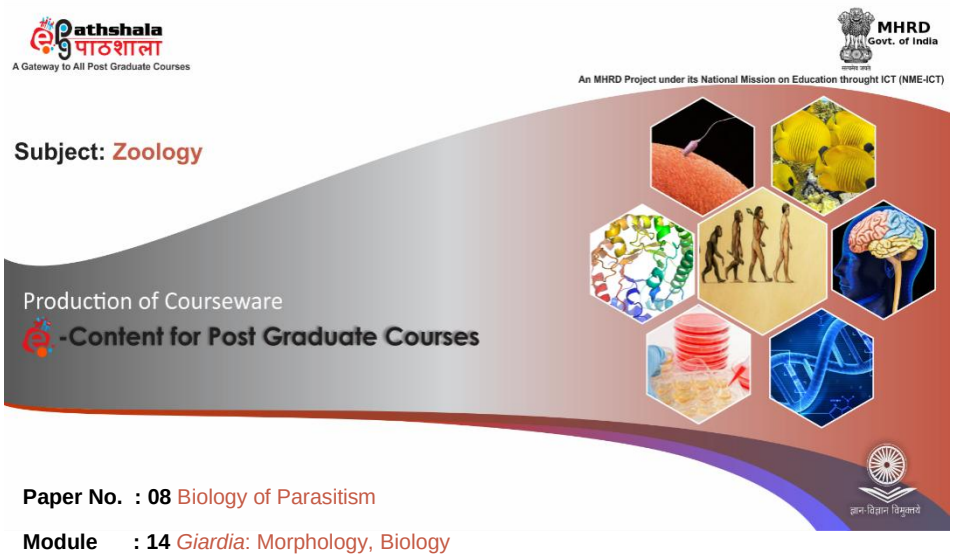

Paper No. : 08 Biology of Parasitism

Module : 14 *Giardia*: Morphology, Biology

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Description of Module	
Subject Name	ZOOLOGY
Paper Name	Biology of Parasitism- Zool 008
Module Name/Title	*Giardia*: Morphology, Biology
Module Id	14; *Giardia*: Morphology, Biology
Keywords	*Giardia, Giardia*sis, trophozoite, cyst

Contents:

1. Learning Outcomes

After studying this module, you shall be able to:

- Know anaerobic flagellated protozoan parasites.
- Learn about taxonomical classification of *Giardia*.
- Identify morphological life-cycle stages.
- Evaluate complex microtubule cytoskeleton of the parasitic protist.
- Analyse *Giardia* Genomics Resource database.

2. Introduction

Giardia is a microscopic parasite that is responsible for the diarrheal illness called as *Giardia*sis. These typical eukaryotic organisms are anaerobic flagellated protozoan parasites of the phylum Sarcomastigophora (Table 1) that colonize and replicate in the small intestines of several vertebrates. *Giardia* is also known as *Giardia intestinalis*, *Giardia lamblia* or *Giardia duodenalis* (Figure 1). This microscopic parasite is generally found outside or in soil, food, or water which has been contaminated by infected humans or animals feces (CDC, 2011). As the parasite can spread in various ways of transmission, the most common of them is via water (such as drinking water and recreational water). The first recorded observation of *Giardia* trophozoite stages is usually credited to scientist Anton van Leeuwenhoek around the year 1681. Even though in 1859, the protist was described in details by Lambl, who wrongly considered the organism in the genus *Cercomonas* and called it *Cercomonas intestinalis.* Almost two centuries later researcher Grassi (between the year 1879-88) and Perroncito documented the acceptable morphological description of *Giardia* cyst and their links with the flagellated forms (Erlandsen SL. and Meyer EA., 1984).

Table 1. Taxonomical classification of *Giardia*.

