Systematic Network and Meta-analysis on the Anti-viral Mechanisms of Probiotics: A Preventive and Treatment Strategy to Mitigate SARS-CoV-2 Infection

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[1]**Abstract**

BACKGROUND: With the alarming rise of infected cases and deaths, COVID-19 is a pandemic, currently affecting 235 countries worldwide. Until now, no curative medicine and vaccine are available against SARS-CoV-2. The causal virus SARS-CoV-2 primarily infects lung cells, leading to respiratory illness ranging in severity from common cold to deadly pneumonia. This, with comorbidities worsens the clinical outcome, particularly for, immunosuppressed individuals with COVID-19. Interestingly, commensal gut microbiota has been shown to improve lung infections by modulating the immune system. Therefore, fine-tuning of gut microbiome with the consumption of probiotics could be an alternative strategy for boosting immunity and treating COVID-19. **METHODS:** Here, we present a systematic biological network and meta-analysis to provide a rationale for implementation of probiotics in preventing and/or treating COVID-19. **RESULTS:** We have identified 90 training genes from the literature analysis (according to PRISMA guidelines) and generated an association network concerning the candidate genes linked with COVID-19 and probiotic treatment. The functional modules and pathway enrichment analysis of the association network clearly show that application of probiotics could have therapeutic effects on ACE2 mediated virus entry, activation of systemic immune response, *nlrp3* mediated immunomodulatory pathways, immune cell migration resulting in lung tissue damage and cardiovascular difficulties and altered glucose/lipid metabolic pathways in the disease prognosis. We also demonstrate the potential mechanistic

[1] Abbreviations: COVID-19, Coronavirus Disease of 2019; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ACE2, Angiotensin-converting enzyme 2; WHO, World Health Organization; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; MERS-CoV, Middle East respiratory syndrome Coronavirus; URTI, Upper Respiratory Tract Infection; ARDS, Acute Respiratory Distress Syndrome; MCODE, Molecular Complex Detection; BiNGO, Biological Networks Gene Ontology tool; IL, Interleukin.

domains as molecular targets for probiotic application to combat the viral infection. **CONCLUSIONS:** Our study therefore offers probiotics mediated novel preventive and therapeutic strategy for COVID-19-warfare.

Keywords: COVID-19, SARS-CoV-2, probiotics, gut-lung axis, biological network analysis, meta-analysis.

1. Introduction

The coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been declared as pandemic by The World Health Organization (WHO). Till date, more than 34.8 million confirmed cases of COVID-19 have been reported in 235 countries, with a total of 1030738 confirmed deaths (https://www.who.int/emergencies/diseases/novel-coronavirus-2019, 04 October 2020, 05:30 GMT+5:30). The death toll has climbed up since no vaccine, antiviral drug or specific treatment for COVID-19 is currently available. The global emergency of the pandemic therefore urgently demands for the investigation on the novel therapeutic strategy effective for SARS-CoV-2.

The positive sense RNA virus SARS-CoV-2 belongs to pathogenic beta-coronavirus. It shares 80% nucleotide sequence identity with SARS-CoV and 50% with MERS-CoV, the other two beta-coronavirus that caused earlier major outbreaks of lethal pneumonia [1]. Epidemiology indicates SARS-CoV-2 is more infectious but less fatal (2–5%) than SARS-CoV (9.4%) and MERS-CoV (34.5%) [2]. However, similar to them, SARS-CoV-2 primarily infects alveolar epithelial cell of the lung, leading to a respiratory syndrome with a variable degree of severity, ranging from upper respiratory tract infection (URTI) to severe interstitial pneumonia and acute

respiratory distress syndrome (ARDS) [3]. The elderly people and immunocompromised individuals with existing medical conditions like diabetes, hypertension and cardiovascular complications are critically affected by the disease [4].

Interestingly, respiratory infections, sepsis and ARDS have been found to be associated with a change in gut microbiota composition indicating their possible role in pulmonary health [5]. Therefore, it can be speculated that a vital cross-talk between the gut microbiota and the lungs through the “gut-lung axis” may impart significant role in SARS-CoV-2 infection [5]. Thus, manipulation of the intestinal microbiota by probiotics appears a potential alternative approach for maintaining health and preventing and/or treating the disease.

Consumption of probiotics has been shown to improve the URTI on numerous human clinical trials [6] (**Table 1**). Probiotics build up a protective mucus barrier, impart healthy immune response to all age group individuals minimizing the probability of infectious disease occurrence [7]. As the specific strains of probiotics, applied with appropriate dosage and mode of administration, can efficiently treat respiratory complications, we hypothesize that application of probiotics could be effective against SARS-CoV-2 infection.

Network analysis offers an effective approach to identify molecular mechanisms and connections between genes and their pathways from dynamic networks [8]. Therefore, in the present network and meta-analysis study, a biological association network is generated concerning training genes of SARS-CoV-2 infection and probiotic treatment. The meta-analysis shows the rational justification for implementation of probiotics approach in respiratory infections. The analysis of

functional modules, pathway enrichment and network topological parameters reveal that probiotics could have tremendous therapeutic potential during the pathophysiological events of COVID-19. The overall network analysis study thus indicates that the application of probiotics at the major domains of the infection mechanism could be benefited in the prevention and treatment of SARS-CoV-2 infection.

