ISSN 0000-0000
9 770000 000003

Quest ©

ज्ञानेन शीलम्

Vol. 4 No. 5

ARIBAS

September- 2016

Quest

Editorial Office
Quest, ARIBAS,
New Vallabh Vidyanagar,
Vitthal Udyognagar - 388121,
Dist- Anand, Gujarat, India.
Phone: +91-2692-229189, 231894
Fax: +91-2692-229189
Email: editor@aribas.edu.in
Website: www.aribas.edu.in

Published By
Director ARIBAS,
New Vallabh Vidyanagar,
Vitthal Udyognagar - 388121,
Dist- Anand, Gujarat, India.

Editorial

The news describes the current situation well, but it may be overly optimistic. Since Jurassic Park came out, paleontologists have joked about finding dinosaurs in amber, since it would contain so much extra information. The finding does reveal a feathered dinosaur tail in 3D for the first time, and offers a unique glimpse into the early evolution of feathers. Older bodies need longer to mend. This reality of aging has been documented since World War I, with the observation that wounds heal slower in older soldiers. Wound healing is known to require specialized immune cells that reside in the skin. The researchers' new experiments showed that following an injury, the keratinocytes at the wound edge talk to these immune cells by producing proteins known as Skints that appear to tell the immune cells to stay around and assist in filling the gap. In a study, describes a new method to non-invasively image the human retina, a layer of cells at the back of the eye that are essential for vision. The research group, was able to distinguish individual retinal ganglion cells (RGCs), which bear most of the responsibility of relaying visual information to the brain.

In the research paper authors work on the burning issue of water pollution by industry. Different methods are their to remove the water pollution. Authors used the Duckweeds as phytoremediation of Pond water, Agro-chemical, Dye and Dairy effluents. Plants act as a nutrient sink, absorbing nutrients from the wastewater. Such systems differ from conventional facilities in that they can achieve a significantly higher level of nutrient removal from the wastewater and remove oxygen consuming substances and pathogenic organisms to an extent comparable to other systems. Malaria is life threatening disease and every year millions of people die due to it. Various new targets have been identified to fight against malaria. Among them *P. falciparum* reticulocyte binding protein shown susceptible advantage to inhibit plasmodium invasion on reticulocyte and in turn inhibit its spreading all over human body. Researchers have proved that antibody against *P. falciparum reticulocyte* binding homologue protein 5 (PfRh5) inhibits *P. falciparum* invasion on reticulocyte.

Index

NEWS AND VIEWS:-

Dinosaur tail trapped in amber sheds light on evolution of feathers 5

Why wounds heal more slowly with age 5

A closer look at the eye: New retinal imaging technique 6

REVIEW ARTICLE:-

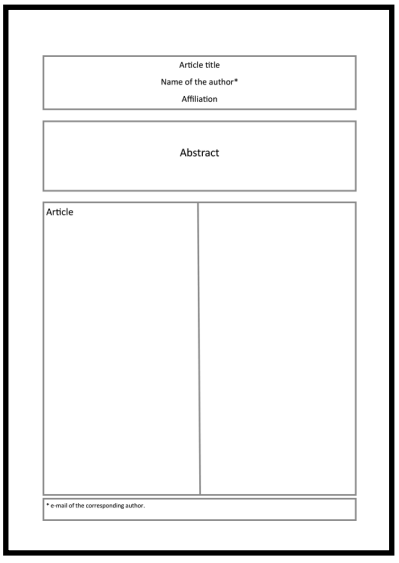
Phytoremediation of Pond water, Agro-chemical, Dye and Dairy effluents using Duckweed 7

PfRh5 Inhibitors: a new strategy to combat malaria 15

Notice to Authors

Manuscripts submitted to Quest should adhere to below mentioned criteria.
Research News: About 400 words (1 page)
Research Article: About 2000 words (4 pages)

Common for all: -
Font: Calibri
Font Size: 14
Columns: 2
Line Spacing: 1
Margin: Narrow
References: 1) In text citing, S No, Superscript.
2) Author's name (s), *Journal name*, **Volume No**, Page No**,** (year).
3) Maximum number of references should not exceed than 25.



Dinosaur tail trapped in amber sheds light on evolution of feathers.

Amber is often prized not just for its golden beauty, but also for the tiny creatures it contains, many of them millions of years old. “Since Jurassic Park came out, paleontologists have joked about finding dinosaurs in amber, since it would contain so much extra information. And now we have a piece of one,” says Thomas Holtz, a vertebrate paleontologist at the University of Maryland in College Park who was not involved in the study.

The finding does reveal a feathered dinosaur tail in 3D for the first time, and offers a unique glimpse into the early evolution of feathers. Amber is a uniquely useful fossilizer, notes Michael Engel, a paleontologist and entomologist at the University of Kansas in Lawrence who was also not involved in the study.

The amber deposits of northern Myanmar harbor one of the most diverse arrays of animals from the Cretaceous period. Paleontologist Lida Xing of China University of Geosciences in Beijing was hunting through an amber market in Myanmar for lizard and insect specimens when a particular chunk caught his eye: Along with the usual scattering of insects, it contained a 3.6-centimeter-long section of a flexible, finely feathered tail. Right away, he knew he had something special.

Xing contacted paleontologist Ryan McKellar of the Royal Saskatchewan Museum in Regina, Canada, and the team used photographs taken through microscopes and computerized tomography scanning (computer-processed combinations of images taken by x-rays at different angles to reveal interior details of the fossil) to study the eight preserved vertebrae and their feathers.

Unlike Archaeopteryx (a 150-million-year-old creature thought by many researchers to be among the very earliest birds) or modern birds, the vertebrae were not fused into a solid rod at the tip of the tail. Instead, the tail in amber is whip like and flexible, bending in several places at once. That, the researchers report online today in Current Biology, suggests that its owner was not a bird but in fact a dinosaur, and likely a member of a group of small two-legged dinosaurs called coelurosaurs. (Jurassic Park fans, take note: Compsognathus—nicknamed “compys” in the movies—are a member of this group.)

Plumage pigments preserved in the amber suggest the theropod was colored chestnut-brown along its dorsal side (the top of the tail), and lighter on its underside. The amber also allowed the researchers to study the structure of the animal’s plumage in 3D.

The feather of the bird you see out your window today has a central shaft, or “rachis,” that branches out into a series of barbs that branch again into fine barbules. In the new specimen, the rachis is relatively thin and flexible compared with the thick, rigid central rachis of modern birds; however, the structure of barbules is complex, with fine tiers of branching as in modern feathers, distributed evenly across the length of the feathers. In all, the structure of the feathers suggests that the animal wasn’t capable of flight, although “it may have been a glider,” McKellar says.

Contributed by Parth Patel
IGBT VI.

Why wounds heal more slowly with age

Older bodies need longer to mend. This reality of aging has been documented since World War I, with the observation that wounds heal slower in older soldiers. Yet until now, researchers have not been able to tease out what age-related changes hinder the body's ability to repair itself.

Recent experiments at The Rockefeller University explored this physiological puzzle by examining molecular changes in aging mouse skin. The results, described November 17 in *Cell*, delineate a new aspect of how the body heals wounds. "Within days of an injury, skin cells migrate in and close the wound, a

process that requires coordination with nearby immune cells. Our experiments have shown that, with aging, disruptions to communication between skin cells and their immune cells slow down this step," says Elaine Fuchs, the Rebecca C. Lancefield Professor and head of the Robin Chemers Neustein Laboratory of Mammalian Cell Biology.

"This discovery suggests new approaches to developing treatments that could speed healing among older people," adds Fuchs, who is also a Howard Hughes Medical Institute investigator. Whenever a wound occurs, the body needs to repair it quickly to restore its protective skin barrier. "Wound healing is one of the most complex processes to occur in the human body," says Brice Keyes, a former postdoc in Fuch's lab and currently a researcher at Calico Life Sciences. "Numerous types of cells, molecular pathways, and signaling systems go to work over timescales that vary from seconds to months. Changes related to aging have been observed in every step of this process." Keyes and Siqi Liu, an immunology specialist and a current Jane Coffin Childs postdoctoral fellow in in the lab, are co-first authors of the *Cell* article.

