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Review Article

Microorganisms: Approaches to Laboratory, Experimental Molecular Biology, Roles in Controlling COVID-19, Advances and Applications

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Abstract

Advances in molecular biology techniques have been made due to biomedical engineering approaches for synthesis of new machineries and biomaterials. There are different molecular techniques that have been used for detection of microbes in order to distinguish their cells on the basis of their characteristics and structural differences of the same group's organism. These molecular techniques are PCR, electrophoresis, DNA fingerprinting, Pulse electrophoresis. Besides these techniques for detection of DNA, PCR demand has become most during pandemic of Covid-19. Real time PCR is the most advanced form of PCR that detection the nucleic acid testing in short time with greater chances of accuracy as compared to the other kit based traditional methods for detection of nuclear segments. Loop-mediated isothermal amplification (LAMP) is used for viral detection through nucleic acid testing with high accuracy and has become popular technique during pandemic of Covid-19. Bacteria can be utilized for human benefits especially for industrial applications such Viruses infect all types of life forms, from animals and plants to microorganisms, including bacteria. As treatment of many diseases, food industry, pharmaceutical industries and medical industry.

Keywords: PCR, RT-PCR, LAMP, Molecular Biology, Infectious diseases.

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Introduction

There are different molecular techniques that have been used for detection of microbes in order to distinguish their cells on the basis of their characteristics and structural differences of the same group's organism [1, 8]. These molecular techniques are PCR, electrophoresis, DNA fingerprinting, Pulse electrophoresis. Besides these techniques for detection of DNA, PCR demand has become most during pandemic of Covid-19. There are many limitations of other methods/techniques such h as time consuming, equipment, sample preparation compared to PCR for Covid-19 [2, 3].

Nature exhibits a variety of pathogens that can cause different diseases as well by interacting with cells of the immune system of living organism body [4, 5]. Microorganisms are one of those organisms that can be for industrial purpose such as bacteria used in yogurts, cheese and baking industry while on the other hand, they are also casing the serious diseases that lead to

deaths of many human populations [3, 6]. A large population of human have has been affected due to bacterial, viral, nematodes attack thus needed to control them. At these processes, greenhouse gases are released to the atmosphere at the same time, so microorganisms also play a key role in relation to climate and climate change. Viruses as well as nematodes also causing different diseases in human and there is need to control the pathogenic microbial infections through chemical, biological and advanced engineering approaches [1, 5].

Microorganisms diversity in spreading the diseases in plants and animals by triggering inflammatory response. They are involved showing immune mechanism with T cells of human body [6, 8]. Bacterial cell wall composed of chemical compound knows as peptidoglycan that can be destroying through the action of antibiotics. But another problem in the modern era is that with increasing the population of pathogens, bacterial also sowing strong resistance against the variety of amitotic and rested

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pharmaceutical products. In addition, microorganisms are able to grow inside animal or plant cells, such as endosymbionts [7, 9]. Sometimes bacterial growth increase at the rate exceeding the normal thus it become difficult to control them with low doses of drugs and increasing the concentrations of pharmaceutical based products leads to cellular toxicity side effects on human overall Bioengineering approach to control the infections caused by microorganisms helpful in order to control their growth at earlier stage thus helpful in diagnosis, controlling the pathogens [8, 17].

PCR Testing for Diagnosis of Covid-19

PCR (Polymerase Chain Reaction) has become the prominent technique for detecting the corona virus family due to specific amplification of the nuclear region [9, 16]. As this technique appears to more advance owing to its different types like real time PCR. Coronavirus as life threatening virus leads to deaths of many patients due to its fast replication inside the cells of human body. Sample for corona virus testing is the most important testing for diagnosis and other critical findings [10]. False positive PCR testing leads to increase the risks of threatening of corona virus. Although, vaccines for corona virus available, but there is need to designed the molecular based laboratory approaches to control the viruses. This virus also affected the global economy of the world [11, 20].

RT-PCR Testing for Diagnosis of Covid-19

Real time PCR is the most advanced form of PCR that detection the nucleic acid testing in short time with greater chances of accuracy as compared to the other kit based traditional methods for detection of nuclear segments [12, 27]. RT-PCR assays based on detecting and quantifying a fluorescent signal generated during amplification do not require post amplification. It has also other advantages over the other molecular biology laboratory techniques wound to fast amplification, less chances of contamination, small amount of sample. There are many strategies needed to enhance the biotechnical and medical products for detection of nucleic acid testing rather than assays or kits based method that have chances for false positive results[13,17].

