralizing FR by contributing their electron to eradicate the unpaired condition of FR and slow down the oxidation process (Rahman, 2007; Mathew et al., 2011). In biochemistry, antioxidants are define as, the substances either enzymatic or non-enzymatic organic molecules, capable of neutralizing the deleterious effects of FR in biological system (Suma, 2014). Antioxidants commonly acts as reducing agents because they are being oxidized in process to halt the oxidative chain reactions in body by eliminating the FR (Kabel, 2014). Low levels of antioxidants cause massive damage to the body; often associated with heart diseases and cancer but abnormal cell division is reduced when antioxidant level becomes normal

(Fang et al., 2002; Mathew et al., 2011). The global natural antioxidants market is expected to grow at rate of 8.4 % annually from 2.22 Billion to 4.14 Billion USD by 2022 (Prakash et al., 2020). In vitro non-enzymatic antioxidant activities (DPPH, ABTS, Iron Reducing Power) are used as initial screening analysis to determine the antioxidant ability of natural as well as synthetic compounds (Tajammal et al., 2017; Yasmin et al., 2020).

In biological system, antioxidants reduce the level of cellular FR in two ways; either by lowering the expressions of those enzymes which are involve in the formation of FR (NAD(P)H oxidase and xanthine oxidase) or by uplifting the expressions of enzymatic antioxidants; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx). The purpose of the present review is to highlight the beneficial role of naturally present antioxidants in minimizing the damage and maintaining the homeostasis of the biological system. Synthetic antioxidants are not being discussed as part of this review.

1. Classification of Antioxidants

Antioxidants are classified based on their existence as natural and synthetic antioxidants. Natural antioxidants are present naturally in human body (endogenous antioxidants) and in plants (exogenous antioxidants). The natural antioxidants found in humans can be further classified on the basis of their nature and activity as enzymatic and non-enzymatic antioxidants (Fig. 1).

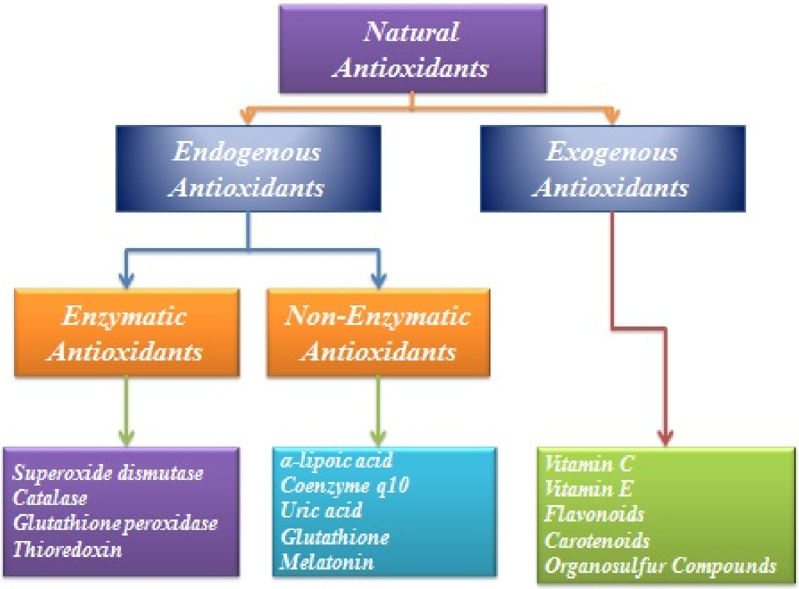

Fig. 1: Schematic Diagram for the Natural Antioxidants and their Major Classes

1.1. Enzymatic Antioxidants and their Role in Biological System

Body protects itself from the harmful effects of FR by using enzymatic antioxidant system as first line of defense and these antioxidant enzymes are essential in reducing the levels of lipid hydro-peroxides and H_2O_2 (major form of ROS). (Masella et al., 2005; Lu et al., 2010). Most abundant group of antioxidants in the living organisms consist of enzymes from the SOD family, which converts superoxide anions to hydrogen peroxide ($O_2^{\cdot-} \rightarrow H_2O_2$) by reduction which in turn decomposes either by GSHPx or by CAT (Rahal et al., 2014). These enzymes function synergistically to neutralize the FR as shown in Fig. 2 (Bonner and Arbiser, 2014).

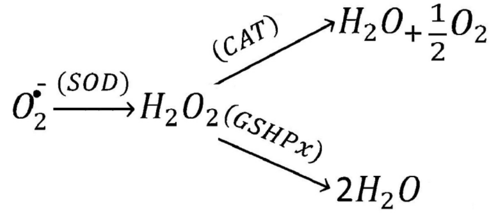

Fig. 2: General Mechanism of Action of Enzymatic Antioxidants (Rahal et al., 2014)

1.1.1 Superoxide Dismutase

Superoxide Dismutase (SOD) an enzymatic antioxidant present in the biological system which reduces oxidative damage and repairs the cells by eliminating superoxide anionic radicals ($O_2^{\cdot-}$) (Limon-Pacheco and Gonsebatt, 2009, Abskharon et al., 2010). It plays vital role in defense of cellular constituents of biological system from toxic side effects of ROS (Johnson and Giulivi, 2005) by converting $O_2^{\cdot-}$ to H_2O_2 (Zelko et al., 2002). Previously it was established that SOD has potential role in suppressing the cell death in ovarian follicles of cultured rats, and transgenic mice by inhibiting NO to be converted into peroxynitrate, which induces apoptosis (Keller et al., 1998). Mostly aerobic cells and extracellular fluids contain SOD with different metal ions which can be Zn, Cu or Mn. In humans, Cu/Zn-SOD form is present in cytosol of cell while Mn-SOD is present in cellular mitochondria (Kabel, 2014). Cu/Zn-SOD form has two similar subunits (32 kDa) and each subunit have binuclear metal clusters of Cu and Zn ions in active sites of enzyme, that provides first line of defense to the system by catalyzing the dismutation reaction of superoxide radical. While Mn-SOD enzyme, is a 96 kDa homotetramer which has one Mn atom in each of its subunit, and it cycles between Mn(III) to Mn(II) form throughout the two-step dismutation process (Rahman, 2007).

1.1.2 Catalase

A well-known catalase named H_2O_2 oxido-reductase have four polypeptide chains in its structure and these chains contain more than 500 amino acids each and four porphyrin haem groups. Peroxisomes of all mammalian cells contain this enzyme except erythrocytes (Kabel, 2014). The optimum pH for CAT in humans is approximately neutral while in other organism it varies from 4 to 11 depending on the species (Bahorun et al., 2006). In mammalian cells, H_2O_2 is produced by various oxidases and acts as a substrate in many biological reactions that produce highly reactive hydroxyl radicals but CAT enhances the body's antioxidant defense mechanism by inhibiting H_2O_2 accumulation (Ho et al., 2004). Two-stage break-down mechanism is used for H_2O_2 in which the haem iron at active site of the CAT enzyme oxidizes and reduces alternatively. In the first step, one H_2O_2 molecule oxidized the haem moiety to an oxyferryl specie while in the second step, second H_2O_2 molecule regenerates the enzyme which acts as reductant and NADPH act as a cofactor. Millions of H_2O_2 molecules decomposed by CAT to water and oxygen and it follows the fashion of a first-order reaction and we know that 1st order reaction rate is dependent on the concentration so here rate is dependent on H_2O_2 concentration (Rahman, 2007). Catalase deficient patients are normal phenotypically but due to excessive tissue damage caused by hydrogen peroxide, tendency to develop progressive oral gangrene is increased (Kabel, 2014).

1.1.3 Glutathione peroxidase

Glutathione peroxidases (GSHPx) are present in cytoplasm as well as in mitochondrial matrix at mM concentrations to remove hydrogen peroxide by cycling between reduced (GSH) and oxidized form of Glutathione (GSSG). GSHPx donates two electrons to reduce peroxides by forming selenoles (Se-OH), and can also reduce fatty acid hydro-peroxides (Limon-Pacheco and Gonsebatt, 2009). There are two forms of Glutathione peroxidase; selenium dependent GSHPx and selenium-independent glutathione-S-transferase (GST). The seleno-protein GSHPx enzyme removes H_2O_2 and oxidize GSH into GSSG (Pham-Huy et al., 2008). Furthermore, in humans, four isoforms of Se-dependent glutathione peroxidases are present which removes the peroxides and works in association with GSH to catalyze the conversion of organic peroxides to alcohol or water. A recent study indicates that the level of enzymes; GSHPx, GST and GSH increases by the administration of ethanolic extracts of A. maurorum in STZ-NA induced diabetic rats due to the presence of flavonoids, it also reduces the high MDA level and oxidative stress; common feature of diabetes (Sheweita et al., 2016).

1.1.4 Thioredoxin System

The thioredoxin system is a thiol-specific antioxidant system of oxido-reductase enzymes which consists of NADPH, thioredoxin (Trx) and thioredoxin reductase (TrxR) (Miranda-Vizuete et al., 2000). Two neighboring cysteine units are present in the active site of Trx which cycle between an active di-thiol form (reduced) to an oxidized disulfide form (Arner et al., 1999). The TrxR carried out the reduction of disulfide in the active sites of Trx with the help of NADPH and the reduced form of Trx is a common reductant of disulfides residues in different proteins (Fig. 3) (Nordberg and Arnar, 2005).

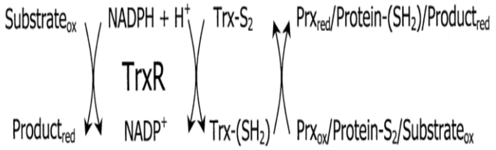

Fig. 3: Thioredoxin System and its Enzymatic Reactions

TrxR reduces the disulfide in Trx by directly consuming the NADPH (Pham-Huy et al. 2008). Trx acts as an effective reducing agent for ROS/RNS in its active state (Kabel, 2014). Antioxidant systems are differentially expressed at the transcriptional level in adult organs and embryonic stages in mice while the Trx antioxidant system is activated in conditions of high oxidative stress (Limon-Pacheco and Gonsebatt, 2009, Holmgren and Lu, 2010).

Until now, three variants of Trx in human are identified which are encoded by different genes. Trx-1 is a 12-kDa variant of Trx and maximally studied gene which is highly expressed in kidneys, lungs and minute amount being present in heart, brain, and testis (Chen et al., 2008). The Trx-2 variant of Trx has 60 amino-acid long peptide chain which signals in mitochondrial translocation and maximal expression of Trx-2 identified in kidneys and heart while minimal levels exist in lungs. SpTrx is a third Trx variant highly expressed in spermatozoa (Bindoli et al., 2009). A conserved active site of -Cys-Gly-Pro-Cys- is present in all variants Trx (Trx-1, Trx-2, and SpTrx) in humans and all other organisms which is important for its antioxidant function as disulfide oxido-reductase for proteins. (Oslowski et al., 2012).

1.2 Non-Enzymatic Antioxidants and their role in Biological System

Non-Enzymatic antioxidants consist of endogenous as well as exogenous antioxidants. The endogenous antioxidants are produced in the human body during metabolic reactions while exogenous antioxidants cannot be produced in the body and must be provided through foods or supplements. These exogenous antioxidants supplementation are important to eradicate the unnecessary internal ROS, while improve the disease resistance capability (Jamshidi-Kia et al., 2020). Microalgae are found to be abundant source of natural exogenous antioxidants, possibly due to presence of phenolic compounds, vitamins, carotenoids and fatty acids (Qiu et al., 2020). These antioxidants reduces the generation of free radicals specially ROS (Table 2).

Table 2: Non-Enzymatic Antioxidants and their Location

Non-Enzymatic Antioxidants	Category	Location/Sources
α-Lipoic acid	Endogenous	Cellular membranes, Cytosol
Coenzyme Q 10	Endogenous	Cellular membranes
Uric acid	Endogenous	Plasma
Glutathione	Endogenous	Cytosol
Melatonin	Endogenous	Cell's mitochondria
Vitamin C	Exogenous	Fruits, vegetables
Vitamin E	Exogenous	Wheat germ, egg yolks and nuts.
Flavonoids	Exogenous	Plants, fruits

1.2.1 α-Lipoic Acid

α-Lipoic Acid (α-LA) is an important endogenous antioxidant which is majorly dispersed in cell membrane and cytosol of both eukaryotic and prokaryotic organisms (Rahman, 2007). Indirectly it maintains the level of antioxidants in cells by increasing the formation of endogenous antioxidants such as GSH (Rajakumar et al., 2016). α-LA and its reduced form; dihydrolipoic acid (DHLA) both are potential antioxidants and involved in scavenging the FR, repairing protein impairment in cytosol of cells which is caused by the oxidative stress, regenerating the antioxidants and chelating the metal ions. DHLA is more potent antioxidant than α-LA and can act synergistically with other antioxidants (Rahman, 2007). α-LA has an significant role in improving insulin sensitivity, mitochondrial oxidative phosphorylation expression, α-oxidation capacity, intracellular ATP production, and lowering the endoplasmic reticulum stress (Lei et al., 2016). Low pH enhances lipids oxidation, for instance, oxidation of linoleic acid and polyunsaturated fatty acids is increased at low pH and occurred rapidly (Goyal and Kaur, 2019). A study revealed that DHLA has a protective effect on the person suffering from Alzheimer's disease by decrease the accumulation of excess iron in the body which in turns reduces the production of ROS (Mudd et al., 2016).