Both skin cells and immune cells contribute to this elaborate process, which begins with the formation of a scab. New skin cells known as keratinocytes later travel in as a sheet to fill in the wound under the scab. The team focused on this latter step in healing in two-month-old versus 24-month-old mice -- roughly equivalent to 20- and 70-year-old humans. They found that among the older mice, keratinocytes were much slower to migrate into the skin gap under the scab, and, as a result, wounds often took days longer to close.

Wound healing is known to require specialized immune cells that reside in the skin. The researchers' new experiments showed that following an injury, the keratinocytes at the wound edge talk to these immune cells by producing proteins known as Skints that appear to tell the immune cells to stay around and assist in filling the gap. In older mice, the keratinocytes failed to produce these immune signals.

To see if they could enhance Skint signaling in older skin, the researchers turned to a protein that resident immune cells normally release after injury. When they applied this protein to young and old mouse skin tissue in a petri dish, they saw an increase in keratinocyte migration, which was most pronounced in the older skin. In effect, the old keratinocytes behaved more youthfully. The scientists hope the same principle could be applied to developing treatments for age-related delays in healing.

- *Contributed by* **Shivam patel**
IGBT VI

A closer look at the eye: New retinal imaging technique

In a study, describes a new method to non-invasively image the human retina, a layer of cells at the back of the eye that are essential for vision. The research group, was able to distinguish individual retinal ganglion cells (RGCs), which bear most of the responsibility of relaying visual information to the brain. Instead of imaging RGCs directly, glaucoma is currently diagnosed by assessing the thickness of the nerve fibers projecting from the RGCs to the brain. However, by the time retinal nerve fiber thickness has changed detectably, a patient may have lost 100,000 RGCs or more.

Rossi and his colleagues were able to see RGCs by modifying an existing technology -- confocal adaptive optics scanning light ophthalmoscopy (AOSLO). They collected multiple images, varying the size and location of the detector they used to gather light scattered out of the retina for each image, and then combined those images. Not only did this technique allow the group to visualize individual RGCs, but structures within the cells, like nuclei, could also be distinguished in animals. If Rossi can achieve that level of resolution in humans, he hopes to be able to assess glaucoma before the retinal nerve fiber thins -- and even before any RGCs die -- by detecting size and structure changes in RGC cell bodies. This technique offers the opportunity to evaluate many cell classes that have previously remained inaccessible to imaging in the living eye.

Contributed by **Dr. Dipika Patel**

"Phytoremediation of Pond water, Agro-chemical, Dye and Dairy effluents using Duckweed"

K.Saroja , Swati narolkar, Mehul Giri*

Ashok & Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Science (ARIBAS), New Vallabh Vidhyanagar,Anand-388001

Abstract

Industrial wastewaters entering a water body represent a heavy source of environmental pollution. It affects both the water quality as well as the microbial and aquatic flora. With competing demands on limited water resources, awareness of the issues involved in water pollution, has led to considerable public debate about the environmental effects of industrial effluents discharged into aquatic environments. Industrial effluents are characterized by their abnormal turbidity, conductivity, high concentrations of NPA (Nitrate, Phosphate, and Ammonia), chemical oxygen demand (COD), dissolved oxygen (DO), total suspended solids (TSS) and total hardness. Waste effluents rich in decomposable organic matter, is the primary cause of organic pollution. Waste waters from dyes, food and beverages, dirty pond water having household wastes and agrochemical industries, the cases chosen are believed to give a broad outline of industrial wastes as well as disposal problems. It is therefore, vital to reclaim and manage these water bodies to its optimum productivity status through some appropriate rural friendly scientific technologies. Effective waste water treatments through 'conventional methods', which rely on heavy aeration, are expensive to install and operate. Hence, there is need to explore some 'non-conventional' methods which are not only economically viable and easy to operate but eco-friendly as well. For this purpose, plant based bio-remediation (phyto-remediation) technology is the most promising option. Any aquatic plant, especially Duckweed (*Lemna minor*) is capable of recovering or extracting nutrients or pollutants and has a fast growth rate coupled with high nutritive value is an excellent candidate for bio-remediation of waste waters. Such plants grow very fast utilizing waste water nutrients and also yield cost effective protein rich plant biomass as a by-product.

INTRODUCTION

One of the most critical problems of developing countries is improper management of vast amount of wastes generated by various anthropogenic activities. More challenging is the unsafe disposal of these wastes into the ambient environment. Water bodies especially freshwater reservoirs are the most affected. This has often rendered these natural resources unsuitable for both primary and/or secondary usage.

Industrial effluent contamination of natural water bodies has emerged as a major challenge in developing and densely populated countries like Nigeria. Estuaries and inland water bodies, which are the major sources of drinking water, are often contaminated by the activities of the adjoining populations and industrial establishments[1].

River systems are primary means for disposal of waste, especially the effluents, from industries that are near them. These effluent from industries have a great deal of influence on the pollution of the water body, these effluent can alter the physical, chemical and biological nature of the receiving water body[2]. Increased industrial activities have led to pollution stress on surface waters both from industrial, agricultural and domestic source[3].Wastes entering these water bodies are both in solid and liquid forms. These are mostly derived from Industrial, agricultural and domestic activities. As a result, water bodies which are major receptacles of treated and untreated or partially treated industrial wastes have become highly polluted. The resultant effects of this on public health and the environment are usually great in magnitude[4].

Phytoremediation is one of the serious efforts to-

* *Corresponding Author: ksaroja@aribas.edu.in*

wards sustainability and has a major role in tackling the problem. The macrophyte based water treatment systems have several potential advantages compared with conventional treatment systems[5].

Treatment systems using aquatic plants consist of shallow reservoirs containing floating or submerged aquatic plants. The best studied wastewater systems are those utilizing duckweed (*Lemna minor*). Generally, treatment systems break into two types based on the dominant plant types. The first type uses floating plants which are distinguished by their ability to meet their need for carbon dioxide and oxygen directly from the atmosphere. Such plants derive their mineral needs from the water.

Phytoremediation is a novel, efficient, environmentally friendly, low-cost technology, which uses plants and trees to clean up soil and water contaminated with heavy metals and/or organic contaminants such as solvents, crude oil, polyaromatic hydrocarbons and other toxic compounds from contaminated environments. This technology is useful for soil and water remediation. Phytoremediation may be conducted superficially in near surface soils, in situ in the deep aquifer, or ex-situ for contaminated liquids treatment (extracted groundwater or surface water). Plants have been used for wastewater treatment applications over the past 300 years, and began to be used for treatment of slurries and metal contamination in the mid 1970s. However, there are still few contaminated sites where phytoremediation has been used for full-scale clean-up.

Throughout the world, and particularly in Asia, farmers have harvested naturally produced aquatic plants for a number of purposes including animal feed, green manure and for their family feed resources. The best known of these include the free floating plants like water lettuce (*Pistia*), water hyacinth (*Eichhcornia*), duckweed (Lemna) and *Azolla* and some deep water plants.

In recent years a commonly occurring aquatic plant, "duckweed", has become prominent, because of its ability to concentrate minerals on heavily polluted water such as that arising from sewage treatment facilities. However, it has also attracted the attention of scientists because of its apparent high potential as a feed resource for livestock (Skillicorn, Paul, 1993). Duckweed grows on water with relatively high levels of N, P and K and concentrates the minerals and synthesises protein. These are the nutrients which are often critically deficient in traditional fodders and feeds given to ruminants and to pigs and poultry particularly where the former depend on agro-industrial by products and crop residues.

Duckweed is the common name given to the simplest and smallest flowering plant that grows ubiquitously on fresh or polluted water throughout the world (Fig.2). They have been botanical curiosities with an inordinate amount of research aimed largely at understanding the plant or biochemical mechanisms. Duckweeds have great application in genetic or biochemical research. This has been more or less in the same way that *Drosophila* (fruit flies) and bread moulds have been used as inexpensive medium for genetic, morphological, and physiological and biochemical researches.