LAMP based Testing for Diagnosis of Covid-19

Loop-mediated isothermal amplification (LAMP) is used for viral detection through nucleic acid testing with high accuracy and has become popular technique during pandemic of Covid-19[14, 18]. LAMP has highest specificity confirmation to target gene, it is considered to be less sensitive than other targets in clinical application and can be used to amplify the target sequence. RT-PCR involves the transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the cDNA. LAMP has also other applications for detection of forensic markers for investigation of criminals and other biochemical analysis [15, 28].

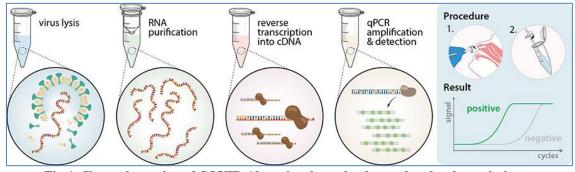


Fig-1: Shows the testing of COVID-19 strains through advanced molecular techniques

Due to multiple applications of RT-PCR, advances in molecular biology techniques have been made due to biomedical engineering approaches for synthesis of new machineries and biomaterials [16]. Although single-reaction RT-PCR removes the need for a separate RT reaction, RNA isolation from clinical samples constitutes a major bottleneck in the diagnostic process, as it remains both manually laborious and expensive. RT-PCR is used for separation and identification of nucleic acid materials using primers thus detects the particular sequence of nucleic acid due to high accuracy in clinical experimentation [17, 31].

Novel Approaches about Roles of Microbes

Bacteria can be utilized for human benefits especially for industrial applications such as treatment of many diseases, food industry, pharmaceutical industries and medical industry. These industries manufactured lots of products in respective to bacterial and microbial enzymes and their applications for discovery of novel compounds [18]. Many of the bacteria in the body play an important role in human survival. Bacteria in the digestive system break down nutrients, such as complex sugars, into forms the body can use. While on the other hand, bacteria also attack on bacterial skin, blood bore infectious and other inflammatory diseases. Besides these, variety of

pathogenic microbes also involved in activating of inflammatory responses [19].

There are different types of bacteria that have gained much attention towards the industrial technology due to its immense applications for synthesis of new products and production of food based materials that can be stored for long periods of time [8]. Bacteria, often lactic acid bacteria, such as Lactobacillus and Lactococcus, in combination with yeasts and molds, have been used for thousands of years in the preparation of fermented foods, such as cheese, pickles, soy sauce, sauerkraut, vinegar, wine and yogurt. These applications emerging the microbial technology for discover of new products in replacing the old pharmaceutical products and hence more economical importance in these days [20-22]. Bacteria can also be used in the place of pesticides in the biological pest control. This commonly involves thuringiensis (also called BT), a Gram-positive, soil dwelling bacterium that leads to control the different types of pests causing different diseases due to changing environment and fast growth of large variety of pests[23].

There are different types of chemicals and drugs used to control the infections caused by microbes that potentially target their cells and hence controlled the specific disease [24]. But due to changing environment and increase the microbial resistance in the modern era, novel drug can be utilized for controlling the specific genes of microorganism that can cause infectious disease. Antibiotics are typically used to treat bacterial infections. However, in recent years, improper and unnecessary use of antibiotics has promoted the bacteria. Antibiotics with strong combinations of drugs are used to control the bacterial infections but due to resistance against the specific drug leads to mechanism of cellular toxicity [25].

Despite of many organisms used for large production of industrial products but due to high cost and poor efficiency of fermentation and cultural method [26]. Algae are major source of products that can be synthesis in pharmaceutical and food industries. Changes in their genetic material leads to production of GMOs that can produce high quality medical based products with low cost and high efficiency. Algae has a high protein content and can be grown to have an even higher protein content. This helps to make algae ideal as an animal feed. Algae also have numerous micronutrients that can enrich standard animal feed [27].

Some species of algae are major source of proteins and minerals have therefore can utilize as for production of food products both industrial and commercial level. Chlorella is major source of proteins, calcium, iron, magnesium, and potassium, vitamins and

vitamin A that help energize your body and help it run more efficiently, from protecting your immune system to fighting off diseases [28]. Algae provide blooms with different colors can attract the useful organisms that can be used for medical purposes due to high protein value and other nutritional aspects. Alga is source of carbohydrate that can be utilized for large scale production of biofuel at industrial scale. Large proportions of industries also depend on algae due to more efficient prediction of pharmaceutical drugs. Proteins concentrations in algae are much favorable as compared to the other microorganisms such as viruses and bacteria and hence less toxic effects and more action on cells [29-31].

Algae are used in different industries for the production of biofuels owing to excellent biochemical composition that leads them as ideal source for biological products in medical as well as pharmaceutical industries [32]. Algae have also great economical importance due to its diversity and species all around the world. Algae store energy in the form of oils and carbohydrates, which, combined with their high productivity, means they can produce of biofuels per acre per year. Thus, algae as potential source of proteins can be utilized for large production of agricultural products for large production of biodiesel and related surplus compounds for burning and agricultural purposes [33].

