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Effect of COVID-19 Pandemic on Quality of Ganga River water in Uttrakhand: A Longitudinal Study

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Abstract

This study aims to show the Indirect effect of SARS Covid-19 on the River water of Ganga flowing in Uttrakhand. The Ganga River has shown a positive sign of quality improvement on many parameters due to the 68 days' nationwide lockdown tobreak the spreading chain of the Covid-19 pandemic in India. During that time, various types of industrially contaminated water and sewage water flowing by the hotel industry were closed as shown in the Abstract figure. The impact could be seen in reduced Biological oxygen demand (BOD) and chemical oxygen demand (COD) and total coliform count and faecal coliform count also found in decreased in number compared to previous studies results from the outcome of these collection points (fig. 1). This paper concludes that River can be rejuvenated if the rule made are strictly followed.

Keywords: SARS-CoV2; Lockdown; Pandemic; Water Quality Index (WQI).

Introduction

Rivers are an important part of the ecosystem. It is the main source of drinking water in our country. Therefore, cleanness of river water is very important for a Nation at present time water pollution is the biggest problem in developing Country. Ganga River was declared a national river in India. At present time, Ganga is also the most polluted river in India. Indian government has taken many initiatives for the cleanness of Rivers. A Big Project known as Namami Gangeyojna is going on. Ganga water pollution is a very challenging task for Govt. of India. The first case of COVID-19 was registered in India on 30th January, subsequently, a nationwide 14-hrs voluntary curfew was observed. On 22nd march 2020 due to an extreme jump in the positive cases. This curfew was extended for another 21 more days from 25th March to 14th April initially. This lockdown resulted in shutting down schools, industries, businesses, markets, religious and social gatherings, and kept people at home. It suspended all forms of travel except in case of emergencies and closed national and international travellers. The lockdown period was extended in many phases till June 8, 2020, and a lockdown of a total of 68 days completed with very strict guidelines, and the nation came to a standstill during this period at the same time there is lots of news coming out about pollution decrease in high levels.[1] This has led to a decline in emissions and restricted the production of industrial waste and other pollutants in the urban and rural centres of India during the duration of lockdown. Ever Since the lockdown applied due to COVID-19 in numerous countries has nearly seized the manufacturing, industrial events, and vehicle movement, a decrease in air pollution across the globe has been reported. According to the Ministry of Ecology and Environment, China, the air quality went up 11% in the category 'good' in as many as 337 cities[2] Scripps Institute of Oceanography reported that the use of fossil fuel would decline by about 10% around the world

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owing to the COVID-19 spread.[3] These upgrades in environmental pollution during lockdown are considered temporary; the current level of river Ganga pollution is reduced from various polluted regions of Ganga.

The water quality of five major rivers in the country, including the Ganga, deteriorated during the coronavirus-induced lockdown due to factors like the release of sewage and no upstream freshwater inflows, the Central Pollution Control Board said on Wednesday. According to the report, titled 'Assessment of Impact of Lockdown on Water Quality of Major Rivers', the quality of water in seven of the 19 rivers monitored by State Pollution Control Boards (SPCBs) improved during the lockdown period.[4]

While aerosol levels over the Indo Gangetic Plains reported a 20 year low during the lockdown as per the satellite data on optical depth measurements published by NASA due to restrictions imposed on industries, surface, and air transport.[5] The impact on water quality in the Ganga River was arguable. Various news reports, as well as social media posts, indicated that 'life seemed to be returning to the river'.[6] It was reported that the lockdown had improved the health of River Ganga, which many projects of the government could not do during the past two decades. The water quality of the Ganga River had witnessed visual improvement since enforcement of the nationwide lockdown started on March 24, 2020, which has led to a reduction in the discharge of industrial effluents into it. The lockdown was extended for more than seven weeks, with its 1.3 billion people instructed to stay home because of the coronavirus outbreak. With people, staying indoors and industries shut during the lockdown period, it is crucial to assess if the water quality in the Ganga River has indeed seen a significant improvement.[7] Researchers observed Signs of rejuvenation and a significant improvement on many parameters in the Ganga River, following nationwide lockdown due to coronavirus pandemic Lockdown period coincides excess rainfall (60 percent above normal), reduced irrigation and power demands in the basin resulting in increased storages and more flow in the river improving the quality Increasing trends of dissolved oxygen (DO) and decreasing trends of biological oxygen demand (BOD) and nitrate (NO3-) concentration River become fit for drinking (Class A) in the upper stretches and for outdoor bathing (Class B) in the middle and lower stretches Ganga river water classified in different classes. Table No.7[8]

Sources of Pollution

The Ganga River pollution made by various sources of pollution and the main source of pollution is municipal sewage, directly dumped in River without any treatment. The source of pollution can be divided into five types, Sewage pollution, Industrial effluent, Agriculture runoff, Religious Activities, and unplanned development.

Sewage Pollution

Discharge of sewage without treatment is 75% of total pollution with millions of liters generated per day in towns along the Ganga.[9] According to the total wastewater generation from 222 towns in the Ganga basin is 8250 MLD, while the treatment facilities are available only for 3500 MLD.[10]

Industrial Effluent

Thousands of Industrial units are situated in the Ganga Basin area and 956 only in Uttar Pradesh and approx. 10-30 industry situated very close to Ganga River in Uttrakhand. Various textiles, sugar mills, synthetic rubber industry, Paper pulp factories, and pesticides production unit. According to an estimate, about 2500 MLD of industrial wastewater is generated in the entire Ganga basin.[11]

Agriculture Runoff

Indo Gangetic plain is one of the most important plains in the world. It is 13% of the total geographical area of the country it includes Uttrakhand, Uttar Pradesh, Bihar, Jharkhand, and West Bengal. About 50% of the total food grain is produced in this region to feed 40% of the population of the country.[12]

Religious Activities

Gangaconsidered as a mokshadyini River means a dip in the Ganga liberates from the cycle of death and rebirth. The Ganga basin has many historical towns like Rishikesh, Haridwar, Garhmukhteshwar, Kannoj, Pyagraj, Mirzapur, Vanaras, and Ganga Sagar. These are important pilgrim centers where several religious activities like fairs take place throughout the year at the Bank of Ganga.[13]

Religious Bathing

Every day millions of peoples take baths throughout the Ganga, however, some of the auspicious days are particularly important when a large number of

people take a dip in the river. Kumbh is the main event for mass ritualistic bathing, which takes place at four places; Haridwar (Har Ki Pauri), Allahabad (Prayag), Nashik (Godavari Ghat), and Ujjain (Shipra Ghat) During Kumbh billions of people take bath at a specific stretch of river. Several devotees and ascetics reside on the bank of river Ganga during the whole Kumbh period. The effect of mass ritualistic bathing on the Ganga water quality during ArdhKumbh, Kumbh, and MahaKumbh, at different places, has been evaluated by various workers.

Temple Waste and Religious Material

More than 1000 tons of flowers and garlands are thrown in the river as an offering during the worship of Ganga as well as those used in the temples nearby. At various places such as Haridwar, Varanasi, etc. splendid evening prayer of Ganga is being held during that the devotees offer flowers and hundreds of floating lighted earthen lamps.[14]

Pollution due to Idol Immersion

Idol immersion is a religious activity that is also responsible for adding several pollutants in the rivers including Ganga. In India, a lot of religious activities take place around the year. Durga Puja is one of the most important festivals celebrated in West Bengal, Bihar, and Uttar Pradesh. In the last 15 years, Lakshmi Puja and Ganesh Chaturthi are also celebrated at an equal pace in Uttar Pradesh and Bihar, which wereoriginally belonging to other parts of India. In these festivals, huge numbers of Durga, Lakshmi, and Ganesha's idols of different sizes (up to 40 ft.) are formed every year and immersed in Ganga at the end of the event. The idols are constructed by plaster of Paris, clay, cloths, small iron rods, bamboo and decorated with different paints such as varnish, watercolors, etc., plastic, and polystyrene that can lead to a significant alteration in the water quality after immersion. Paints that are used to color these idols contain various heavy metals/metalloids, such as As, Cd, Cr, Hg, and Pb which are known carcinogens.[15]

Dead body cremationin India it is believed that death and cremation along the banks of the Ganga, particularly in Varanasi, releases the soul from the cycle of rebirth and the soul shall directly go to heaven. Due to this religious belief, thousands of dead bodies are being cremated every day on the bank of Ganga.[16]

Material and Methods

Graphical Abstract

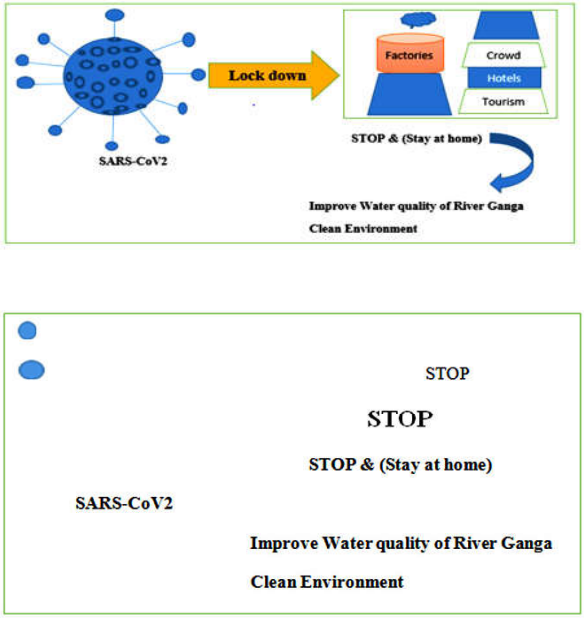

Fig.1: A Graphical Abstract of Covid -19 effects.

