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Prebiotics, Probiotics and Nutraceuticals

Springer

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Kambaska Kumar Behera • Renu Bist •
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Editors

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Dedicated
To
ALL COVID WARRIORS
OF THE WORLD

Foreword

It gives me immense pleasure to write a foreword to the book *Prebiotics, Probiotics and Nutraceuticals* edited by Dr. Kambaska Kumar Behera (Fakir Mohan University, Balasore, Odisha, India) and his collaborators (Dr. Renu Bist (Rajasthan University, Rajasthan, India), Dr. Sangita Mohanty (ICAR-NRRI, Cuttack, Odisha, India) and Dr. Manojit Bhattacharya (Fakir Mohan University)). The book edited by this young and dynamic research group has attempted to cover all emerging fields of knowledge in the era of climate change with a special focus on Covid-19. As technologies develop, many conventional problems of food, environment and health have been alleviated. However, the continuous increase in the global population and the current scenario of the pandemic have been providing a screaming alert on global immunity to the future. It is true that technology revolution has contributed greatly to improve the quality of life, but it is also obvious that world pandemic issues have emerged from technology exploration. In this context, the research ethos has been increasing among the scientific community for providing bioactive compounds as an immune stimulant to enhance immunity to various diseases among the human population. The knowledge generation and/or exploration and application under this particular subject is huge and highly diversified as compared to other emerging scientific-cum-technological domain. To prepare and present for the young generation of specialized readers, the title *Prebiotics, Probiotics and Nutraceuticals* seems to be bold and mega-sized, and also exploratory. Encouragingly, Dr. Behera, other editor(s) and author(s) have been successful in touching upon almost all perceivable title points, to give 'completeness' to the volume.

The contributors of this book are mainly from reputed academic and scientific institutions across India. This book shall be considered as one of the best knowledge sources on the present status, trends and approaches of nutrition and food supplements. This volume is unique in providing practical knowledge and ideas in employing nutrition for human health to fight against pandemics and shall be used as a text for students, researchers, experts and policy makers. I hope this book

Prebiotics, Probiotics and Nutraceuticals will contribute a lot to the development of ideas on balancing sustainability and utility in the field of Food and Nutritional Biotechnology.

School of Earth Sciences & Natural Resource Management, Mizoram (Central) University
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Uttam Kumar Sahoo

Preface

The book *Prebiotics, Probiotics and Nutraceuticals* accelerates the molecular path of whole or parts of living organisms to produce or improve the immune system and processes to fight against diseases and specially sudden pandemics. It is a rapidly evolving branch of natural sciences which started with the creation of the first recombinant gene, 30 years ago in the field of molecular farming. These nutritional supplements are used in different ways, changing the way we live by improving the foods we eat, the beverages we drink and the medicines we take. They also have enhanced other aspects of our lives through the intake of Prebiotics, Probiotics and Nutraceuticals to fight many dreadful diseases such as arteriosclerosis, cancer, diabetes, Parkinson's and Alzheimer's diseases. The application of these nutrition in the food supplements of humans is one of the many aspects of biotechnology that has great impact on the society to fight against pandemics. By the year 2050, it is expected that more than 10 billion people will be living on this planet, and it is also believed that there may not be enough resources to feed the world population (UNFPA 1995) for healthy life. Hunger and malnutrition already claim 24,000 lives a day in developing countries such as Asia, Africa and Latin America (James 2003). Malnutrition, however, is not exclusive to developing nations. Many people of developed countries though considered well fed often suffer from improper nourishment resulting in poor mass immunity making the population vulnerable or susceptible to communicable diseases as evident during recent pandemic. Through nutrition biotechnology, scientists can enhance resistance to diseases and environmental stresses, afflicting the human society. Recent developments in the biotechnology of nutrition will allow the production of more nutritious, safer, tastier and healthier food. Advances in genetic engineering are revolutionizing the way we produce and consume food, and it is quite possible that in the next decade a large percentage of the food we eat will be bioengineered for safeguarding human health. Recent advances in Prebiotics, Probiotics and Nutraceuticals of biotechnology encompass continuously evolving methods or materials, for generating energy to non-toxic cleaning nutritional products. It is that innovation which reduces waste by changing patterns of production and consumption through DNA engineering. The book *Prebiotics, Probiotics and Nutraceuticals* contains 15 chapters and covers most of the core nutrition of both plant and animal origin, isolated and detected by our distinguished scientists. The objective of the book is to draw the attention of our

future budding scientists, researchers and policy makers for the exploration and development of *Prebiotics, Probiotics and Nutraceuticals* in the frontier area for social welfare.

Balasore, Odisha, India — Kambaska Kumar Behera
Jaipur, Rajasthan, India — Renu Bist
Cuttack, Odisha, India — Sangita Mohanty
Balasore, Odisha, India — Manojit Bhattacharya

Acknowledgements

It is my privilege to express my deep sense of gratitude to Prof. (Dr.) Uttam Kumar Sahoo, Senior Professor & Dean, SES & NRM Mizoram (Central) University, Aizawl (Mizoram), for his kind consent to write the foreword for this volume.

I acknowledge the assistance, encouragement, excitement and efforts of the contributors in organizing and publishing this book. The excellent contents and uniqueness of each individual chapter will make this book as a textbook on the use of nutrition biotechnology and its frontier area for a moderately long term, which usually does not occur with books associated with this genre. I thank all the contributors for their understanding and patience. There are several people who deserve special thanks, but few of them are my beloved co-editor Dr. Manojit Bhattacharya, Dr. Renu Bist and Dr. Sangita Mohanty for their unconditional support and cooperation in making the project successful and fruitful.

Words are not enough to thank all my friends and colleagues who also inspired a lot to complete this work.

Last but not least, I would like to assert my intricate appreciation of gratitude and best regards to my loving family members for their incessant support and cooperation.