Viruses are most leading infectious bodies that can replicate rapidly due their their fast entry into their particular host then cause the specific disease [34, 35]. It has different hosts so that it can easily infect cells of living systems such as HIV, hepatitis, cotton leaf curl viruses and many other types of viruses exhibit different properties due to its specific nature. A virus is a submicroscopic infectious agent that replicates only inside the living cells of an organism. Viruses infect all types of life forms, from animals and plants to microorganisms, including bacteria [32]. There are different viral drugs available that can targeted the viral cells but sometimes viruses show resistance assist the specific drugs[36].

CONCLUSION

Although, viruses are most life threatening organism that causes serious diseases in human especially COVID-19. There is need for the effective medicines and vaccines for through control at molecular and cellular level. Microorganisms are most important group that can be utilized for the production of industrial products such as bacteria used in industries for cheese and bread production, yogurt and many other pharmaceutical applications. Algae as source of proteins used for food preparation in different industries. Each microorganism also cause specific disease in respective to their particular host. There is need to designed drugs and pharmaceutical products

that can control their growth at cellular and molecular level.

REFERENCES

- 1. De Azevedo, J. L., & Quecine, M. C. (Eds.). (2017). Diversity and benefits of microorganisms from the tropics. Springer.
- 2. Gobbetti, M., Cagno, R. D., & De Angelis, M. (2010). Functional microorganisms for functional food quality. Critical reviews in food science and nutrition, 50(8), 716-727.
- 3. Curtiss III, R. (1976). Genetic manipulation of microorganisms: potential benefits and biohazards. Annual review of microbiology, 30(1), 507-533.
- 4. Chaudhry, Q., Blom-Zandstra, M., Gupta, S. K., & Joner, E. (2005). Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment (15 pp). Environmental Science and Pollution Research, 12(1), 34-48.
- 5. Rychert, J. (2019). Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. Journal of Infectiology, 2(4).
- Rijkers, G. T., Bengmark, S., Enck, P., Haller, D., Herz, U., Kalliomaki, M., ... & Antoine, J. M. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: current status and recommendations for future research. The Journal of nutrition, 140(3), 671S-676S.
- Corb Aron, R. A., Abid, A., Vesa, C. M., Nechifor, A. C., Behl, T., Ghitea, T. C., ... & Bungau, S. (2021). Recognizing the benefits of pre-/probiotics in metabolic syndrome and type 2 diabetes mellitus considering the influence of akkermansia muciniphila as a key gut bacterium. Microorganisms, 9(3), 618.
- Tamime, A. Y., Saarela, M. A. K. S., Sondergaard, A. K., Mistry, V. V., & Shah, N. P. (2005). Production and maintenance of viability of probiotic microorganisms in dairy products. Probiotic dairy products, 3, 39-63.
- Dharaneedharan, S., & Heo, M. S. (2016). Korean Traditional fermented foods-a potential resource of beneficial microorganisms and their applications. Journal of Life Science, 26(4), 496-502.
- Perlmutter, J. I., & Bordenstein, S. R. (2020). Microorganisms in the reproductive tissues of arthropods. Nature Reviews Microbiology, 18(2), 97-111
- 11. Kumar, D., Malviya, R., & Sharma, P. K. (2020). Corona virus: a review of COVID-19. EJMO, 4(1), 8-25.
- 12. He, F., Deng, Y., & Li, W. (2020). Coronavirus disease 2019: What we know?. Journal of medical virology, 92(7), 719-725.