Domain	**Eukaryota**
Kingdom	Protista
Subkingdom	Protozoa
Phylum	Sarcomastigophora
Subphylum	Mastigophora
Class	Zoomastigophora
Order	Diplomonadida
Family	Hexamitidae
Genus	*Giardia*

Cysts (parasite protected by an outer shell) are resistant forms and are accountable for transmission of parasite that causes disease *Giardia*sis. Both cysts and trophozoite forms (known as diagnostic or indicative stages) can be traced in the contaminated faeces. Cysts of *Giardia* occur in the aquatic environment all over the world and their population density is related to the level of fecal pollution or water pollution mainly done by humans (LeChevallier MW, *et al.*, 1991). Kofoid and Christiansen projected the names *G. lamblia* (Kofoid, C. A., and Christensen ED., 1915) and *G. enterica* (Kofoid, C. A., and Christensen ED., 1920). The genus is named after French zoologist and applied entomologist Alfred Mathieu Giard. The species name *G. lamblia* was extensively used during 1970s but in 1980s, some scientist used the name *G. duodenalis*, while in the 1990s, other researchers encouraged the name *G. intestinalis* (Kulda, J., and Nohy´nkova´ E., 1996). *G. lamblia* has a distinct nucleus and nuclear membrane, well-developed cytoskeleton, and endomembrane system, but lacks other eukaryotes-like organelles, such as nucleoli, mitochondria, peroxisomes, and any of the components of oxidative phosphorylation (Adam RD, 2001).

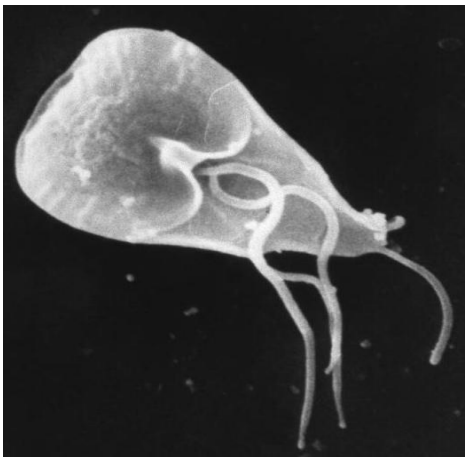

Figure 1. Scanning electron micrograph of flagellated *Giardia lamblia* protozoan parasite responsible for causing the diarrheal *Giardia*sis

(Image Source: Wikimedia Commons http://phil.cdc.gov/PHIL_Images/8698/8698_lores.jpg).

3. Taxonomy of *Giardia*

Presently in the scientific literature five species of *Giardia* are recognized, including the 03 species proposed by F.P. Filice in 1952, that are *G. muris* in rodents, birds, and reptiles, *G. intestinalis* (or *G. duodenalis*, *G. lamblia*) in mammals (include humans), rodents, and reptiles, *G. agilis* in amphibians, *G. ardae* in the great blue heron (Erlandsen SL., *et al.*, 1990) and straw-necked ibis (Forshaw D., 1992; McRoberts KM., *et al.*, 1996), and *G. psittaci* in the budgerigar (Erlandsen SL and Bemrick WJ., 1987) (described in Table 2). Based on the molecular comparison (ribosomal RNA) of small subunit 18S rRNA

sequences, *G. lamblia* is proposed as one of the most ancient eukaryote, along with *T. vaginalis* and the microsporidia (Sogin ML., *et al*., 1989).

Table 2. Hosts and morphology of different *Giardia* species (compiled from Adam RD., 2001).

Species	Host Organisms	Morphological features noted by Light microscopy	Morphological features noted by Electron microscopy	Molecular Data
G. agilis	Amphibians	Long and slender, tear-drop like median structure	--	Not available
G. lamblia	Many mammals such as humans	Pear shaped, one or two transverse(s) and claw shaped median body	--	Clade with multiple genotypes
G. muris	Rodents	Short and rounded median body	--	Distant from *G. lamblia*
G. psittaci	Psittacine birds	Features same as *G. lamblia*	Partial ventro-lateral flange, no marginal groove	Not available
G. ardeae	Herons	Features same as *G. lamblia*	Ventral disk and caudal flagellum alike *G. muris*	Nearer to *G. lamblia* species than *G. muris* species
G. microti	Voles and muskrats	Features same as *G. lamblia*	Cysts contains 02 trophozoites with mature ventral disks	Related to genotype of *G. lamblia*

Giardia lamblia was named after Scientist Alfred Mathieu Giard because of his extensive findings on this parasite (Adam RD, 2001). In order to understand this parasite better below is a detailed classification.