I acknowledge Mr. Gaurav Singh and Ms. Raman Shukla from Springer Nature, for their constant support and cooperation for publication of this volume.

Contents

About the Editors

Kambaska Kumar Behera, Ph.D. & Postdoc is a distinguished academician and researcher, published more than 100 research papers and six books of international and national repute. Born in Odisha, Dr. Behera has studied in Utkal University for his M.Phil. and Ph.D. degrees and owned the distinction of DBT-Postdoctoral Fellowship. He has worked on different research capacities in various national research institutes of India viz. CSIR, ICAR and NIT. He presently works as ASSISTANT PROFESSOR in the P.G. Department of Botany at Fakir Mohan University, Balasore, Odisha.

Renu Bist, Ph.D. currently serves as an **Associate Professor** in the Department of Zoology at the University of Rajasthan, Jaipur, India. As an academician and researcher, she supervised eight Ph.D. students and has 45 publications in journals of high repute. She is the author of two books of international and national repute. She is born in Udaipur, Rajasthan, and completed her M.Sc. and Ph.D. degrees in Mohanlal Sukhadia University, Udaipur. She has almost 15 years of teaching and research experience.

Sangita Mohanty, Ph.D. is an IARI Alumni and S. P. Raychowdhury Gold medallist, presently working as Senior Scientist, Crop Production Division, ICAR-**National Rice Research Institute**, Bidyadharpur, Cuttack, Odisha, India. She has more than 15 years of research experience in both international and national research organizations. She obtained her master's and doctoral degrees in soil science and agricultural chemistry from Indian Agricultural Research Institute, New Delhi. She has published more than 50 research articles in high-impact journals, book chapters and books of

national and international repute. She has been deputed as visiting researcher to the University of Aberdeen, Scotland, UK, with NEWS India-UK Senior fellowship. She has handled more than ten research projects as lead and co-investigator and guided several Master science students in his research capacity in the field of soil nutrient management.

Manojit Bhattacharya, Ph.D. & Postdoc successfully completed his doctoral degree (Zoology) at Vidyasagar University, West Bengal, India. He published 60 research articles in peer-reviewed, high-impact journals and two books (as co-author). He has more than 8 years of operational experience in freshwater resources mapping, fish genomics and computational biological platforms. Currently, he serves as an Assistant Professor of Zoology at Fakhir Mohan University, Balasore, Odisha. Dr. Bhattacharya has wide expertise in different aspects of advanced molecular biology and bioinformatics.

Potentiality of Probiotics in Inactivation of Tetrodotoxin

1

Rudra Prasad Nath and Jayanta Kumar Kundu

Abstract

Tetrodotoxin (TTX) is a powerful neurotoxin principally spotted in the liver, skin and gonads of puffer fishes. TTX is also reported from different other organisms like goby, newts, frogs, blue-ringed octopus, gastropods, starfishes and xanthid crabs. TTX is more toxic than that of potassium cyanide (KCN), and the human nervous system will suffer badly when exposed to TTX. Mild exposure to TTX may lead to different problems like headache, lack of sensation of the lips, etc. Intoxication of TTX influences vomiting tendency, dizziness, lack of sensation, itching, increased heart rate, lowered blood pressure and paralysis of the skeletal muscles and diaphragm and at last can cause demise by arrestation of breathing. Toxicity of TTX is fairly widespread among Asian countries like Japan, China, Thailand, India, Bangladesh, etc. TTX can never be destroyed after cooking or refrigerating or by digestive juices inside the alimentary canal because it is thermostable and acid-stable. TTX is an effective neurotoxin which can particularly obstruct voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted. Toxicity of tetrodotoxin (TTX) can be neutralised by some probiotics like exopolysaccharide (EPS) extract of a few lactic acid-producing bacteria. TTX detoxification ability of EPS of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 was proven by different experiments. The entire assessment proved the significance of EPS of bacteria in the neutralisation of TTX. Exopolysaccharide (EPS) extracts from *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 were properly examined. Different types of investigations like high-performance liquid

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chromatography (HPLC) and thin-layer chromatography (TLC) were done to confirm the presence of glucose in most of the EPS of these bacteria.

Keywords

Tetrodotoxin (TTX) · Probiotics · Exopolysaccharide (EPS) · Puffer fish

1.1 Introduction

Tetrodotoxin (TTX) is especially an effective toxin present in tissues like the liver, skin, ovaries and testes of puffer fishes (Chau et al. 2011). Tetrodotoxin is named so because of its chief occurrence in puffer fishes of order Tetraodontiformes (Goto et al. 1965). TTX is not only reported from puffer fishes but also found in goby (Noguchi and Hashimoto 1973), California newts (Bucciarelli et al. 2014), frogs (Noguchi and Arakawa 2008), xanthid crabs (Noguchi et al. 1986), gastropod molluscs (Yang et al. 1995), blue-ringed octopus (Yotsu-Yamashita et al. 2007) and starfishes (Noguchi and Arakawa 2008). There is no such phylogenetic alliance between these TTX-containing animals. TTX is accumulated in different tissues of different organisms (Table 1.1) (Noguchi and Arakawa 2008).

TTX is predominantly an effective neurotoxin which can particularly barricade voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted (Narahashi 2001; Benzer 2007). There was a controversy about the endogenous or exogenous property of TTX. Endogenous means that TTX is made up by puffer fish by its own, whereas exogenous is where TTX is isolated from the exterior and accumulated in puffer fish (Noguchi and Arakawa 2008). Several experiments recommended that puffer fish toxin is exogenous. The manufacture of TTX is done principally by marine bacteria and diverse parasitic or symbiotic bacteria which can be directly gathered inside the different tissues of

Table 1.1 Different TTX-containing animals (source: Noguchi and Arakawa 2008)

Animals	Toxic tissues
Arthropoda Xanthid crab (Family: Xanthidae)	Whole body
Mollusca Blue-ringed octopus (*Octopus maculosus*) Gastropoda (*Babylonia japonica*)	Posterior salivary gland, digestive gland
Echinodermata Starfish (Genus: *Astropecten*)	Whole body
Fish Puffer fish (Family: Tetraodontidae) Goby (*Gobius criniger*)	Liver, skin, gonads, etc. Viscera, skin, muscle, etc.
Amphibia California newt (Genus: *Taricha*) Frog (Genus: *Atelopus*)	Viscera, skin, muscle, egg, etc. Skin, ovary, egg, etc.

