DRUG DISCOVERY

Isolation, cloning and transformation of glucan binding protein (GBP) gene

Ravikumar Patil HS[1✉], Vasantha Naik T[2], Vijay Avin BR[3], Sayeswara HA[4]

[1]Department of Biotechnology, G.M. Institute of Technology, Davangere-577066, Karnataka, India
[2]Department of Botany, D.R.M. Science College, Davangere University, Davangere-577066, Karnataka, India
[3]Department of P.G. Studies and Research in Biotechnology, Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga-577203, Karnataka, India
[4]Department of Zoology, Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga-577203, Karnataka, India

✉**Corresponding author**:
Department of Biotechnology, G.M. Institute of Technology, Davangere-577066, Karnataka, India, E-mail: patil_varuni@reddifmail.com

Citation
Ravikumar Patil HS, Vasantha Naik T, Vijay Avin BR, Sayeswara HA. Isolation, cloning and transformation of glucan binding protein (GBP) gene. *Drug Discovery*, 2021, 15(35), 16-22

ABSTRACT
This work was to isolate the glucan binding protein (gbp) gene from *Streptococcus mutans*, it possesses at least four glucan binding proteins GbpA, GbpB, GbpC, and GbpD, each of which appears to be immunologically and biochemically distinct using various processes. Firstly, the organism was cultured using the brain heart infusion broth media. Later the isolation of the gene was carried out using several processes such as the ones beginning from electrophoresis, isolation of the total protein and genomic DNA etc. Then, cloning was carried out using *Escherichia coli* as the host and the competent cells were prepared. In the second part, the competent cells were cultured in a media containing IPTG, X-gal, ampicillin and the transformed cells were differentiated from the recombinant cells by the color developed by the colonies. In later stages, the gbp gene that was isolated was sequenced and the query sequence that was obtained was analyzed using bioinformatics tools like BLAST and the similarity search was carried out in order to find and derive its various applications.

Key words: *Streptococcus mutans*, glucan binding protein (gbp), PCR, competent cells.

Abbreviations: CHAP - cysteine, histidine-dependent amidohydrolase/peptidase.

1. INTRODUCTION
Streptococcus mutans (S.mutans), strain mttc 890, is a Gram-positive, facultative anaerobic bacterium commonly found in the human oral cavity and is a significant contributor to tooth decay. *S.mutans* is a bacterium that inhabits the human oral cavity. It produces

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

plaque and acids that break down tooth enamel and cause dental caries (Loesche, 1996). *S.mutans* is that a great deal of interest was generated when researchers began studying dental caries in detail (Clark, 1924; Benjavongkulcharist et al., 2007).

The cariogenic potential of *S. mutans* is manifested by the organism's ability to ferment various carbohydrates, producing large amounts of acid, and by its ability to participate in the formation of dental plaques (Loesch, 1996; Jeevarathan et al., 2007). Two groups of bacteria are responsible for initiating caries: *S.mutans* and *Lactobacillus*. Initially, it may appear as a small chalky area that may eventually develop into a large cavitation. Secreted products of *S.mutans* that play a role in bacterial accumulation include glucosyltransferases, their glucan products, and glucan binding proteins. It possesses at least four glucan binding proteins GbpA, GbpB, GbpC, and GbpD, each of which appears to be immunologically and biochemically distinct (Banas, et al., 1990; Sato et al., 1997; Shah and Russel, 2004). Clinical studies showed that GbpB was the antigen most commonly recognized by antibodies in saliva of young children, and the natural immunoglobulin. A response to GbpB after initial exposure to *S. mutans* may modulate infection. Systemic or mucosal immunization of rats with GbpB or GbpB-derived peptides induced protective immunity to dental caries, indicating that GbpB may be involved in virulence of *S. mutans*. It shares extensive amino acid homology with putative peptidoglycan hydrolase from *S. agalactiae* and *S. pneumonia* (Smith et al., 1994; Smith and Taubman, 1995). In the *S. mutans* genome, *gbpB* is flanked by genes encoding proteins involved in cell shape determination, and this gene order is conserved in other gram-positive bacteria. Further sequence analyses showed that GbpB contained homology to the cysteine, histidine-dependent amidohydrolase/peptidase (CHAP) superfamily, whose members hydrolyze gamma-glutamyl-containing substrates (Rigden et al., 2003). The main objectives of this research are Isolation of glucan binding protein (gbp) gene from *Streptococcus mutans* the major causative agent of dental caries by total protein isolation method and its confirmation by SDS-PAGE and the isolation of genomic DNA. Elution of the DNA from the gel and its amplification using PCR and cloning of the gene using *Escherichia coli* as the host and pUC18 as the vector. Gene transformation using Calcium chloride mediated gene transfer method and the uptake of DNA by competent cells and plasmid expression. Gene sequencing and its analysis using BLAST and its homology modeling.