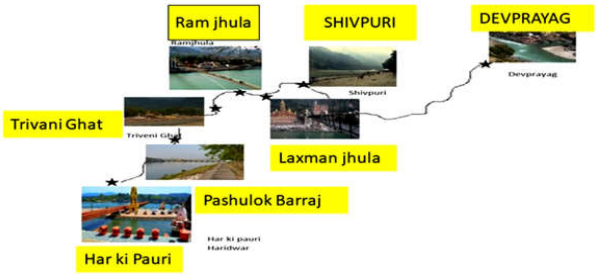

Fig. 2: Showing the View of All Collected Ganga water Samples Sites.

Study Area

In this study, the water quality of the Ganga River has been assessed between DevPrayag to Haridwar located at Uttrakhand during the lockdown period April to May 2020 for water quality testing we have used some physicochemical and Microbiological parameters and compared them with pre lockdown water quality data of February 2019.

River Water Sample Collection Procedure

- According to the requirement of test protocol sample nature, are different and water Samples were collected in a triplicate form.
- A two different new sterile plastic containers.
- Note-sample was collected in triplicate from

each site and then pours & mix in a particular site labelled container with a waterproof marker. The date, time, site & nature of the specimen should be mentioned on this slip.

Table 1: Water samples collection procedure in different containers.

1st Container (1 litre) For Physio-chemical Analysis	2rd Container (1 Litre) Microbiological Analysis
Water sample collected from 0.5m depth from the surface of river using a clean plastic bucket, transfer in to labelled container and transported to the laboratory on ice and stored in a deep freezer (-200c) till analysis.	Water sample collected from 0.5m depth from the surface of River using a sterile sample bottle. • Aseptically remove the cap and cover of the sterile sample bottle. • Face the mouth of the bottle upstream. • Plug the neck downwards about 30 cm below the water surface • Tilt the neck slightly upwards to let it fill completely before carefully replacing the cap and cover

Water samples were collected during the lockdown in all phases between April to May 2020 from five study designed sampling sites. Showing in fig. no. 2 Water sampling sites of the Ganga River flowing in Uttrakhand were nominated on the source of catchment characteristics and sources of anthropogenic involvement along its course of a run. Among water sampling sites of Ganges, two sites were of hilly regions Devpyag (GD), Shivpuri (GS), and others five sites were Laxman Jhula Rishikesh (GL), Ram Jhula (GR), Triveni Ghat (GT), Barrage (GB) and Haridwar (GH) of Uttrakhand. From every sampling site, water was collected in triplicates at the depth of 10 cm below the superficial water level; water samples were mixed and poured in two separate sterile polyethylene containers. Each container filled with water sample was tightly sealed and labelled with a site of water sampling and mode of specific analysis viz physicochemical & microbiological analysis described in tab. No.1 Water sample containers were transported and stored in an icebox shield until analysed in laboratories.

Physiochemical Analysis

All Ganga river water samples collected in polyethylene sterile containers from eleven different sites were checked for physicochemical analyses using a specific methodology. pH, temperature, conductivity, and dissolved oxygen were measured by HACH HQ40D portable multipara meter two channels advanced digital meter. Dissolved Oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), and total solid solutes were measured by Wrinkle's methods, volumetric analyser, and titration methods.[17]

Microbiological Analysis of Water samples

The precise identification of pathogenic bacteria is tremendously challenging; the coliform group of organisms is used as an indicator of the presence in the wastewater of pathogenic organisms. Coliform bacteria are found in the intestinal tract of human beings. The coliform group of bacteria includes genera Escherichia. (According to Environmental protection Agency) the contaminant level of Coliform count should be zero per 100 ml of water for drinking purposes and for the bathing purpose the faecal coliform should be less than 500 /100 ml of water. Most probable number test (MPN Test) – Included presumptive test, confirmation test, and complete test. In the MPN method by the use of sterile pipette 10 ml, 1ml, and 0.1ml of water samples from sterile containers were inoculated in a 50 ml test tube having 10 ml double strength Lauryl Tryptase (LT) broth medium and 5 ml single strength and 5 ml LT broth respectively. All tubes were incubated for 24 hours at 37°C. After the growth of mixed culture, they were inoculated in the Broth culture tube for confirmation test viz. Brilliant Green Bile Broth (BGBB), EC Broth, and Tryptone water, Azide Dextrose for a complete test. Selenite F Broth, Alkaline peptone water was used to a culture of some specific bacteria like Vibrio cholera and salmonellae typhi species, growth present was then subcultured on Xylose Lysine Deoxy Cholate (XLD), Thiosulfate Citrate-Bile Salt Sucrose (TCBS) Agar culture plate respectively. Various morphological characteristics of improved isolates, colony morphology shape, color, arrangement, biochemical tests, and Gram staining were carried out for the identification of isolates. The water samples were also checked for Fungi using two methods, direct plate and dilution plate with the use of two types of growth media Sabouraud's dextrose agar (SDA) and potato dextrose agar (PDA) incubate for 7 days on 25^0c.

For Salmonella and Shigella Culture Procedure:

Firstly takes the 20-25 ml of Ganga water sample and centrifuged at 1520x g for 15 min and after that pour off all supernatant except 1-2 ml bottom part and re-suspend the pellet and add 8 ml selenite F or GN Broth and incubate for 24hrs at 35^0C examine to the plate and streak on the TSI slant incubate at

37^0C for overnight confirm by oxidase and catalase test.

For Faecal Streptococci Count

Inoculate loop full of a sample was taken from a positive presumptive tube in an Azide dextrose Broth (selective media for faecal streptococci) and incubate on 45^0C for 24 hrs. If the turbidity is, found then streak loop full material on bile Esculin Agar at 44-45^0C for 24hrs. Presence of brownish black colonies with brown halos sign of confirmation presence of streptococci.

For Vibrio Species

In this procedure, we have used alkaline peptone water with added colistin and incubate 6-8 hrs. at 35^0C if the turbidity occurred then streak the loop full material on TCBS Agar (Thio sulphate Citrate bile salt sucrose Agar) look for yellow colonies on TCBS Agar and typical haemolytic colonies on sheep Blood Agar if these color colonies found then identification process required by agglutination with Antisera.

WQI

The water quality index is the way to represent the water quality only. While computing WQI there are three steps, which we have to be taken. Firstly, we assigned the weight (wi) for each selected parameter as per their relative importance in describing the water quality for drinking purposes. The assigned weight remains within a range of 1 to 5.

Secondly, we calculate the relative weight (Wi) for all chooses parameters with help of the following equation.

$Wi = wi / \sum wi$ (i =1 to n)

Where Wi is the relative weight, wi is the assigned weight to the chosen parameters.

Final and last step, we go to calculate the quality scale (qi) for each parameter by following the equation give below.

$qi = (Ci / Si) \times 100$

Where,

Qi is the quality rating,

Ci is the trace concentration of each chemical parameters

Si is the standard.[18]

For computing the WQI, firstly we have to determine the sub-index (SI) for each selected parameters with the help of the following equation

$SIi = Wi \times qi$

$WQI = \sum SIi\text{-}n$

The Calculated WQI values are categorized into five categories: excellent water (W<50); Good Water (WQI=50-100); Poor Water Quality (WQI=200-300) and water unsuitable for drinking (WQI>300)(19). The results of the water quality index calculation shown in table no 2, 3 and 4.

Statistical Analysis

The statistical analysis work done by using paired t test in Graph pad prism software version 5.0.

Results and Discussion

All Physicochemical and microbiological analyses were carried out at Namami Gange Research Unit, AIIMS Rishikesh. Pre lockdown data analysis was done in Feb-march 2019 and during the lockdown period, Ganga water samples were collected every lockdown phase I to IV and analysed.The parameters such as pH, TDS, Conductivity, BOD, and DO were found within acceptable limits in water samples collected before and during a lockdown. The Chemical oxygen demand of Ganga water samples collected from Rishikesh Triveni ghat and Barrage siteswas found in high amount in Pre lockdown period data andCOD had decreased during a lockdown. In our study, the physicochemical parameters result (table. 2) during the lockdown period showed better improvement in Ganga water quality.

Table 2: Variations of Microbiological parameters (MPN, TCC, FCC, E.coli count and FSC) in collected Ganga water samples of Pre-lockdown and During Lockdown periods.