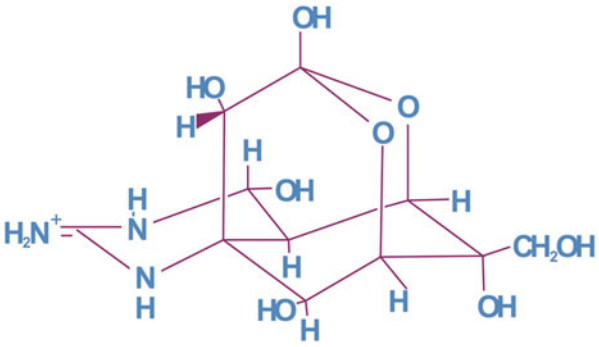

Fig. 1.1 Structure of TTX. (Source: Arakawa et al. 2010)

puffer fishes like the liver, skin, gonad, etc. (Noguchi and Arakawa 2008). Tetrodotoxin is basically a metabolic product of the host. TTX can be manufactured by different symbiotic bacteria, viz., *Vibrio alginolyticus*, *Pseudoalteromonas tetrodonis*, *Alteromonas tetraodonis*, *Shewanella algae*, *Shewanella putrefaciens*, etc. (Matsui et al. 1990; Yasumoto et al. 1986). TTX can be stored inside the puffer body via bioaccumulation process, and that is why toxicity of puffer fishes may fluctuate from season to season. Different tissues of puffer fishes are differentially toxic due to differential storage by bioaccumulation. These symbiotic bacteria survive in the intestinal lining of the puffer fishes (Noguchi et al. 1986a, b).

Many studies exposed that TTX is much more toxic than that of potassium cyanide (KCN) (Kaplan 2006) and the human nervous system suffered badly due to exposure to TTX (Tambyah et al. 1994). Mild introduction to TTX may lead to different problems like headache, lack of sensation of the lips, etc. Intoxication of TTX influences vomiting tendency, dizziness, lack of sensation, itching, increased heart rate, lowered blood pressure and paralysis of the skeletal muscles and diaphragm and at last can cause demise by arrestation of breathing (Cheng et al. 1968). Toxicity by TTX is fairly widespread among Asian countries like Japan, China, Thailand, India, Bangladesh, etc. (Noguchi and Ebesu 2001). TTX can never be destroyed after cooking or refrigerating or by digestive juices inside the alimentary canal because TTX is thermostable and acid-stable (Kuromi et al. 1979). There is no readily available antidote till date to overcome tetrodotoxin poisoning, but instant supportive management and watchful administration of atropine or neostigmine can restrain lethality (Xu et al. 2005).

6-Hydroxyl (-OH) groups and a guanidinium group which has positive charge are found in molecular structure of tetrodotoxin ($C_{11}H_{17}O_8N_3$) (Fig. 1.1) with a molecular weight of 319 Da (Hwang et al. 2007). Tetrodotoxin is not an alkaloid, a carbohydrate, or a steroid, and it is not like any conventional amino acid. From the seventeenth century, cases of puffer fish poisoning have been recorded (Clark et al. 1999) and are still a biggest threat although cases are declining (Arakawa et al. 2010; Kungsuwan 1993). Majority of puffer fishes have elevated level of TTX accumulation in tissues like the testis, liver, ovary, skin, etc. (Hwang and Noguchi 2007; Monrat et al. 2011; Anraku et al. 2013).

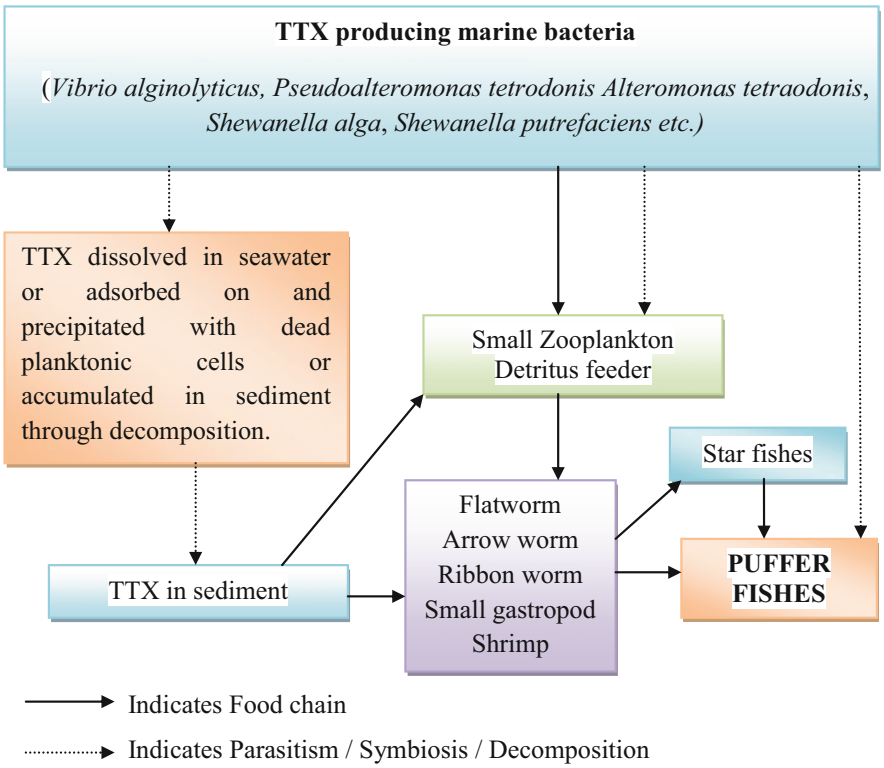

Fig. 1.2 Mechanism of TTX accumulation in puffer fishes (source: Noguchi and Arakawa 2008)