Domain: **Eukaryote** Members of this domain possesses true nuclei and a nuclear membrane. **Kingdom: Protista** Members of this kingdom are eukaryotic and are generally unicellular organisms even though few multicellular organisms also fall into this kingdom (like algae). A major key feature of Protista is that they are aquatic environment organisms.
Phylum: Sarcomastigophora Organisms are flagellated, and typically 10 to 20 micrometer in size. These include either free-living or parasitic protozoan.
Class: Zooflagellates Zooflagellates are also known as Zoomastigophora means 'whip-bearing animal'. The main feature of this class is how organisms use their flagella (one or more) for its movement.
Order: Diplomondida The order includes mostly parasitic organisms. These organisms lack mitochondria or Golgi apparatus, but have mitosomes instead. A mitosome is an organelle located in few

unicellular eukaryotic organisms and localization of iron sulfur cluster assembly proteins (IscU, IscS, ferredoxin) have been reported within them.

Family: Hexamitidae Organisms contains 06 or 08 flagella, two true nuclei and are bilaterally symmetrical.

Genus: *Giardia* The genus *Giardia* includes anaerobic parasites that feed and replicate in the small intestine of infected human beings and other mammals. The life-cycle of organisms is divided into two stages, first the actively swimming stage- trophozoite and the second stage - cyst that feeds on the host mammals and are responsible for transmission of parasite that causes *Giardia*sis disease.

4. Morphological stages of *G. lamblia*

Giardia lamblia, a ubiquitous intestinal pathogen of mammals, is one of the members of earliest diverging eukaryotic lineages.The life cycle of *Giardia* is uncomplicated as the environmentally robust cysts of the parasite (present in the human faeces) are transmitted to a new human host mainly by waterborne transmission (Feely DE, *et al.*, 1990; Meyer EA, 1994; CDC-DPDx, 2013; Figure 2).

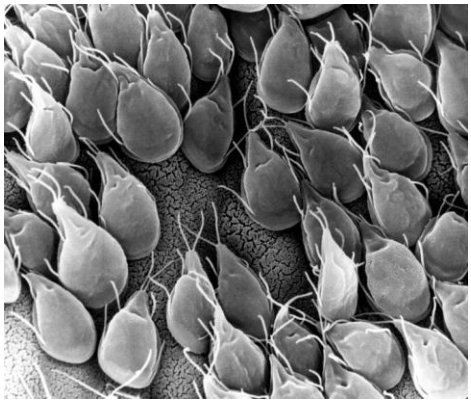

Figure 2. A scanning electron micrograph of the surface of the small intestine of a gerbil (desert rats) infected with Giardia trophozoites

(Image source: Wikimedia Commons https://commons.wikimedia.org/wiki/File:Giardia-spp.--infected--gerbil-intestine.jpg)

G. lamblia has two morphological stages: the trophozoite and the cyst (Figure 2 and 3). In new host's duodenum the trophozoite stages emerge from the infective cyst which undergoes a mitotic division (to form 02 trophozoites). Thus through excystation, each cyst produces two trophozoites. With the help of adhesive disc, each of the two trophozoites remains attached to the epithelial cells and start feeding on the epithelial cells. In the lumen of the proximal small intestine, trophozoites multiply and remain as free or attached form (by a ventral sucking disk) to the mucosa. The trophozoites undergo rapid division (divide within 72 hours) by mitotic division in the intestinal lumen. In diarrhoea condition, many of the trophozoites get detached from the epithelial cells and excreted out with the intestinal contents, but they do not survive extensively outside the host body. However, encystation process occurs where trophozoites

get transformed into cyst stage as the parasites transit toward the colon and excretes with the faeces. These cyst forms can survive for long in the external environment and causes person-to-person transmission of the infective parasite.