2. MATERIALS AND METHODS

Centrifuge (Remi pvt lmt, India), Horizontal Electrophoresis Chamber (Geni, Bangalore) Vertical Electrophoresis Chamber (Geni, Bangalore), UV Transilluminator (Chromous biotech, Bangalore), PCR Machine/ Thermal cycler (Long-gene made). All the chemicals used were analytical grade procured from SD-fine chemicals, Bangalore, India.

2.1. Culturing of *Streptococcus mutans*

Pure culture of *S. mutans* was procured from NCCI, Pune, India. And subcultures are maintained for further process according to the standard protocol (Fig.1).

Figure 1
Streaked culture of *S.mutans*

2.2. Isolation of total protein

1.5ml of culture was taken in two vials and centrifuged at 10,000 rpm for 3mins. After centrifugation, the supernatant (cell free extract) was discarded and the pellet was taken. To the pellet, 50µl each of lysis buffer was added and was kept for incubation in hot water bath at 45°C for 20mins. Re-centrifugation was carried out at 10,000 rpm for 10mins. The supernatant (cell lysate) was taken

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

and the pellet was discarded. 100μl of supernatant was taken and 30μl of sample buffer was added and kept in boiling water bath for 2-10mins. This sample was used further in SDS-PAGE.

2.3. Isolation of genomic DNA by phenol chloroform method

1.5ml of culture was taken in two vials and centrifuged at 10,000 rpm for 3mins. Supernatant was discarded and to the pellet 0.3ml of lysis buffer was added and incubated for 10mins at room temperature. Then, 0.3ml of saturated phenol and 0.3ml of choloroform were added and incubated at 55°C for 10mins and centrifuged at 10,000 rpm for 10mins. Carefully the upper layer was separated into a new tube and equal volume of chloroform isoamyl alcohol mixture was added. Then 1/20th volume of sodium acetate was added and vortexed 2-3 times and centrifuged at 10,000 rpm for 10mins. Carefully the upper layer was separated into a new tube and chilled absolute alcohol was added to observe the fibrous DNA and then centrifuged at 10,000 rpm for 10mins. The dry DNA pellet was dissolved in TE Buffer.

2.4. PCR polymerase chain reaction

PCR mix was prepared for amplification by adding all the contents in a separate vial. All these were added in a tube called thin walled PCR tube. Once the PCR mix was added in the tube, it was kept in the thermal cycler and the PCR program was set up according the conditions mentioned above. After the PCR was completed, the DNA was run in low melting point agarose gel along with the lambda DNA EcoR1/ Digest marker and visualized using the UV Transilluminator. If a single dark, orange colored band was seen, then the DNA was found to be amplified.

2.5. Cloning

From the media that was inoculated and kept on the shaker, nearly 1ml of culture was taken and inoculated into the fresh media that was removed from the refrigerator and kept at room temperature. This was kept on the shaker and left for 3-5 hrs and simultaneously the things mentioned above that were to be sterilized were kept for autoclaving. The contents required preparing the restriction digestion mixture and the vector mixture were taken in two separate thin walled tubes and kept for incubation for 1 hr at room temperature. After sterilization, 500μl of ampicillin was added to the media and from this 25ml was taken and poured into one petri plate. To the remaining 25ml, 25μl of IPTG and 50μl of X-gal were added and poured into another petri plate and both these plates were left to solidify. The sub-cultured culture which was kept on the shaker for 3-5 hrs was taken and its OD was measured at 660nm and the 10ml broth which was taken in test tube and sterilized was taken as the reference and the OD was set to 0(zero). The ligation mixture was prepared by adding the contents mentioned above and kept for overnight incubation at room temperature.