There are two ways by which TTX can be accumulated inside the body of puffer fishes. *In the first way*, TTX may be dissolved in saltwater or precipitated with deceased planktons or accumulated inside sediment in the course of breakdown and decomposition. Through the food chain, the primary consumers such as zooplankton, detritus feeder, flatworm, arrow worm, ribbon worm, small gastropod and shrimp will feed on dead planktonic cell on the sediment that consists of TTX followed by the secondary consumers like puffer fish, large gastropod, etc. feed on the primary consumers. As a consequence, the secondary consumers acquire TTX in their body. *The second way* is the TTX-producing marine bacteria like *Vibrio alginolyticus*, *Pseudoalteromonas tetrodonis*, *Alteromonas tetraodonis*, *Shewanella algae*, *Shewanella putrefaciens*, etc. which perform as parasite or build symbiotic relationship with zooplanktons and detritus feeder (Noguchi and Arakawa 2008). Through the food chain, the entire consumers will get the TTX. The illustration is shown in Fig. 1.2 (Noguchi and Arakawa 2008).

Several TTX derivatives have been isolated from puffer fishes, newts, frogs etc. till date. (Yotsu-Yamashita et al. 2007). These organic compounds are reported to possess biological activities like antitumor, antimicrobial, analgesic, etc. (Rajamanikandan et al. 2011; Narahashi 2001). TTX is applied as a potential pain

relief, and the analgesic activity of TTX is studied to reduce extreme cancer pain, anxiety and drug abuse (Hagen et al. 2008; Joshi et al. 2006; Marcil et al. 2006; Shi et al. 2009). TTX is largely an efficient neurotoxin which can particularly blockade voltage-gated Na^+ channels present at the outer layer of the nerve membrane. So, transport of sodium ions through the cell membrane is blocked, and progression of nerve impulse is halted. This action takes place in the peripheral motor, sensory and autonomic nerves (Shi et al. 2009). The function of electrical impulses is to transmit information from the brain to different parts of the body. These signals work from dendrite to axon inside the nerve cell. The signals engage in the flow of K^+ ions and Na^+ ions via axon, along the plasma membrane of the nerve. Nerves require K^+ ions and Na^+ ions together to create specific electrical communication called as action potential. K^+ ions and Na^+ ions are travelling interior and exterior of the plasma membrane causing changes and carrying electrical message, but tetrodotoxin can be coupled with sodium ion, stopping its movement. Therefore, "message-sending" method will be deactivated, and the impulse certainly not reaches to its destination (Fig. 1.3) (Shi et al. 2009).

α-Subunits of Na^+ channel which is voltage-gated are made up with four similar domains like DI, DII, DIII and DIV. Every domain has six α-helical sections (1–6). Section 4 is brown in colour and denoted as voltage sensors. Locations of phosphorylation via protein kinase C (PKC) are denoted by brown-coloured boxes, and protein kinase A (PKA) is symbolised by yellow-coloured circles. Fast inactivation gate is positioned at intracellular loop connecting DIII and DIV and is denoted as H in green oval configuration. Helixes 1, 2 and 3 are fluorescent green in colour in this pictorial depiction. In between blue-coloured fifth helix and sixth helix, specific loops known as P-loops are positioned. The inner ring is represented by pink colour, and the outer ring is coloured by green in the picture. Mechanism of action of TTX is accompanied by amino acids between the pore of the outer ring and inner ring (Fig. 1.4) (Nieto et al. 2012).

Till date no specific antidote has been developed to prevent tetrodotoxin poisoning. Certain effective method is developed to curtail those hazards allied with TTX intoxication. In different researches, the roles of many probiotics are studied to overcome TTX toxicity. Lactic acid bacteria (LAB) are found to be the most successful to conquer TTX intoxication in several cases. Probiotics are actually live, good, helpful bacteria found in certain foods or supplements which keep the gut healthy. Probiotics can offer plentiful health benefits. Different foods and food supplements which are treated as probiotics are considered to be secure for many people; nevertheless, individuals having low immunity or past illness record should avoid the use of probiotics. In a few incidents, placid side effects like trouble of the stomach, diarrhoea, gastritis and allergic reactions may happen (Doron and Snydman 2015; Singhi and Kumar 2016; Durchschein et al. 2016).

Studies proved that exopolysaccharide (EPS) was one of the main compounds which bacteria produce to confer their resistance to harsh conditions or toxic environment. Research also exposed the piece of evidence that TTX was isolated from toxic tissues of puffer fishes by the process of conventional fermentation of Japanese. LAB has considerable the role in this procedure. Lactic acid bacteria may