- Zu, Z. Y., Jiang, M. D., Xu, P. P., Chen, W., Ni, Q. Q., Lu, G. M., & Zhang, L. J. (2020). Coronavirus disease 2019 (COVID-19): a perspective from China. Radiology, 296(2), E15-E25.
- 14. Liang, H., & Acharya, G. (2020). Novel corona virus disease (COVID-19) in pregnancy: What clinical recommendations to follow?.
- Xie, X., Zhong, Z., Zhao, W., Zheng, C., Wang, F., & Liu, J. (2020). Chest CT for typical coronavirus disease 2019 (COVID-19) pneumonia: relationship to negative RT-PCR testing. Radiology, 296(2), E41-E45.
- 16. Treibel, Thomas A., Charlotte Manisty, Maudrian Burton, Áine McKnight, Jonathan Lambourne, João B. Augusto, Xosé Couto-Parada, Teresa Cutino-Moguel, Mahdad Noursadeghi, and James C. Moon. "COVID-19: PCR screening of asymptomatic health-care workers at London hospital." The Lancet 395, no. 10237 (2020): 1608-1610.
- 17. Poon, L. L., Wong, O. K., Luk, W., Yuen, K. Y., Peiris, J. S., & Guan, Y. (2003). Rapid diagnosis of a coronavirus associated with severe acute respiratory syndrome (SARS). Clinical chemistry, 49(6), 953-955.
- Arevalo-Rodriguez, I., Buitrago-Garcia, D., Simancas-Racines, D., Zambrano-Achig, P., Del Campo, R., Ciapponi, A., ... & Zamora, J. (2020). False-negative results of initial RT-PCR assays for COVID-19: a systematic review. PloS one, 15(12), e0242958.
- Islam, Nazrul, Stephen J. Sharp, Gerardo Chowell, Sharmin Shabnam, Ichiro Kawachi, Ben Lacey, Joseph M. Massaro, Ralph B. D'Agostino, and Martin White. "Physical distancing interventions and incidence of coronavirus disease 2019: natural experiment in 149 countries." bmj 370 (2020).
- Fidan, V. (2020). New type of corona virus induced acute otitis media in adult. American journal of otolaryngology, 41(3), 102487.
- 21. Singh, B. K. (2009). Organophosphorus-degrading bacteria: ecology and industrial applications. Nature Reviews Microbiology, 7(2), 156-164.
- 22. Saichana, Natsaran, Kazunobu Matsushita, Osao Adachi, Ivo Frébort, and Jitka Frebortova. "Acetic acid bacteria: A group of bacteria with versatile biotechnological applications." Biotechnology advances 33, no. 6 (2015): 1260-1271.
- 23. Teusink, B., & Smid, E. J. (2006). Modelling strategies for the industrial exploitation of lactic acid bacteria. Nature Reviews Microbiology, 4(1), 46-56.
- Juturu, V., & Wu, J. C. (2012). Microbial xylanases: engineering, production and industrial applications. Biotechnology advances, 30(6), 1219-1227.
- 25. Ryan, P. M., Ross, R. P., Fitzgerald, G. F., Caplice, N. M., & Stanton, C. (2015). Sugar-coated:

- exopolysaccharide producing lactic acid bacteria for food and human health applications. Food & function, 6(3), 679-693.
- Baruah, R., Das, D., & Goyal, A. (2016). Heteropolysaccharides from lactic acid bacteria: current trends and applications. J Prob Health, 4(141), 2.
- 27. Priyadarshani, I., & Rath, B. (2012). Commercial and industrial applications of micro algae—A review. Journal of Algal Biomass Utilization, 3(4), 89-100.
- Chojnacka, K., Wieczorek, P. P., Schroeder, G., & Michalak, I. (Eds.). (2018). Algae biomass: Characteristics and applications: Towards algaebased products (Vol. 8). Springer.
- 29. Sivakumar, R., & Rajendran, S. (2013). Role of algae in commercial environment. International Research Journal of Environment Sciences, 2(12), 81-83.
- 30. Kadam, S. U., Tiwari, B. K., & O'Donnell, C. P. (2013).Application of novel extraction technologies for bioactives from marine algae. Journal of agricultural and food chemistry, 61(20), 4667-4675.
- 31. Beaumont, M., Tran, R., Vera, G., Niedrist, D., Rousset, A., Pierre, R., ... & Forget, A. (2021). Hydrogel-Forming Algae Polysaccharides: From Seaweed to Biomedical

- Applications. Biomacromolecules, 22(3), 1027-1052.
- 32. Xin, X., Huang, G., & Zhang, B. (2020). Review of aquatic toxicity of pharmaceuticals and personal care products to algae. Journal of Hazardous Materials, 124619.
- 33. Röhder, L. A., Brandt, T., Sigg, L., & Behra, R. (2014). Influence of agglomeration of cerium oxide nanoparticles and speciation of cerium (III) on short term effects to the green algae Chlamydomonas reinhardtii. Aquatic toxicology, 152, 121-130.
- 34. Hoffmann, E. M., & Bauknecht, N. (1999). A dye binding assay for the quantification of soluble and cell-bound acidic polysaccharides produced by red algae. Analytical biochemistry, 267(2), 245-251.
- Perez, M., Nolasco, N. A., Vasavada, A., Johnson, M., & Kuehnle, A. (2015). Algae-mediated valorization of industrial waste streams. Industrial Biotechnology, 11(4), 229-234.
- Bele, S. D., Patil, S. S., Sharmila, S., & Rebecca, L. J. (2014). Isolation and partial purification of lipase and protease from marine algae. J Chem Pharm Res, 6, 1153-1156.
- 37. Claus, G. (1970, April). Potential ties of Explo tlng Marine Algae. In Offshore Technology Conference. OnePetro.