Duckweeds belong to four genera; *Lemna, Spirodela, Wolfia* and *Wolffiella*. About 40 species are known worldwide and all these species have flattened minute, leaf like oval to round "fronds" from about 1mm to less than 1cm across (Fig. 2). Some species develop root-like structures in open water which either stabilise the plant or assist it to obtain nutrients where these are in dilute concentrations. When conditions are ideal in terms of water temperature, pH, incident light and nutrient concentrations they compete in terms of biomass production with the most vigorous photosynthetic terrestrial plants doubling their biomass in between 16 hours and 2 days depending on the conditions.

Duckweed was successfully used for improving pond water quality by many workers[6]. The experimental studies conducted led to understand that Duckweed plant efficiently removes 75% phosphate from pond water.

MATERIALS AND METHODS

Area under investigation

In the present study, a comparison has been made

Table-1: The various processes through which plants can incorporate pollutants

TYPE	PROCESS INVOLVED	POLLUTANTS TREATED
Phyto-extraction	The plants are used to concentrate metals in parts harvestable (leaves and roots).	Cadmium, cobalt, chromium, nickel, mercury, lead, selenium, zinc
Rhizo-filtration	Plant roots are used to absorb, precipitate and concentrate heavy metals from contaminated liquid effluents and to degrade organic compounds.	Cadmium, cobalt, chromium, nickel, mercury, lead, selenium, zinc radioactive isotopes, phenolic compounds
Phyto-stabilization	Metal-tolerant plants are used to reduce the mobility of metals and prevent their passage to groundwater or the air.	Lagoons rid of mineral deposits. Proposed for phenolic and chlorinated compounds.
Phyto-stimulation	Root exudates promote the development of microorganisms (bacteria and fungi) capable of biodegrading compounds.	Petroleum hydrocarbons and polyaromatic, benzene, toluene, atrazine, etc.
Phytovolatilization	Plants take up heavy metals and organic compounds, bind or modify them and release the by-products into the atmosphere via transpiration.	Mercury, Selenium and chlorinated solvents (tetraclorometano and trichloromethane)
Phyto-decomposition	Both aquatic and terrestrial plants capture organic compounds and store them or decompose them to less toxic or non-toxic byproducts.	Munitions products (TNT, DNT, RDX, nitrobenzene, nitrotoluene), atrazine, chlorinated solvents, DDT, phosphate pesticides, phenols and nitriles, etc.

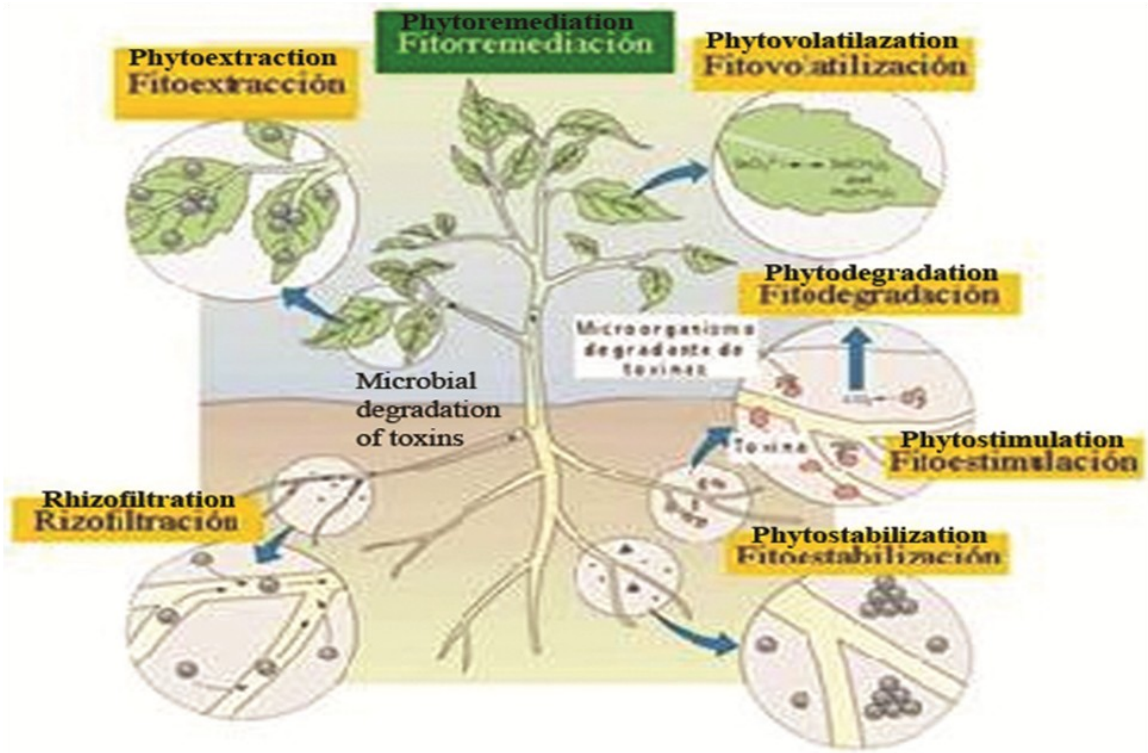


Fig 1: Types of phytoremediation process through which plants can incorporate pollutants

Fig 2: Duckweed in polluted water

Plant used in the present study

Lemna minor (Duckweed)

METHODS

Estimation of Dissolved Oxygen by Winkler's method
Estimation of Nitrate using Brucine sulphate method
Estimation of Phosphates using Stannous chloride method
Estimation of Ammonia using Sodium nitroprusside method
Estimation of Chemical Oxygen Demand (COD)
Measurement of surface temperature and pH
Batch process for reduction of dye effluent concentration using glass column

RESULTS AND DISCUSSION

The experimental setup was designed for the estimation of various parameters for five weeks at an interval of one week each. The physiochemical results observed are as follows:

Dissolved Oxygen (DO)

The initial DO content of the Pond water sample was found to be 5.23 mg/l, upon duckweed's application and after two weeks the level of DO reduced to 1.57 mg/l and from third week onwards up to fifth week the DO level steadily increased to 3.04 mg/l. Similarly, the initial DO content of agrochemical effluent was 4.531 mg/l, which after two weeks reduced to 2.12 mg/l, and finally the DO level was increased to 1.86 mg/l. Dairy and Dye effluent's DO was found nil in the initial stage, which might be due to the uptake of DO by microbes present in the sample.

Chemical Oxygen Demand(COD)

The reading of Blank's titrant value came as 2.5 ml, therefore, the results of COD among the different samples is shown in Table-2

From the above observation (Table:2), Duckweed is found to be efficient in reducing the COD of Dairy effluent with 83.33%compared to that of Pond and Agrochemical samples with 40% and 21.4% respectively (Table:2)

Estimation of Nitrate (NO_3^-) , Phosphate (PO_4^-) and Ammonia (NH_4^+)

The readings (O.D) of all the samples, Pond, Dairy and Agrochemical from 0^{th} week (initial) to the 5^{th} week (final) upon Duckweed application is provided in (Table-3) and their concentrations is given in (Table- 4)

According to the observation Duckweed is capable of reducing the major environmental polluting parameters like Nitrate, Phosphate and Ammonia from the Pond water sample, with nitrate's conc. reducing from 0.091 µg/ml to 0.048 µg/ml, Phosphate's conc. from 0.057µg/ml to 0.002µg/ml, and Ammonia conc. from 0.403µg/ml to 0.012µg/ml which is maximum upon Duckweed's application within 6 weeks (Fig.3).

Table 2: Percentage reduction in COD of various samples.

Samples	Unit	Initial Concen-tration	Final Concen-tration	Percentage Reduction
Pond	mg/l	2000	1200	40
Dairy	mg/l	4800	800	83.33
Agrochemical	mg/l	2800	2200	21.4

Table -3:O.D from initial to final week of various samples.

