A NOBLE AND INNOVATIVE SOLUTION TO BLOOD SHORTAGE

Yaswobanta Kar [1], Saswati Das [2], Preetha Bhadra *, Bishwambhar Mishra*
Yaswobanta Kar: 3rd year Biotechnology, Centurion University of Technology, Bhubaneswar
Saswati Das: 3rd year Biotechnology, Centurion University of Technology, Bhubaneswar
Preetha Bhadra: Assistant Professor, Department of Biotechnology, Centurion University of Technology, Bhubaneswar.
Bishwambhar Mishra: Assistant Professor, Department of Biotechnology, Centurion University of Technology, Bhubaneswar.

Abstract-Review of literature has showed that there is a high demand for type "O" blood. Type-o positive is the most transfused blood type as it can be given to any patients with RH positive blood type due to absence of antigens and presence of both antibodies. Meanwhile type o-negative is a universal donor and can be given to patient of any blood type due to the absence of any kind of antigens. The eight different blood types that can be categorized into four groups based on their antigens: A, B, AB, and O with the help of presence on antigens and antibodies which are responsible for the detection of the foreign objects and deal with it. Our aim was to convert the AB blood group to O blood group. Thus it can be safely transfused to anyone without any risk. We have extracted the total DNA from human gut using metagenomics and expressed in *E. coli*. It eventually led to focus on the mucosal lining of the human gut, which contains sugars that similar in structure to blood antigens. They were then able to "use *E. coli* to select for DNA containing genes that code for enzymes that can cleave sugar residues." then multiple the enzymes and found that it was capable of removes the Gal or Gal-NAc residues that determine the antigens of type-A and type-B blood, which essentially makes the blood type-O.2) select specific glycoside hydrolase families of interest and create a phylogenetically distinct sub-families of their genes. From each sub-family select a representative gene and expressed it in *E.coli.*

Key words: **Blood Group, E.coli, Human Gut, Metagenomics, Phylogenetics**

1. Introduction:

The discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of the ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now study one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations.

The immune system forms antibodies against whichever ABO blood group antigens are *not* found on the individual's RBCs. Thus, a group A individual will have anti-B antibodies and a group B individual will have anti-A antibodies. Blood group O is common, and individuals with this blood type will have both anti-A and anti-B in their serum. Blood group AB is the least common, and these individuals will have neither anti-A nor anti-B in their serum [1]. The ABO locus has three main alleleic forms: A, B, and O. The A allele encodes a glycosyltransferase that produces the A antigen (N-acetylgalactosamine is its immunodominant sugar), and the B allele encodes a glycosyltransferase that creates the B antigen (D-galactose is its immunodominant sugar).

Whereas type A or B blood can only receive transfusions from (or make donations to) others with the same blood type, medical professionals can use type O blood with any of the three other major blood types: A, B, or O. Type AB, the other of the four major blood types, works in reverse; it can receive blood from any other type but only donate to others who are type AB [2]

The O allele encodes an enzyme with no function, and therefore neither A or B antigen is produced, leaving the underlying precursor (the H antigen) unchanged. These antigens are incorporated into one of four types of oligosaccharide chain, type 2 being the most common in the antigen-carrying molecules in RBC membranes. Some of the other enzymes involved in the earlier stages of ABO antigen synthesis are also involved in producing antigens of the Hh blood group and the Lewis blood group [3,4]. In 1982, a group of researchers found a promising lead using an enzyme isolated from certain types of unroasted coffee beans. Scientists collected the beans, trimmed the B antigens off red blood cells, and effectively created type O blood. Unfortunately, it only worked within the parameters of a very controlled set of conditions, and was largely inefficient [5-7].

Withers' research centers on an as yet unexplored protagonist: gut bacteria. By extracting sugars from proteins on the gut wall, called mucins, the team learned it could add the enzymes to type A negative blood, thereby turning it into type O negative — the universal donor. "This technique could broaden the utility of the current blood supply because O type blood can be donated to anybody [8-12]. The routine practice of blood typing and cross matching blood products should prevent adverse transfusion reactions caused by ABO antibodies. However, clerical error can result in "the wrong blood" being transfused into a patient, an error which can result in the death of the patient [7,8].