Study Location		Microbiological Indicators					
Site	**Timing**	**MPN count**	**Total Coliform count**	**Faecal coliform count**	**E.coli Count**	**Faecal streptococci count**	**Salmonella, Shigella, V. cholaerae, Fungal (SDA,PDA**
GD	Pre Lockdown	–	–	–	–	–	No growth
	During Lock down	–	–	–	–	–	No growth

GS	Pre Lockdown	–	–	–	–	–	No growth
	During Lockdown	–	–	–	–	–	No growth
GL	Pre Lockdown	111 ± 12.7	87 ± 8.4	26.3 ± 2.3	35.3 ± 5.1	–	No growth
	During Lockdown	–	–	–	–	–	No growth
GR	Pre Lockdown	140 ± 14.1	111 ± 12.7	41.6 ± 1.8	71.3 ± 5.1	04	No growth
	During Lockdown	71.3 ± 5.1	41.6 ± 1.8	20.6 ± 0.4	21.3 ± 1.2	–	No growth
GT	Pre Lockdown	1100 ± 00	460 ± 00	230 ± 14.1	240 ± 00	–	No growth
	During Lockdown	453 ± 12.4	245 ± 3.2	208 ± 2.8	216 ± 6.2	–	No growth
GB	Pre Lockdown	2400 ± 00	1100 ± 00	376.6 ± 117	376 ± 117	–	No growth
	During Lockdown	1100 ± 00	460 ± 00	376± 117	210 ± 00	–	No growth
GH	Pre Lockdown	140 ± 14.1	111 ± 12.7	111 ± 12.7	140 ± 14.1	–	No growth
	During Lockdown	41.6 ± 1.8	26.3 ± 2.3	20.3 ± 0.4	24.6 ± 2.3	–	No growth

Table 3: Computing WQI for Pre Lock down.

Parameters	Weight (wi)	Relative Weight Wi=wi ∑wi	Ci Pre Lockdown	Si	qi=(Ci/Si)×100	Sub Index SI=Wi×qi
pH	4	0.5	7.17	7	102.43	51.21
TDS	4	0.5	120.3	500	24.06	12.03
	∑wi=8	∑Wi=1				∑SI=63.24

WQI=∑SI=63.24

Table 4: Computing WQI for during Lock down.

Parameters	Weight (wi)	Relative Weight Wi=wi ∑wi	Ci Post Lockdown	Si	qi=(Ci/Si)×100	Sub Index SI=Wi×qi
pH	4	0.5	7.25	7	103.57	51.8
TDS	4	0.5	154.3	500	30.86	15.43
	∑wi=8	∑Wi=1				∑SI=67.23

WQI=∑SI=67.23

Table 5: Showing the water quality category of both sampling periods.

WQI Value	Water Quality	Sample Description	Pre Lock down	During Lock down
<50	Excellent	NA	×	×
50-100	Good Water	During Both Study period WQI	✓	✓
100-200	Poor water		×	×
200-300	Very Poor Water		×	×
>300	Water Unsuitable for Drinking		×	×

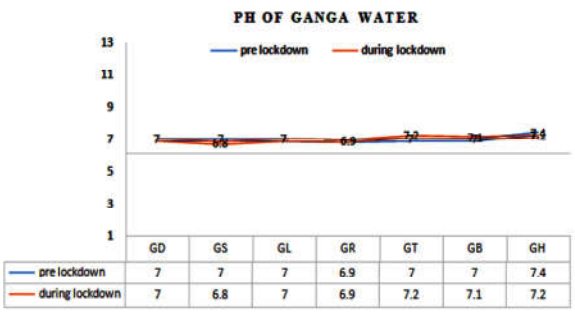

Fig. 3: Variation in pH during and pre lockdown stage of Ganga Water samples from seven different sites.

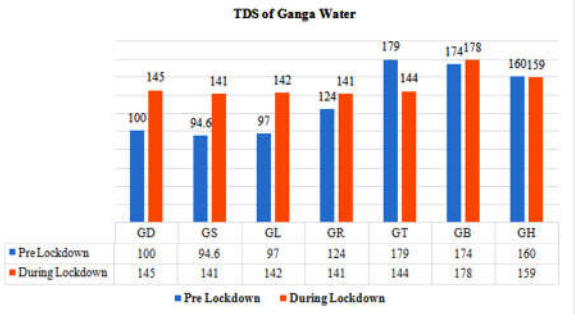

Fig. 4: Variation in TDS during and pre lockdown stage of Ganga Water samples from seven different sites.

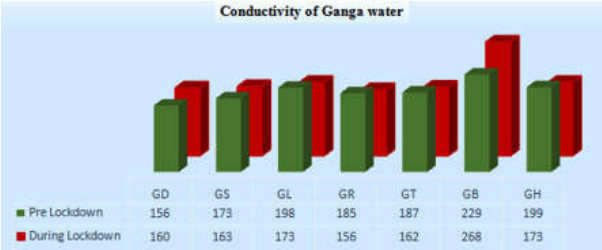

Fig. 5: Variation in conductivity during and pre lockdown stage of Ganga Water samples from seven different sites.

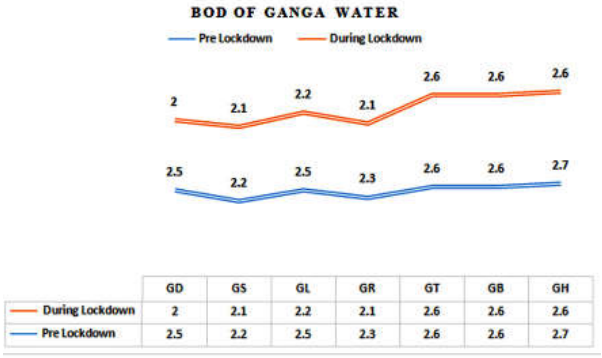

Fig. 6: Variation in BOD during and pre lockdown stage of Ganga Water samples from seven different sites.

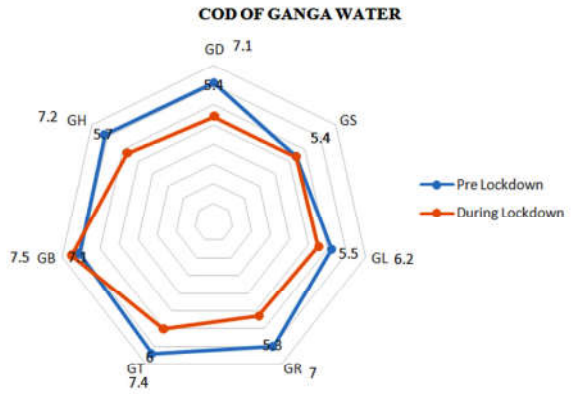

Fig. 7: Variation in COD during and pre lockdown stage of Ganga Water samples from seven different sites.

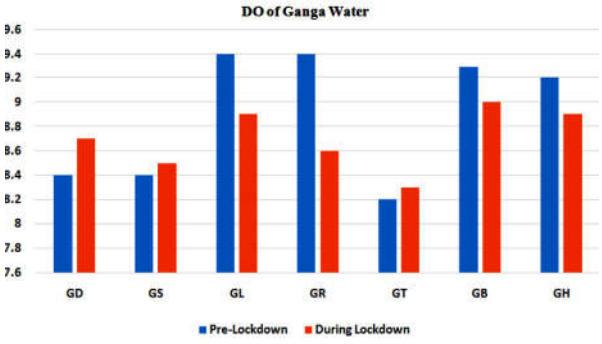

Fig. 8: Variation in DO during and pre lockdown stage of Ganga Water samples from seven different sites.

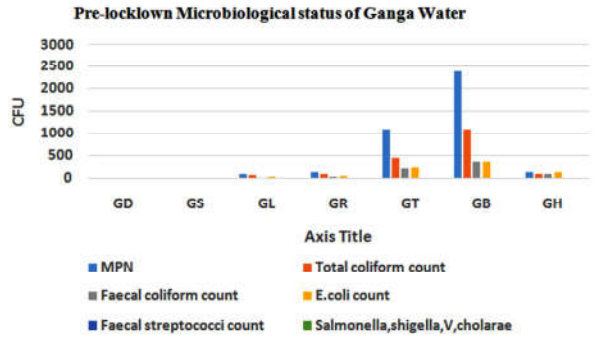

Fig. 9 (a): Variations in Bacterial count of different Ganga water collected samples in pre-lockdown.

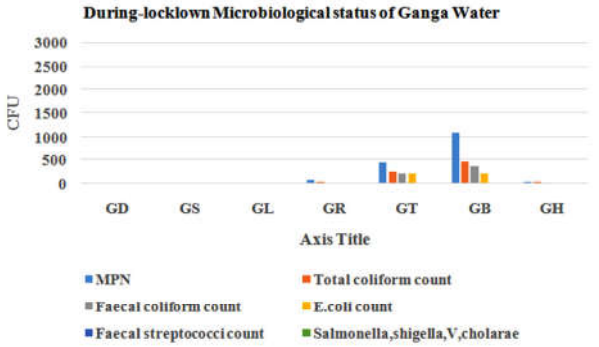

Fig. 9 (b): Variations in Bacterial count of different Ganga water collected samples During-lockdown period.