1.2.2 Coenzyme Q10

An endogenous antioxidant; synthesized in intercellular region by using tyrosine as fundamental building blocks (Pahari et al., 2016). It exists biochemically in redox form in all the biological tissues, ubiquinol (reduced) and ubiquinone (oxidized) form which is useful biomarker of oxidative stress. In the reduced form, it holds electrons loosely; it can give up easily to neutralize the FR. Reduced form of CoQ10 displays strongest antioxidant action (Maladkar et al., 2016) by inhibiting lipid peroxyl radicals production and even neutralize the already present FR (Carocho and Ferreira, 2013). It protects membrane phospholipids from damage caused by peroxides and FR-induced oxidation (Sarmiento et al., 2016) and also involved in the prevention of liver fibrosis by lowering the oxidative stress, inflammation, and hyper-insulinemia (Tarry-Adkins et al., 2016). Normally CoQ10 performs its regular function in electron transport chain (mitochondria), while acts as antioxidant in plasma and cell membranes where it prevents the lipid peroxidation (Ernster and Dallner, 1995). It restores the cellular functions of the antioxidant enzymes which are altered due to the oxidative stress induced by hydrogen peroxide and lowers the ROS production in response to it. It also enhances the defense capacity of the cellular antioxidants through both intrinsically scavenging the FR and activating the Nrf2; regulates the antioxidant expression in cells which protects from hydrogen peroxide induced oxidative toxicity (Fliedel et al., 2016).

1.2.3 Uric Acid

Uric acid is produced in humans as the end product of purine metabolism, the most copious antioxidant found in human plasma having robust scavenging activity for the carbon-centered and peroxyl radicals (Fabbrini et al., 2014). It prevents the excess production of the Oxo-hem oxidants that leads to severe metabolic effects, formed during the reaction of peroxides and hemoglobin. Uric acid is also a potent scavenger of the FR mostly scavenges singlet oxygen and hydroxyl radicals thus prevents the lysis of erythrocytes by carrying out the peroxidation reactions (Carocho and Ferreira, 2013). Uric acid while acting as antioxidant also exerts its neuro-protective effect by decreasing the circulating (blood) concentrations of the malondialdehyde (MDA) and matrix metalloproteinase (MMP), the lipid peroxidation marker and blood-brain barrier marker respectively (Llull et al., 2016). Recent studies revealed that the level of uric acid is reduced in major depressive disorder (MDD), anxiety and autoimmune diseases, i.e. polymyositis and dermatomyositis (PM/DM) which leads to up-regulation of oxidative stress in the biological system (Black et al., 2016, Chen et al., 2017).

A novel antioxidant mechanism is identified which uses uric acid to maintain the oxidants balance in body by p53-SLC2A9 pathway. SLC2A9 is a transporter of uric acid and associated with p53 gene which reduces the level of ROS in the biological system by transporting uric acid (Itahana et al., 2015). In the extracellular fluid, it remarkably scavenges the peroxynitrite ($ONOO^-$) but for complete scavenging activity it requires ascorbic acid and thiols which specifies its crucial role in scavenging the peroxynitrite (Fig. 4) (Nimse and Pal 2015).

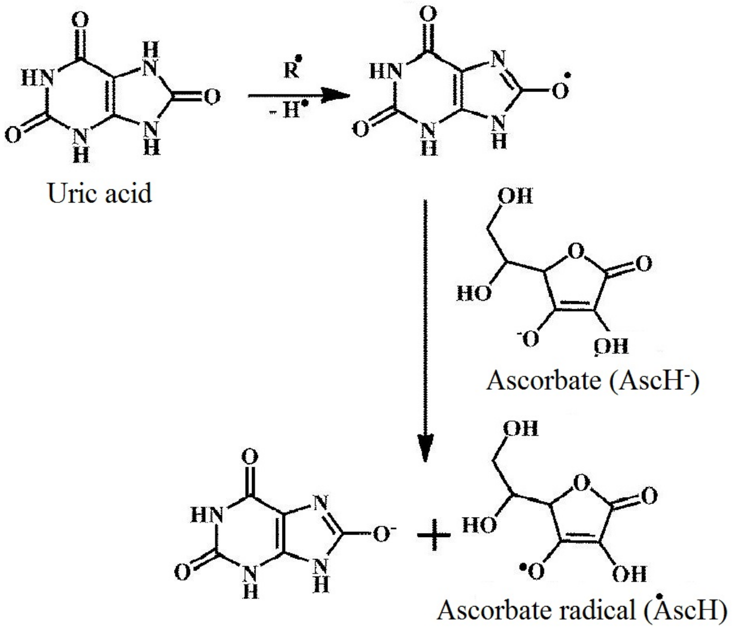

Fig. 4: Radical Scavenging Mechanism of Uric Acid (Fabbrini et al. 2014).

But irrespective of the protective properties of uric acid, its elevated level in the body commonly accompanied with higher risk for many diseases which includes cardiovascular disease and gout (Sautin et al., 2007). In a recent study it was concluded that uric acid also provides protection form the NSAID-induced enteropathy in the serum and intestinal lumen through its antioxidant action (Yasutake et al., 2017).

1.2.4 Glutathione

Glutathione (GSH) is a cysteine-containing tri-peptide, present in the cells cytosol in human body (Carocho and Ferreira, 2013). Cysteine is the sulfur containing amino acid and the thiol group (-SH) is responsible for its reducing action because it cycles between its oxidized form and reduced form (Birben et al., 2012). It is most important cellular antioxidant because of the presence of sulfhydryl group and high availability in biological system (Raffa et al., 2011). Three enzymes; glutathione reductase (GR), glutathione oxidase, and GSHPx are present in the GSH catalytic cycle hence, GSHPx and Glutathione oxidase converts GSH to GSSG by oxidation reactions while the regeneration of GSH from GSSG is carried out by glutathione reductase (GR) in an NADPH-dependent process (Fig. 5) (Raffa et al. 2011).

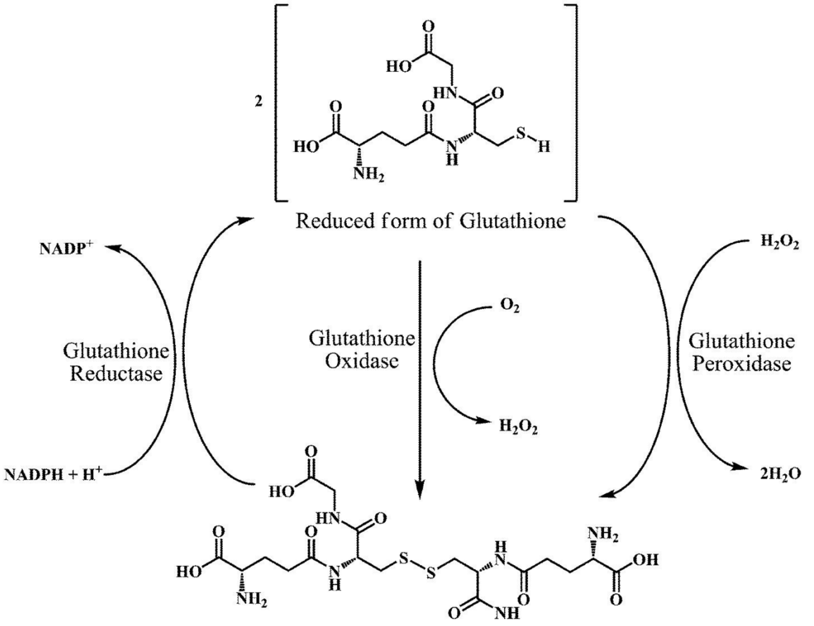

Fig. 5: Interconverting Process of GSH (Raffa, et al. 2011)

GSH in combination with these antioxidant enzymes provides a primary antioxidant defense mechanism against the harmful effects of ROS by maintaining cellular redox state (Baeza, Fdez-Tresguerres et al., 2010). GSH enhances ROS neutralization by its oxygen radical scavenging ability and helps in reducing the oxidative stress related damage to the cells (Nimse and Pal, 2015). GSH antioxidant system provides protection against the oxidant-induced cell death either autophagy or apoptosis, low levels of GSH is related to higher levels of oxidants and FR which consequently enhanced apoptosis.

1.2.5 Melatonin

Melatonin; "N-acetyl-5-methoxytryptamine", a indole containing neurohormone, synthesized in the pineal glands but large concentration are found in the mitochondrial portion of the cells, has many protective effects on various physiopathological functions (Rahman 2007; Mathew et al., 2011). It is effective in reducing the oxidative stress in the body by reducing the pro-oxidant enzymes lipoxygenases. It directly scavenges ROS/RNS and increases the synthesis process of enzymatic antioxidants indirectly (Torres et al., 2015). Via its anti-radical mechanisms, it chelates the transition metals which carry out the Fenton/Haber-Weiss reactions, where it decreases the toxic hydroxyl radicals generation and toxicity of noxious prescription drug (Reiter et al., 2016). It has various distinctive anti-oxidative features as compared to other antioxidants, including the cascade pathway for scavenging numerous free radicals, because its secondary and tertiary metabolites also neutralizes several harmful ROS/RNS derivatives (Pieri et al., 1994). One molecule of melatonin is capable of scavenging up to 10 molecules of ROS/RNS therefore, cascade reaction strengthens its capability as a potential antioxidant (Nohl et al., 2005). Melatonin is also useful in sickle cell anemia management either individually or in combination with N- acetyl cysteine (Silva et al., 2015) while N-acetyl cysteine is effective in schizophrenia and in pulmonary fibrosis (Brieger et al., 2012). It was also suggested that the derivate of melatonin having o-halogenated and di-halogenated aromatic side chain exhibits effectual antioxidant properties by significantly protecting the neuronal cells and scavenging ROS/RNS (Brewer 2011; Gurer-Orhan et al., 2016). Melatonin has extensive role in the immune and neuropsychiatric systems while it also regulates the bioenergetics (Zhao et al., 2019). Melatonin up-regulated the Nrf2 signaling pathway by lowering the oxidative damages due to FR and plays a potent role as neuroprophylactic against Aluminum-induced neurotoxic effect in rats (Sadek et al., 2019).

1.2.6 Vitamin C

Vitamin C is a water soluble substance generally known as ascorbic acid, acts as efficient antioxidant in the biological system and necessarily be obtained from exogenous sources in diet. It acts as a strong radical reducing agent in cell's cytoplasm (Limon-Pacheco and Gonsebatt, 2009) where it neutralizes ROS; it remains in reduced form by reacting with GSH in the cells. It works in combination with vitamin E to reduce ROS while regenerating vitamin E (reduced form) in the cell membranes and prevents from heart diseases (Pham et al. 2008; Sesso et al., 2008). It acts as oxygen scavenger by catalyzing the oxidation at low levels (<100 mg/kg) while it also maintains the balance between Fe^{2+} and Fe^{3+} by chelating the Fe^{2+} ions (Brewer, 2011). It terminates the chain reaction of lipid peroxidation and in consequence to this termination it converts into ascorbate radical. When two ascorbate radicals reacts with each other one ascorbate and dehydroascorbate molecule is generated. Dehydroascorbate cannot contribute in the antioxidant system therefore it is transformed into ascorbate by addition of two electrons by oxidoreductase (Fig. 6) (Nimse and Pal, 2015).

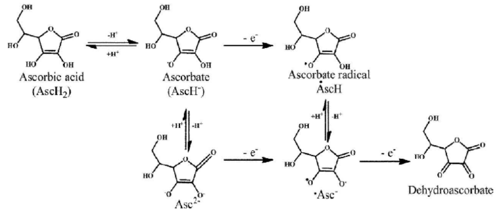

Fig. 6: Radical Scavenging Mechanism of Vitamin C (Nimse and Pal 2015)

1.2.7 Vitamin E

Vitamin E is the lipid soluble substance, mostly abundant in plants and known as α-tocopherol. In biological system, it acts as potent antioxidant because it protects the cell membranes from lipid peroxidation reactions by quenching the lipids free radicals and their intermediates (Traber and Atkinson 2007; Kabel 2014). It terminates the peroxidation process by giving its phenolic hydrogen to peroxy radicals, thus it acts as chain breaker in cell membranes (Carocho and Ferreira, 2013; Niki, 2014). The resultant tocopheroxyl radical is relatively stable under normal conditions and remains unreactive thus it cannot initiate lipid peroxidation chain reaction; vital criterion of a good antioxidant (Nimse and Pal, 2015). It can also inhibit oxidation of protein by reducing the formation of α-aminoadipic and α - glutamic semi-aldehydes from oxidized myofibrillar proteins (Brewer, 2011). It was documented that a-tocopherol is utmost effective peroxyl radical scavenger in vitamin E family kinetically, and quantitatively, in human vitamin E is the major antioxidant in plasma and red blood cells (Stocker, 2016). Furthermore, high intake of vitamin E may be related to the decrease in the clinical progression of early Alzheimer's disease symptoms and with a lower occurrence of Alzheimer's disease (Sinyor et al., 2020).