2.6. Preparation of competent cells

2ml of culture was taken in two separate tubes. These tubes were centrifuged at 6,000 rpm for 10mins. The supernatant was discarded and the pellet was re-suspended in 1ml of sterile cold Calcium chloride. This was left in cold condition for 15mins with occasional shaking. After 15mins, it was again centrifuged at 6,000 rpm for 10mins. Supernatant was discarded and the pellet was dissolved in 0.25ml of Calcium chloride. And was stored at a temperature of -20°C and the competent cells were used whenever necessary.

2.7. Calcium chloride mediated gene transfer method

Fresh culture of host was taken and subcultured. The subcultured culture was grown for 3-5 hrs till an optical density of 0.3- 0.5 was obtained and then centrifuged. Pellet+ calcium chloride mixture was left in cold condition for 15-30mins and was centrifuged again to obtain the competent cells. The competent cells and rDNA were taken in a tube and were exposed to a temperature of 42°C for 2mins and this was called as heat shock. After 1min, it was immediately transferred to 4°C and fresh media was added and incubated at 37°C for 1 hr. After incubation, the cells obtained were called transformed cells. These were inoculated on one plate containing ampicillin (transformed cells and ampicillin) and the competent cells were inoculated on another plate containing ampicillin (competent cells and ampicillin) and were incubated at 37°C for 24 hrs. Then the plates were observed for the development of colonies.

2.8. DNA uptake by competent cells and plasmid expression

Out of the 25μl of recombinant DNA, 15μl was taken and the remaining 10μl was kept aside in another tube. Out of two tubes containing 250μl each of competent cells, only one tube was used and the other was kept aside which was later used for inoculation.

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

The media and the plates were removed from the refrigerator and kept at room temperature. 15µl of rDNA and 250µl of competent cells were taken in a single tube and kept in ice for 30mins and then heat shock was given by keeping the tube in hot water bath maintained at 42°C for 2mins and immediately transferred back to ice and kept for 10-15mins. After that it was taken to the laminar air flow unit and 0.5ml of the pre-sterilized media kept in the test tube was added and incubated by keeping in water bath maintained at 37°C for 1 hr and vortexed after every 10-15mins. Then the contents of this tube were added to the plate containing media, ampicillin, X-gal and IPTG. The 250µl of competent cells which was kept aside was added to the plate containing media and ampicillin only and this plate was not shaken too much. Both the plates were kept in the incubator and incubated at 37°C for 24 hrs and were observed for the development of colonies. 0.5ml of media was added to each tube of cells which were pre-warmed immediately after heat shock was given by keeping in a hot water bath maintained at 42°C for 2mins. The tubes were then incubated in a hot water bath at 37°C for 1 hr. After incubation, the tubes were returned to ice.

2.9. Sequence analysis and homology modeling

The homepage of BLAST was opened and the BLAST option was selected. Out of the five types displayed such as the BLAST-P, BLAST-N etc., the BLAST-N option was selected. The query sequence was pasted in the space provided in the FASTA format and the option against which the query had to be compared was selected such as the human, mouse, non- redundant etc., At the bottom of the page, the option: highly similar sequences was selected and finally the BLAST option was clicked upon. The sequences obtained from BLAST including the query sequence were downloaded and the sequences were arranged in FASTA format in the note pad. This file was uploaded and the multiple sequence alignment was performed using the CLUSTAL software.

3. RESULTS

3.1. Protein profiling

Sodium Dodecyl Sulphate- Poly Acrylamide Gel Electrophoresis (SDS-PAGE) was conducted and the gel was observed for the formation of bands (Fig. 2). The result was that, dark blue colored bands were formed against a clear background. This confirmed the presence of glucan binding protein in the sample that was used and this result stands to be the major one for the proceeding of the project with the other methods mentioned.