Time / Week	Pond			Dairy			Agrochemical		
	Nitrate	Phosph-ate	Ammonia	Nitrate	Phosphate	Ammonia	Nitrate	Phosphate	Am-monia
0	0.061	0.144	0.104	0.17	0.129	0.046	0.373	0.085	0.049
1	0.045	0.112	0.093	0.151	0.119	0.023	0.232	0.054	0.036
2	0.04	0.093	0.086	0.126	0.105	0.019	0.187	0.048	0.031
3	0.032	0.079	0.071	0.095	0.088	0.015	0.138	0.04	0.024
4	0.079	0.026	0.018	0.047	0.063	0.009	0.109	0.029	0.015
5	0.071	0.005	0.003	0.034	0.031	0.001	0.069	0.024	0.009

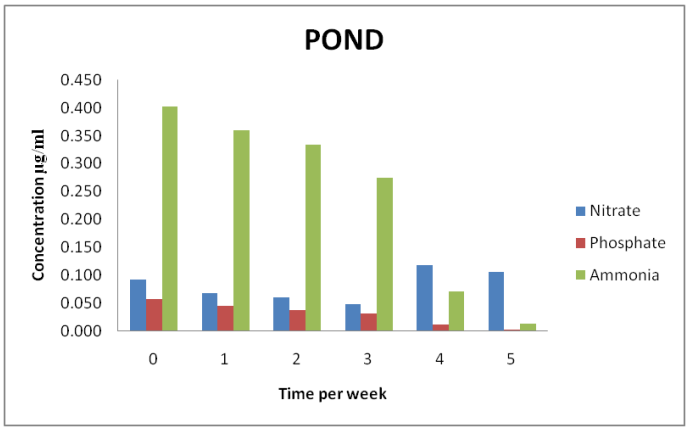


Fig 3: Changes in removal of NPA during 6 weeks of Pond water.

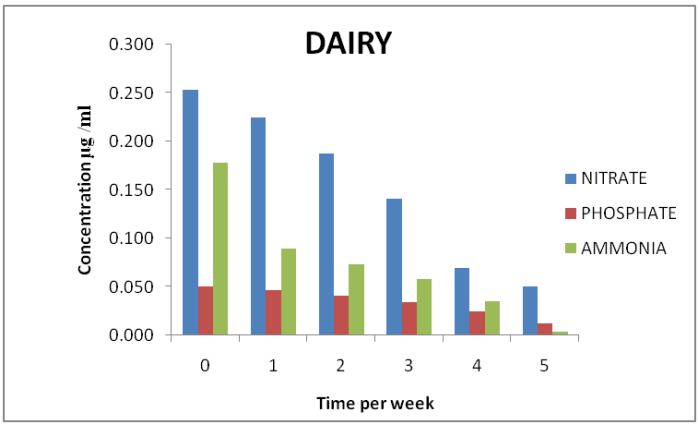


Fig 4: Changes in removal of NPA during 6 weeks of Amul Dairy effluent.

According to the observation Duckweed is capable of reducing the major environmental polluting parameters like Nitrate, Phosphate and Ammonia from the Pond water sample, with nitrate's conc. reducing from 0.091 µg/ml to 0.048 µg/ml, Phosphate's conc. from 0.057µg/ml to 0.002µg/ml, and Ammonia conc. from 0.403µg/ml to 0.012µg/ml which is maximum upon Duckweed's application within 6 weeks (Fig.3). There was found to be significant reduction in concentration of parameters within Amul Dairy's sample, with Nitrate's conc. falling from 0.253µg/ml to 0.051µg/ml, Phosphate's conc. came down from 0.051µg/ml to 0.012µg/ml and Ammonia's conc. dropping down from 0.178µg/ml to 0.004µg/ml as it can be depicted from the results (Fig.4).

Table 4: Concentration from initial to final week of different samples.

Time/ Week	Pond			Dairy			Agrochemical		
	Nitrate	Phos-phate	Ammo-nia	Nitrate	Phos-phate	Ammo-nia	Nitrate	Phos-phate	Ammo-nia
0	0.091	0.057	0.403	0.253	0.051	0.178	0.555	0.033	0.190
1	0.067	0.044	0.360	0.225	0.047	0.089	0.345	0.021	0.140
2	0.060	0.036	0.333	0.188	0.041	0.074	0.278	0.019	0.120
3	0.048	0.031	0.275	0.141	0.035	0.058	0.205	0.016	0.093
4	0.118	0.010	0.070	0.070	0.025	0.035	0.162	0.011	0.058
5	0.106	0.002	0.012	0.051	0.012	0.004	0.103	0.009	0.035

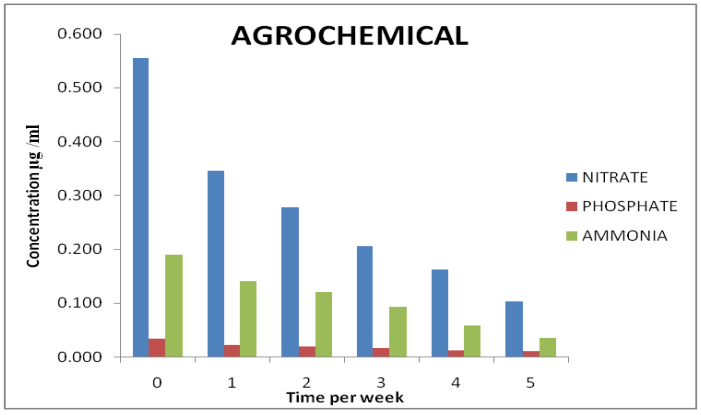


Fig 5: Changes in removal NPA during 6 weeks of Agrochemical effluent.

There was found to be significant reduction in concentration of parameters within Amul Dairy's sample, with Nitrate's conc. falling from 0.253µg/ml to 0.051µg/ml, Phosphate's conc. came down from 0.051µg/ml to 0.012µg/ml and Ammonia's conc. dropping down from 0.178µg/ml to 0.004µg/ml as it can be depicted from the results (Fig.4). Similarly, the Duckweed was found efficient in reducing parameters for the Agrochemical sample as it can be understood from figure.5. The Nitrate conc. was reduced to 0.103µg/ml from 0.555µg/ml, and so for Phosphate's conc. from 0.033µg/ml to 0.009µg/ml, and lastly the Ammonia's concentration were brought down from 0.190µg/ml to 0.035µg/ml. Therefore, as stated from the researchers that Duckweed plant with its ability to reduce environmentally hindering parameters, it is found successful in reducing maximally the Nitrate and Ammonia from all of the three samples and thus bringing it down to a normal range compared to Phosphate's concentration.

Duckweed takes up the above parameters as nutrient requirements for its own growth and on the contrary cleans up the aquatic environment.

Table 5: Reduction in pH in selected samples.

SAMPLES	pH Reduction
Pond	9.19 to 8.37
Dairy	9.10 to 7.76
Agrochemical	8.93 to 8.34
Dye	9.04 to 8.68

pH determination

The maximum pH reduction was found in the Dairy sample compared to that of Pond, Dye and Agrochemical sample (Table-5) thus it can be stated that Duckweed is also capable in normalizing the pH of an effluent without itself getting deteriorated.

Surface Temperature

There was no significant changes found in the surface temperature of the effluents, hence all of the samples temperature were in and around 33° C for throughout the period of 6 weeks.

Reduction of Dye effluent using glass column

Duckweed was fitted in a column filled with dye effluent which was kept under continuous circulation mode by providing aeration for efficient reduction, therefore the reduction of following parameters are provided in Table-6. Thus, according to the results above it can be concluded that Duckweeds are also capable in reducing the dye effluent provided that when there is a continuous supply of air for better treatment.

Table 6: Reduction in NPA and COD of Dye effluent.