In our study, the microbiological analysis showed no microbial contamination before and during the lockdown in the first two collection sites i.e., Devpryag and Shivpuri.In our third collection site Laxman Jhula having some microbial contamination before lockdown water samples. and there was no microbial contamination during the lockdown phases. There was a 50% decrease in microbial contamination from other sample collection sites such as Ramjhula, Trivenighat, Barrage, and HarkiPauri Haridwar. In Prelockdown period feb 2019 we found some pathogenic Bacteria like Acenatobactor, Klabsiallae, Pseudomonas and Enterococci found that time but we found E. coli during Lockdown periods collected water samples. It's a positive sign on behalf of microbial contamination in river water of Ganga. Water quality Index the pre lockdown and during lockdown are approximately equal calculated values ∑SI=67.23 for pre lockdown and during lockdown value was ∑SI=67.23 and water quality found in good condition with the Normal range (50-100) table No. 6. Our study aims to bring out the effect of lockdown on the water of the Ganga River in Uttrakhand. Thisstudy mainly focused on the effect of lockdown on the water quality of River Ganga. Results of the study intelligibly announce

that there was significant devaluation in water pollution indicators during this lockdown these results of the study are similar to other studies.A lot of reports showing improvement of water quality of Indian River like Hindon, Yamuna, Cauvery, and Ganga has Improved during the Current lockdown due to Covid-19 crisis.[20] There was also a reduction in bacteriological count in the Ganga River during a lockdown.while aerosol levels over the Indo Gangetic plains reported a 20 year low during the lockdown due to restrictions imposed on Industries, surface, and air transport.[21] A questionnaire based survey found that the population of the plain area had a high incidence (75%) of water-borne disease as compared to the hilly area (20%) (Table 4). The estimated result of hazard quotient (HQ) and hazard index (HI) for non carcinogenic concern related to adult and child by ingestion of Ganga River water.[22]

Conclusion

Our studyindicates that all parameters of water quality assessment have significantly improved that it has shown that Ganga auto purification increased very fast. Its shows a positive angle of Lockdown Covid-19 on nature. The Government of India has spent thousands of crores of rupees on many cleaning programs for rejuvenation of the Ganges for the last several years and is doing so further. However, the Ganges has not yet been completely clean. There has been a pleasant experience also during this so called trial based pandemic era. Human activities were restricted in making an addition to waterpollution by doing so called holy celebration or ritual activities. In the lockdown period, the public was restricted mostly in some boundaries, home, and town, so they could not make a frequent visit for pilgrimage and tourism. Due tothis, Ganga water quality from devprayag to Laxman Jhulahas changed from Class B to Class A. Now it has become potable for drinking water sources without conventional treatment.

Conflict of interest: No conflict of interest

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Review Article

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Epidemiology and Diagnosis of Bluetongue Virus in India

Koushlesh Ranjan

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Abstract

Bluetongue (BT) is an economically important, vector-borne, non-contagious, viral disease of ruminant animals. It is caused by the Bluetongue virus (BTV) and belongs to the genus *Orbivirus* under the family *Reoviridae*. The clinical form of the disease is reported in sheep, white-tailed deer, bighorn sheep, etc. The subclinical infections are reported from camelids, cattle, and goats. Due to its high morbidity, mortality, and huge economic loss to the livestock sector, BT is listed as a multispecies disease by World Organization for Animal Health (OIE). The diagnosis of BTV is required for the identification of etiological agents and control of disease which is essential for the international trade of livestock and its products. Some of the diagnostics techniques such as reverse transcription-polymerase chain reaction (RT-PCR), competitive enzyme-linked immunosorbent assay, agar gel immunodiffusion assay are OIE recommended tests for BTV diagnosis at the international level. For BTV control, serological and vector surveillance, vector control, mass vaccination of susceptible animals, etc. are used.

Keywords: Bluetongue virus; Vector; Culicoides; Serotype.

Introduction

Bluetongue (BT) is a viral disease of ruminants caused by the Bluetongue virus (BTV) of the genus *Orbivirus* under the family *Reoviridae*. The genome of BTV consists of 10 segments of dsRNA surrounded by icosahedral capsid. BTV encodes 7 structural (VP1-VP7) as well as 5 non-structural (NS1-NS5) proteins (Stewart et al. 2015). BTV infection led to huge economic losses which are associated with high morbidity, mortality, abortions, fetal abnormalities, stillbirths, reduced milk yield, etc. Due to severe economic loss, trade restrictions are imposed on live ruminant animals, their products, and germplasm from BT-infested to BT-free countries (Gethmann et al. 2020). BT is also listed under the multispecies disease section by World Organisation for Animal Health (OIE) (OIE, 2008). The clinical form of the disease is mostly reported from pronghorn antelope, white-tailed deer, llamas, alpacas, and sheep. However, goats, cattle, and camelids usually remain asymptomatic or may exhibit the sub clinical form of the disease (Schulz et al. 2012). The major route of transmission of BTV to the susceptible host is by the biting of *Culicoides* spp. (Benelli et al. 2017). However, BTV transmission may also take place via alternative routes such as oral, venereal, and transplacental transmission (Saminathan et al., 2020). Based on differences in the segment-2 genome sequence, there are 28 different BTV serotypes have been identified globally (Bumbarov et al. 2020). The BTV-27 was identified in France in 2014 in goat (Schulz et al. 2016) and BTV-28 from live-attenuated lumpy skin disease and sheep pox vaccines in Israel (Bumbarov et al., 2020). Being India a vast country with a tropical climate, huge ruminant animal population,

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and presence of *Culicoides* vector, several BTV serotypes have been reported (Rao et al. 2016). In a current review paper, the epidemiology of BTV along with its diagnosis and control has been discussed especially in the Indian scenario.

BTV Epidemiology in India

BT is endemic in the Indian subcontinent and causes huge economic losses to the livestock sector. Most of the BTV outbreaks in India were reported in cross-breed and exotic breeds of sheep. However, native breeds of sheep were also found susceptible to BTV in south India. Due to the endemic nature of BT in India, several BTV serotypes have been reported. Out of 28 serotypes of BTV, 23 serotypes (except serotype 22 and 25-28) have already been reported from different regions of India by virus isolation and identification of BTV specific neutralizing antibodies (Ranjan et al. 2015; Krishnajyothi et al. 2016; Hemadri et al. 2017). Previous study suggested the seropositivity of cattle, buffalo, goat, camel, and Mithun for BTV specific antibodies without exhibition of clinical form of the disease (Karam et al. 2018). Studies also suggested that most of the BTV serotypes were isolated from the southern states of India (Krishnajyothi et al. 2016; Hemadri et al. 2017).

Isolation of BTV Serotypes in India

Since the first reported case of BT in India in the 1960s, several BTV outbreaks have been recorded throughout the country. Bhambani and Singh (1968) successfully isolated the BTV in sheep in Uttar Pradesh without confirming the virus serotype. Subsequently, BTV-2 was isolated from Tamil Nadu in 1982 (Maan et al. 2012). Later on, several other serotypes were also reported in India such as BTV-2 (Ranjan et al., 2012), BTV-9 (Ranjan et al., 2013), BTV-10 (Prasad et al., 2013), BTV-16 (Dadawala et al., 2013; Ranjan et al., 2014) and BTV-23 (Ranjan et al., 2017), etc.

With the advancement in molecular techniques, other BTV serotypes such as BTV-1 from aborted and stillbirth foetuses of goats from Gujarat (Chauhan et al., 2014), BTV-12 from sheep flocks in Andhra Pradesh (Rao et al., 2015), BTV16 from goats in Tamil Nadu (Minakshi et al., 2012), BTV-16 from sheep in Karnataka (Ranjan et al., 2016), etc. were isolated. In 2010, BTV-24 was identified from sheep in Telangana during BT outbreaks. The vp2 gene sequence analysis revealed its close similarity with western BTV-24 isolates indicating the entry of exotic serotype in the Indian subcontinent (Krishnajyothi et al. 2016). Similarly, BTV-5 was isolated in Karnataka in sheep during 2010-2011 (Hemadri et al. 2017).

The first generation capillary sequencer can also be used for complete gene sequencing of BTV serotypes. In one of the studies, a capillary sequencer was used for complete gene (vp2, vp5, and ns1) sequencing of the Indian isolate of BTV-16. The result revealed that vp2 and vp5 gene was of eastern topotype origin whereas ns1 gene was belonging to western topotype, indicating the reassortment in BTV-16 in India (Kumar et al., 2013). Next-generation sequencing (NGS) or deep sequencing techniques has revolutionized molecular research. The NGS-based machines are much faster than first-generation capillary sequencing machines (Minakshi et al., 2014). The complete genome sequencing and subsequent phylogenetic analysis have assisted in the identification of several reassortant BT viruses having both eastern wells as western topotype genome segments. Full genome sequencing of BTV-2 revealed the presence of nine genome segments of the eastern topotype and one segment (Segment-5) of the western topotype in origin, indicating the genomic reassortment (Maan et al., 2012). Similarly, a reassortant strain of BTV-2 was isolated in India which showed segment-5 and segment-9 of western topotype origin and segment-2 of eastern topotype origin from other BTV-2 strains. The segment-6 of this virus was found closer to the eastern topotype strain of BTV-1 which indicated the reassortment in outer-capsid proteins (VP2 and VP5) (Maan et al. 2015). The study also revealed that the reassortment between genome segments of eastern and western origin may result in the origin of BT viruses with enhanced virulence which may cause a subsequent outbreak in Indian sheep breeds (Maan et al., 2012).

Diagnosis of Bluetongue

Diagnosis of animal viral diseases is essential for a successful control, eradication of disease, and reduction in economic losses (Hamblin, 2004; OIE 2008). BT can be tentatively identified by its clinical sign. Laboratory diagnosis of BTV depends mainly on the identification of antigens and antibodies. The antigen identification assays include virus isolation either in cell lines or embryonated chicken eggs (ECEs), immunofluorescence test, virus neutralization test, reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, sandwich enzyme linked immunosorbent assay (s-ELISA) (OIE 2008; Rojas et al. 2019), etc.