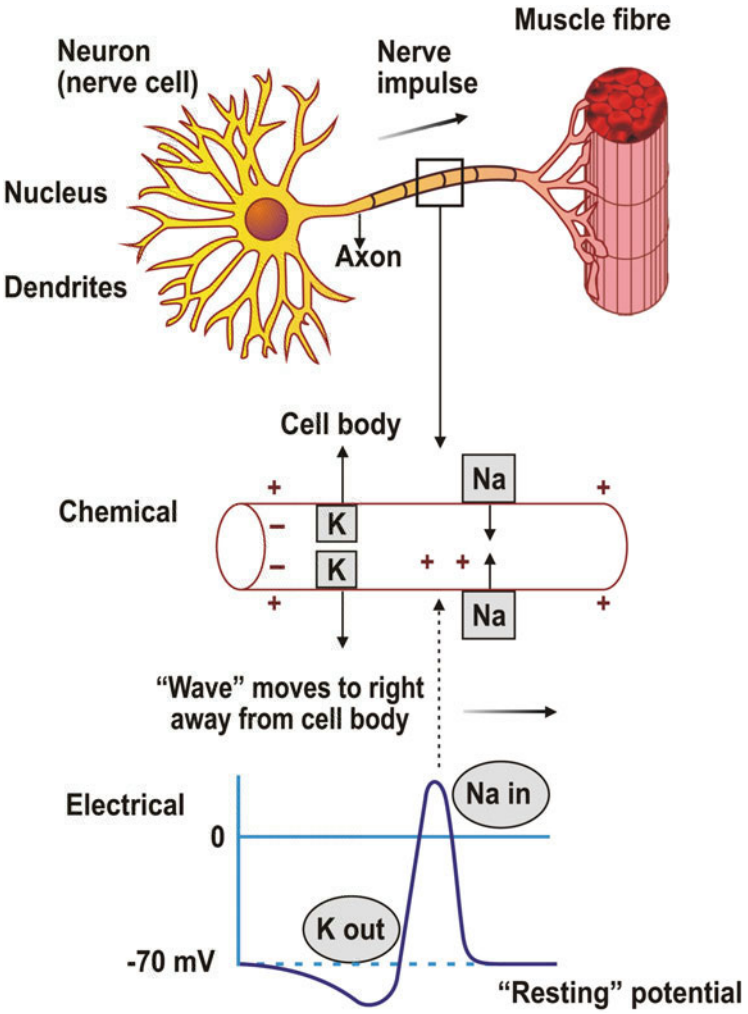

Fig. 1.3 Mechanism of generating action potential due to action of tetrodotoxin (source: Shi et al. 2009)

be of two types, i.e. homopolysaccharide bacteria and heteropolysaccharide bacteria, to keep them from adverse culture environment like desiccation, osmotic pressure, predation by protozoans, phagocytosis, antibiotics or toxic compounds and phage attack (Patel and Prajapat 2013; Rodriguez et al. 2003). There are quite a lot of heteropolysaccharides categorised in accordance with their structure, molecular weight, composition, function, etc. Production of exopolysaccharide from LAB is robustly subjective to culture setup (Iliev et al. 2006). Chronic gastritis can be checked by the exopolysaccharide made from *Streptococcus thermophilus* CRL 1190 (Rodriguez et al. 2009). Potential antioxidant properties were seen in the

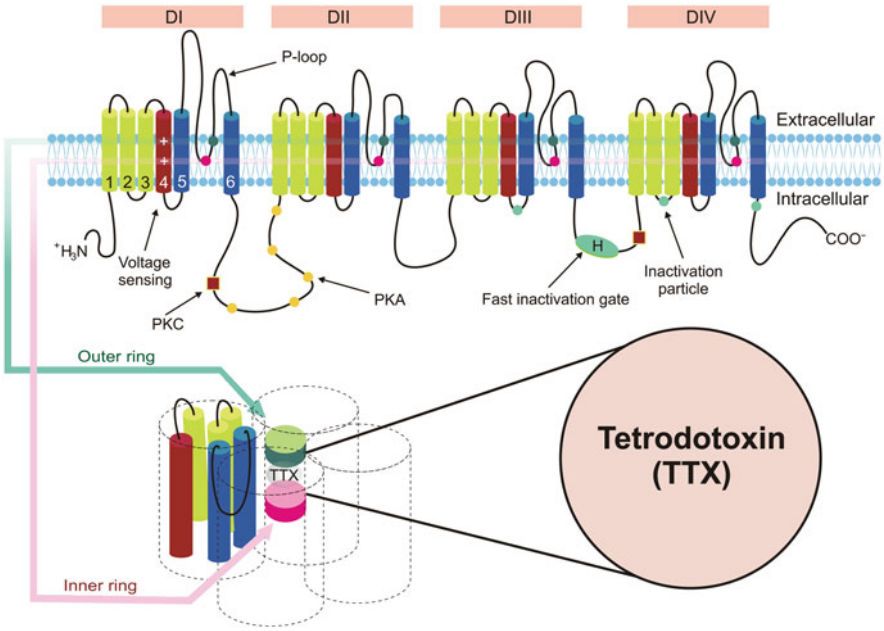

Fig. 1.4 Pictorial representation of interaction of α-subunit of Na^{+} channel and coupling site of TTX (Nieto et al. 2012)

exopolysaccharide made up from two *Lactobacillus* species like *Lactobacillus plantarum* NTU 102 and *Lactobacillus paracasei paracasei* NTU 101 (Liu et al. 2011). TTX detoxification ability of EPS extracted from *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 is proved. The entire assessment proves the significance of EPS of bacteria in neutralisation of TTX. EPS yielded from the samples of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 is experimented (Tu et al. 2014).

Detoxification properties of different strains of *Lactobacillus* sp. bacteria including *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are determined in the direction of mycotoxins like deoxynivalenol, zearalenone, aflatoxin B1, fumonisins, etc. which are present in various contaminations of food (Chlebicz and Śliżewska 2020).

1.2 Results and Discussion

TTX detoxification abilities of different lactic acid bacteria, viz., *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3, were tested in different studies. EPS is being produced by several bacteria in response to tough situation and caries out a variety of activities (Hall-Stoodley et al.

2004). EPS can combine directly to antibiotic and decrease the level of toxicity or put off active sites for binding of antibiotics. Different methods are applied like high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The investigation confirmed the presence of glucose in the majority of EPS of *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 (Tu et al. 2014). *Lactobacillus rhamnosus* PN04, *Lactobacillus plantarum* PN05 and *Leuconostoc mesenteroides* N3 have equivalent output of exopolysaccharide but, their water solubility is varied mostly due to disparity of functional groups, directing to the dissimilar relation with different atoms as well as water (Tu et al. 2018).