α-tocopherol–OH + LOO$^{\cdot}$ → α-tocopherol–O$^{\cdot}$+ LOOH

1.2.8 Vitamin A

Vitamin A; retinol, is a carotenoid formed in the liver from the cleavage reaction of α-carotene by α-carotene-15,15α-dioxygenase enzyme. The antioxidant role of vitamin A was firstly identified by Monaghan and Schmitt, as it protects the lipids from rancidity. Its beneficial role is to protect the human low density lipoprotein from copper-stimulated oxidation (Nimse and Pal 2015). It combines with peroxyl radicals due to its radical scavenging activity and detoxify the toxic species before the radicals initiate the peroxidation chain reactions (Fig. 7) (Carocho and Ferreira, 2013).

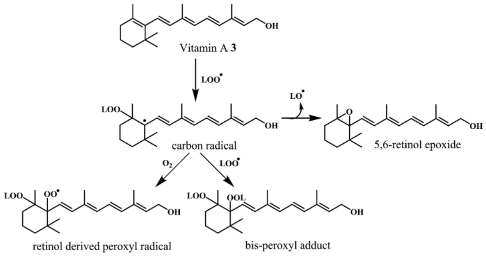

Fig. 7: Radical Scavenging Mechanism of Vitamin A (Carocho and Ferreira 2013)

1.2.9 Flavonoid

Flavonoids are group of compounds found in the plants and exhibits potential the antioxidant activities in the biological system when taken in through diet. These compounds share similar diphenylpropane ($C_6C_3C_6$) skeleton and their antioxidant abilities are owing to the presence of phenolic groups, hence they works as superoxide radical scavengers, reducing agents, singlet oxygen quenchers, hydrogen donators and even as metal chelators (Carocho and Ferreira, 2013). The presence of multiple hydroxyl groups (-OH) enhances their antioxidant potential as compared to those compounds with has only one group and ortho- 3,4-dihydroxy moiety also increases their antioxidant activity (Brewer, 2011). Furthermore, they are also involves in activating the antioxidant enzymes, lowering the oxidative stress due to ROS/RNS and increasing the levels of GSH, α-lipoic acid, CoQ 10 and uric acid in the body (Carocho and Ferreira, 2013). The hydroxyl groups at the C-5 and C-7 positions of flavonoids plays important role in xanthine oxidase (enzyme) inhibition because xanthine oxidase generates superoxide free radicals through catalysis of hypoxanthine and xanthine (Esatbeyoglu et al., 2017). Their indirect impact is to influence the gene expression of antioxidant enzymes by modulation of Nuclear Factor κB (NF κB) and Nuclear factor like 2 (Nrf2), redox sensitive transcription factors, that induces the activation of genes encoded for the antioxidant proteins (Dajas, 2016). Two new natural flavonoids were isolated from aerial parts of Ononis angustissima L.,, i.e. (3S)-7-hydroxy-4′-methoxy-isoflavanone 3′-β-d-glucopyranoside and kaempferol 3-O-β-d-glucopyranoside-7-O-(2‴-acetyl)-β-d-galactopyranoside, which exhibits excellent antioxidant activity against ROS/RNS in the biological system (Mezrag et al., 2017).

1.2.10 Carotenoids

Carotenoids are lipid soluble colored compounds, present in plants and microorganisms. Conjugated double bonds are present in their structure which is responsible for their antioxidant activities due to delocalization of unpaired electrons. Antioxidant efficiency of the carotenoids for quenching the singlet oxygen and peroxy radicals is due to presence of conjugated double bonds in the molecule (Rahman, 2007). Carotenoids are important compounds because of their peroxyl radical scavenging activity by which they protect the cell membranes and lipoproteins from the oxidation reactions of free radicals. β-Carotenes are naturally occurring orange-colored carotenoid, exhibits potential antioxidant activity by quenching the singlet oxygen with higher efficiency (Brewer, 2011; Nimse and Pal, 2015; Imai et al., 2016). Lutein; most predominant carotenoids, is a eminent antioxidant and free radical scavenger with biological properties such as prevention of cardiovascular diseases (Ranard et al., 2017). Furthermore, in vivo antioxidant activities of β-carotene represented that it significantly reduces the oxidative stress (El-Baz et al., 2019).

1.2.11 S-allylcysteine (SAC)

SAC; organosulfur compound, is the most abundant water-soluble derivative of garlic and exhibits antioxidant property (Fig. 8) (Shi et al., 2015).

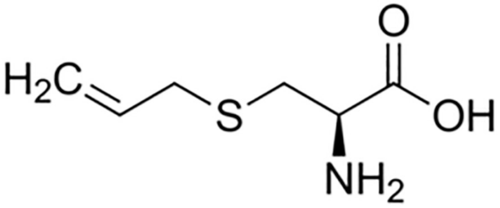

Fig 8. Structure of S-allylcysteine (Shi, et al. 2015)

SAC reduces oxidative stress and the accumulation of ROS, endoplasmic reticulum stress, mitochondrial dysfunction while enhances the functional activities of mitochondrial SODs, CAT, GSHPx, GST and the levels of GSH (Borek 2001; Colín-Gonzalez et al., 2015). SAC administration reduces the products of lipid peroxidation chain reaction and enhances the non-enzymatic antioxidants such as GSH, in the livers of diabetic rats (Naidu et al., 2016). Its exposure to the primary neurons provides protection against oxygen and glucose deprivation-induced oxidative insults and plays important role in activating the antioxidant Nrf2 signaling pathway in this way it protects the neurons from ischemic injury (Ashafaq et al., 2012; Shi et al., 2015). SAC was also involved in the up regulation of Heme oxygenase-1 (HO-1); defensive enzyme with anti-inflammatory property, through activation of the Nrf2 expression thus enhances its protective antioxidant effect (Baluchnejadmojarad et al., 2017). It was further evaluated that the derivatives of SAC also exhibited the neuroprotective effect which confirmed the antioxidant role of organosulfur compounds in biological system (Imai et al., 2016). SAC showed adequate therapeutic effect against diabetic nephropathy by reducing the oxidative stress and down regulation of inflammatory factors (Uddandrao et al., 2019).

CONCLUSION

Free radicals (FR) are the by-products of metabolic processes, associated with various physical and biochemical changes in human body. ROS/RNS induced oxidative stress leads to many deleterious effects including damage the proteins, lipids and DNA. If this stress exceeds the protection limit afforded by antioxidants then it subsequently increases the risk for various disorders such as cancer, inflammatory disorder, rheumatoid arthritis, cardiovascular disorder, diabetes etc. Therefore, balance between antioxidants and oxidants (FR) are crucial for the maintenance of biologic system. Naturally occurring antioxidants protects the body from the negative effects of FR and have beneficial impact on the human health. They may react directly with the ROS/RNS to eliminate their unpaired condition by donating electrons or they may indirectly decrease their formation either by lowering the expressions of enzymes that generate the free radicals or by enhancing the expressions of antioxidant enzymes which includes SOD, CAT, GSHPx, GSH and CoQ 10. Vitamin A, Vitamin E, Vitamin C, flavonoids and carotenoids are natural exogenous antioxidants which act as "chain breakers" for FR-induced oxidative reactions. The common feature of chain-breaking antioxidants is that they have one or more −OH groups which donates the $H^{\cdot}$ to the oxidizing molecules. Flavonoids contains more than one aromatic ring and hydroxyl groups while ascorbic acid and α-tocopherol each have only one aromatic ring thus have lesser hydroxyl groups in their structure and have positive impact in reducing effects of FR in the biological system. Level of exogenous antioxidants must be maintained through diet because the natural antioxidants play a significant role in defending the living beings form the harmful effects of the oxidants.

CONFLICT OF INTEREST STATEMENT

It is declared that authors have no conflict of interest.

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AUTHORS' CONTRIBUTIONS

MMS is involved in manuscript preparation along with MA; MARB in charge of ideas and supervision of this review article.

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In-Vitro Antifungal Efficacy of *Zingiber Officinale*

Sana Iqbal*, Urooj Akhtar, Shoaib Siddiqui

Department of Biology, Lahore Garrison University, Lahore, Pakistan.
Corresponding Author's Email: si9706825@gmail.com

ABSTRACT:

Synthetic medicines are being replaced by those derived from natural resources because of cost burden and side effects of synthetic medicines. Zingiber officinale (Ginger) has been act as home remedy against the microbial infections. Its fresh juice has radio protective action that could be helpful in avoiding gamma radiation which exert harmful effects during the period of cancer treatment. It exhibited chemo defensive and anti-neoplastic action as well as has been useful to cure the inflammation in the body. Present study investigated in vitro antifungal efficacy against Aspergillus niger, Aspergillus oryzaea, Fusarium oxysporum and Fusarium tricinctum using disc diffusion technique. Maximum inhibitory zone against Aspergillus niger produced after 96 hours i.e. 46.66 ±2.5mm and minimum after 48 hours i.e. 41.66 ± 2.5mm. Maximum inhibitory zone against Aspergillus oryzaea was produced after 96 hours i.e. 23.33±1.73 mm and minimum at 48 hours i.e. 17.66 ± 1.73 mm. Maximum inhibitory zone against Fusarium oxysporum was produced after 96 hours i.e. 24.00 ± 1.5mm and minimum at 48 hours i.e. 18.00 ±1.5mm. Maximum inhibitory zone against Fusarium tricinctum was produced after 96 hours i.e. 38.33 ± 2.00 mm and minimum at 48 hours i.e. 35.00 ± 2.00 mm. These concentrates may have been proved to be effective antimicrobials and can be exploited in controlling superfluous microbial growth.

Keywords: *Aspergillus oryzaea, Aspergillus niger, Fusarium tricinctum, Fusarium oxysporum, Zingiber officinal, Antimicrobial activity, Inhibitory activity.*

INTRODUCTION

Zingiber officinal (Ginger) is herbal plant that has been cultivated broadly throughout the world for various purposes. Rhizome or root part of ginger is medicinal properties and aids in various ailments e.g. motion sickness, vomiting, gastrointestinal ulcers, diabetes, fever, nausea, arterial tension, dry mouth/xerostomia, rheumatoid arthritis, migraine headache, sore throat, cancer and other minor respiratory problems (Teles et al., 2019) . Ginger has been in use since ancient times due to its antiseptic properties for thousands of years in Asian cultures (Ernst and Pittler, 2000; Weil, 2005). Europe is famous for the ginger and is a daily use spice and a natural medicine (Sasidharan and Menon, 2010). Due to its (*Zingiber officinale*) medicinal properties, consumption of wild ginger rhizome to normalize menstruation and heart beat is well known among inhabitants of America (Holtmann et al., 1989).

Ali et al. (2008) reported that *Zingiber officinale* is a medicinal plant widely used for a broad range of unrelated diseases e.g. cramps, hypertension, arthritis, indigestion, constipation, sprains, sore throats, muscular aches, pains, dementia, vomiting, rheumatism, fever and other infectious diseases all over the world. Curative plants are used as pharmaceuticals, nutraceuticals, cosmetics and food supplements (Sharma et al., 2010). There are some qualities of Ginger. A few include simply available, universally suitable, relatively low-priced and well accepted by public. *Zingiber officinale* is member of the Zingiberaceae family (Sharma et al., 2010). Tuberous and non-tuberous rhizomes have sturdy aromatic and medicinal properties (Chen et al., 2008).

Zingiber officinale extract obtained are reported to repress the growth and stimulate apoptosis of variety of cancer types including colon, oral, skin, cervical, ovarian, breast, renal, prostate, pancreatic, liver, gastric and brain cancer. *Zingiber officinale* have antioxidant, anti-inflammatory, anti-mutagenic actions and biological efficacies (Srinivasan, 2014). Therefore, it is not wrong to say that it can be used to treat the bacterial infections (Tan and Vanitha, 2004). It is abundantly grown in several countries such as Indonesia, India, Japan, China, Nigeria, Australia (Queensland), Jamaica, Sierra Leone and other West Indies islands. Ginger is considered to act directly on the digestive system for controlling queasiness (Foster, 2011) and motion sickness (Holtmann et al., 1989).

Zingiber officinale has great value as a trendy medicine against nausea during conceiving (Langner et al., 1998). Different types of problems e.g. upset stomach, bloating, flatulence, heartburn, dyspepsia, sickness, colic, diarrhea, gas and hungriness can be cured by *Zingiber officinale*. Ayurvedic medicinal system suggests Zingiber officinale as increasing agent for digestion of food (Ali et al., 2008). *Zingiber officinale* is considered as relief for various kinds of pains e.g. low back pain, arthritis pain, stomach, muscle soreness, menstrual pain and chest pain and as well as helpful to alleviate cough, upper respiratory tract diseases and bronchitis. It (*Zingiber officinale*) is suggested for joint problems due to its anti-inflammatory action (Shukla and Singh, 2007). Active ingredients of ginger are helpful for laxative and antacid medication also protect from skin burn. Regulating the body circulation and lowering high blood pressure are also included in their uses. There are many applications of *Zingiber officinale* e.g, flavoring compound in foodstuffs and as fragrance in soaps as well as in cosmetics (Alam, 2013). *Zingiber officinale* contains the element caprylic acid, which has powerful antifungal activity. Gingerols present in it (*Zingiber officinale*) have analgesic, sedative, antipyretic, antibacterial and gastro intestinal tract motility effects (Azu et al., 2007). *Zingiber officinale* has strong aromatic and medicinal properties (Chen, 2008). It has been exploited for the cure of many infectious in many countries (Ali et al., 2008; Tan and Vanitha, 2004). The spicy taste of *Zingiber officinale* is due to gingerols, shagaols and zingerone (Mascolo et al., 1998) and therefore possess a broad array of pharmacological characteristics (Ficker et al., 2003; Habsah et al., 2000). Unfortunately, East Pakistan is now separated and is has a big source of this crop yield. Now in Pakistan extensive amount of *Zingiber officinale* is imported from foreign countries. So, there is necessity to develop appropriate regions in Pakistan for the cultivation of this expensive crop (Ahmad et al., 1983).