The RNA-Polyacrylamide gel electrophoresis

(RNA-PAGE) is a sensitive and low cost technique for the detection of genome segments of BTV. Several modifications in RNA-PAGE and silver staining for the identification of the BTV genome have been done by researchers. One such modification was carried out as the development of a novel staining method for RNA-PAGE where ultrasensitive eriochrome black t-silver staining (EBT-SS) was used to stain BTV RNA-PAGE. The novel staining protocol was found eight times more sensitive than routine silver staining of BTV RNA-PAGE (Minakshi et al., 2013). Several other studies have also highlighted the importance of RNA-PAGE in the identification of BTV genome segments (Ranjan et al. 2015; Rojas et al. 2019).

The virus neutralization test (VNT) is commonly used for serotyping of newly isolated BTV serotypes. VNT is the gold standard assay for serotyping of BTV isolates (OIE, 2008). The major disadvantage of this assay is the requirement of standard reference sera of known BTV serotypes which may be difficult especially during the first time outbreak of newer serotypes (OIE, 2008).

The sandwich ELISA (s-ELISA) or antigen-capture ELISA (Ag-ELISA) is a very sensitive assay and is commonly used in the laboratory for the detection of antigen. The MAb-based s-ELISA of a detection limit of 104 TCID50/ml was developed for direct detection of BTV in infected serum samples (Ten Haaf et al., 2017).

The immunofluorescence assay is a reliable assay for the detection of BTV serotypes. In immunofluorescence assay, MAbs or primary antibodies are subjected to bind with a secondary antibody which is tagged with specific fluorochromes viz., fluorescein isothiocyanate (FITC) to give detectable fluorescence (Rojas et al., 2019). For the identification of BTV, MAbs against group-specific VP7 protein have been widely used.

The segment 2 (vp2 gene) based primers in RT-PCR are commonly used for the identification of BTV serotype. The RT-PCR is a highly sensitive technique and can detect up to ten infectious BTV particles in cell culture grown virus (Prasad et al., 1999). Similarly, nested PCR is even more sensitive and may detect up to five BTV particles (Ayanur et al. 2016).

With the advancement in first generation capillary sequencing and next generation sequencing (NGS) technologies, a paradigm shift occurred in the detection of serotype and topotype of BTV (Maan et al., 2012). Genome sequencing generates a huge amount of sequencing data which can be used for various purposes such as designing serotype-specific primers which can be used for screening of newer BTV serotypes. The only disadvantage of sequencing technology is that it is not a cost-effective technique and is also not available in all the research institute/laboratories.

Real-time PCR is much sensitive than conventional PCR assay. It can identify the very low level of BTV specific RNA from cell culture-grown viruses, viraemic animals, a semen sample, etc. (Saminathan et al. 2020). Segment 1, segment 2, segment 5, and segment 10 based real-time PCR assay have been successfully used for diagnosis of BTV (Toussaint et al. 2007; van Rijn et al. 2012). Segment 5 (ns1 gene) of BTV is the highly conserved genome segment. Therefore, segment-5 based real-time PCR is commonly used for the identification of BTV serotypes (Vishwaradhya et al. 2013).

Apart from a direct antigen or nucleic acid detection, BTV can also be diagnosed in the animal sample by detection of BTV specific antibodies by agar gel immunodiffusion (AGID), competitive ELISA (c-ELISA), indirect ELISA (i-ELISA), serum neutralization test (SNT), etc (Rojas et al. 2019). The c-ELISA, RT-PCR, and AGID are sensitive assays and recommended by OIE for the diagnosis of BTV in international trade (Rojas et al. 2019).

The AGID is a serological assay where soluble BTV antigen is precipitated by a specific antibody in a medium made up of transparent agarose gel. AGID assay is usually used for diagnosis of group specificity of BTV using antibodies against the conserved VP7 protein of BTV (Chandel et al. 2003).

For BTV specific antibody detection, ELISA is a reliable technique. For antibody detection, different formats of ELISA are used. The c-ELISA is commonly used to detect the BTV specific antibodies in ruminant animal sera (Rojas et al. 2019) because it is a more specific, sensitive, and rapid method than other serological assays such as AGID, plaque neutralization assays, and CFT (Kramps et al. 2008). It is also extensively used for monitoring BTV during national and international trade (Rojas et al. 2019). In one of the experiments, VP7 protein based antigen capture c-ELISA was described to detect antibodies against EHDV and BTV where VP7 antigens were expressed in the baculovirus expression system (Mecham and Wilson 2004).

The other ELISA format commonly used for BTV specific antibody detection is i-ELISA. The i-ELISA is a rapid technique for the detection and quantification of antibodies in serum samples (Chand et al. 2019; Rojas et al. 2019). The NS3 antigen-based i-ELISA has been employed for the

differentiation of BTV vaccinated animals to the BTV infected ones. In infected animals, higher levels of NS3 specific antibodies are detected in comparison to vaccinated animals. Thus, i-ELISA can be used for the DIVA strategy whereas c-ELISA can't be used for DIVA (Rojas et al. 2019). The major disadvantage of i-ELISA is that it requires species-specific secondary antibody conjugates, which may create a practical problem for routine sero-diagnosis of BT like multi species disease (Chand et al. 2017).

Control and Prevention of BTV

BTV outbreaks can be controlled by mass vaccination of susceptible ruminant animals, vector control by use of insecticides, vector repellents and attractants (decoy host), and larviciding agents, serological as well as vector surveillance and sentinel program (Mayo et al. 2017; van Rijn, 2019; Ranjan et al., 2019). In India, a pentavalent inactivated adjuvant vaccine having serotypes 1, 2, 10, 16, and 23 has been developed and licensed for the BT control program (Reddy et al. 2010).

Conclusions and Future Perspectives

BT is an endemic disease in India due to the hot and humid climate which favors Culicoides breeding. Most of the BTV serotypes (23 out of 28 reported) have been identified from India probably due to the reassortment of BTV genome segments, a large number of ruminant animals, and the Culicoides vector population. BTV study in India suggested that regular sero surveillance programs should be maintained to monitor the emerging, re-emerging, and endemic nature of BTV serotypes. The data from such surveillance programs are a gold mine for the development of an area specific multivalent vaccine that can be used as a tool for the control of BTV in endemic regions. Moreover, for effective BT control, vector control programs need to be executed in endemic regions. Simultaneously, the role of wild ruminants and vectors in wildlife habitats should also be monitored from an epidemiological point of view for successful BTV control because wild ruminants may act as a reservoir host for BTV along with vector maintenance. Moreover, the interface of domestic and wild ruminants near the forest areas should also be monitored to control the spread of BTV to domestic ruminants.

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A Review on Tannase and its Applications

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Abstract

Tannin acyl hydrolase (E.C. 3.1.1.20) or Tannase is a type of hydrolase enzyme that catalyzes the hydrolysis of tannin and is converted to gallic acid and glucose. Natural tannins occur in various parts of plants such as leaves, roots, bark and fruits. Tannins are distributed in animals, insects and microorganisms. However, microbial tannase is one of the great attention in many industries. Gallic acid is one of the industrial and therapeutic molecules that is widely used in various applications. Tannase is also applicable in feed, pharmaceutical, brewing, chemical and beverage industries. Furthermore, tannase plays a significantly important role in biodegradation or reduction of tannin from the tannery effluent. The present review apprises a brief description of tannase and its industrial applications.

Keywords: Tannin; Tannase; Gallic acid; Glucose; Tannery effluent.

Introduction

Tannases represent a group of enzymes, that are produced by microbes, plants and animals. However, tannase from microbial sources is preferred over other sources for industrial. The catalytic reaction of tannase involves the hydrolysis of ester bond and depside bond present in various substrates i.e., tannic acid, complex tannin, gallic acid esters, epigallocatechin gallate, methyl gallate, ethyl gallate, n-propyl gallate to produce gallic acid (3, 4, 5-tri hydroxybenzoic acid) and glucose. Various parameters such as temperature, pH, metal ions, activators, inhibitors and substrate specificity affect the enzyme activity.

Tannase enzymes are used in the food, brewing, and pharmaceutical industries. Gallic acid production is one of the most important commercial applications of tannase. Apart from that, they are extensively used in the food industry, especially in instant tea production, where it enhances the extractability and cold water solubility of key compounds. Another important application of tannase is the removal of haze formation and unflavored phenolic compounds from beer and wine and fruit juices. The quality of fruit juices also can be improved by the tannase enzyme.

Industries require novel microbial strains for enzyme production using easily available inexpensive raw materials and trying to evolve various dimensions of tannase such as novel sources of tannase, new microbes with high tannin transformation ability, new substrate specificities, low cost production, less expenditure on the purification strategies and the potential use of tannase in different industries has been shown by researchers through out the world. The current review articles explain in detail the structure, purification and application of tannase produced by microbes.

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Tannins

The meaning of Tannin or Tanna (German word) is oak, which refers to the use of tannins from the wood/bark of oak or fir trees that were used in the tanning process (Barbehenn and Constabel, 2011; Tomak and Gonultas, 2018). Tannins are well known large, complex, natural plant derived polyphenolic biomolecules that are found in the bark, stem, buds, wood, leaves, fruits and roots. Tannin plays a very important role in protecting plants from microorganisms and other predators (Khanbabaee and van Ree, 2001; Sharma, 2019). In a few countries, tannin rich plant extracts serve as traditional medicines for the treatment of cancer, diarrhea, inflammation, dieresis, hemorrhagic stroke and skin (Dai, 2010 and Działo, 2016).