Exopolysaccharide derived from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 has specific ability of detoxification of TTX when amalgamated with cuprous oxide; on the other hand, the EPS of *Lactobacillus plantarum* PN05 without help of cuprous oxide individually can counteract TTX. Dissimilar molecular configuration of exopolysaccharide made up from these three *Lactobacillus* strains may result to disparity (Tu et al. 2018).

Different observations revealed that the exopolysaccharide acquired from *Lactobacillus plantarum* PN05 could be applied alone to avoid TTX contamination. On the other hand, the exopolysaccharide yielded from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 demonstrated TTX neutralisation capability while they are coupled to copper ion. Exopolysaccharide has long sequence polymer, but TTX encloses quite a lot of strong functional residues. Exopolysaccharide configuration of both *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 in existence of copper ion includes different functional groups like N–H and O–H that can react with copper and form a multifaceted configuration which is capable of binding to TTX through several hydrogen (H) bonds (Fig. 1.5), and then TTX becomes unable to develop toxicity anymore. In accordance with FTIR data, the exopolysaccharide derived from *Lactobacillus plantarum* PN05 is found to be methylated. Electrical density of oxygen molecule turns into more negative by this methyl group which subsequently permits the oxygen molecule to bind with the hydrogen molecule present within hydroxyl (–OH) groups of tetrodotoxin (Fig. 1.6). This type of communications may take place more rapidly than the dealing of copper ion with nitrogen molecule of tetrodotoxin directing to inactivation of tetrodotoxin in the absence of copper ion (Tu et al. 2018).

1.3 Conclusion

From different studies, it is clear that exopolysaccharide derived from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 when complexed to cuprous oxide assists fairly to detoxify tetrodotoxin which is particularly observed on mouse model. Cuprous oxide alone is referred to as a toxic compound; so the mixture of cuprous oxide and exopolysaccharide obtained from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 should be considered as toxic as well. On the other hand, exopolysaccharide yielded from *Lactobacillus plantarum* PN05 can

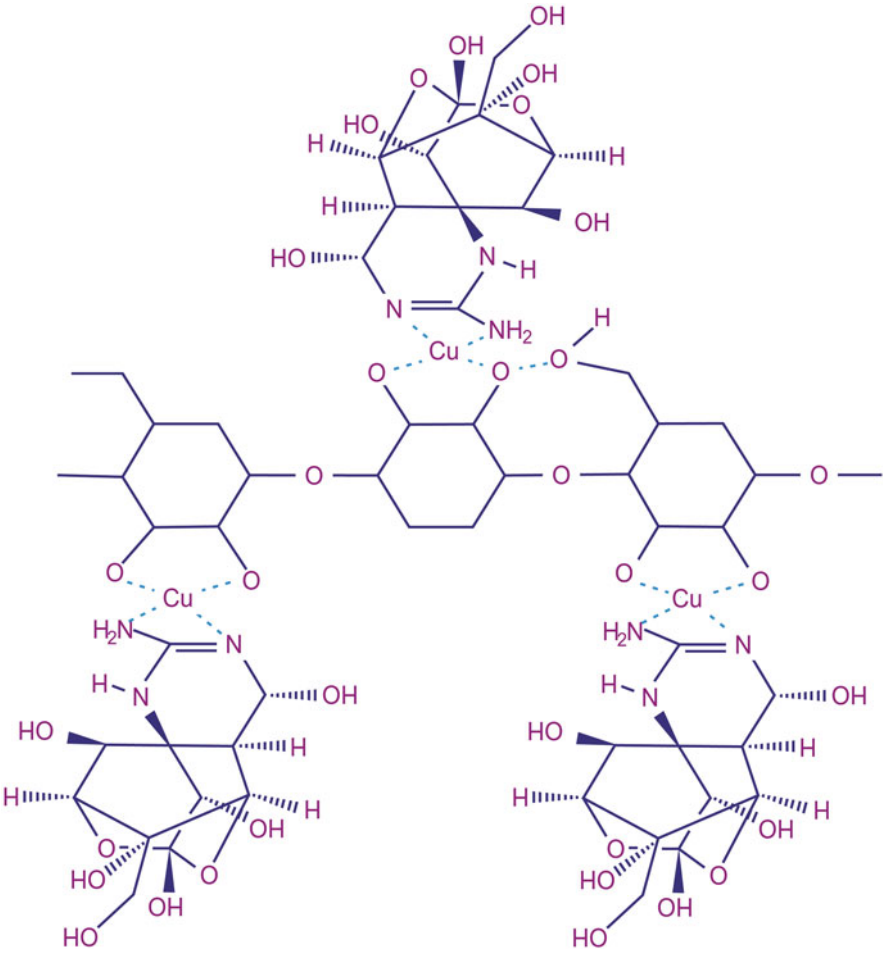

Fig. 1.5 This model depicts relation of TTX and EPS from *Lactobacillus rhamnosus* PN04 and *Leuconostoc mesenteroides* N3 via copper bridges (Tu et al. 2018)

detoxify tetrodotoxin by its own in experimental mice model. Consequently, more studies possibly will disclose the promising ways of neutralisation of tetrodotoxin in human also.