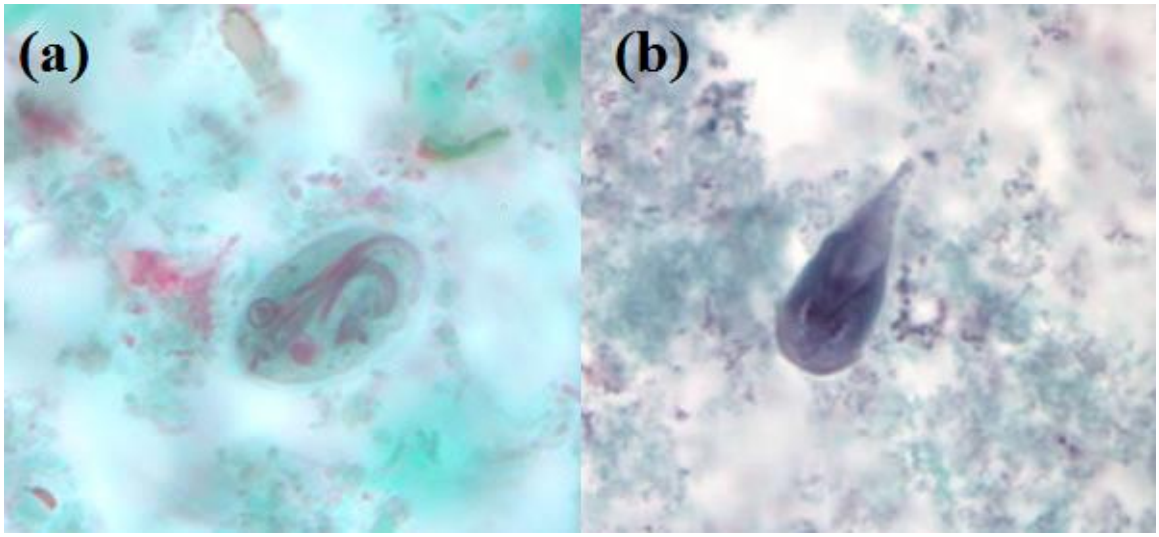

Figure 3. *Giardia duodenalis*
(a) cyst stage and (b) trophozoite stage stained with trichrome (a three-colour staining protocol).
(Image source: CDC-DPDx, 2013 http://www.cdc.gov/dpdx/*Giardia*sis/index.html)

4.1 Trophozoite structure

The pear shaped *G. lamblia* trophozoites are bilaterally symmetrical, and bear two nuclei without nucleoli that are positioned anteriorly. They are around 10 to 12 µm long and 5-7 µm broad with wide front and much attenuated posterior end (John DT and Petri W., 2006). In this stage, the parasite is relatively flattened. A large sucking disk is present on the anterior ventral side for its attachment to the host intestinal mucosa. The trophozoites also have two median bodies and 04 pairs of flagella (namely anterior flagella, caudal flagella, posterior flagella and ventral flagella) (Adams R., 1991) (Figure 4). Cytoplasm contains lysosomal vacuoles, ribosomal and glycogen granules. Golgi complexes are noticeable in encysting trophozoites as stacked membranes indicative of Golgi complexes have been confirmed (Lanfredi-Rangel A., *et al.*, 1999).

4.2 Cyst structure

The environmentally stable cyst is egg-shaped, 8-14µm long and 7-10µm broad (John DT and Petri W., 2006). During excystation, each of the ingested cysts gives rise to two trophozoites. However after encystation, each organelle of the parasite duplicates, hence single cyst now encloses 04 nuclei, 04 median bodies, 08 pairs of flagella even though the organelles arrangement pattern is not clear. Cysts are covered by an outer filamentous and an inner membranous (having two membranes) layers. The outer surface of the cyst wall is roofed by a network of 7- to 20-nm filaments and 04 major proteins have been

reported (that are 29 KDa, 75kDa, 88kDa, and 102 kDa in size) (Erlandsen, S. L., *et al.*, 1990). The sugar constituent of the outer structural section is chiefly galactosamine in the form of *N*acetylgalactosamine (GalNAc). They have a metabolic rate of only 10 to 20% with respect to trophozoites. Ethanol stimulates the respiration of both cysts and trophozoites while glucose stimulates only trophozoite respiration.

4.3 Cytoskeleton and Motility

Complex microtubule (MT) cytoskeleton of this parasitic protist is vital for its motility, attachment to host surface, intracellular transport, cellular multiplication and transitioning between its two morphological stages (the cyst and the trophozoite). The *Giardia* microtubule cytoskeleton is made up of both unique elements (such as the median body, ventral disc, funis and axoneme-associated elements) and general structures of flagellated protists (08 flagella and 02 mitotic spindles) (Dawson SC., 2010).