Diseases caused by infectious organisms including viruses, bacteria, protozoa, fungi and different multicellular parasites are called as infectious diseases (Zubairu and Gwa, 2020). Currently, plant infections are limiting factor in crop production. Crops infections lead to field losses and become popular as world population increases. About 50% of the deaths in equatorial countries happen due to infectious diseases (Khosravi and Behzadi, 2006).

The present study was focused on evaluation of the antifungal efficacy of fresh juice of *Zingiber officinale* against four different fungi i.e. *Aspergillus niger, Aspergillus oryzaea,* Fusarium oxysporum and Fusarium tricinctum to determine the most suitable, non-hazardous and inexpensive method for controlling fungal strains.

MATERIALS AND METHODS

Study Area

The present study was conducted in Department of Biology, Lahore Garrison University, Lahore, Pakistan and University of the Punjab, Lahore, Pakistan to study the Isolation, Identification, Antifungal susceptibility pattern of *Aspergillus niger, Aspergillus oryzaea, Fusarium oxysporum and Fusarium tricinctum.* These strains were obtained from Institute of Agricultural Sciences; University of the Punjab, Lahore. The pure cultures were sub cultured on Potato Dextrose Agar (PDA) and kept at 4^{o}C until ready for the further analysis.

Preparation of the Zingiber officinale fresh juice

The rhizome was washed with fresh water. The outer layer of the *Zingiber officinale* was peeled through knife and the fat part of the plants was rewashed with distilled water. Ginger was cut into different sections. About 30.0gm of the ginger rhizome was used to form juice. After making juice, it is ready for its antifungal efficacy (Adeshina et al., 2012).

Disc Diffusion assay

This method for antimicrobial susceptibility test was done to ensure the antifungal action of the plant extracts. Fungal cultures were placed on potato dextrose agar plates uniformly using a cotton swab. The disc diffusion assay was performed according to the method of (Tepe et al., 2005). The discs were dipped into different plant extracts and then placed on the dried potato dextrose agar plates for the susceptibility test. Each plate containing one disc incubated at 30°C and then checked the zone of inhibition after different time intervals.

Experimental design

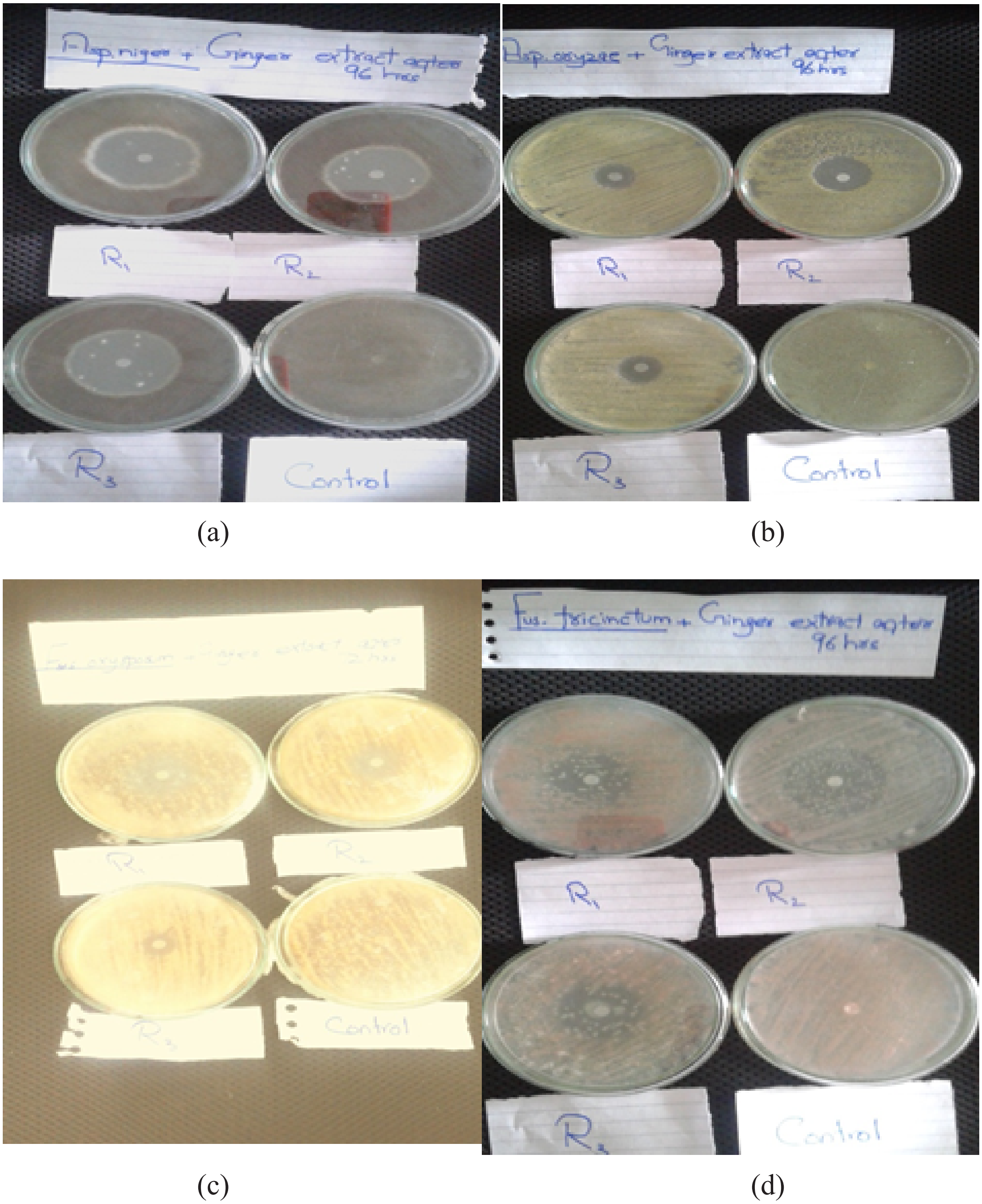
Antifungal assay of prepared petri plates having discs of fresh juice (*Zingiber officinale*) was done to observe the results. Petri plates were labeled properly as R1, R2, R3 with fourth test tube being the control. Zone of inhibition was observed after 48 hours. Plates showing zone of inhibition were captured and measured in millimeters.

STATISTICAL ANALYSIS

The results were mentioned as Mean ± Standard Deviation (S.D). Data were assessed through analysis of variance (IBM SPSS version 21).

RESULTS AND DISCUSSION

Zingiber officinale has an antifungal activity against all fungus. Fig. 1 shows zone of inhibition against such fungi with three replicates and control, after 96 hours. Fig. 2 shows the graphical representation of antifungal activity after different time intervals and it indicates that *Aspergillus niger* had maximum growth while *Aspergillus oryzaea* had minimum growth after 96 hours. Fig. 1 represents that mean inhibition zone (mm) of *Zingiber officinale* against *Aspergillus niger* is greater than other fungus. Fig. 2 represents that inhibition zone increases with the passage of time i.e. 48, 72 and 96 hours. Table 1 describes that maximum inhibitory zone against *Aspergillus niger* produced after 96 hours i.e. 46.66 ±2.5mm and minimum after 48 hours i.e. 41.66±2.5mm. Maximum inhibitory zone against *Aspergillus oryzaea* was produced after 96 hours i.e. 23.33 ± 1.73 mm and minimum at 48 hours i.e. 17.66 ± 1.73 mm. Maximum inhibitory zone against *Fusarium oxysporum* was produced after 96 hours i.e. 24.00 ± 1.5mm and minimum at 48 hours i.e. 18.00 ±1.5mm. Maximum inhibitory zone against *Fusarium tricinctum* was produced after 96 hours i.e. 38.33 ± 2.00 mm and minimum at 48 hours i.e. 35.00 ± 2.00 mm.



(a) (b)

(c) (d)

Fig. 1: Zone of inhibition produced against (a) Aspergillus niger (b) *Aspergillus oryzaea (c) Fusarium oxysporum (d) Fusarium tricinctum* with four replicates after 96 hour.

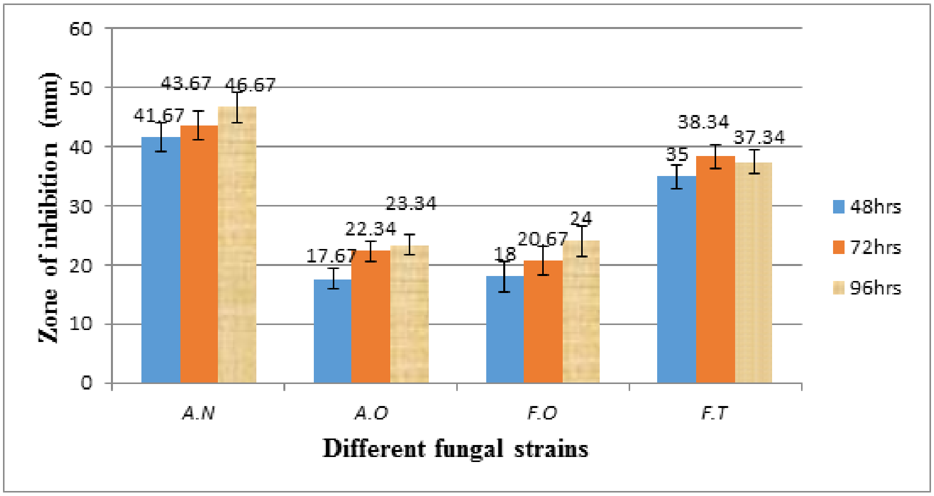


Fig. 2: Activity of Ginger (*Zingiber officinale*) against various fungi i.e. A.N (*Aspergillus niger*), A.O (*Aspergillus oryzaea*), F.O (Fusarium oxysporum) and F.T (*Fusarium tricinctum*) after different time intervals.

The above results showed that *Zingiber officinale* fresh juice had the best antifungal activity against the test organisms such as Aspergillus spp. and Fusarium spp. The fresh juice of *Zingiber officinale* produced the maximum value of zone of inhibition i.e. 46.66±1.5mm against *Aspergillus niger* and showed minimum value i.e. 17.66±1.73mm against *Aspergillus oryzaea* among all the fungal strains. This very high antifungal value is due to the compounds having strong antifungal potential present in the extract. This is in conformity to work by Atai et al. (2009), who observed similar activity of ethanol extract of *Zingiber officinale* (Ginger) on Candidia with relation to that of conventional antibiotics. Several modern studies prove that *Zingiber officinale* has antimicrobial properties against many bacteria, fungi and viruses. Investigators have compared its efficiency with viable recommendation antifungal disc and results show that *Zingiber officinale* is more powerful than antibiotics (Saad et al., 2014).

The susceptibility of ginger extract also confirms observation by Sasidharan and Menon, (2010), who performed an antimicrobial action of the oils (*Zingiber officinale*) against, Pseudomonas aeruginosa, *Aspergillus niger, Bacillus subtilis, Candida albicans, Trichoderma spp, Pencillium spp. and Saccharomyces cerevisia* by disc diffusion method .They analyzed a broad appliance of ginger oil in the healing of many bacterial and fungal infections. The results of Pratibha and Rajendra, (2016) also supports present work who investigated the antimicrobial efficacy of *Zingiber officinale* (Ginger) and concluded that chloroform extract of ginger exhibited highest zone of inhibition as 25.75 mm against tested microbial strain. Our results also similar to work of Supreetha et al. (2011) who evaluated the inhibitory effect of *Zingiber officinale* extract on candida albicans under controlled conditions and found ethanol extract of *Zingiber officinale* had inhibitory effect against that fungus. The search work of Saada et al. (2015) also confirms present study but they worked on bacteria using fresh and powdered ginger extract by performing same antimicrobial test. They concluded that in comparison to tested antibiotics, fresh and powdered ginger extracts showed a higher antibacterial effect against *Streptococcus mutans* ginger oil in the healing of many bacterial and fungal infections. The results of Pratibha and Rajendra, (2016) also supports present work who investigated the antimicrobial efficacy of *Zingiber officinale* (Ginger) and concluded that chloroform extract of ginger exhibited highest zone of inhibition as 25.75 mm against tested microbial strain. Our results also similar to work of Supreetha et al. (2011) who evaluated the inhibitory effect of *Zingiber officinale* extract on Candida albicans under controlled conditions and found ethanol extract of *Zingiber officinale* had inhibitory effect against that fungus. The search work of Saada et al. (2015) also confirms present study but they worked on bacteria using fresh and powdered ginger extract by performing same antimicrobial test. They concluded that in comparison to tested antibiotics, fresh and powdered ginger extracts showed a higher antibacterial effect against *Streptococcus mutans.*

CONCLUSION

Our results suggest that *Zingiber officinale* can serve as strong antifungal agent and its consumption could be useful in the prevention of diseases. Therefore, adding ginger in preserved foodstuff can eventually increase the useful life and also keep the value of preserved food. Further research is needed towards phyto-chemical analysis of compounds present in the extracts of ginger and sugarcane which could possibly be subjugated for pharmaceutical use. Because of active phytochemicals present in *Zingiber officinale,* this plant can be helpful in designing antifungal drugs.