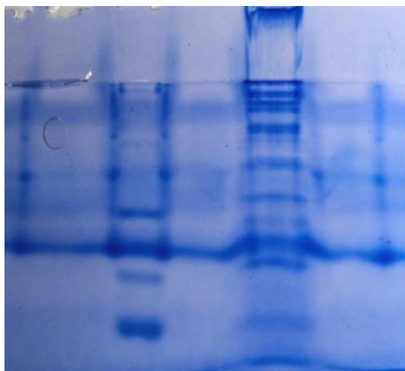

Figure 2

Band formation in gel; after SDS-PAGE was conducted

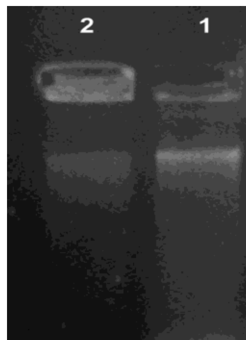

Figure 3

Band formation in low melting agarose after AGE was conducted

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

3.2. DNA isolation

After the confirmation of the glucan binding protein, the DNA was isolated and was amplified using Polymerase Chain Reaction and the amplified product was run in low melting point agarose and observed using UV Transilluminator (Fig.3). The result was that, DNA appeared as an orange colored band and on comparing with the marker- lambda DNA EcoR1/Digest and its molecular weight was found to be approximately 21,226 bp. This DNA was then eluted out of the gel and cloned using *E.coli* as the host. Competent cells were prepared and Calcium chloride mediated gene transfer was carried out and observed for the development of colonies. The competent cells and rDNA were incubated and taken on the media containing ampicillin, IPTG, X-gal and observed for the development of colonies. The result was that, the transformed cells developed blue colored colonies as they contained only the vector and not the rDNA where as the recombinant cells looked colorless or white in colour as they contained rDNA (Fig.4). After the gbp gene was sequenced, the query sequence was obtained and its length was found to be 2kb. The query sequence was analyzed using BLAST and the graphical summary was obtained as follows and the query sequence was found to have 100% homology with *Streptococcus mutans* strain SJ32 glucan binding protein B gene and 99% homology with the *Streptococcus mutans* NN2025 DNA complete genome. The query sequence obtained was shown in Table 1. The homology results of the query sequence are shown in Table 2.

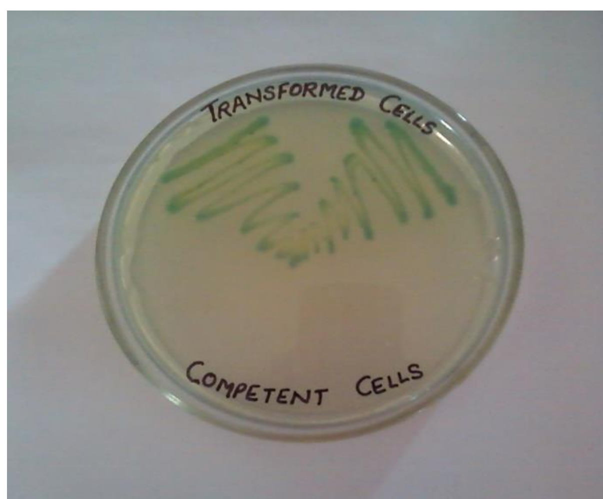

Figure 4
Recombinant Screening

Table 1 Sequencing of gbp gene (Source: Bioserve Biotechnologies (India) Pvt. Ltd. Length: 1225 b)