If a recipient who has blood group O is transfused with non-group O RBCs, the naturally occurring anti-A and anti-B in the recipient's serum binds to their corresponding antigens on the transfused RBCs. These antibodies fix complement and cause rapid intravascular hemolysis, triggering an acute hemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute renal failure, and death.

Anti-A1 is a less significant cause of transfusion reactions and does not appear to fix complement.

Our research has based on the isolation of gut bacteria and with help of this change the different type of blood group to O negative blood group.

2. Material and Methods:

The chemicals PBS buffer, NaCl, 1% Methanol as a solvent, AflB1, and AflG1 from Sigma –Aldrich. Distilled water and isolated the RBC with the standard Protocol [Hanson, 2008].

2.1. RBC isolation:

The 5 ml peripheral venous blood sampled on Na_2EDTA as anticoagulant and centrifuge (Eppendorf) at 500xg for 10 min at 4 degrees. Aspirate supernatant (plasma) and add PBS to erythrocyte pellet cells from the top layer containing WBCs were removed. Centrifuge erythrocytes at 500xg for 10 min at 4 degrees C. this has repeated for two more times for a total of 3 washes of the blood and collect the RBC pellet. In all of the experiments, we have used RBC preparations with less than 1% other blood cells.

2.2. Microbial Culture:

Liquid agar media (LB)(Sigma Aldric) was prepared and the autoclaved and cool to room temperature and transfer approximately 1 mL of overnight *Ideonella sakaiensis* culture to the flask and incubated overnight in a shaker (REMI). Sterile plates are being made with LB media and transfer the bacteria and incubated for overnight to get maximum number of bacteria.

2.3. ROS analysis:

Membrane fluidity of blood cells was shown to have a decisive role in the direct cell to cell contact and the modulation of the activity of membrane enzymes and to be affected by the increased release of ROS [14].

For the measurement of the changes in the reactive oxygen of cells, DCF-DA was added to a 2 ml of RBC suspensions. The cell suspension with DCF-DA was incubated for 30 minutes at 37^0 C. The measurement was done at 540 nm on an average of three.

2.4. Fluorescence Anisotropy:

The fluorescence anisotropy of PBMC was assessed by the determination of TMA-DPH steady-state fluorescence polarization after the cell membrane exterior phospholipid layer permeation of the probe [15-17].

For the measurement of the changes in the TMA-DPH fluorescent properties following the membrane permeation, we added 2.5µMTMA-DPHto a 2 ml of RBC suspension an aliquot of TMA-DPH stock solution in DMSO to get 2.5 µM TMA-DPH in the measuring cuvette.The cell suspension with the fluorescent probe was incubated for 30 minutes at 37°CThe measurement has been done between excitation and emission state, 360 nm and 430 nm respectively (on an average of three).

3. Result and Discussion:

3.1. ROS analysis:

The ROS analysis is the cell viability test based on the presence of mitochondria. The ROS binds to the mitochondria of a viable cell and changes the density accordingly. The triplicate analysis report of ROS in Fig:2 has clearly shown that the changes of number of RBCs are due to the changes of the structural deformation of Hb with the interaction of the Gut Virus and the enzymes and it has gradually increased by the increment of the time of interaction and also with the increment of the concentration of the Gut Virus and the enzymes. It has also be found that there is a similarity with the results after 6 hrs of incubation with the mycotoxins which indicates the structural changes of Gut Virus and the enzymes.