Based on diversified structures and properties of tannins, they are classified into three major classes and two subclasses: (1) Hydrolysable tannins (2) Condensed tannins and (3) Complex tannins (Figure 1). Hydrolysable tannins can be defined as the types of tannins that are hydrolysed into gallic acid and ellagic acid in the presence of acid or alkaline conditions, hot water and enzymes. Hydrolysable tannins are further classified into two subclasses based on their hydrolysable products i.e. (i) Gallotannins and (ii) Ellagitannins. Hydrolysable tannins are mostly found in berries, grapes, nuts, coffee, tea, fruits and wine. The second class is condensed tannin or non-hydrolysable tannin. They are flavanoid tanning substances that belong to oligomeric and polymeric proanthocyanidins. Condensed tannins are obtained from cocoa, various fruits like pears, apples, green grapes, legumes such as chickpeas, red kidney beans and peas, respectively (Costain, 2001; Selma et al.,2009). The third class of tannin is complex tannin. Complex tannins or non-classified tannins have catechin units that are glycosidically bound to the gallic acid (gallotannins) or ellagic acid (ellagitannins). Several sources of complex tannins are camelliatannins A and B (Camellia japonica L), malabarin A (Melastoma malabathricum) and acutissimin A. (Okuda and Ito, 2011).

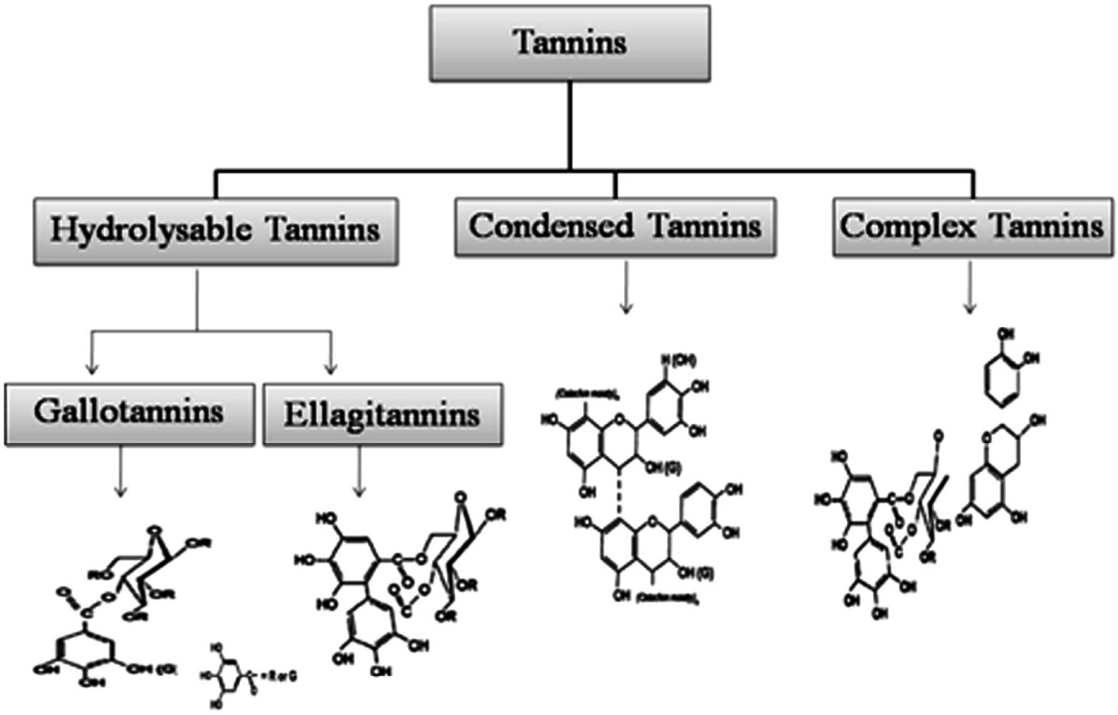


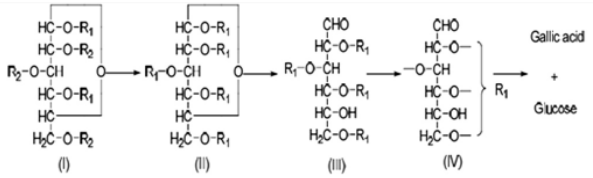
Fig. 1: Major Classes of Tannins.

Hydrolysable tannins can be helpful in the leather, wine and plastic manufacturing industries (Guo et al.,2020; Motta et al.,2020). They can be used as antimutagenic, anticancer, antioxidant, wood preservative, anti-corrosion agents for metals. (Tomak and Gonultas, 2018; Byrne et al., 2019).

Tannase

Tannase (E.C. 3.1.1.20) or tannin acyl hydrolase (TAH) is an explored group of hydrolases family. The catalytic reaction of tannase involves the hydrolysis of ester bond and depside bond present in various substrates i.e., tannic acid, complex

tannin, gallic acid esters, epigallocatechin gallate, methyl gallate, ethyl gallate, n-propyl gallate to produce gallic acid (3, 4, 5-tri hydroxybenzoic acid) and glucose (Lal and Gardner, 2012). Several intermediate molecules (Figure 2) are formed during the hydrolytic breakdown of tannic acid into gallic acid and glucose by tannase enzyme i.e (II) 1, 2, 3, 4, 6, - pentagalloyl glucose, (III) 2,3,4,6 tetragalloyl glucose and (IV) monogalloyl glucose (Lekha and Lonsane, 1997).



(R1-gallic acid; R2–m-di gallic acid; I-tannic acid, II-1,2,3,4,6-pentagalloyl glucose; III - 2,3,4,6 tetragalloyl glucose and IV- monogalloyl glucose)

Fig. 2: Mechanism of hydrolysis of tannic acid by Tannase (Lekha and Lonsane, 1997)

The novel biotechnological approach of gallic acid is the production of trimethoprim. Gallic acid has emerged as a basic intermediate of trimethoprim containing bacteriostatic and broad-spectrum features. (Anderson et al., 1980; Misro et al., 1997; Mukherjee and Banerjee, 2003). The enormous applications of gallic acid such as in the manufacture of ink, dye and paper,in photographic development, in the tanning process of leather are found in many sectors of various industries.

Tannase Structure

Ren et al. (2013) reported the monomeric three-dimensional structure of tannase from Lactobacillus plantarum SICC 1.15 (Figure 3). This tannase contains 50,747 Da molecular weight and 469 amino acid residues. However, the crystal structure of tannase consists of two domains such as α/β-hydrolase domain (residues 4-204 and 396-469) and lid domain (residues 205-395). The α/β-hydrolase domain contains six α helices such as α1-α4 and α 12- α13 and nine β-stranded sheets such as β1-β7 and β 12-β13. Among the α helices, α2- α4 and α12 are present on one side of the sheet, while α1 and α13 are present on the opposite side. The other domain known as the lid domain contains seven α-helices such as α5- α11 and two β-stranded sheets such as β 8- β9 and β10-β11. The two β-stranded sheets lie between the β 7-12 strands. The active site of the tannase enzyme is revealed in the α/β-hydrolase domain. A deep tunnel is formed by the juxtaposition of α/β-hydrolase and lid domain. The tunnel wall is formed by three amino acid residues as Ser163, Asp419 and His451, known as a catalytic triad. Along with the catalytic triad (Ser163, Asp19 and His451), tannase also contains serine residue Ser163 that is located in the pentapeptide motif i.e.GXSXG or Gly161-X-Ser163-X-Gly165 (where X = any amino acid residue) between β6 and α6. The active site of the tannase consists of three charged residues as Lys 343, Glu357 and Asp421. These three charged residues play a major role in the hydrolysis and binding of galloyl moiety of the substrate.

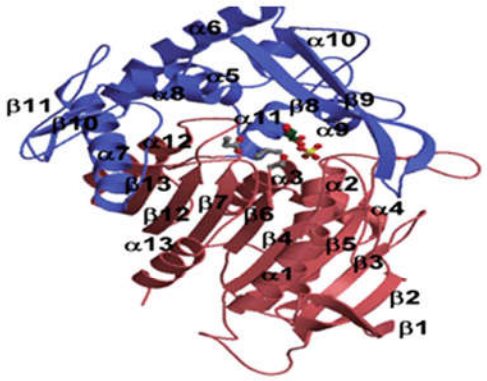


Fig. 3: Structure of L. plantarum tannase. The structure contains α/β- hydrolase domain in red ribbon view and lid domain in blue-ribbon view (Matoba et al.,2013)

Sources of Tannase

Tannase can be found in prokaryotic and eukaryotic cells. It is widely distributed in animals, plants and microorganisms. Among various sources, microbial strains play a crucial role in tannase production because of their great stability over plants and animals. Bacteria, molds and yeasts produce 30%, animals produce 8% and plants produce 4% industrial enzymes.