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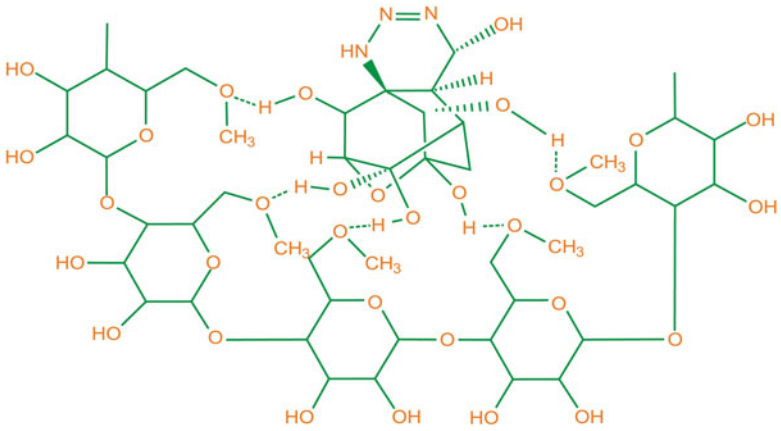

Fig. 1.6 The model shows interaction between TTX and EPS derived from *Lactobacillus plantarum* PN05 (Tu et al. 2018)

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Therapeutic Strategy for the Deterrence of COVID-19 with Relevance to Probiotics

2

Shubhita Mathur and Renu Bist

Abstract

The epidemiology of novel corona virus disease (COVID-19) is attributed by cough, fever, fatigue, headache, sore throat, gastrointestinal disorders, and pneumonia. The frequency of COVID-19 infection is accelerating at great speed globally, and till date, there is no specific vaccine or drug available for prevention or cure of the disease. Therefore, certain measures are required to check the outbreak of this pandemic immediately. Current chapter concerns with the development of certain probiotics including a single or mixed culture of live microorganisms that could maintain the intestinal or lung microbiota of humans. Besides being beneficial bacteria, probiotics also possess antiviral activity. The stimulation of the immune system through probiotics is one of the approaches that have been emphasized for fighting against the viral infections. The immunomodulatory activities of probiotics include the enhancement of the phagocytic receptors like CR1, CR3, FccRI, and FcaR; induction of APC-derived pro- and anti-inflammatory cytokines such as IL-10, IL-12, IL-17, TNF-α, and IFN-α against foreign antigens; and an increase in the microbicidal function of neutrophils. The most common probiotics include *Lactobacillus*, *Bifidobacterium*, *Leuconostoc*, *Pediococcus*, and *Enterococcus*. *Lactobacillus* and *Bifidobacterium* are widely used in yogurts and other dairy products. Probiotics exert antiviral activity by production of antiviral in inhibitory metabolites.

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Probiotics · COVID-19 · SARS-CoV-2 · Clinical trials · Hypoxemia · ARDS

2.1 Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 was first reported in Wuhan, China, in December 2019. Subsequently, the disease has spread worldwide. Coronaviruses (CoVs) are spherical or pleomorphic, enveloped, positive-stranded RNA viruses (+ssRNA) accompanied by long surface spikes. These crown-like viruses belong to the family *Coronaviridae* and order *Nidovirales*. The CoVs consist of four genera within the subfamily *Orthocoronavirinae*: *Alphacoronavirus* (alpha CoV), *Betacoronavirus* (beta CoV), *Deltacoronavirus* (delta CoV), and *Gammacoronavirus* (gamma CoV) (Yang and Leibowitz 2015; Li et al. 2020a, b). Among these, the alpha CoV and beta CoV have emerged as major human pathogens since they have the ability to cross animal-human barriers (Coleman and Frieman 2014; Zhu et al. 2020). Till date, seven groups of human coronaviruses (hCoVs) have been recognized that can infect humans. This includes the beta-genera CoVs such as severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV, hCoV-HKU1, and hCoV-OC43, the α-genera CoV such as hCoV-NL63, and the novel coronavirus that causes COVID-19 which is officially named as SARS-CoV-2 (Weiss 2020; Gorbalenya et al. 2020; Zhu et al. 2020). The ability of this virus to mutate and infect nonimmune populations has emerged as an ongoing global threat. Globally, as of 6 December 2020, there have been 65,870,030 confirmed cases of COVID-19, including 1,523,583 deaths, according to the World Health Organization (WHO) COVID-19 dashboard. There are no clinically approved medicines or vaccines which could provide therapy for COVID-19. In 2001, the definition of "probiotics" was reframed by the Food and Agriculture Organization of the United Nations (FAO) and the WHO as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Probiotics are used to impart useful functions to gut microbial communities by promoting microbial diversity within human microbiome. The inflammation of the gut and other disease phenotypes are averted as probiotics reinstate the composition of the gut microbiome. These live microorganisms are used to treat microbial deficiencies in the form of microbial supplementation. The representative symptoms of COVID-19 include fever, cough, myalgia, fatigue, and pneumonia (Guan et al. 2020; Huang et al. 2020). The less familiar symptoms include sputum production, headache, hemoptysis, and diarrhea (Huang et al. 2020). Few patients with COVID-19 exhibited intestinal microbial dysbiosis and decreased probiotics such as *Lactobacillus* and *Bifidobacterium*; hence, nutritional and gastrointestinal function must be evaluated for all patients. The application of prebiotics or probiotics can aid in the regulation of the balance of intestinal microbiota and, thus, reduces the risk of secondary infection.

2.2 Pathogenesis of COVID-19

The transmission of COVID-19 causing virus is predominantly via infective respiratory droplets. The mucosal surfaces of the host (eyes, nose, and mouth) get exposed to these droplets when an infected person is in close contact with someone who is actively coughing or sneezing (Li et al. 2020a, b). The incubation period of COVID-19, also known as "presymptomatic period," is 5–6 days but can be up to 14 days. COVID-19 patients at the time of admission in hospital exhibit a fever and dry cough, diarrhea, muscle and/or joint pain, nausea, headache/dizziness, struggle for breathing, and coughing up of blood (Tay et al. 2020).

Briefly, following three phases of COVID-19 correlates to different clinical stages of the disease (Wu and McGoogan 2020; Mason 2020).

Phase 1: Asymptomatic State (Mason 2020)

This phase comprises of initial 1–2 days of infection. During this phase, the inhaled virus SARS-CoV-2 replicates and fixes itself to epithelial cells in the nasal cavity. The main receptor and cofactor for SARS-CoV-2 entry in cells are angiotensin I-converting enzyme 2 (ACE-2) and transmembrane serine protease 2 (TMPRSS2) (Mason 2020; Hoffmann et al. 2020).