O.D.	Nitrate	Phosphate	Ammonia
Initial	1.922	0.94	0.121
Final	1.16	0.108	0.062
Conc.	Nitrate	Phosphate	Ammonia
Initial	2.86	0.368	0.468
Final	1.73	0.042	0.24

In all of the original water samples such as Pond, Dairy, Dye and Agrochemical effluents, the dissolved oxygen (DO) level initially decreased from 5.23 mg/l to 1.07 mg/l and then it increased to 2.08 mg/l for Pond water sample and for Agrochemical effluent the DO level initially decreased from 4.53 mg/l to 1.86 mg/l and then it increased to 2.54 mg/l. But in the presence of Duckweed, the DO initially decreased from 5.23 mg/l to 1.57 mg/l and then increased to 3.04 mg/l for Pond water and for Agrochemical effluent in the presence of Duckweed, the DO initially decreased from 4.53 mg/l to 2.12 mg/l and then increased to 2.86 mg/l, thus, there was no DO found for Dye and Dairy effluent respectively. The reduction of DO may be due to the decomposition of organic matter by aerobic bacteria. Later, the DO starts to increase. When compared to the original water samples the DO level is more in the presence of Duckweed at the end of the experiment. This may be due to (1) Supply of Oxygen by Duckweed plants. (2) Atmospheric diffusion. Similarly, the original water samples had Chemical Oxygen Demand (COD) level initially in the range (2000, 4800, 2400 and 2800) mg/l for Pond, Dairy Dye and Agrochemical effluents respectively, which in the presence of Duckweed finally reduced to (1200, 800, 1200 and 2200) mg/l respectively. This reduction in the COD is due to the ability of Duckweed to absorb organic substances or in other words the Duckweed bio accumulates these substances in its own cells for breakdown and assimilation in plant itself. The phosphate removal in all of the effluents in presence of Duckweed after 5 weeks was measured as 96.4 %, 76.4 %, 88.5 % and 72.7 % respectively. The maximum phosphate removal was measured in Pond water sample in the presence of Duckweed, the reduction might be due to the following reason (1) may be due to phosphate uptake by *Duckweed* plant and assimilation into plant protein, (2) Adsorption on plant leaves, (3) Chemical precipitation and (4) Microbial uptake. The nitrate removal in all of the samples in presence of Duckweed after 5 weeks was recorded as 47.2%, 79.8%, 39.55% and 81.4% respectively, thus Duckweed is found efficient in nitrate removal thus it can be regarded as a boon as it can remove high nitrate content from agrochemical effluent thereby helps in controlling eutrophication in water reservoirs and can be used as fertilizer itself. The ammonia removal was recorded maximum in Dairy effluent with 97.75 %, followed by in Pond sample with 97 % removal of ammonia nitrogen in the presence of Duckweed, thus, there was moderate reduction in Agrochemical and Dye effluent, thus Duckweed satisfactorily removes ammonia provided only that the conditions are suitable to it. The pH value is decreased from 9.19 to 8.37, 9.10 to 7.76, 8.93 to 8.34, and 9.04 to 8.68 for Pond, Dairy, Agrochemical and Dye samples respectively; this might be due to respiration by Duckweed.

CONCLUSION

Effluents from various chemical industries and village ponds are rich source of nutrients like nitrate and phosphate which can be recovered by phytoremediation. It is an affordable technology utilizing plants as environmental cleansers in wastewater management. On one hand manure and fertilizers are getting costlier day by day and on the other hand we have resources like village ponds where the much needed nutrients are lying free of cost. Therefore, recovering this valuable nutrient resource and recycling into some productive system makes sense both ecologically and economically.

An eco-friendly approach of duckweed culture in nutrient rich village ponds will not only help in free of cost extraction of nutrients (which otherwise pollute the water and go waste) in the form of protein rich duckweed but also bio-remediate the village ponds and other effluents and make them a more suitable water resource for aquaculture. Bioremediation will not only augment contribution of village ponds to total aquaculture production of any developing countries like India but also generate em-

ployment opportunities and additional food security for its rural population. Moreover in view of increased pressure on land over the years for production of food and fodder (due to ever increasing population, urbanization, industrialization etc.), utilization of an alternate resource for the purpose makes sense.

Sewage treatment systems with duckweeds are simple lagoons. Cultivated in such systems, these plants act as a nutrient sink, absorbing nutrients from the wastewater. The ions is been removed permanently from the effluent as the plants are harvested. Such systems differ from conventional facilities in that they can achieve a significantly higher level of nutrient removal from the wastewater and remove oxygen consuming substances and pathogenic organisms to an extent comparable to other systems.

References:

1. Sangodoyin, A. Y. Characteristics of control of industrial effluents-generated pollution. *Env. Mgt. Heal.* **6,** 15–18 (1995).
2. Sangodoyin, A.Y. Groundwater and Surface Water Pollution by Open Refuse Dump in Ibadan, Nigeria. *J. Discov. Innov.* **3,** 24–31 (1991).
3. Ajayi S.O. and Osibanji O. D. A. P. Pollution studies on Nigeria Rivers 11; Water quality of some Nigerian Rivers. Environ. Pollut. *J. Appl. Technol. Environ. Sanit.* **3,** 87–95 (1981).
4. Osibanjo, O. D. A. P. and G. A. M. Osibanjo, Daso A P. and Gbadebo A M. The impact of industries on surface water quality of River Ona and River Alaro in Oluyole Industrial Estate, Ibadan. J. Appl. Technol. Environ. Sanit. **10,** 696–702 (2011).
5. Anima Priya, K. A. and G. P. “Assessing the potentials of Lemna minor in the treatment of domestic wastewater at pilot scale”. Environ. Monit. assessment, Springer Journal, **184,** 4301–4307 (2012).
6. Vanitha, S., Nampoothiri, N. V. N., Sivapragasam, C. & M, A. M. For improving pond water quality. **4,** 14–18 (2013).

PfRh5 Inhibitors: a new strategy to combat malaria

Aakash Patel[1], Janki Thakker[2], Pinakin Dhandhukia[1]*

[1]Ashok & Rita Patel Institute of Integrated Studies in Biotechnology & Allied Sciences (ARIBAS), New Vallabh Vidhya Nagar-388121 (Gujarat) India

[2]PD Patel Institute of Applied Sciences, Charotar University of Science and Technology

Abstract:

Malaria is life threatening disease and every year millions of people die due to it. Anti-malarial drugs are available in market but the major problem with drugs is *P. falciparum* drug resistance property so new drugs has to be synthesize to combat malaria. Various new targets have been identified to fight against malaria. Among them *P. falciparum* reticulocyte binding protein shown susceptible advantage to inhibit plasmodium invasion on reticulocyte and in turn inhibit its spreading all over human body. Researchers have proved that antibody against *P. falciparum reticulocyte* binding homologue protein 5 (PfRh5) inhibits *P. falciparum* invasion on reticulocyte.

Introduction

Malaria is the tropical disease with the highest global mortality in childrens and adults. The penetration of malaria is shown to have graduate increased between 10,000 and 5,000 years ago when there were the beginning of agriculture and more human settlements. During this period, the numbers of both the human population and the mosquito vector increased, resulting in higher spread of malaria [1]. In 2010, there were an estimated 219 million cases of malaria and 660,000 deaths worldwide, with children under five years and pregnant women the most vulnerable [2]. Over 81% of cases and 91% of deaths were in Africa, with the majority of the remaining being in India, Southeast Asia and South America. According to the World Malaria Report 2011, over 70 percent of the country's 1.2 billion population faces the risk of malaria infection, with an estimated 310 million people - one-third of the total - facing the "highest risk" [3].

Causative agent of malaria

Malaria is caused by a type of parasite known as Plasmodium. This is a microscopic parasite transmitted by certain species of mosquitoes. Although there are numerous types of Plasmodia parasites, only four viz. *Plasmodium falciparum, plasmodium vivax, plasmodium ovale, plasmodium malariae* cause malaria in humans..

**Corresponding Author: pinakindhandhukia@aribas.edu.in*

The Plasmodium parasite is mainly spread by female Anopheles mosquitoes, which are night-biting mosquitoes. In India malaria is generally caused by falciparum species of plasmodium parasite.