Plants derived tannase

Tannin-rich plants are great sources for tannase production. Various parts of plants such as bark, woods, stems, roots, fruits, leaves and seeds contain a high amount of tannin. Different plant species like Acacia katechu, Agrimonia eupatoria, Diospyros kaki, Hamamelis virginiana, Monochaetum multiflorum, Syzygium cumini, Terminalia chebula and Quercus robur were reported to be efficient tannase producers (Shen et al.,2006; Granica et al.,2013; Zhange et al., 2011). Plants produce a variety of acids such as chebulinic acid, gallic acid, and hexahydroxyphenic acid and a large quantity of sugar, during the growth phase. As fruits become mature/ ripen, tannase esterified these acids with sugar (i.e glucose) and form complex tannin (Lekha

and Lonsane, 1997).

Animals and Insects Derived Tannase

The ruminal mucosa of several animals such as koalas (Phascolarctos cinereus) (Osawa, 1992), goats (Capra hircus) (Brooker et al., 1994; Nelson et al., 1995), and horses (Equus caballus) (Nemoto, 1995) contain a small amount of tannase that degrade tannin from the nutritional resources and narrow down harmful effects of tannin on animals (Mosleh et al., 2014). Tannase can be extracted from the larva of some insects such as Indian Mole Cricket (Gryllotalpa krishnani), Cynipid, Salix caprea (Mani, 1966). The larva of the insects contains other digestive enzymes such as invertase and diastase. The role of digestive enzymes is to act on the large molecules of plant cells and convert them into simple forms and help insects in digestion. Tannin present in the leaves of plants precipitates with digestive enzymes and inactivates them. Tannase from the larva of insects acts on tannin and protects the digestive enzymes (Mani, 1966).

Microbially derived tannase

Many microorganisms such as bacteria, fungi, yeast are known to be tannase producers. Tannase producing microbes utilize tannin as a sole source of carbon and transform tannin into glucose and gallic acid.

Bacterial tannase

For the past few decades, the most significant approach to obtain tannase is from bacterial source compared to yeast and fungal sources due to its high genetic manipulation, biochemical diversity, stability in an extreme environment, and extracellular enzyme production (Jana et al., 2014, Aguilar and Gutierrez Sanchez, 2001). In earlier reports, many bacterial strains such as Achromobacter, Klebsiella, Bacillus, Corynebacterium., Citrobacter, Streptococcus, Leuconostoc Pediococcus, Pantonea and Streptomyces were able to produce tannase. Out of 21 different bacterial genera, Lactobacilli are the most dominant tannase producing bacterial genera among others (Chandrasekaran and Beena, 2013).

Hydrolysable tannins such as gallotannins and ellagitanins are hydrolysed by bacterial tannase (Bhat et al., 1998). Glucose and gallic acid are the hydrolysed products of tannin through tannase. Gallic acid is decarboxylated by the enzyme gallate decarboxylase and converted to pyrogallol. The pyrogallol is converted into pyruvic acid, cis- aconitic acid and 3-hydroxy-5-oxo-hexanoate (Figure 4). These three molecules enter and circulate into the TCA cycle. 3-hydroxy-5-oxo-hexanoate is the breakdown product of dihydro phloroglucinol. Dihydro phloroglucinol and phloroglucinol are produced through catalytic actions of diphloroglucinol hydrolase and phloroglucinol isomerase from pyrogallol.

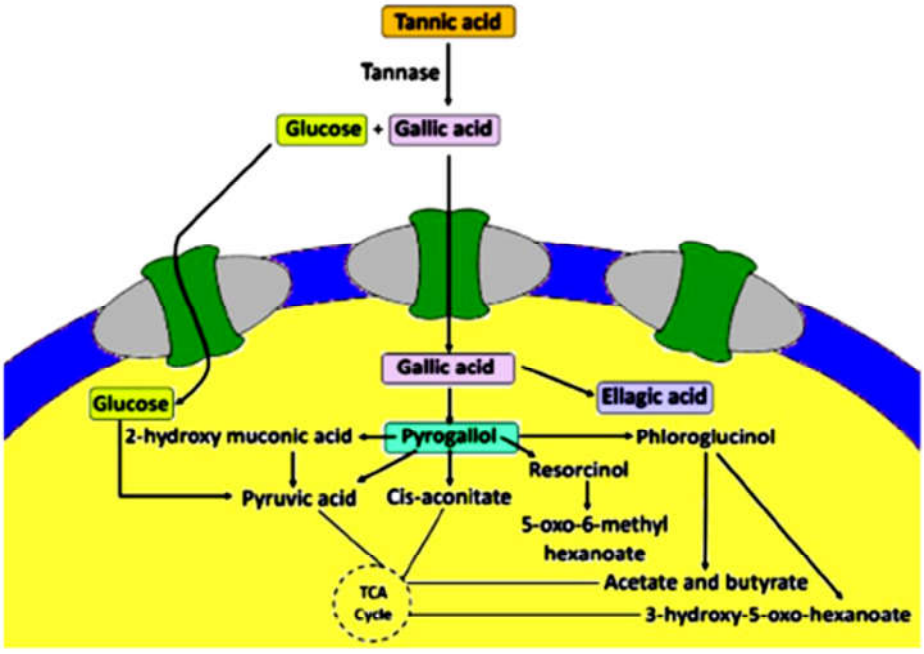


Fig. 4: Pathway showing Tannin degradation by bacterial tannase (Jana et al., 2014)

Fungal tannase

Fungal tannase have better degrading ability for hydrolysable tannin and condensed tannin (Belmares-Cerda et al., 2003). Out of 20 different genera of fungi, Aspergilli and Penicillia are the most dominant groups of tannase producers (Raghuwanshi, 2012). Among 2o different fungal genera, 27 species of Aspergillus, 24 species of Penicillium, 4 species of Trichoderma and 3 species of Fusarium are known as tannase producers. Several fungal species such as Mucor sp., Paciliomyces sp., Hyalopus sp. and Neurospora sp. are also reported as tannase producers. The pathway of tannin degradation by fungal tannase is shown in Figure 5.

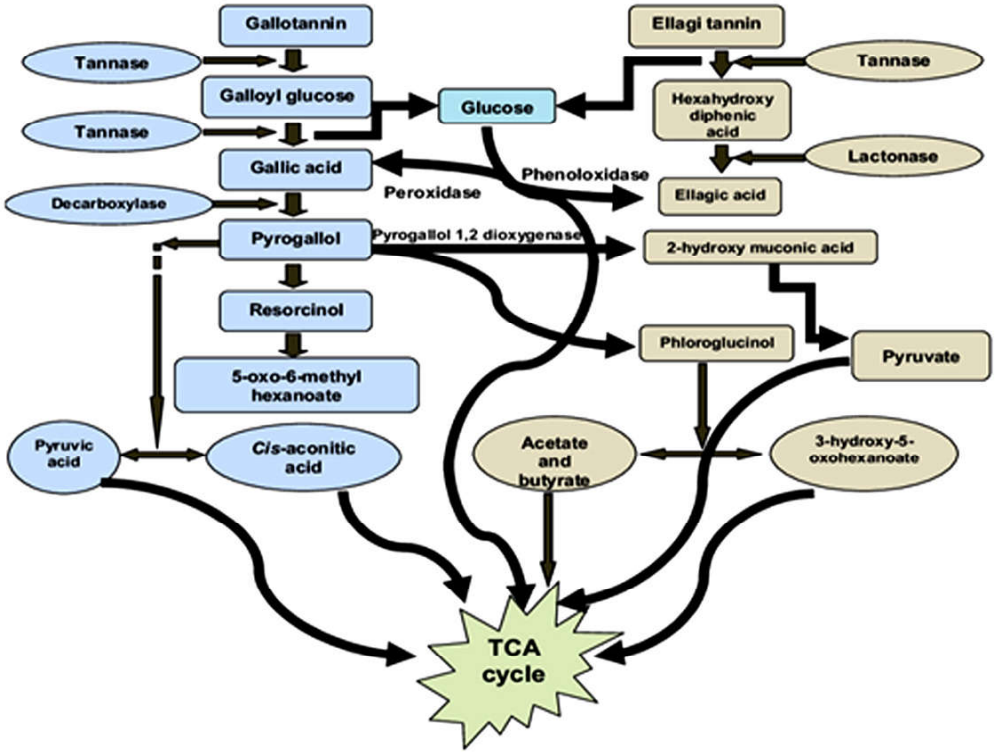


Fig. 5: **Pathway showing Tannin degradation by fungal tannase (Banerjee and Mahapatra, 2012)**

Yeast Tannase

Yeast tannases are less efficient for degrading naturally obtained tannin compare to tannic acid (Deschamps et al.,1983). Nowadays, researchers develop conventional and biotechnological approaches for the production of tannase from yeast due to its short growth period and easy accessibility towards molecular genetics. Various yeast species such as Candida sp., Pichia sp., Debaryomyces hansenii, Mycotorula japonica and Saccharomyces cerevisae are reported as tannase producers.

Actinomycetal Tannase

Various actinomycetal species such as Streptomyces sviceus (Wang,et al.,2018), Streptomyces gancidicus (Tripathi et al., 2021), Streptomyces sp. AL1L (Roy et al., 2018) are reported as tannase producers.