gcgcagattgcgtcacaagattctaaaatcaacaacttgaccgcacaacagcaagcagcacaagcacaagttaat
acgattcaaggacaagtaagtgctttacagacacaacaagctgaattacaagctgaaaatcaaagacttgaagctc
agtctgctactttgggtcaacaaattcaaacactttcaagcaaaattgttgcacgtaatgaatctttgaagcaacaagct
cgtagtgctcaaaaaagtaacgcagctaccagctatattaatgctatcattaattcaaaatcagtttctgatgctattaat
cgtgtttcggctattcgtgaagttgtatctgctaatgaaaaaatgcttcaacaacaagagcaagataaagcagctgttg
agcaaaagcaacaagaaaatcaagcagcaattaatactgttgcagctaatcaggagacaattgctcaaaataca
aatgctttaaatacacagcaagctcaattagaagcagcacaactaaacttgcaagctgaattgactactgcacaag
atcaaaaagctactttagttgctcaaaaagcggcagcagaggaagctgcacgccaagcagcagcggcacaagc
ggcagcagaagctaaggccgcagcagaagcaaaagctttacaagaacaagcagcgcaagcacaggtagcag
caaataataatactcaagctacagatgcttctgaccaacaagcagcggcagctgataacactcaagcagcacaaa
caggtgattcaactgagcagtcagcagcgcaagcagtaaataattctgatcaagaaagtactacagcaacagaag
cacaaccatcagcttctagtgcttcgacagcggctgtagcagctaatacttcttctgctaatacatatccagcagggca
atgtacttggggtgttaaatcattagctccttgggtaggaaactactggggtaatggtggacaatgggcagcaagtgc
agcagcggcaggatatagagttggttctacaccttcagctggagctgtagctgtatggaatgatggcggttatggaca
cgttgcttatgttacaggtgttcaaggtggccaaattcaagttcaagaagctaactatgcaggtaaccaatctattggta
actaccgtggttggtttaatccaggtagtgtaagctatatctatccaaactaatttaatattataaaaaggtaag

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS

Table 2 Homology results of the query sequence

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AY046410.1	Streptococcus mutans strain SJ32 glucan-binding protein B gene, complete cds	2263	2263	100%	0.0	100%
AP010655.1	Streptococcus mutans NN2025 DNA, complete genome	2246	2246	100%	0.0	99%

3.3. nBLAST analysis at NCBI

Sequence matches with AY046410.1 *Streptococcus mutans* strain SJ32 glucan-binding protein B gene, complete cds Score = 2263 bits (1225), Expect = 0.0 Identities = 1225/1225 (100%), Gaps = 0/1225 (0%) Strand=Plus/Plus. The present study designed for the isolation of glucan binding protein (gbp) gene from *Streptococcus mutans* which is the major causative agent of dental caries worldwide and also included its cloning and transformation.

4. DISCUSSION

Hence the work was designed where in, it consists of two parts. Firstly, it involves culturing of *Streptococcus mutans* using the brain heart infusion broth, isolation of total protein, SDS-PAGE, isolation of genomic DNA, PCR and AGE, gel elution, cloning using *E.coli* as the host and pUC18 as the vector, preparation of competent cells, calcium chloride mediated gene transfer, DNA uptake by competent cells and finally the plasmid expression where in the competent cells and the rDNA were taken on the media containing IPTG, X-gal and ampicillin. Further, the incubation resulted in the development of blue colored colonies by the transformed cells and colorless colonies by the recombinant cells. Therefore it can be said that the cells that contained the rDNA appeared colorless or white in color and the cells that contained only the vector appeared blue.

It has been proposed by several authors that secreted products of *S.mutans* that play a role in bacterial accumulation include their glucan products, and glucan binding proteins. It possesses at least four glucan binding proteins GbpA, GbpB, GbpC, and GbpD, each of which appears to be immunologically and biochemically distinct. Secondly, it involves the sequencing of the gbp gene and the analysis of the query sequence obtained by aligning it with the target sequences using bioinformatics tools like BLAST and its homology modeling in order to find similar sequences as that of the query sequence. The gene was cloned using *E.coli* as the host and pUC18 as the vector in order to obtain rDNA and then the competent cells were prepared and were plated along with the rDNA on the media containing IPTG, X-gal and ampicillin. Further, the incubation resulted in the development of blue colored colonies by the transformed cells and colorless colonies by the recombinant cells. Hence this shows that the cells which did not contain rDNA appeared blue in colour whereas the recombinant cells appeared colorless or white in colour as they contained the rDNA. Taking into consideration the result of the sequence analysis of the query sequence, it can be concluded that the query sequence has 100% homology with *Streptococcus mutans* strain SJ32 glucan binding protein B gene and 99% homology with the *Streptococcus mutans* NN2025 DNA complete genome and finds application in production of vaccines and in the field of diagnosis.