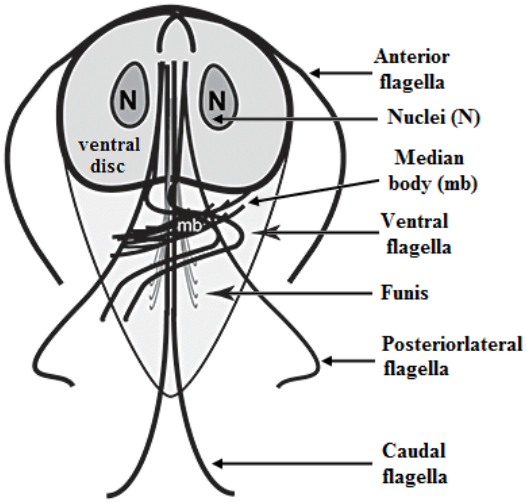

Figure 4. Schematic description of the primary elements of the teardrop shaped *Giardia* trophozoite microtubule cytoskeleton (Image adapted from: Dawson SC., 2010).

The non-membranous and functionally unknown median body (MB) is a semi-organized microtubule arrangement present on the dorsal part of *Giardia* trophozoites, following anterior ventral disc (Piva B., Benchimol, M., 2004). Ventral disc is a unique and highly structured microtubule structure essential for parasite virulence as it helps the parasitic protist to attach and colonize at the intestinal microvilli (Elmendorf, H.G., *et al.*, 2003). The ventral disc includes 03 main components: (a) concave spiral microtubules arrangement; (b) microribbons (trilaminar structures); and (c) crossbridges. Four bilaterally symmetrical flagellar pairs (anterior, caudal, posteriolateral and ventral) are present. The anterior axonemes exit at the anterior end while the caudal axonemes exit at the extreme posterior part of the trophozoite. The ventral axonemes exit at the ventral disc posterior end (known as ventro-lateral flange region). The angled posteriolateral axonemes exit posterior to the ventral disc. Each axoneme consists of radial spokes, dynein arms and the central microtubule pair. The 08 basal bodies are present between the two nuclei (n). The funis has been reported to help maintaining *Giardia* l cell shape or flexion of the

posterior end of the organism during detachment process (Benchimol, M *et al.*, 2004). However the exact function of funis still remains elusive.

5. The *Giardia* Genome Project

The divergence of *Giardia lamblia* from prokaryotes to eukaryotes as traced by phylogenetics trees are gaining importance to decode genetic mechanisms that led to rise of eukaryotic cells. The *G. lamblia* genome bears eukaryotic features, like linear chromosomes with telomeres (multiple tandem repeats of the 5-mer TAGGG similar to other eukaryotic organisms) (Adam, R. D., *et al.*, 1991). The haploid genomic size of *G. intestinalis* (syn *G. lamblia* and *G. duodenalis*) was approximately 11.7 Mb and first reported by Morrison HG., et al (Morrison HG., et al, 2007). Each nucleus of the *G. intestinalis* isolate WB encloses a diploid set of 05 chromosomes inherited independently (Bernander R., *et al.*, 2001). Scientists have identified 5012 protein coding genes; the majority of these protein coding genes are conserved as compared to the earlier sequenced WB and GS strains genome (Jerlström-Hultqvist J., *et al.*, 2010). Like eukaryotic nucleosomes histone proteins, *G. lamblia* also has all four of the core histones (H2a, H2b, H3 and H4), which are very similar to their eukaryotic counterparts but not particularly to the Archaea's histone-like proteins (Wu, G., *et al.*, 2000). The initial molecular and genomic studies on *G. lamblia*, recommended a GC-rich genome. The comparison of G + C content of the overall assembly in various *Giardia* strains predicted 47%, 47% and 49% for P15, GS and WB strains respectively (Jerlström-Hultqvist J., *et al.*, 2010).

5.1 Giardia Genomics Resource: Giardia Data Base (Giardia DB)

Giardia lamblia is an amitochondriate protist which is environmentally transmitted and human pathogen causing diarrhoea condition. A major health concern causing enormous worldwide burden of human diarrheal diseases, but still now the basic biology and metabolic processes of this eukaryotic parasite are not clear. *Giardia* DB a pathogen-database maintained by 'The EuPathDB Project Team' under the NIAID-funded EuPath DB Bioinformatics Resource Center (BRC) is available online (http://*Giardia*db.org/*Giardia*db/) (Figure 5). The *Giardia* DB 1.0 version was released on 1 October 2007 and updated versions are available now (*Giardia* DB 5.0 released on 10 September 2014). *Giardia*DB 1.1 release contains the genome and annotation information for the *Giardia lamblia* ATCC50803, WBC6 isolate, generated at the MBL and published in 2007 (Science. 317: 1921-1926). The new data available includes: All genes query, 10 SAGE tag libraries, Microarray data and Proteomics data from trophozoites, and contig coordinates. The *Giardia lamblia* genome has only 12 million base pairs spread over five chromosomes. In addition no virulence factor has been recognized in the genome. The genome analysis of this parasitic protist is vital for understanding the origins of nuclear genome organization, various metabolic pathways, features of the prokaryotic / eukaryotic divergence, parasite-host interactions and cellular mechanisms to evade host immune systems.