Life-cycle of Plasmodium

The malaria parasite exhibits a complex life cycle involving an insect vector (mosquito) and a vertebrate host (human). The malaria parasite develops both in humans and in the female Anopheles mosquitoes. In human, parasite reproduce through asexual type of reproduction[4] whereas in mosquitoes, it reproduce through sexual reproduction[5]. The size and genetic complexity of the parasite mean that each infection presents thousands of antigens (proteins) to the human immune system. The parasite also changes through several life stages even while in the human host, presenting different antigens at different stages of its life cycle (Figure 1). Understanding which of these can be a useful target for vaccine development has been complicated. In addition, the parasite has developed a series of strategies that allow it to confuse, hide, and misdirect the human immune system[6].

Treatment of malaria

Malaria is usually over-diagnosed on the basis of symptoms alone, especially in endemic areas like India, China because of this non-specificity of symptomatology. Around two million laboratories confirmed cases of malaria are reported in the country annually. Out of the total malaria cases, 40-50% are of *P. falci-*

parum. It is observed that *P. falciparum* infection may lead to complications in 0.5% to 2% of cases. Mortality may result in about 30% of such cases if timely treatment is not given. A single dose of chloroquine may save the life in *P. falciparum* cases by averting complications[7]. In recent studies, chloroquine-resistant *P. falciparum* malaria has been observed with increasing frequency across the country.

Various drugs are listed in below table 1 which are currently using in treatment of moderate to severe level of malaria disease. Most common drug for malaria treatment is quinine and their derivatives like chloroquinine[7]. Its effect on plasmodium is much significant. Rational approach of malarial treatment is using ''artimisinin'' in mix compound form. Artimisinin is a secondary metabolite produced by Chinese plant. This treatment is called as Artimisinin based Combination Therapies (ACTs), is the best treatment for falciparum malaria[8,9]. Implementation of these therapies has lagged behind due to various factors such as high cost, strain resistance etc [10].
Table 1 contains information regarding various core compounds utilized for preparing drugs to cure malaria. Some antibiotics like doxycycline, tetracycline are also utilized in primary treatment of *P. falciparum* infected patients[11]. Various isomers and analogs of quinine are frequently utilize in malaria prescription. Quinine, artemisinins, chloroquine, lumefantrine, tetracycline, atovaquone, sulphadoxine, clindamycin, proguanil act on erythrocytic stage of the parasite thereby terminating clinical illness. Primaquine, pyrimethamine, proguanil act on primary tissue forms of plasmodia which initiate the erythrocyte stage and they block further development of the infection.

Plasmodium falciparum
Development of resistance
''A *plasmodium* strain have ability of anti-malarial drug resistance, survive and multiply despite of the administration and exposure of a drug given in doses equal to or higher than its minimum inhibitory concentration'' [12].

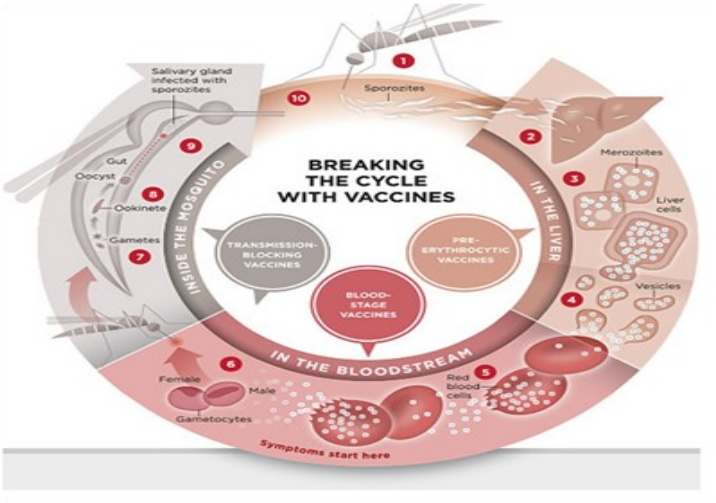


Figure 1: Life cycle of Anti-malarial drug resistance arises due to spontaneously occurring mutations that affects activity and structure of the drug target at molecular level in *P. falciparum* or might be affect the access of the drug to that target[13]. Various factors relating to parasite, drug ,human host interaction contribute to the spread and development of *P. falciparum* drug resistance. As far as large number of drug compounds are used, the likelihood that exposure of parasite to inadequate drug levels rises and resistance mutants are readily selected[14,15].

Rh5pf as target to halt Infection process
Infection of *P. falciparum* is multi step process in which erythrocyte invasion is central to the pathogenesis of malaria. The asexual blood stage of the parasite life cycle multiplies rapidly within the erythrocytes and is major responsible for the malaria manifestation. The invasive merozoite forms are released from mature schizont stages and these rapidly invade new red blood cells in a process mediated by a cascade of events that includes multiple receptor–ligand interactions[16,17]. Interaction of merozoite with reticulocyte leads to reorientation and the tight junction is develop with the host cell by an array of receptors with the parasite ligands that finally linked to an actin -myosin motor. The tight junction is move along with the surface of the invading merozoite by virtue of the force generated by the motor untill membrane fusion at the posterior end of the parasite resulting in internalization within a parasitophorous vacuole[16]. Invasion requires series of extracellular recognition events between erythrocyte receptor and ligands on merozoite[18,19]. Among *P. falciparum* merozoite proteins,

that are involve in erythrocyte invasion, most attention has focused on two major parasite protein families*: Erythrocyte Binding Antigens* (EBA's) and *Reticulocyte Binding Homologous proteins* (Rhs)[20]. EBA and Rhs appears to require high affinity interaction. Erythrocyte binding like protein consisting of EBA-175, EBA-181 (known as JESEBL) and EBA140 (known as BAEBAL)[21] which binds to glycophorin A and glycophorin c respectively[22,23].

The reticulocyte binding like-proteins of *P. falciparum* includes PfRh1, PfRh2a, PfRh2b, PfRh3, PfRh4, PfRh5. This proteins are situated at the neck of the rhoptries in the merozoite[24]. Among them PfRh3 is a pseudogene at least in the strains that are analyzed till date[25]. PfRh1 binds to reticulocyte in sialic acid dependent manner and the properties of this receptor has been characterized but still need to identify. *P. falciparum* have very small numbers of cell types that can be invaded within the blood. Though having a restricted host cell specificity *P. falciparum* can adopt different patterns of multiple ligand-receptor interaction thus providing a mechanism of phenotypic variation to evade host immune response and the polymorphic nature of the human erythrocyte. Among EBA and PfRh families, PfRh family plays a significant role in recognition and invasion of the host erythrocyte and it allows different parasite strains to use alternate receptor. *P. falciparum*'s all strain contain PfRh1 expression, however it levels can vary dramatically as a result of gene amplification. PfRh2a and pfRh2b present in many strains but do not all express proteins. Some strains are able to activate PfRh4 protein resulting in switch in receptor utilization for merozoite invasion. PfRh5 and PfRh2b have been implicated in sialic acid independent pathway of erythrocyte invasion[26]. Alignment of amino acid sequences of the PfRh proteins indicate small but significant degree of sequence conservations (approx.20%) among members and have conservative module that is repeated in all other PfRH family members thus likelihood of common fold. Among all of EBA and RH family, PfRh5 is a novel protein that binds to reticulocyte because it can't be deleted in any *P. falciparum* strains and thus its apparently essential in all parasite strains[17,27]. Native and recombinant PfRh5 have shown to bind erythrocyte through glycosylated receptor that is resistant to trypsin, chy-