Tannase Assay

Diversified analytical methods such as UV-spectrometric (Parmentier, 1973 and Iibuchi et al.,1967), colorimetric (Haslam and Tanner, 1970), photometric (Chen,1969), titrimetric (Freudenberg et al., 1927; Nishira, 1961; Haslam and Stangroom, 1966), chromatographic (Jean et al., 1981) etc. are reported. All these methods used for assay of tannase are based on either determination of residual tannic acid (Deschamps et al., 1983) or formation of gallic acid, a byproduct of enzymatic hydrolysis of tannic acid.

Solid state and Submerged Fermentative production of tannase

Fermentation is the metabolic process in which complex compounds are converted into simple compounds by using various microorganisms such

as bacteria and fungi. The fermentation process is classified into two broad classes as solid-state fermentation (SSF) and submerged fermentation (SmF) based on the type of substrates used in the process.

Solid-state fermentation or SSF is defined as a process in which microbes utilize natural substrates or non-soluble inert supports as a nutritional source and can grow in the presence of low water availability or absence of water. SSF is a favorable method for tannase production due to its simple operating system, less water consumption and low-cost production (Aguilar et al., 2001 and Barrios González, 2012). Selwal et al. (2010) found maximum tannase activity in the presence of amla (Phyllanthus emblica) and keekar (Acacia nilotica) leaves by Pseudomonas aeruginosa IIIB. Ding et al. (2020) used mixed strains of Bacillus subtilis, Aspergillus niger and Saccharomyces cerevisiae for the production of tannase during solid-state fermentation of tea residue.

Submerged fermentation or SmF is defined as a process in which microbes utilize dissolved or suspended nutrients from the liquid medium (Frost, 1987). Among the various fungi, Penicillium verrucosum and Aspergillus niger degraded tannin from the coffee pulp and tea residue, respectively under SSF and produced tannase (Bhoite and Murthy, 2015 and Sharma et al.,2014). Submerged fermentation is more preferable comparedd to solid-state fermentation for the production of bacterial and fungal tannase in various industries. Bacillus megaterium showed maximum tannase activity (10.77 U/mL/ min.) in the presence of 1% tannic acid concentration under SmF (Tripathi et al.,2016).

Tannase Purification

Most of the produced enzymes are extracellular or intracellular through the fermentation process. Cells are directly separated from the fermentation broth for the recovery of the extracellular enzyme, wherein the case of intracellular enzyme cells are disrupted by the mechanical or non mechanical methods. The purification process mostly depends upon the need for the enzyme in the market. Purification of the enzyme is carried out to recover a high yield with the greatest catalytic activity and purity. Downstream processing is essential to remove the impurities from the end product. It is a multistep process in which various methods such as Salting out or solvent precipitation, Ultrafiltration, electrophoresis and chromatography methods could be employed. The initial step for purification of an enzyme is ammonium sulphate precipitation. The role of ammonium sulphate in purification is to stabilize the protein. Various types of precipitating agents like 2 ethoxy 6-9 diamino acridiniiun lactate or rivanol, as well as such polymers like dextran, PEG (polyethylene glycol) and polyvinyl alcohol, were used for the precipitation of tannase (Sharma et al., 1999 and Aoki et al., 1976). The second step of enzyme purification is a desalting process or dialysis. Dialysis is a process in which small molecules are removed from a mixture of molecules. The process was performed by using a semipermeable membrane. The third step applicable in tannase purification is ion exchange chromatography. The protein separated by ion-exchange chromatography is based on its charge that interacts with an oppositely charged ion-exchange matrix. Many researchers have used anion exchangers such as DEAE-Sephadex, DEAE-cellulose, DEAE-Sephadex A-50, and DEAE Sepharose for purification of tannase due to its acidic nature (Bhardwaj et al., 2003, Mahendran et al., 2006, Sharma et al., 1999). The fourth or last step of enzyme purification is gel permeation or size exclusion chromatography. The gel beads used in gel filtration chromatography are prepared from Sepharose, Agarose, Vinyl polymers, Polyacrylamide and Sephadex. Sephadex G-100, Sephadex G-200 and Sephadex G-150 were used as a gel in gel filtration chromatography for tannase purification (Beena et al.,2010, Ramirez–Coronol et al.,2003 and Sharma et al., 1999).

Biochemical Properties and Kinetics of Tannase

Different tannases obtained from different microbial sources are characterized and have some biochemical properties that give information related to the nature and structure of the enzyme. Various parameters such as temperature, pH, metal ions, activators, inhibitors and substrate specificity affect the enzyme activity. Microorganisms express the gene for the enzyme at specific pH. The optimum fungal and bacterial tannase biosynthesis has been reported in 3-8 pH range. However, in certain reports, the optimum pH of 8 and 8.9 was reported for bacterial tannase (Belur et al.,2010 and Matsuda et al., 2016). The optimal pH for actinomycetal and yeast tannase was 6 and 4.5, respectively (Roy et al., 2018 and Pan et al., 2020). Tannase from A. fumigates was stable at pH 4.0 and it lost enzyme activity at pH 8.0 (Batra and Saxena, 2005). Mahapatra et al. (2005) reported that mostly fungal tannase required an acidic environment to become active. Many researchers reported the optimum temperature range of some tannase-producing microorganisms

was between 30-70 °C (Raghuwanshi et al., 2011; Beniwal et al., 2013 and Govindarajan et al., 2018). The native structure of tannase might be affected by the presence of metal ions due to an increase in the ionic strength of the solution (Chaitanyakumar and Anbalagan, 2016). At a high concentration of metal ions, tannase activity was decreased. In the previous kinds of literature, tannase activity was enhanced in the presence of sodium chloride (Mondal et al., 2001; Aftab and Hamid, 2016). The catalytic activity of the enzyme is affected by the surfactants (Prasad et al., 2012). Sodium lauryl sulfate (SDS) showed the stimulatory effect on tannase produced from B. subtilis at 1% (v/v) concentration, while Tween 80, Tween 60, DMSO (Dimethyl Sulfoxide), Sodium azide, β-mercaptoethanol, EDTA, PMSF (phenylmethylsulfonyl fluoride) and Triton X-100 showed a negative effect on tannase activity (Jana et al., 2013). Various polar and nonpolar organic solvents also affect tannase activity. Several nonpolar solvents such as hexane, toluene and benzene enhanced tannase activity. However, few polar solvents such as methanol, isoamyl alcohol and butanol inhibited tannase activity (Beniwal et al., 2010; Valera et al., 2015).

Applications of Tannase

Tannase is an important industrially relevant and versatile enzyme that has tremendous applications such as in the manufacturing of tea, wine, beer, food, pharmaceutical, chemical, brewing and leather industries. Moreover, the tannase enzyme is also used for the bioremediation of toxic wastes from tannery effluents.

Gallic acid Production

Gallic acid or 3, 4, 5-trihydroxybenzoic acid is an organic compound that is widely used in pharmaceutical and chemical industries. Gallic acid is obtained from various sources such as plants and microorganisms. Gallic acid is present in a plant as free molecules or it can be produced from the tannase-producing microorganisms by hydrolysis of tannic acid or tannin-rich substrates. 3, 4, 5 trimethoxy benzaldehyde is the intermediate component during the manufacturing of trimethoprim from gallic acid. Trimethoprim is effective against gram-negative and gram-positive microorganisms when it synergistic with sulfonamide. Trimethoprim inhibits the dihydrofolate reductase enzyme and prevents the synthesis of folic acid. Folic acid plays a very important role in the synthesis of DNA. Gallic acid serves as an antiapoptotic, antiviral, antimutagenic and anticancer agent (Badhani et al., 2015).

Instant tea Preparation and Tea Cream Solubilization

Tea is the most highly consumed and favorable beverage worldwide, after water. There are so many health benefits of tea such as it helps to reduce the risk of cancer, heart disease and diabetes. When tea leaves are treated with tannase. It improves the flavor of tea with better acid stability and cold water solubility (Natarajan et al., 2009). It also enhances the bright reddish color due to the formation of epitheaflavic acid from epicatechin and gallic acid. Haze and tea cream formation during the preparation of tea beverages should be removed by tannase treatment.

In Beverages

The application of tannase in beverage industries is to remove water insoluble precipitates and undesirable tannin which is responsible for haze formation and bitter taste (Boadi and Neufeld, 2001). Additionally, tannase plays a major role in the removal of tannins and phenolic compounds from the different fruit juices and influences the flavor and maintains the color stability of the juice during the long period of preservation (Aguilar et al., 2007).

In Animal feed and Cell wall Digestion

The quality of animal feed can be also improved by the digestibility of the plant cell wall. Tannase acts on crosslinks of plant cell wall polymers that contain ferulic acid dehydrodimers. Tannase hydrolyzes diethyl diferulates and improves the quality of animal feed (Garcia-Conesa et al., 2001).

In the Biological Treatment of Tannery Effluent

The tannery industry is one of the largest industries and most important for the tanning of leather in India. The tanning process is necessary to prevent the leather from decomposition and to impart the color of the leather. Moreover, it causes environmental pollution during the discharge of tannery wastes to sewage or natural bodies. Besides these, tannin present in the effluent also inhibits the microorganisms. Various chemical and biological methods are used to remove tannin and other phenolic compounds from the tannery effluent. Recently, biodegradation technology is widely used for the treatment of tannery effluent and other types of industrial wastes. Tannase treatment is an eco-friendly treatment that helps to remove tannin and decrease pollution load such as BOD and COD from industrial wastes or wastewater (Zhao et al., 2017).

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