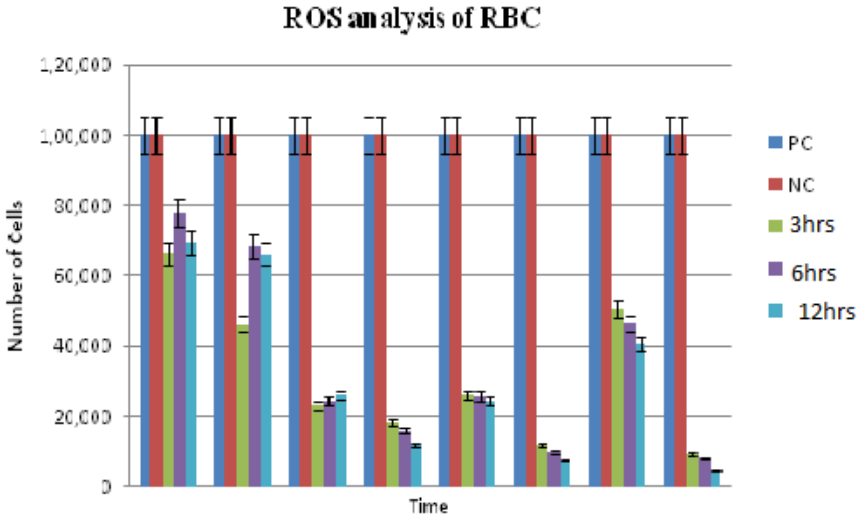

Fig 1: ROS analysis of RBC with the treatment with AflG1 and AflB1

3.2. Fluorescence anisotropy:

The fluorescence spectra have been studied for all the samples (triplicate) and shown in the Fig: 4 The decreasing value of the fluorescence anisotropy leads to the conclusion about the interaction between the Hb and the gut bacteria got minimal due to the lower concentration and not any significant changes are noticed with the increment of the exposure time of this mycotoxin. But on the other hand, the changes in structure of Hb due to the addition of gut bacteria is similar as in the samples treated with other enzymes in a particular time frame which implies that the conformational change of gut bacteria to enzymes occurs and revives.

This is also a confirmatory test that the gut bacteria to enzymes have shown significant changes in the structure of Hb and as the values are decreased, the changes in the structural configuration of gut bacteria and enzymes changes to the minimal energy state.

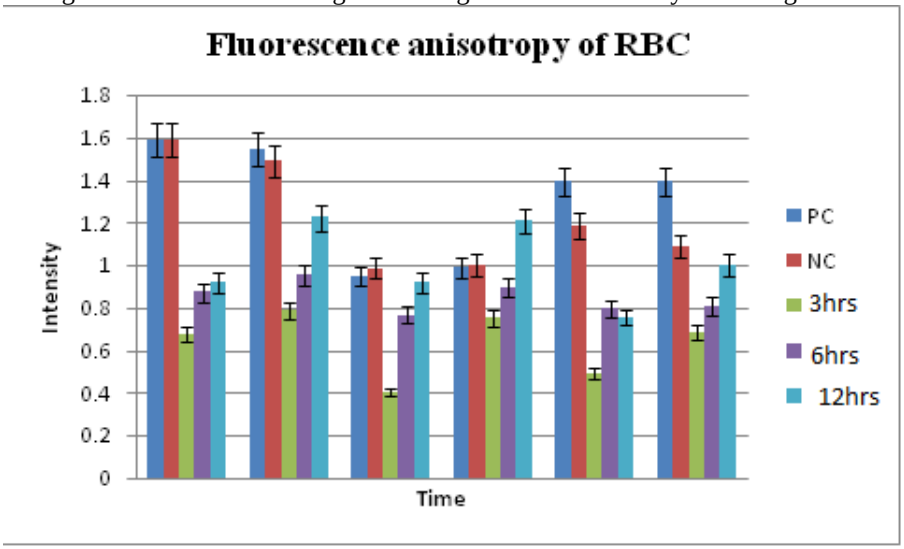

Fig: 2: Fluorescence anisotropy of RBC with the treatment of gut bacteria.

4. Conclusion:

Antibiotics should be administered before and during surgery, the antibiotics of choice currently being under process. If the wound is closed primarily, the antibiotics are stopped on the third postoperative day. If the wound is closed secondarily, the antibiotics are continued for another three days after this procedure.

By these experiments we have got O group by removing all other antigens from RBCs. It has an effect on recent days activities and the research program this may be a helpful part of the struggling the shortage of blood .

5. Acknowledgement:

We like to thank Paramedical department of Centurion University of Technology, Bhubaneswar for supporting us with all the equipments and helping us collection of blood and sample preparation. We also want to thankful to our university for every kind of financial support.

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