Table 1: Drugs used in malaria treatment

	Core Com-	**Drug name**
1	Aminoquinolines,	Chloroquine, Hydroxychloroquine, Amodiaquine
2	8- aminoquinolines,	Primaquine, Tafenoquine, Bulaquine
3	Arylamino alcohols,	Quinine
4	Methanols I. 4-quinoline methanol II 9-phenanthrene methanol	Mefloquine Halofantrine, Lumefantrine
5	Biguanides	Proguanil
6	Diamino-pyrimidines	Pyrimethamine
7	Antimalarial endoperoxidases I.First generation endoperoxidases (Artemisinin derivatives) II.Second generation endoperoxidases a. Trioxanes b. Tetroxanes	Artesunate, Artemether, Arteether
8	Hydroxynaphthoquinone	Atovaquone
9	Benzonaphthyridine deriative	Pyronaridine
10	Antibiotics	Sulfonamides,Tetracycline, Doxycycline,Clindamycin, Azithromycin

motrypsin, neuraminidase treatment[27,28]. Screening a large library of erythrocyte proteins, it is found that Ok blood group antigen, BASIGIN, is a receptor for PfRh5. Besigin (BSG) is a member of the immunoglobulin superfamily (IgSF) and has a role in many biological functions including embryo implantation, spermatogenesis and retina development. BSG presents in both long (three IgSF domains, BSG-L) and short (two IgSF domains, BSG-S) splice isoforms and although BSG-L was used in the screen, BSG-S is thought to be the major isoform expressed on erythrocytes (figure 2). Binding experiments using domain deletions established that PfRh5 could interact with BSG-S and this required both domains since neither of the two BSG-S IgSF domains were individually able to bind PfRh5. Determining whether PfRh5-BSG interaction is required for invasion, purified pentamerized soluble BSG-S added into invasion assays to specifically compete with the membrane-bound receptor. In the result BSG-S strongly inhibited invasion in a dose-dependent manner relative to controls which included each of the two non-binding BSG-S IgSF domains added individually[29]. It shows PfRh5 directly interacted with BSG-S and BSG-L using purified proteins and surface plasma resonance. Strong inhibition was also observed across multiple strains when soluble BSG-L was added , although this was slightly weaker for the 3D7 strain. Soluble forms of BSG consisting of the extracellular regions are known to have biological effects such as up regulation of matrix metalloproteases[30]. To rule out an indirect effect of exogenous BSG on invasion, two independent purified anti-BSG monoclonal antibodies (MEM-M6/6 and TRA-1-85) which could both block the PfRh5–BSG interaction in vitro added to invasion assays. These high affinity reagents gave a potent invasion blocking effect that was saturable at very low antibody concentrations (IC50 ~ 0.5 µg/ml), consistent with binding and occluding a specific surface receptor of typical abundance (~10^4 to 10^6 molecules per cell)

Pre-adsorbtion of the MEM-M6/6 antibody with soluble monomeric BSG specifically relieved the inhibition, ruling out any indirect effect of the antibody on non- BSG targets; furthermore, MEM-M6/6 did not affect intra-erythrocytic *P. falciparum* development[29]. Invasion was quantified using flow cytometry and a fluorescent DNA dye to stain parasites[31]. To independently confirm the essentiality of BSG as a *P. falciparum* invasion receptor, a genetic approach by differentiating erythrocytes from hematopoietic stem cells transduced with lentiviruses containing either an shRNA targeting *BSG* or a scrambled shRNA control (pLKO) have been performed. *BSG*-targeted erythrocytes showed a reproducible knockdown to approximately 50 to 60% of cell surface BSG levels relative to the pLKO control.

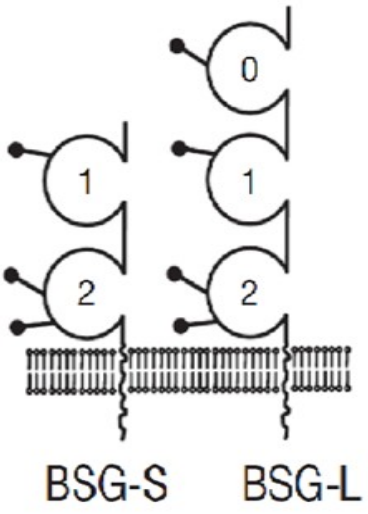


Figure 2: Domain structure of BESIGIN isoforms

PfRh5 is expressed as a 63 kDa protein in the asexual parasite

The members of the PfRH protein family have no obvious domain structures, such as the Region II cysteine-rich domains in the EBL family. However, the N-terminal amino acids of the PfRH ligands are the most highly conserved in the sequences of these proteins. Additionally, this region contains most of the proteins' cysteine residues, which could reflect the presence of binding sites. Thus, focused on the N-terminus of PfRh5 and chose a 143-aa sequence of PfRh5 from Asn-31 to Val-174 on the basis of a Clustal alignment of PfRh5 with the phylogenetically close PvRBP1[32] (~22% overall similarity). This region of RH5, moreover, also exhibited significant homology with the RBC-binding domain of PfRH4[33]. The recombinant protein was expressed as a GST fusion protein of ~43 kDa in *E. coli* and purified to homogeneity, using glutathione-Sepharose 4B. Antiserum raised in rabbits against the purified protein was used to identify na-

tive PfRh5 in asynchronous parasite extracts, by immunoprecipitation and immunoblotting. It shows the results of these assays, in which a 63 kDa protein is clearly seen in the 3D7 parasite extracts, the position corresponding to the predicted molecular mass of PfRh5. The protein does not appear to undergo processing, as no lower MW bands are apparent on either the blot or the autoradiograph. Similar results were obtained from parasite extracts from Dd2 and HB3, indicating that all these strains synthesize PfRh5, with no perceptible difference in size[26].

PfRh5 binds to erythrocyte

Having established its location at the invasive apical end of the merozoite and homology with other known RBC binding proteins, various assays are performed to determine whether native PfRh5 binds to RBCs. The native full-length PfRh5 was isolated from [^{35}S] methionine/cysteine-labeled culture supernatants (HB3 strain) that contained merozoites released from infected erythrocytes in the absence of target erythrocytes. Studies have shown that extracellular merozoites release parasite proteins into the culture, and such culture supernatants can be a source of parasite ligands that bind erythrocytes. Thus, [^{35}S] methionine/cysteine-labeled spent merozoite supernatants were used as the source of RBC-binding proteins, and when the eluate was immunoprecipitated with anti-RH5 antiserum, a dominant band at ~63 kDa was seen. Thus, PfRh5 appears to be an adhesin that participates in invasion by binding to the RBC surface.

PfRh5 as one of the malaria vaccine candidate

No vaccine has yet proven effective against the blood-stages of *P. falciparum*, which cause the symptoms and severe manifestations of malaria. Recently found that PfRh5, a *P. falciparum*-specific protein expressed in merozoites, is efficiently targeted by broadly-neutralizing, vaccine-induced antibodies. Antibodies against PfRh5 efficiently inhibit the *in vitro* growth of short-term-adapted parasite isolates from Cambodia, and that the EC_{50} values of antigen-specific antibodies against PfRh5 are lower than those against PfAMA1. Since antibody responses elicited by multiple antigens are speculated to improve the efficiency of blood-stage vaccines, experimental detailed assessments of parasite growth inhibition by antibodies against PfRh5 in combination with antibodies against seven other merozoite antigens. It is found that antibodies against PfRh5 act synergistically with antibodies against certain other merozoite antigens, most notably with antibodies against other erythrocyte-binding antigens such as PfRh4, to inhibit the growth of a homologous *P. falciparum* clone. A combination of antibodies against PfRh4 and basigin, the erythrocyte receptor for PfRh5, also potently inhibited parasite growth. This methodology provides the first quantitative evidence that polyclonal vaccine-induced antibodies can act synergistically against *P. falciparum* antigens and should help to guide the rational development of future multi-antigen vaccines[34]. Antibodies against PfRh5 can act synergistically with antibodies against other merozoite antigens. Systematically combined purified anti-PfRh5 rabbit IgG in GIA assays with purified rabbit IgG against seven other merozoite antigens – PfAMA1, PfMSP1 (a construct containing the conserved blocks of sequence 1, 3, 5 and 12 followed by both of the dimorphic forms of the 42 kDa C-terminus, $MSP1_{42}$), PfEBA175, PfRH2, PfRH4, Pf38 and Pf Rhoptry Associated Protein 3 (PfRAP3) are used to detect synergy, the GIA effect of a fixed concentration of anti-PfRh5 IgG with or without the addition of a range of concentrations of IgG against the other merozoite antigens is measured[34]. For each combination of antibodies, the predicted GIA that would be achieved by the two components having an independent, additive effect by using the definition of Bliss additivity[35].