SUMMARY OF RESEARCH

1. Isolation of glucan binding protein (gbp) gene from *Streptococcus mutans* the major causative agent of dental caries was performed by total protein isolation method and its confirmation by SDS-PAGE and the isolation of genomic DNA was done.
2. Elution of the DNA from the gel and its amplification using PCR and cloning of the gene using *Escherichia coli* as the host and pUC18 as the vector was performed.
3. Gene transformation using Calcium chloride mediated gene transfer method and the uptake of DNA by competent cells and plasmid expression.
4. Gene sequencing and its analysis using BLAST and its homology modeling was studied.

FUTURE ISSUES

In future, this work can be expanded by varying particular media components which are responsible for the optimal growth of *Streptococcus mutans* and results in the isolation of large amounts of gbp gene. Also the analyzed sequence can be submitted to the

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

NCBI- National Centre for Biological Information, which is stored in its database and can be retrieved for other beneficial aspects. And one of the major futures prospective stands to be the modification of the genetic make-up of *Streptococcus mutans* using Recombinant methods and making it resistant to the acidic conditions such that it does not become pathogenic under prolonged acidic conditions.

Acknowledgment
We would like to acknowledge the Principal, GMIT College, Davangere, Karnataka for providing facility to carry out the present research work.

Funding:
This study has not received any external funding.

Conflict of Interest:
The authors declare that there are no conflicts of interests.

Peer-review:
External peer-review was done through double-blind method.

Data and materials availability:
All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Banas JA, Russell RR, Ferretti JJ. Sequence analysis of the gene for the glucan-binding protein of *Streptococcus mutans*, *Infect immune,* 58(3), 1990, 667–673
2. Benjavongkulchai E, Luengpailin S, Ajdicacute D, Banas JA. Manganese Affects Streptococcus mutans Virulence Gene Expression, *Caries Res*. 2007, 41(6), 503-11
3. Clark WB, Gibbonser J. Influence of salivary components and extracellular polysaccharide synthesis from sucrose on the attachment of *Streptococcus mutuns* 671 *5* to hydroxyapatite surfaces, 1997, 514-523
4. Jeevarathan J, Deepti A, Muthu M, Rathna Prabhu V, Chamundeeswari G. Effect of fluoride varnish on Streptococcus mutans counts in plaque of caries-free children using dentocult SM strip mutans test: A randomized controlled triple blind study. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, 2007, 25, 157-163
5. Loesche WJ. Microbiology of Dental Decay and Periodontal Disease. In: *Baron's Medical Microbiology*, 1996, 4th edition
6. Rigden DJ, Jedrzejas MJ, Galperin, MY. Amidase domains from bacterial and phage autolysins define a family of gamma-D, L-glutamatespecific amidohydrolases, *Trends Biochem Sci*. 2003, 28(5), 230-4
7. Sato Y, Yamamoto Y, Kizaki H. Cloning and sequence analysis of the *gbpC* gene encoding a novel glucan-binding protein of *Streptococcus mutans*, *Infect immune*, 1997, 65(2), 668–675
8. Shah DS, Russell RR. A novel glucan-binding protein with lipase activity from the oral pathogen *Streptococcus mutans*, Microbiology, 2004, 150(6), 1947–1956
9. Smith DJ, Taubman MA. Development of salivary IgA antibody to oral streptococcal antigens associated with virulence, *Adv Exp Med Biol*. 1995, 371B, 1141–1143
10. Smith DJ, Akita H, King WF, Taubman MA. Purification and antigenicity of a novel glucan-binding protein of *Streptococcus mutans*, Infect immune, 1994, 62(6), 2545–2552

Peer-review
External peer-review was done through double-blind method.

Article History
Received: 07 November 2020
Reviewed & Revised: 08/November/2020 *to* 10/December/2020
Accepted: 11 December 2020
Prepared: 14 December 2020
Published: January 2021

Publication License

This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note

We recommended authors to print article as color digital version in recycled paper. Discovery Scientific Society will not provide any prints for subscription.

© 2021 Discovery Scientific Society. All Rights Reserved. ISSN 2278–540X EISSN 2278–5396 | OPEN ACCESS