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Proteomic Analysis of Cr (VI) Reducing Synechocystis sp. AHZ-HB-MK and its Mutant Obtained by Gamma Irradiation

Saiqa Razi*1, Shahida Hasnain[2], Byung-Gee-Kim[3]

[1,2]Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan,
[3]School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea
Corresponding Author's E-mail: saiqarazi@ymail.com

ABSTRACT:

A chromium resistant Synechocystis species, AHZ-HB-MK (DQ 381960) was used in this study. It was aimed to characterize this strain at molecular level and to produce certain mutants with better chromate reduction abilities. The strain was irradiated with a 60Co source in a dose range of 0.5, 1, 2, 5, 10 and 20 Gy at different stages of growth (after 5, 15 and 30 days) of incubation. The selected derivatives were characterized morphologically, physiologically and biochemically. Chromium reduction activity and the amount of Cr (VI) accumulated within the cells of all of the selected derivatives were estimated. Protein profiling was done to study the impact of gamma rays on the proteins. Protein profiling proved that in the case of cultures irradiated after 5 days of incubation, maximum protection of proteins to radiation damage was observed at 0.5, 1, 2 and 5 Gy. Maximum expression of stress proteins was observed in the derivative obtained at 2 Gy. This derivative was labeled as Synechocystis MK-TR. Identification of these proteins was done by mass-spectrometry. Seventy-four peptides were identified from ten selected protein bands. In these peptides sll0170 a heat shock protein, slr2076 a 60kD chaperonin and sll0416 identified as 60kD chaperonin 2 and GroEL2. Whereas sll0170 was also a heat shock protein 70, slr1198 was identified to be an antioxidant protein and slr1516 was annotated as superoxide dismutase which may be involved in the chromate resistance in this strain.

Key words: *Chromium Reduction, Gamma radiation, Mutagenesis, Mass spectrometry*

INTRODUCTION

Heavy-metal resistance mechanisms is exist in various species of bacteria (Nies, 1999; Navarro et al., 2013; Nanda et al., 2019). In stress, the working of few proteins is repressed or vanished and that of others is boosted or induced (Los et al., 2008). Many types of environmental stresses such as osmotic stress, thermal stress, heavy metal stress, ionizing radiation, baric stress, oxidative stress, and hypoxia/ischemia have been shown to cause deleterious changes in protein conformation (Somero, 1992; Kempner, 1993; Borkan and Gullans, 2002; Hochachka and Somero, 2002; Kasprzak, 2002; Rodriguez-Rojas et al., 2020). Induction of proteins under metal stress has been reported in various microbial strains. Induction of Fe stress proteins results in certain phenotypic variations including decrease in growth rate and change of pigmentation have been reported by Fe stress-induces. In few studies, assemblage of Fe binding ligands (siderophores) have also been observed. A key protein produced during Fe stress was found to be an IdiA (iron deficiency-induced protein) homologue (Webb et al., 2001). The adaptive response in Sulfolobus solfataricus to its exciting environments was investigated by examining the changes in its protein range. It was reported that few Sulfolobus on exposure to NiSO4 show considerable protein alteration in their countenance levels (Salzano et al., 2007). Ivanina et al. (2008) reported that cadmium resistance may involve in the synthesis of numerous cellular proteins that are protective in nature and glutathione like amino acids. Similarly, cadmium exposure in oyster cells results in a dose-dependent increase. In a finding of a study at the highest tested Cd level (2000 µmol l-1) the rate of protein synthesis increased up to 150% as compared to control. The way of chromium (Cr) toxicity in yeast (Saccharomyces cerevisiae) is involved in oxidative damage to cellular proteins (Sumner et al., 2005). Perhaps in this mechanism of chromate-induced toxicity the reactive oxygen species (ROS) are produced during enzymatic chromate reduction (Cervantes et al., 2001). For removing ROS, bacteria mostly use a strategy to modify expression of genes as they persuade genes encoding antioxidant enzymes and proteins. A Cr (VI) stressed bacteria strain Shewanella oneidensis MR-1 showed a 2 to 5-fold generation of katG-1 (so0725, catalase/peroxidase hydroperoxidase), katB (so1070, catalase), and so4640 (antioxidant AhpC/Tsa family protein) at the 60- and 90-min time exposure (Brown et al., 2006). A recent study by Seleiman et al. (2020) reported that metal resistant microbes have the ability to enhance plant defense responses.

Understanding the cellular effects of radiation-induced oxidation requires the unraveling of basic molecular events, which particularly harm protein's important cellular functions (Gillard et al., 2007). The effect of gamma radiation on the protein profiles of Synechocystis AHZ-

HB-MK was studied. For polypeptide profiling parental strain and one of its representative mutants from each dose giving maximum chromium reduction potential, was grown in BG 11 liquid medium (Rippka et al., 1979) for 10 days. After that Cr (VI) was supplemented in the medium at a concentration of 100 µgml-1. The strains were exposed to Cr stress for three days. After three days, crude extracts of treated and untreated cells were analyzed at the proteome level using one-dimensional SDS-polyacryalmide gel electrophoretic approach.

MATERIALS AND METHODS

Preparation of Protein Sample

Proteins were extracted from 100 ml of cultures by repeated freeze-thawing, sonication and vortex mixing (Lyra et al., 1997). From cell extracts, the concentration of proteins was estimated by the Coomassie Blue protein assay at 595 nm utilizing bovine serum albumin as a standard (Bradford, 1976; Yang et al., 2020).

Electrophoresis of Protein Samples

1-D gel electrophoresis of protein samples was carried out by using methods of Vandamme and Kersters, (1994).

Trypsin Digestion

After de-staining, the bands were visualized under light box. Ten bands were selected for Trypsin digestion. Each band was cut into slices. All slices approximately containing the same protein content. Trypsin gel was used to absorb proteins from the slices. The trypsin digest was performed following the method of Shevchenko et al. (1996). Four volumes of 50 mM NH4HCO3 buffer solution and trypsin were added to obtain a final amount of 1µg per 50 µg proteins. In all cases, acetonitrile was used to extract tryptic peptides from the gel particles.

Mass Spectrometry

Nanoflow liquid chromatography was used to separate mixtures of tryptic peptides. Further analysis was carried out by high mass-accuracy tandem mass spectrometry (LC MS/MS) on a QSTAR pulsar quadrupole time-of-flight hybrid mass spectrometer (Thermo Finnigan, San Jose, CA). Sequences of tryptic peptides were imitative by information-dependent acquisition of fragmentation spectra of multiple-charged peptides. Acquired spectra were investigated against the CyanoBase protein database (http://bacteria.kazusa.or.jp/cyanobase /Synechocystis/index.html) using Mascotsearch algorithm. Different parameters such as maximum of one missed trypsin cleavage, cysteine carbamidomethylating, methionine oxidation, and a maximum 0.2 Da error tolerance in both the MS and MS/MS data (40 ppm after dynamic recalibration). In the study only tryptic peptides were considered. All the sequences were manually verified against the raw mass spectrometric data using accepted rules for peptide fragmentation in a quadrupole-TOF hybrid mass spectrometer. On average, 30% of the proteins were identified by a single tryptic peptide; to all other proteins more than one tryptic peptide was found.

RESULTS

Protein profile of AHZ-HB-MK and its irradiated derivatives after 3rd day of exposure to 100 µg ml-1 Cr (VI) Twenty proteins bands were observed in this strain under control condition. The bands were of ~ 110, 135, 132, 120, 110, 104, 63, 58, 52, 48, 45, 38, 36, 34, 30, 27, 25, 23, 20, 18, and 14 KDa (Table 1, Fig. 1). At third day of exposure to Cr VI, ten proteins of ~ 104, 63, 58, 52, 35, 27, 25, 20, 18, and 14 KDa were up regulated and gave more expression as compared to control. Other six proteins of ~135, 132, 120, 48, 45, 30 and 25 KDa were down regulated and their expression was reduced under Cr VI stress while no change was observed in proteins of ~ 38, 34, and 20 as compared to control. In derivatives obtained by irradiation after 5 days of culturing, ten out of twenty proteins of ~ 104, 63, 58, 52, 35, 30, 27, 20, 18 and 14 KDa were up regulated as compared to non-irradiated control at 0.5, 1, 2, and 5 Gy. Maximum expression was observed at 2 Gy. Under Cr stress proteins of ~ 104, 63, 58, 35, 20, 18 and 14 gave more expression as compared to their respective controls and the non-irradiated parental strain. (Table 2, Fig. 2). All proteins gave a reduced expression under normal as well as Cr-stress condition in derivatives obtained by irradiation at 10 and 20 Gy as compared to non-irradiated control. Proteins of ~ 51 and 45 KDa disappeared after irradiation at all doses in all cultures. In case of cultures irradiated after 15 days of incubation, up regulation of proteins of ~ 104, 63, 58, 35, and 18 KDa was observed at 0.5, 1, 2 and 5 Gy. Under Cr stress these proteins showed more expression as compared to control. Proteins of ~ 48, 45, 38, 34, 30, 25, 24, and 14 KDa were down regulated as compared to non-irradiated control and were observed only with silver staining. All proteins were down regulated at 10 and 20 Gy under normal as well as under Cr-stress as compared to non-irradiated control (Table 2). In case of cultures irradiated after 30 days of incubation only seven bands of ~ 63, 58, 52, 35, 20, 18 and 14 KDa were observed at all doses with silver staining. Under Cr stress condition these proteins were down regulated as compared to control. Proteins of ~ 135, 132, 120, 110, 104 and 48 KDa were disappeared in these cultures under control and Cr stress conditions.

Table 1: Protein profile of chromium resistant Synechocystis AHZ-HB-MK under control conditions

MWt. (KDa)	**Protein Profile**	
	***Synechocystis* AHZ-HB-MK**	
	No. of Bands	**Detail**
<100 KDa	5	135, 132, 120, 110, 104
100-70 KDa	0	0
69-50 KDa	3	63, 58, 52
49-40 KDa	2	48, 45
39-30 KDa	4	38, 36, 34, 30
29-20 KDa	4	27, 25, 23, 20
19-15 KDa	1	18
>15 KDa	1	14
Total	20	

Fig. 1: Total Proteins from Synechocystis AHZ-HB-MK analyzed by SDS-PAGE on a 12.5% (w/v) polyacrylamide gel. Cr VI, 100µgml-1 was added to the growth medium to induce expression of stress proteins and the cultures were incubated for 3 days at 28 °C. Ten micrograms of protein were applied to each lane. The position of protein molecular lass markers is indicated on the left and represented as M in the middle of the gel.

Lane 1: Non-irradiated Control (without Cr); Lane 2: Non-irradiated Control (with Cr VI)

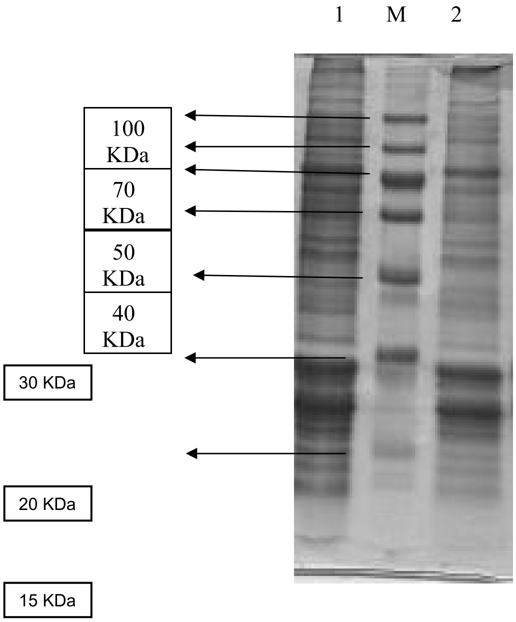

Table 2: Protein profiles of chromium resistant *Synechocystis* AHZ-HB-MK and its mutants obtained after gamma radiation under control and after 3rd day of exposure to hexavalent chromium (100 µg ml^{-1})

	Dose (Gy)	Treatment	Protein profile (~KDa)																			
Irradiated after 5 days of incubation	0	cont	135	132	120	110	104	63	58	52	48	45	38	36	34	30	27	25	23	20	18	14
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
	0.5	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	1	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	2	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	5	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	10	cont	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
	20	cont	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
Irradiated after 15 days of	0.5	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
	1	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐

	2	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
	5	cont	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
		+Cr	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	☐	☐	☐	☐
	10	cont	---	---	---	---	---	☐	☐	☐	☐	**☐**	☐	**☐**	☐	☐	**☐**	---	**☐**	**☐**	**☐**	☐
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Irradiated after 30 days of incubation	0.5	cont	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	☐	---	---	---	☐	☐
		+Cr	---	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
	1	cont	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
		+Cr	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
	2	cont	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
		+Cr	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
	5	cont	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
		+Cr	---	---	---	---	---	**☐**	**☐**	**☐**	☐	☐	☐	☐	☐	☐	**☐**	---	---	☐	**☐**	☐
	10	cont	---	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	---	---	☐	☐	☐
		+Cr	---	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	---	---	☐	☐	☐
	20	cont	---	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	---	---	☐	☐	☐
		+Cr	---	---	---	---	---	☐	☐	☐	☐	☐	☐	☐	☐	☐	---	---	---	☐	☐	☐

Key: ☐ =Up regulated

☐=Downregulated

☐= Same as non-irradiated control

--- = Disappeared

Protein profile analysis of these strains revealed the fact that many proteins were stimulated at low doses like 2 Gy in cultures irradiated at an early growth stage i.e. after 5 days of incubation. These proteins gave a very strong expression under chromate stress resulting in the ultimate enhancement of chromate reduction potential. For the identification of these up regulated proteins LC MS/MS analysis was done in the derivative of AHZ-HB-MK irradiated after 5 days of incubation obtained at 2 Gy. This derivative was labeled as Synechocystis MK-TR. Seventy-four peptides were identified from ten selected protein bands. These proteins were categorized into 11 categories (Fig. 3) based on their expected functions. Out of this long list of identified peptides, six peptides are of particular interest: sll0170, a heat shock protein, slr2076 identified as 60kD chaperonin and sll0416 identified as 60kD chaperonin 2 and GroEL2. sll0170 is also a heat shock protein 70 and slr1198 was identified to be an antioxidant protein while slr1516 was identified as superoxide dismutase. The increase in the amount of soluble protein and peroxidase enzyme content at this dose is also correlated to the induction of these proteins.