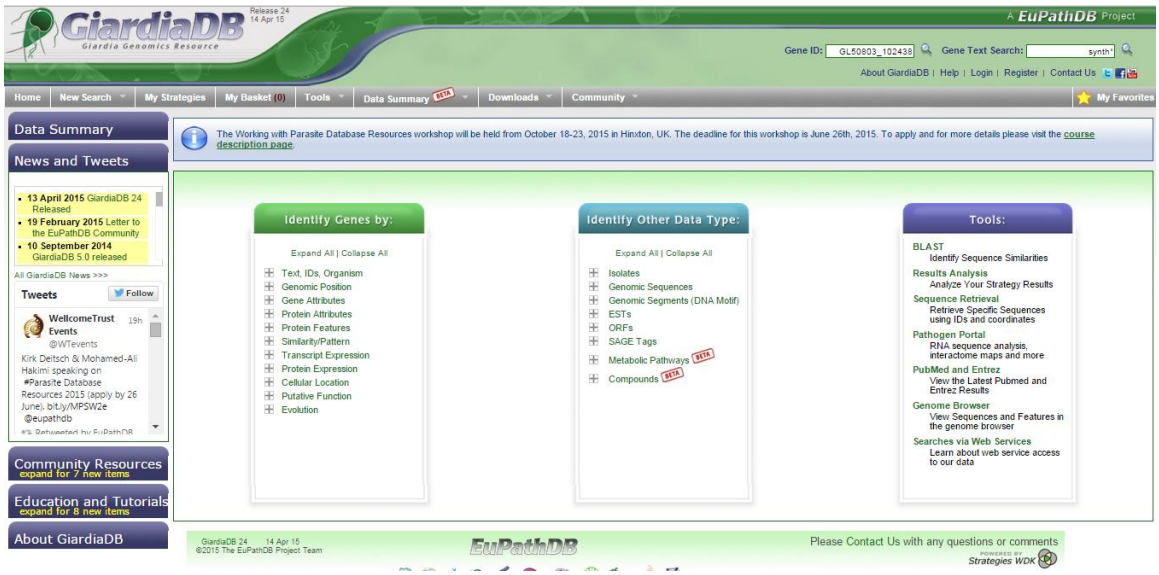

Figure 5. Screenshot of the *Giardia* DB (http://*Giardia*db.org/*Giardia*db/) **online database**

6. Summary

Giardia lamblia (also called as *Giardia intestinalis*, *Giardia duodenalis*) a flagellated unicellular eukaryotic parasite commonly causes waterborne outbreaks of diarrhea and occasionally food-borne diarrhea globally. The diarrhea disease caused by the giardial species is known as giardiasis. *Giardia lamblia* have two major stages in the life cycle: the cyst and the trophozoite. Infection in the mammalian host (such as humans) is caused by cyst ingestion via contaminated water or food or direct faecal-oral contact. Human giardiasis can be separated into two disease phases- acute giardiasis and chronic giardiasis. The acute phase is usually short-lived and abdominal cramps. While in chronic giardiasis, the patient suffers from weight loss with frequent pale or yellow stools. This binucleated flagellated protozoan alternates between an actively motile trophozoite (12 - 18 µm in length, up to 10 µm in breadth and 2 -4 µm in thickness) and a smaller infective, resistant cyst (measuring about 10 µm x 8 µm). The *G. lamblia* WB strain genome is of 11.7 Mb with 4889 annotated coding sequences, with no reported virulence factors in them. Whole genome-wide data tools helps to reveal insights to establish phylogenetic associations, host specificity and virulence genes, evolutionary history of these microbial pathogens, understand metabolic or biochemical processes and provides information for novel drug targets.