The discovery of full-length PfRh5 as a conserved and antibody-susceptible antigen has renewed hope that this challenge may be surmountable that multiple laboratory-adapted parasite lines and naturally-circulating parasite isolates are susceptible to anti-PfRh5 IgG, which neutralizes parasites at concentrations that are comparable to or, in many cases, lower than anti-PfAMA1 IgG. Furthermore, it has frequently been suggested that multi-antigen blood-stage vaccines may induce antibodies that act synergistically.

Reference

1. Carter, R. & Mendis, K. N. Evolutionary and Historical Aspects of the Burden of Malaria. *Clin. Microbiol. Rev.* **15,** 564–594 (2002).

2. WHO. *WHO World Malaria Day,25th April.* (2013).
3. ICMR. *Indian Malaria Annual Report*. (2010).
4. Rosenberg, R. Malaria: some considerations regarding parasite productivity. *Trends Parasitol.* **24,** 487–491 (2008).
5. Sinden, R. E. Malaria, sexual development and transmission: retrospect and prospect. *Parasitology* **136,** 1427–1434 (2009).
6. PATH accelerating malaria vaccine. (2012).
7. Sullivan, D. J., Gluzman, I. Y., Russell, D. G. & Goldberg, D. E. On the molecular mechanism of chloroquine's antimalarial action. *Proc. Natl. Acad. Sci.* **93,** 11865–11870 (1996).
8. Health, T. W. Artemisinins in malaria treatment in the UK. *Drug Ther. Bull.* **49,** 129–132 (2010).
9. Ali, S. A., Zare, Mohammadpour Payman, H. & Tayefi-Nasrabadi, H. Effects of different concentrations of artemisinin and artemisinin-iron combination treatment on Madin Darby Canine Kidney (MDCK) cells. *Interdiscip. Toxicol.* **5,** 30–7 (2012).
10. World Health Organization. THE USE OF ANTIMALARIAL DRUGS Report of an Informal Consultation. (2000).
11. Briolant, S., Fusaï, T., Rogier, C. & Pradines, B. Tetracycline Antibiotics in Malaria. *Open Trop. Med. Journal, 2008.* **1,** 31–46 (2008).
12. World Health Organization. Resistance of parasite. (1973).
13. Peters, W. Changing pattern of antimalarial drug resistance. *J. R. Soc. Med.* **82 Suppl 1,** 14–17 (1989).
14. National Institute of Malaria & Research. Guidelines for Diagnosis and Treatment of Malaria in India. (2009).
15. Watkins, W. M. & Mosobo, M. Treatment of Plasmodium falciparum malaria with pyrimethamine-sulfadoxine: selective pressure for resistance is a function of long elimination half-life. *Trans. R. Soc. Trop. Med. Hyg.* **87,** 75–78 (1993).
16. Triglia, T., Tham, W.-H., Hodder, A. & Cowman, A. F. Reticulocyte binding protein homologues are key adhesins during erythrocyte invasion by Plasmodium falciparum. *Cell. Microbiol.* **11,** 1671–87 (2009) .
17. Cowman, A. F. & Crabb, B. S. Invasion of red blood cells by malaria parasites. *Cell* **124,** 755–766 (2006).
18. Muramatsu, T. Basigin: a multifunctional membrane protein with an emerging role in infections by malaria parasites. *Expert Opin. Ther. Targets* **16,** 999–1011 (2012).
19. Gaur, D., Mayer, D. C. G. & Miller, L. H. Parasite ligand-host receptor interactions during invasion of erythrocytes by Plasmodium merozoites. *Int. J. Parasitol.* **34,** 1413–1429 (2004).
20. Iyer, J., Grüner, A. C., Rénia, L., Snounou, G. & Preiser, P. R. Invasion of host cells by malaria parasites: a tale of two protein families. *Mol. Microbiol.* **65,** 231–49 (2007).
21. Adams, J. H. *et al.* The Duffy receptor family of Plasmodium knowlesi is located within the micronemes of invasive malaria merozoites. *Cell* **63,** 141–153 (1990).
22. Mayer, D. C. G., Mu, J., Feng, X., Su, X. & Miller, L. H. Polymorphism in a Plasmodium falciparum Erythrocyte-binding Ligand Changes Its Receptor Specificity. **196,** 1523–1528 (2002).
23. Lobo, C., Rodriguez, M., Reid, M., Lustigman, S. & Dc, W. Glycophorin C is the receptor for the Plasmodium falciparum erythrocyte binding ligand PfEBP-2 (baebl) Brief report Glycophorin C is the receptor for the Plasmodium falciparum erythrocyte binding ligand PfEBP-2 (baebl). 4628–4631 (2013). doi:10.1182/blood-2002-10-3076
24. Triglia, T. *et al.* Identification of Proteins from Plasmodium falciparum That Are Homologous to Reticulocyte Binding Proteins in Plasmodium vivax. *Infect. Immun.* **69,** 1084–1092 (2001) .
25. Taylor, H. M. *et al.* Plasmodium falciparum homologue of the genes for Plasmodium vivax and Plasmodium yoelii adhesive proteins, which is transcribed but not translated. *Infect. Immun.* **69,** 3635–45 (2001).
26. Rodriguez, M., Lustigman, S., Montero, E., Oksov, Y. & Lobo, C. A. PfRH5: a novel reticulocyte-binding family homolog of plasmodium falciparum that binds to the erythrocyte, and an investigation of its receptor. *PLoS One* **3,** e3300 (2008).
27. Hayton, K. *et al.* Erythrocyte binding protein PfRH5 polymorphisms determine species-specific pathways of Plasmodium falciparum invasion. *Cell host microbe* **4,** 40–51 (2008).
28. Baum, J. *et al.* Reticulocyte-binding protein

homologue 5 - an essential adhesin involved in invasion of human erythrocytes by Plasmodium falciparum. *Int. J. Parasitol.* **39,** 371–380 (2009).

29. Crosnier, C. *et al.* Basigin is a receptor essential for erythrocyte invasion by Plasmodium falciparum. *Nature* **480,** 534–537 (2011).

30. Guo, H., Zucker, S., Gordon, M. K., Toole, B. P. & Biswas, C. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. *J. Biol. Chem.* **272,** 24–27 (1997) .

30. Guo, H., Zucker, S., Gordon, M. K., Toole, B. P. & Biswas, C. Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. *J. Biol. Chem.* **272,** 24–27 (1997) .

32. Galinski MR, Medina CC, Ingravallo P, B. J. A reticulocytebinding protein complex of Plasmodium vivax merozoites. *Cell* 1213–1226 (1992).

33. Taylor, H., Grainger, M. & Holder, A. Recombinant Plasmodium falciparum reticulocyte homology protein 4 binds to erythrocytes and blocks invasion. *Infect. Immun.* 5779–5789 (2002).

34. Williams, A. R. *et al.* Enhancing blockade of Plasmodium falciparum erythrocyte invasion: assessing combinations of antibodies against PfRH5 and other merozoite antigens. *PLoS Pathog.* **8,** e1002991 (2012).

35. Bliss, C. I. The toxicity of poisons applied jointly. *Ann. Appl. Biol.* **26,** 585–615 (1939).


"We
are committed to nation
through our quality teaching
and research keeping students in
focus along with involvement of
our employees and continual
improvement in all areas."
ज्ञानेन शीलम्
ARIBAS
A CVM Institute

ज्ञानेन शीलम्

Do send us your comments and suggestion at e-mail:

quest@aribas.edu.in

ASHOK & RITA PATEL INSTITUTE OF INTEGRATED STUDY & RESEARCH IN BIOTECHNOLOGY AND ALLIED SCIENCES

P.O. Box No. 61, New Vallabh Vidyanagar, Vitthal Udyognagar - 388121, Dist- Anand, Gujarat, India.

Phone: +91-2692-229189, 231894 Fax: +912692-229189