Fig. 2: Total Proteins from Synechocystis AHZ-HB-MK derivatives obtained in cultures irradiated at 5 days of incubation (a) at 2 Gy (b) at 5 Gy, analyzed by SDS-PAGE. The position of protein molecular markers are indicated on the right.

a) Lane 1 and 2 MK at 2 Gy without and with Cr stress respectively
b) Lane 3 and 4 MK at 5 Gy without and with Cr stress respectively

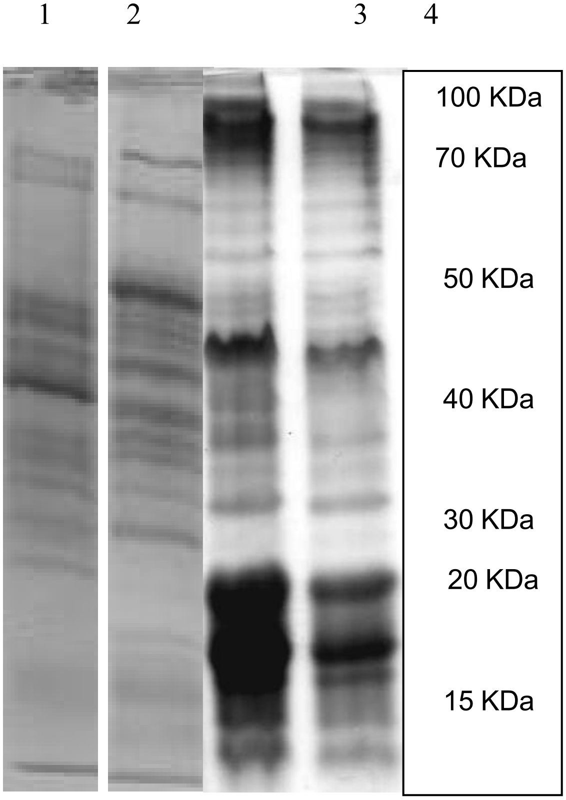
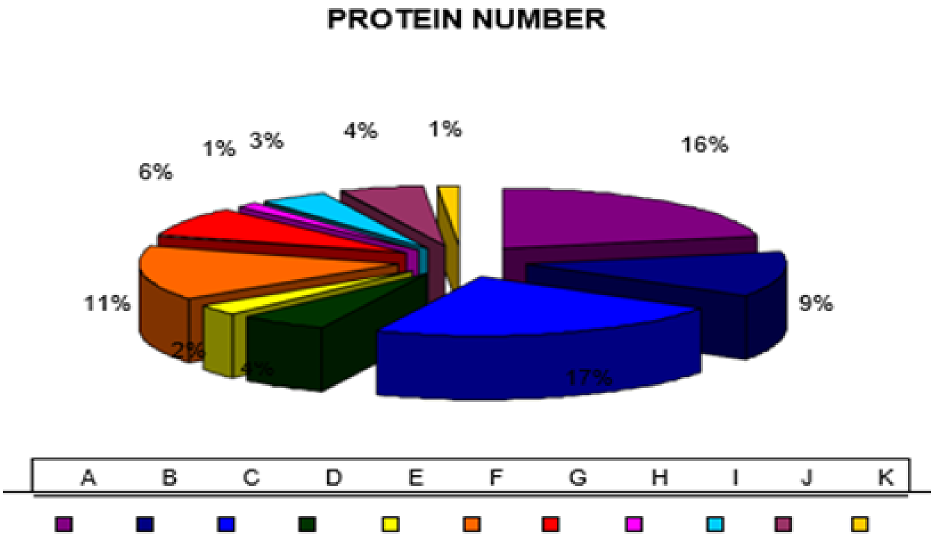


Fig. 3: Categories and relative proportion of the proteins identified in the proteome of Synechocystis MK-TR. The 74 proteins identified from 10 bands were divided into categories based on their expected functions. The percentage of proteins identified in each category is given.



Key:

A=unknown proteins 16%

B=proteins involved in carbohydrate metabolism 9%

C=energy metabolism 17%

D=amino acid biosynthesis 4%

E=protein synthesis 2%

F=post-translational modification of proteins 11%

G=nucleotide metabolism 6%

H=lipid metabolism 1%

I=reductases 3%

J=cell motility and secretion and cell envelope biogenesis 4%

K=inorganic nutrient transport and metabolism 1%

DISCUSSION

High- and low-dose irradiation-response of proteins was investigated using proteomics on one-dimensional (1D) SDS-PAGE with and without chromium stress. Four types of responses were observed in protein profiles of Synechocystis species MK after irradiation at different doses and different stages of growth.

i. Up regulation
ii. Down regulation
iii. Disappearance, and
iv. No change as compared to non-irradiated control

Studies of protein countenance have the potential to expose the mechanism of metal reduction and estimate how bacterial cells react these toxic compounds upon exposure. This study was investigated to identify those proteins up regulated during the reduction of Cr (VI) that may be part of the reduction pathway(s). The roles of the proteins identified were further investigated by using appropriate mass spectrometric analysis. Cells used an important protective mechanism that is expression of heat shock genes in response to a variety of stressors. Organisms such as bacteria, plants, and animals have proteins which are present in highly conserved form one of the important heat shock protein is GroEL. It is a protein of 58,000 amu which lumped together into two stacked rings each of seven subunits and possess an additional ring of seven, 10,000 amu GroES subunits. Together, this complex has been shown to renature and makes functional proteins that have been misfolded as a result of cellular stress (Turner and Robinson, 1995). Transcription of GroEL(58 KDa) in response to alterations in the environment prevents protein aggregation and misfolding which increased in case of Cyanobacteria, and clearly observed in the present study. In addition, one antioxidant protein and Superoxide dismutase has also been identified in the present derivative. It is observed that Synechocystis sp. Strain PCC 6803 inevitably evolves superoxide dismutase, designated as SodB which plays a significant role in averting oxidative damages that caused by radiation (Kim and Suh, 2005). Free radicals such as O2o-, Ho, HO2o OHo and H2O2 are formed as a result of irradiation which have indirect effect on biological systems and cause damage. Presence of superoxide dismutase supports the fact that the oxidative free radicals are being prevented from causing indirect damage. Presence of this enzyme at low dose is significant as at higher doses the damage by direct effect (direct hit) is more pronounced as compared to indirect effect. Radiation mutagenesis has thus proved to be a successful tool in the identification of proteins involved in chromate resistance. It appears that the sequestration of the metal with these proteins detoxify it, and reduced its detrimental effects on the cell. To create Cyano-bacterial strains which express these proteins at very high levels would be possible through genetic engineering and it will be potentially valuable for attempts at bioremediation (Silver and Ji, 1994). Further studies with reference to

Synechocystis sp. isolates has been performed which showed thiols might play a role in metal resistance (Y?lmaz et al., 2020).

CONCLUSION

Synechocystis MK-TR, the selected derivative of AHZ-HB-MK produced after exposure to gamma radiation at 2 Gy dose has the highest chromate reduction ability. Mass spectrometric analysis of this strain indicated that it has certain proteins which express very strongly when subjected to Cr stress and enable it to overcome this stress. The present strain is considered as the 'target strain' with better morphology, physiology and biochemical properties and useful for future perspectives as bioremediant to treat Cr- contaminated soils and wastewater.

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An Update on the Spread of COVID-19 in Pakistan

Haleema Sadia*, Fatima Bibi, Aizaz Ali, Muhammad Naeem Shahwani

Department of Biotechnology, BUITEMS, Quetta, Pakistan
Corresponding Author's E-mail : sadiahaleema377@gmail.com

ABSTRACT:

In Pakistan, the outbreak of Covid-19 was announced by the Ministry of Health on February 26, 2020 and a single covid-19 case in the city of Karachi was confirmed. The number of cases started to multiply rapidly in Pakistan, although a strict lockdown was observed from 15th March 2020 until May, 2020. The most prevalent number of cases were in Punjab, decreasing to Sindh, KPK, Balochistan, Gilgit Baltistan, Islamabad, Azad Jamu, and Kashmir in fewer numbers. Pakistan had more chances of threats as its border is near to China, but on the contrary to the statistics proposed by WHO, the number of cases started to reduce in Pakistan from August 2020. This may be due to the hot climatic conditions of the country. According to CDC and WHO, 16th August, 2020, in Pakistan, out of 288041 confirmed cases, 6162 deaths occurred and 265624 cases have been recovered, which is a positive indication towards the recovery of other patients too. WHO has praised the strategies adopted by Pakistan to cope with this novel disease. However, it is also necessary to keep on maintaining the SOPs suggested by WHO in advance if the infection reappears.

Keywords: *COVID-19, Ministry of health, Pakistan, WHO, SOPs*

INTRODUCTION

Emergence and Spread

The outbreak of covid-19 emerged from the city of Wuhan, China, in December 2019, and it was rapidly spread to the whole world (Zhu et al., 2019). It was treated as a case of pneumonia with uncertain etiology. At the initial stage, it was investigated in respiratory cases and diagnosed by PRC (People's Republic of China) Centre for Disease Control (CDC) as pneumonia, and named as Novel Coronavirus Pneumonia (NCP) (Ren et al., 2020). Coronavirus is an infectious disease, and its main target area is human respiratory system. Chinese scientists named this virus as 2019-nCoV. It was named as severe acute respiratory syndrome coronavirus-(SARS-Cov-2) by the International Committee on Taxonomy of Virus. Hence, it was named by the World Health Organization (WHO) as Pneumonia Coronavirus illness 19 (COVID-19) (WHO, 2020). This covid-19 was announced as a 6th strength of crisis services (SPHEC) on January 30, 2020 by World Health Organization (WHO). At initial, it was not the case of a severe outbreak as compared to past coronavirus outbreaks like Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Zhou et al., 2020). Covid-19 was the third coronavirus outbreak, which effected 209 nations around the globe. Americans (38%) were on top of the list in positive cases followed by South-East Asians (32%), Europe (21%), East Mediterranean (6%), Africa (1%), Western Pacific (1%), and others. (WHO, 2020).

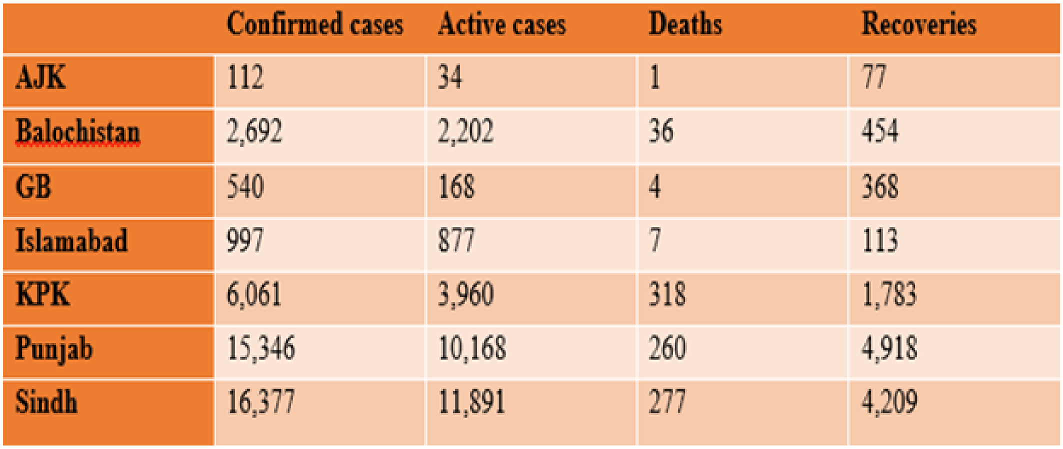
Covid-19 Spread in Pakistan

In Pakistan, the outbreak of Covid-19 was announced by the Ministry of Health on February 26, 2020. At that time, it was confirmed a single covid-19 case in the city of Karachi. After that, a series of cases had been confirmed in Karachi and Federal Islamabad (Ali, 2020). In less than 15 days, the count was more than twenty confirmed cases, and a considerable number belonged to Sindh province. It was announced that all the positive cases had resulted from the people those travelled from Iran, Saudi Arabia, and China to Pakistan. Pakistan understood the danger of pandemic alarmingly by neighborhood transmission on 13 March 2020, when the first nearby transmission case was accounted from Sindh, in a patient who was 52 years of age with no universal voyaging history (https://www.geo.tv/latest/274482-pakistan-confrms-frst).