Phase 2: Respiratory Tract Response

A robust immune response initiates upon proliferation and migration of virus down the respiratory tract (Fig. 2.1) (Yang et al. 2020). However, expression of type I interferons (IFNs) by infected human monocyte-derived dendritic cells (DCs) and macrophages is inhibited by SARS-CoV (Cheung et al. 2005; Spiegel et al. 2005). The chemokine ligand CXCL10 is useful as disease marker in SARS (Tang et al. 2005; Rockx et al. 2009; Mason 2020). The blood of SARS-CoV-infected patients shows an increased expression of CXC chemokine ligand 10 (CXCL10) and C-C motif chemokine ligand 2 (CCL2) (Wong et al. 2004).

Phase 3: Respiratory Failure, "Happy" Hypoxemia, and Succession to ARDS

The low oxygenation index exhibited by SARS-CoV-2-infected patients leads to severe respiratory failure. As infection progresses, virus infects alveolar type II cells. Multifocal bilateral patchy shadows and/or ground glass opacities are observed in the chest computed tomography (CT) scans of infected patients (Mason 2020; Li et al. 2020a, b). A sensation of "uncomfortable, difficult, or labored" breathing is known as dyspnea. Several patients exhibit distinct arterial hypoxemia but lack relative signs of respiratory distress without the expression of dyspnea. This phenomenon in the infected patients is described as silent or "happy" hypoxemia (Tobin et al. 2020; Couzin-Frankel 2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients have characteristic viral pneumonia leading to acute respiratory distress syndrome (ARDS). Diffuse alveolar damage is caused by ARDS. During acute stage, alveoli exhibit hyaline membrane formation followed by interstitial widening, edema, and then fibroblast proliferation in the organizing stage. As disease progresses, COVID-19 ARDS patients exhibit fibrosis in the lungs (Gibson et al. 2020).

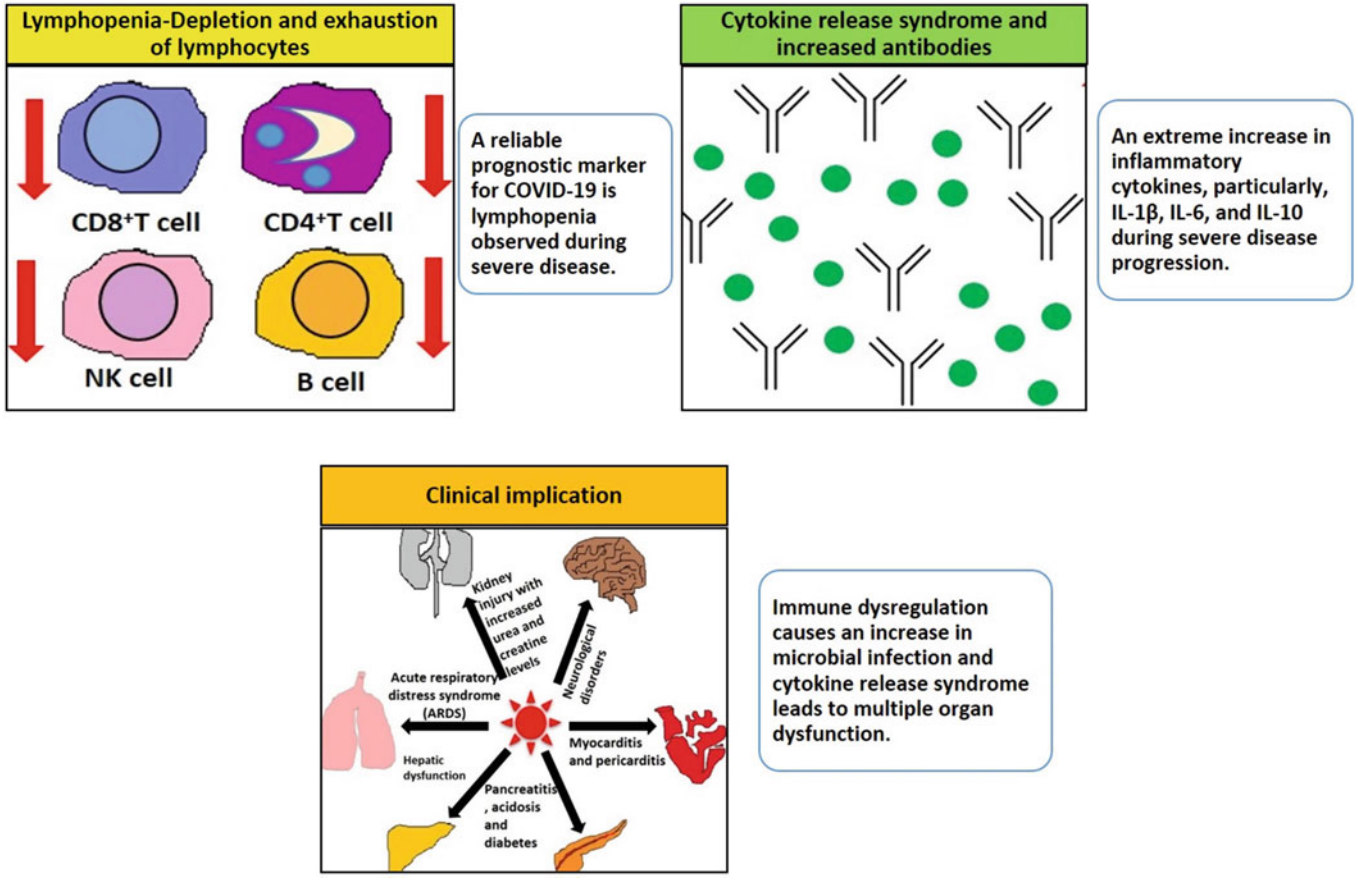

Fig. 2.1 Immunopathology and clinical implications of SARS-CoV-2 during disease progression