Pakistan began taking exceptional choices from the announcing of clench hand nearby transmission instance of COVID-19. National Security Panel (NSC) chose social separating in the nation, and Instructive establishments were reported to be shut all through the nation just as fixing of fringes with Iran and Afghanistan on 13 Walk 2020. National Security submitted a formulated National Activity Plan for COVID-19 as a strategy record for guaranteeing that every core value for episode readiness, control, and relief was followed (https://www.nih.org.pk/novel-coranavirus-2019-ncov/). According to the headings of NSC, significant preventive measures were taken to guarantee insignificant national dismalness and mortality. Readiness was controlled by accessible and required wellbeing assets, observation systems, reaction potential and by scaling up of the calculated instrument. Isolate focuses were set up in Taftan at Pak-Iran fringe to distinguish and isolate the Pakistani nationals coming back from Iran, which has been demonstrating exponential development of the sickness (https://www.geo.tv/latest/273964 Pakistan-alert). Screening offices were built up at air terminals likewise alongside the instrument of following contacts of tainted cases. Up to 18 April,, 2020, there are 163 isolate focuses in Pakistan that have been built up with a bed limit of 23557 and 25 diagnostics communities. The testing limit was under 500/day during early Walk 2020, which has been elevated to 6500/day (Sultan and Khan, 2013).

Day by day growth

In Pakistan, the infection movement information is observed and distributed by the National Establishment of Wellbeing (NIH) through the live dashboard (http://covid.gov.pk/stats/pakistan). Just 21 cases were accounted for on 12th March 2020 when Pakistan announced the strategy of Lockdown to diminish the danger of disease spread. The coming days were very compromising as the patient number was continually expanding in all districts of Pakistan. On 20 March 2020, the quantity of positive cases has crossed the figure of 500, and within the next two days, the number of cases reached to 1000 on 22 March 2020. At this stage, the illness movement indicated a sharp ascent on a regular routine, and Pakistan Government demonstrated the danger of having around 50,000 patients till 25 April, 2020 according to a report submitted to the Incomparable Court of Pakistan on 5 April, 2020 (Hashim, 2020). On 11 April, 2020, the confirmed positive cases were accounted to be 5038. The most noteworthy day by day increment among the initial fifty days of infection movement was seen on 6 April, 15 April, 16 April and 17 April, 2020 with every day new cases 577, 543, 488, and 465 respectively. Forward-thinking, a sum of 40,151, affirmed instances of covid-19 had been accounted for in Pakistan by the world wellbeing association with 873 passing's (WHO, 2020). To date pandemic statistics of covid-19 of different provinces/territories is summarized in Table 1.



	Confirmed cases	Active cases	Deaths	Recoveries
AJK	112	34	1	77
Balochistan	2,692	2,202	36	454
GB	540	168	4	368
Islamabad	997	877	7	113
KPK	6,061	3,960	318	1,783
Punjab	15,346	10,168	260	4,918
Sindh	16,377	11,891	277	4,209

According to the above data from the National Institute of Health Pakistan, the Sindh province appears with the highest number of confirmed cases i.e. 16,377, whereas Azad Jamu Kashmir reports the lowest number i.e. 112 confirmed cases with only 1 death till date. The highest mortality rate is reported in the KPK region i.e. 318 cases. AJK, GB, and Islamabad seem to be combating well enough against mortality compared to the other provinces and display the highest recovery rates. The graph below shows the daily prevalence data of Covid-19 in Pakistan as of Apr 21, 2020 to May 17, 2020 (Fig. 1).

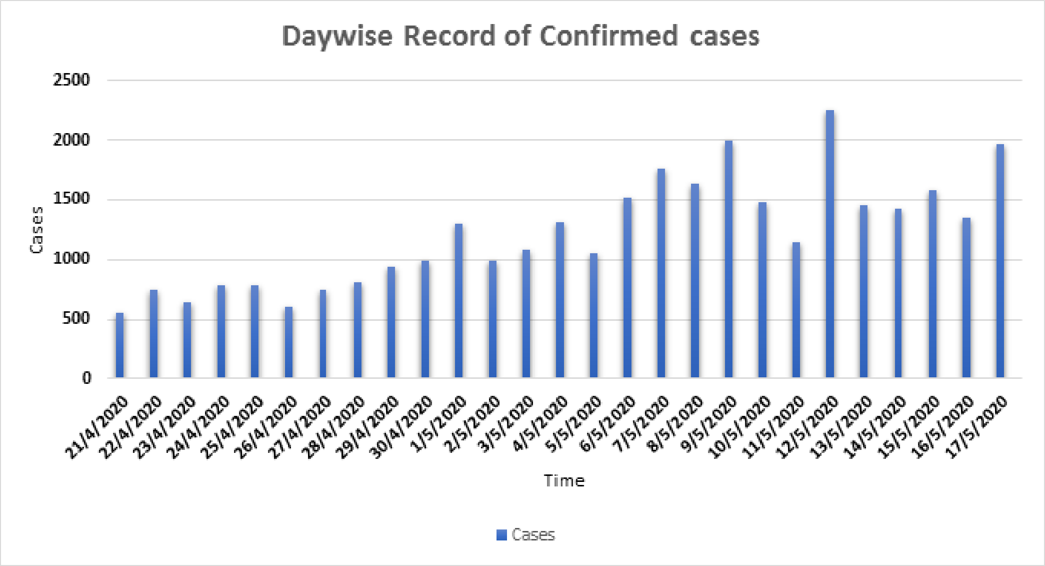


Fig. 1: Daywise data of confirmed Covid-19 cases in Pakistan

It can be seen that the new number of positive cases had increased on a daily basis, and it predicts an alarmingly dangerous situation in the future if precautionary measures are not kept as serious as it should be. The significant number of cases which has been increased since the outbreak, needs a well planned national level policy and action strategy to control this growth of newly affected cases.

Threats to Pakistan and Possible Reasons

The event of a gigantic episode is relied upon in Pakistan because of an absence of assets and deficient analytic and human services habitats, which will be cataclysmic for Pakistan likewise, Pakistani individuals appeared to be relatively at a higher hazard to the plague of COVID-19 than a large portion of the world, because of close land nearness and a mutual fringe of 272 miles with China and 596 miles with Iran (https://www.thenews.com.pk/tns/amp/606911-controlling-coronavirus). More cases are conceivable in the coming days for a few reasons; the social insurance framework is progressively delicate and has no agreeable assets to deal with the condition. The realities showed that the pace of transmission of COVID-19 in Pakistan gives off an impression of being little if we contrast with different nations. It has been proposed that the low pace of contamination might be credited to numerous reasons, for example, humid conditions, sweltering climate, tropical conditions, far-reaching BCG inoculations, and moderately government's prudent steps. At this stage, no examination has been found about coronavirus, and it has been found that there is a considerable connection between coronavirus and environmental conditions, and it can play a vital role in spreading coronavirus in the suspending particles of air. It has published that outright dampness unequivocally influences influenza transmission having drier situations being increasingly positive for the transmission of season's cold virus than out colder situations (Lowen and Steel, 2014). Pakistan has a blistering climate like Saudi Arabia, and due to that environmental changes, it may have possibly lessen spread of the virus in that way. There was a rapid increase in cases after Eid ul-Fitar, a religious festival in Pakistan dur to reduction in social distancing practices during the festival. The most prevalent cases were in Punjab, decreasing to Sindh, KPK, Balochistan, Gilgit Baltistan, Islamabad, Azad Jamu, and Kashmir in fewer numbers.

Under such a scenario when the number of cases was increasing rapidly, It was recommended the correct advances ought to be taken to control the circumstance for example, remaining at homes, lockdown, social distancing, utilizing sanitizers, and face mask when essential. If bend leveling can't be accomplished up to finish of May 2020, it was expected that the quantity of positive case would reach to a number that won't be sensible according to the present limit of National Health System of Pakistan along these lines, therefore a severe execution of preventive and screening must be made through the nation. Contrary to the statistics proposed by WHO, the number of cases started to lower in Pakistan from August, 2020.

Factors that caused the lowering of numbers might be the invulnerable levels as considering the climate of Pakistan, a healthy individual's resistant framework is efficiently more regrettable in winter than summer. One speculation has concentrated on melatonin, which has some safe impacts and is balanced by the photoperiod, which changes regularly. Another theory with more proof is that of nutrient D levels, which depend, to some extent, on bright light presentation. The best proof for the pertinence of this theory is that nutrient D supplementation diminishes the occurrence of intense respiratory disease, as indicated by one research (Coussens, 2017). Pakistani people are having a basic hygienic level and they have been prone to the viral diseases, so it might have caused a resistance against the more evolving viruses.

According to CDC and WHO, 16th August, 2020, in the Pakistan out of 288041 confirmed cases, 6162 deaths had occurred and 265624 cases have been recovered, which is a positive indication towards the recovery of other patients. The WHO has praised the strategies adopted by Pakistan to cope with this novel disease.

CONCLUSION

Covid-19 started from Wuhan, and it has been spread to the whole world, including Pakistan. Full lock down and strict SOPs are required to tackle with this pandemic in Pakistan. However, due to poor Pakistani economy, smart lock down will be applied in near future. The number of emergency clinics and isolate offices being are not satisfactory as required and predicted by WHO. Contrary to the statistics proposed by WHO, the number of cases started to lower in Pakistan from August, 2020. This may be due to the start of more hot days, or due to Pakistani people who are on basic hygienic levels and have been more prone to the viral diseases, resulting in resistance against the more evolving viruses. It is necessary to maintain the SOPs suggested by WHO, CDC USA, and NIH Pakistan.

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Antibiogram of Uropathogenic Bacterial Isolates from Urine Samples

Muhammad Ahsan Ali Rana*[1], Asmara Imtiaz[2], Kiran Ahmad[2], Iqra Shafiq[2]

[1]Department of Zoology, University of the Punjab, Lahore,
[2]Department of Biology, Lahore Garrison University, Lahore
***Corresponding Author's E-mail: Rana66pk@yahoo.com**

ABSTRACT:

Most of the bacteria of Enterobacteriaceae family are pathogenic and cause urinary tract infections, Pneumonia and diarrheal diseases. It was noticed that antimicrobials resistance of bacteria is increasing day by day that brought expanded healthcare costs due to morbidity and mortality from treatment failures. In the present study three of gram negative bacteria Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis of Enterobacteriaceae family were considered. Antibiotic susceptibility of these bacteria was checked against clinical samples by Kirby Bauer test commonly known as disc diffusion method. Eleven commercially available antibiotics i.e. Fosmomycin (FOS), Imipenem (IMP), Tazobactum (TZP), Meropenem (MEM), Amikacin (AK), Tobramycin (TOB), Nnitrofurantoin (F), Doxycycline (DO), Norfloxacin (NOR), Gentamycin (CN), and Nalidixic Acid (NA) were used to check efficacy against selected microbes. Comparison of results produced by these bacteria showed variability in resistance pattern as FOS>IPM>MEM>AK>F>F>TZP>CN>TOB>DO>NOR>NA. Fosmomycin, Imipenem, Amikacin and Meropenem were found as most effective one among all selected antibiotics.

Key words: *Antimicrobial resistance, Escherichia coli, Klebsiella pneumonia, gram-negative bacteria, Proteus mirabilis*

INTRODUCTION

Cows belong to the family bovidae (Dobson and Kamonptana, 1986) and crossbred cows are with pure bred parents of two different breeds, varieties or populations. Crosses occur within a single species (Gillah et al., 2013). Cows are important sources of food and income. Cross bred cows get mature at the age of 16 months and produce more milk while local cows

INTRODUCTION

Enterobacteriaceae is a family of gram negative bacteria and most of them prefer to live in the intestines of animals and humans. Many species of Enterobacteriaceae are pathogenic and cause urinary tract infections (UTI), pneumonia and diarrheal disease (Caroline, 2000; Edlin and Shapiro, 2013; Kara, 2014). The species are also common for wound infections and other nosocomial infections (Micheal, 2006; Anbazhagan, 2010). Bacteria possess an enzyme known as beta –lactamases that used to reduce efficacy of antibiotics (Martinez et al., 2000). Moreover, resistance towards antimicrobial agents has spreaded health care costs because morbidity and mortality from treatment failures. More and irregular use of antibiotic is the major reason for the process of developing antibiotic resistance (Davies and Davies, 2020). According to Laura (2009), and Lopez-Jacome et al. (2019) utilization of improper drugs, consumption of excessive antibiotic, less hygienic conditions in health care centers and exposure to the resistant gene carrying microbes are few of the components to bring about antimicrobial resistance. Dider (2004) has reported transformation, conjugation and mutations can also result in antibiotic resistance. Many microbes have characteristic resistance towards many antibiotics and carry the resistance gene and transfer to offspring (Jessica, 2014). Moisture, pH and CLSI (Clinical and Laboratory standard institute) standards are factors affecting the antibiotic susceptibility testing.

Antimicrobial susceptibility testing is the most established and most uniform duty performed by the clinical microbiology laboratory. The history and development of antibiotic susceptibility testing (AST) goes back in the late nineteenth century and with the passage of time development occurred by discovering new methods to get the interpretations of AST in short span of time (Poupard, 1994; Sykes, 2001; John, 2007). Ditch plate method introduced by Fleming to test antimicrobial activity (Jagdishchandra et al., 2019; Kuhekar et al., 2019). Later Fleming also developed the broth dilution method and used pH indicator instead of turbidity (Poupard, 1994). Later Vincent used filter paper discs incorporated by Penicillin (Vincet, 1994). Frank used agar dilution method separating microbes into two categories susceptible and resistant (Steers, 1959; Tolhurst, 1963). In Kirby Bauer Method antibiotic susceptibility testing is carried out in clinical laboratories to check the susceptibility of microorganisms isolated from clinical samples. The microorganisms are getting resistant to different antibiotics day by day because of modifications in their structure. Kirby Bauer method is used because it is an effective and simple method (Bbauer, 1966; Lawrence, 1972; Turnidge 2007). The antibiotics target the bacterial cell. Antibiotic mainly inhibit the synthesis of DNA, cell wall, RNA and proteins. In 1962 Nalidixic acid was discovered and was introduced for clinical use in 1967. It was effective and induce cell death to inhibit the growth of bacteria (Bradford, 2001; Peglers, 2007). The objective of the present study was to focus urine samples especially from Enterobacteriaceae family and to determine their susceptibility against various antibiotics.

MATERIALS AND METHODS

For the isolation of pathogenic bacteria urine sample of 20 patients were collected from the CMH and Chugtai Hospital. Pathogens were isolated by using standard spread and streak plate methods on cystine–lactose–electrolyte-deficient agar (CLED) (Fallon et al., 2002). Inoculated plates were incubated at 37 °C for 18-24 hours. After incubation, the plates were observed for the presence of Klebsiella pneumonia, Escherichia coli and Proteus mirabilis. Their presence was further confirmed by gram staining. Kirby-Bauer test, commonly known as the disk-diffusion method was followed to assess antibiotic susceptibility (Ouno et al., 2013). Antibiotic discs was placed on the surface of inoculated agar plates. Mueller Hinton agar plates were used for these isolated bacteria to test the antibiotic susceptibility. Each disc pressed down to ensure complete contact with the agar surface and antibiotic discs on the agar surface was equally distributed. Total seven discs were placed on the 90 mm agar plates because some drugs diffuses without any delay. After that the plates were inverted and placed in an incubator set to 37 °C for 18-24 hours. After incubation each plate were examined. The diameters of zones of complete inhibition including the diameter of the disc were measured using ruler. Faint growth of tiny colonies was observed with the help of magnifying glass.

RESULTS

From all collected samples ten bacteria were isolated on CLED agar and three of them were identified as Klebsiella pneumonia, Escherichia coli and Proteus mirabilis. All three types' bacteria were found as gram negative rods. However, Klebsiella pneumoniae appeared as whitish-yellow and very mucoidy colonies on CLED agar. While, Escherichia coli and Proteus mirabilis showed deeper colored center yellow colonies and translucent blue colonies on the same agar. Susceptibility test was carried out by dilution method. The minimum concentration of antibiotics was used to kill the microorganisms by diluting it in the broth that have been tested. In Kirby Bauer method, sterilization of glassware was done and plates were inoculated with Mueller Hinton agar and wrapped to avoid any contamination. In the set of experiments observed for antibiotic susceptibility, the zones of inhibition from all the respective bacteria were observed and recorded in Table 1.

The average effectiveness of each antibiotic was calculated by the following formula:

Effectiveness of each antibiotic = Total number of sensitive zones obtained × 100

Total no of sets taken

The efficacy of each antibiotic was recorded in the following manner i.e.

FOS> IPM> MEM> AK> FOS>F> TZP> CN> TOB> DO> NOR>NA.

Average behavior of different antibiotics against various bacterial isolates was shown in (Table 2).

Table 1: Resistant and general trend of susceptible behavior of bacterial isolates against different antibiotics

Sr.no	**Name Of Antibiotic**	**Abbreviation of Antibiotic**	**Class of Antibiotic**	**General trend of Susceptible zone**	**Amount of Antibiotic in disc**
1	Tazobactum	TZP	Miscellaneous	=21mm	10/100µg
2	Doxycycline	DO	Tetracycline	=14mm	30 µg
3	Amikacin	AK	Aminoglycosides	=17mm	30 µg
4	Gentamycin	CN	Aminoglycosides	=15mm	10 µg
5	Tobramycin	TOB	Aminoglycosides	=15mm	10 µg
6	Imipenem	IPM	Carbapenem	=23mm	10 µg
7	Meropenem	MEM	Carbapenem	=23mm	10 µg
8	Nitrofurantion	F	Miscellaneous	=17mm	300 µg
9	fosmomycin	FOS	Miscellaneous	=16mm	200 µg
10	Nalidixic acid	NA	Quinoline	=19mm	30 µg
11	Norfloxacin	NOR	Quinoline	=17mm	10 µg

Table 2: Average percentage effectiveness of each antibiotic towards Escherichia coli, Klebsiella pneumonia, and Proteus mirabilis

Antibiotics	**Average % effectiveness of antibiotic in *E.coli***	**Average % effectiveness of antibiotic in *K. pneumonia***	**Average % effectiveness of antibiotic in *P. mirabilis***
TZP	34/44 (100)=77%	12/18(100)=67%	6/9(100)=67%
DO	12/44(100)=27%	6/18(100)=33%	3/9(100)=33%
TOB	16/44%(100)=36%	9/18(100)=50%	0/9(100)=0%
CN	19/44%(100)=43%	13%18(100)=72%	5/9(100)=45%
AK	40/44(100)=91%	15/18(100)=83%	7/9(100)=78%
MEM	42/44(100)=95%	42/44(100)=95%	8/9(100)=89%
IPM	42/44(100)=95%	17/18(100)=94%	8/9(100)=89%
NOR	9/44(100)=20%	5/18(100)=28%	----
NA	5/44(100)=11%	3/18(100)=17%	----
FOS	43/44(100)=98%	16/18(100)=89%	----
F	35/44(100)=80%	12/18(100)=67%	----

DISCUSSION

Antibiotics are becoming less effective against bacteria with the passage of time due to development of different resistance mechanisms that bacteria followed to inhibit the effect of antibiotics. The present study was performed to find the changing behavior of the urine isolated pathogenic bacteria towards the antibiotics and to check the resistance and susceptibility of bacteria (Aslam et al., 2018). The sets of experiments were performed by using eleven commercially available antibiotics and were used against three types of bacteria i.e. Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis. Mueller Hinton agar was used in this experiment because it supports the growth of all types of bacteria. The average values of these antibiotics were taken in the percentage where Imipenem (IMP), Meropenem (MEM), Fosmomycin (FOS) showed highest activity against the microbes. Norfloxacin, Nalidixic acid and Doxycycline showed the least activity against the microbes. Gentamycin, Amikacin and Tobramycin belongs to the class of aminoglycosides, are highly reactive against many bacteria (Krause et al., 2016). Antimicrobial resistance in the E. coli has increased worldwide with the passage of time. The isolates of E.coli obtained from urine sample showed high resistance rate to erythromycin, teteracycline and amoxicillin as described in previous studies conducted in Northeast Ethiopia and in other parts of the world (Kibret and Abera, 2011). E. coli isolated from urine sample of female patients show significance variations in resistance rates, for ampicillin 29.4 to 43.5 percent. Klebsiella species are resistant to penicillin and have the ability to acquire resistance against third generation of cephalosporins. In late onset Klebsiella pneumoniae were found to be susceptible to Fluoroquinoloness and Carbapenems however only half of the Klebsiella pneumoniae were susceptible to Amikacin.

Quinolone resistance in Enterobacteriaceae is usually the result of chromosomal mutations (Hooper and Jacoby, 2015). Quinolone resistance has been reported in Klebsiella pneumoniae and E. coli. P. mirabilis has been found resistant to Nitrofurantoin and tetracycline.

CONCLUSION

Antibiotic susceptibility of 11 of the commercially available antibiotics were assessed against Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis isolated from clinical urine samples. Fosmomycin, Imipenem, Meropenem and Amikacin were found as the most effective antibiotics against urine isolated bacteria. However, there is a need for in depth study in this field to discover further effective remedies for overall improvement of the health of general public.

CONFLICT OF INTEREST

Author's declare there is no conflict of interest.

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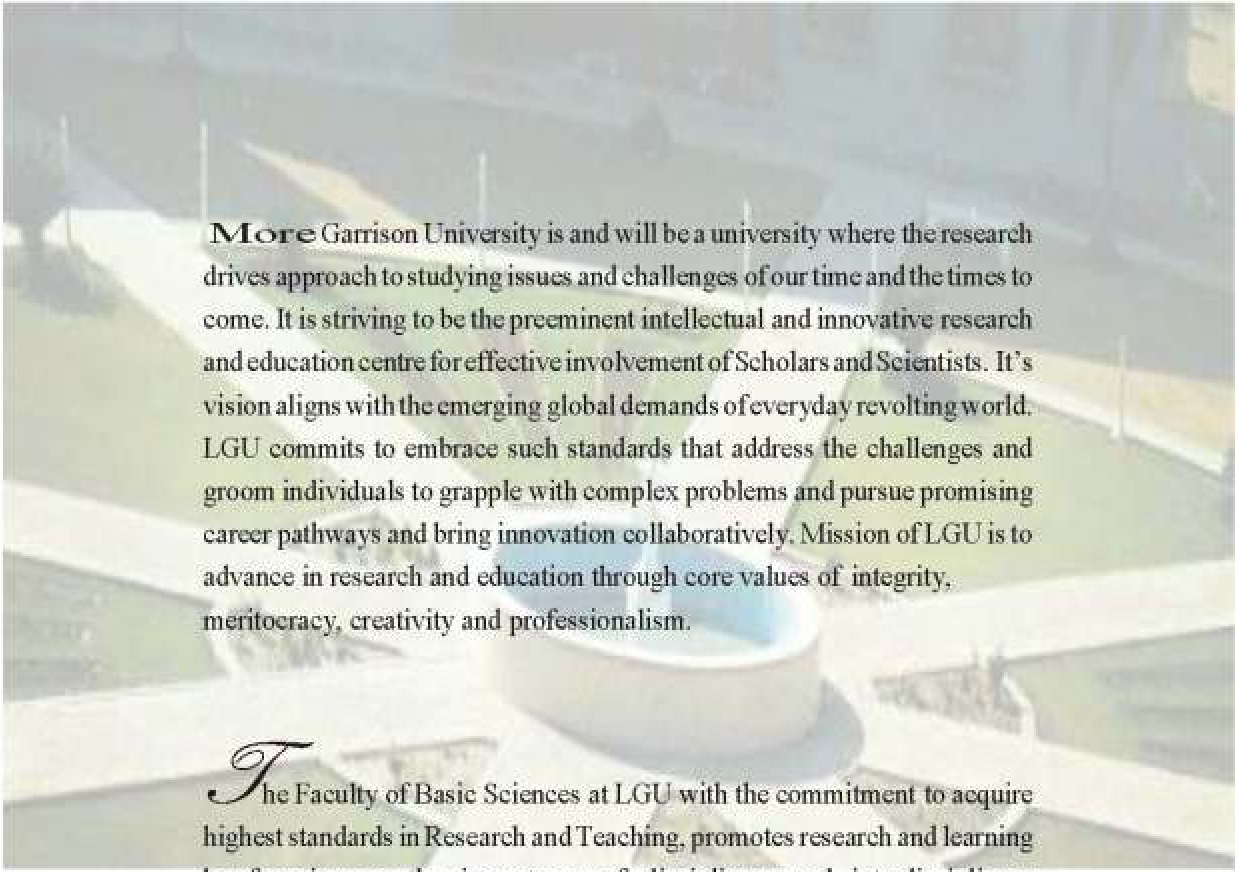

More Garrison University is and will be a university where the research drives approach to studying issues and challenges of our time and the times to come. It is striving to be the preeminent intellectual and innovative research and education centre for effective involvement of Scholars and Scientists. It's vision aligns with the emerging global demands of everyday revolting world. LGU commits to embrace such standards that address the challenges and groom individuals to grapple with complex problems and pursue promising career pathways and bring innovation collaboratively. Mission of LGU is to advance in research and education through core values of integrity, meritocracy, creativity and professionalism.

The Faculty of Basic Sciences at LGU with the commitment to acquire highest standards in Research and Teaching, promotes research and learning by focusing on the importance of disciplinary and interdisciplinary approaches. LGU Journal of Life Sciences aims to disseminate research through publications. It fosters integration through encouraging a collaborative approach in the diverse fields of Life Sciences.

LAHORE GARRISON UNIVERSITY, LAHORE