2. Materials and Methods

Despite of the genomic similarity with the SARS-CoV, high-throughput data and an unambiguous model for SARS-CoV-2 immunopathology is unavailable. The literature search for the study was performed according to PRISMA guideline (**Fig. 1**). The rational for probiotics as a cure of the global pandemic was justified by the meta-analysis study (**Fig. 2, 3**). Further, a network-analysis study was considered to evaluate the probable bioprotective mechanisms of probiotics against SARS-CoV-2 infection. The analysis of the network modules revealed functionally related genes and provided key domains for the potential mechanism of probiotics against SARS-CoV-2 infection. The entire methodology is summarized in **Fig. 4**.

2.1. Rational for application of probiotics as potential preventive and an alternative treatment strategy for SARS-CoV-2 infection: A meta-analysis

The meta-analysis was conducted to analyse similar studies that are already available in the literature. In our study concerning probiotic treatment on URTI patients, Q-statistics is used to assess whether all effect sizes in the sample of single studies are homogeneous (belong to the same population) and I^2 index indicates the degree of heterogeneity. Further, the impact of heterogeneity on the pooled

estimates of the individual outcomes of the meta-analysis was assessed by the forest plot. The effects of probiotics depend on specific strains, age-group, clinical dosage and the mode of administration. Clinical studies with randomized double-blind placebo controlled human trials were screened. The overall effects of specific probiotic strains were measured as mean difference (MD) or standardized mean difference (SMD), effects size, 95% confidence interval (CI) and weight percentage for studies, by using random-effects model. The mean change *i.e*. standard deviation in the number of healthy individuals and patients was used to calculate the MD between the probiotics (or synbiotics) and the placebo groups. The statistical analyses were performed using metapython. The p value of Q statistic < 0.05 was defined as an indicator of heterogeneity and data were considered heterogeneous for I^2 value higher than 40%. Begg's funnel plot was performed to examine the publication bias. All the reported p values were two-sided and p values ≤0.05 were regarded as statistically significant for all included studies.

2.2. Literature search strategy: data-mining

The literature study was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (**Fig. 1**). The comparatively unexplored functional genes (training genes) relevant to the viral infection were detected from the structured databases by data-mining strategy. The primary search strategy included manual analysis of the published literature indexed in the electronic search database PubMed archive (http://www.ncbi.nlm.nih.gov/pubmed/) using reference management software EndNote X7 (Bld 7072) (endnote.com). The search terms or MeSH terms used in EndNote included "probiotics" AND "coronavirus." In the scarcity of the available

studies regarding SARS-CoV-2 immunopathology and anti-viral probiotic application against them, the literature search was continued using NCBI PubMed database. The search terms or keywords used were “probiotics” ∩ “COVID-19”, “probiotics” ∩ “severe acute respiratory syndrome coronavirus 2”, “probiotics” ∩ “SARS-CoV-2” and “probiotics” ∩ “coronavirus”. The search was limited to the studies conducted on humans and available literature from April 18, 2020 to June 8, 2020 was considered for the present study. Therefore, data-mining considered the immunopathology of SARS-CoV-2 infection and application of probiotics to mitigate the disease severity. This approach allowed us to pull out the training genes associated with novel coronavirus infection and probiotic treatment. The top-ranked genes were thus selected and used for text-mining.

2.3. Association network construction and selection of the candidate genes: text-mining

An efficient and integrated combination of data-mining and text-mining approach was used to retrieve documents eliminating biasness towards vigorously investigated disease phenotypes. The unstructured biomedical texts were regained with simultaneous construction of interaction network by text-mining strategy. Thus, the candidate genes associated with SARS-CoV-2 infection and anti-viral probiotic treatment were constructed in the form of association network by screening the training genes from literature based on the text-mining approach. An open-source bioinformatic tool Cytoscape and the metasearch plug-in Agilent Literature Search were used to visualize the molecular interaction networks, integrating the gene expression profiles of the respective training genes. Agilent Literature Search software fetched documents based on the entered query using multiple text-based

search engines which are parsed into sentences. The software generated the association network based on a lexicon set definition which defines the gene names of the parsed sentences as “concepts" and the interaction terms of interests as "verbs". The network was generated by extracting an association for every parsed sentence containing at least two "concepts" and one "verb" or known interaction term. The gene names and association terms represented nodes and edges respectively of the text-mining based network, generated through Cytoscape. In this work, Cytoscape 3.7.1 App and Agilent Literature Search 3.1.1 beta (LitSearch version 2.69) were used to analyze the existing data source from the published literature available in the PubMed database. The training genes were used as "search terms"; Max Engine Matches was set at 10; the "use context," and the "concept lexicon restrict search" options were selected as search controls; "Homo sapiens" was used as "extraction control."

2.4. Functional module determination: MCODE analysis

Any disease pathogenesis involved complex interaction of biological events that were represented by the disease concerning association network. Identification of tightly interconnected nodes or genes from a very densely connected network were useful for understanding significant biological events in terms of modules and the interconnections between them. The association network generated with SARS-CoV-2 pathogenesis candidate genes, were subdivided into modules by Molecular Complex Detection (MCODE) plug-in of Cytoscape according to local neighbourhood density. The extracted modules were graphically displayed as an isolated, more considerable dense region with functionally similar genes. The network view of the clusters enabled to understand the local topology and functional features with

respect to the whole network consisting of several other candidate genes and edges. Network modules with MCODE score more than three, and a minimum of four nodes were considered as significant and carried forward for further analysis of functional annotation.

2.5. Quantitative data synthesis: Network Analysis

The network topology parameter details of the association network were obtained by analysing the association network with the NetworkAnalyzer tool in Cytoscape. The node degree and clustering coefficient of the individual node genes together represented the number of connections associated with a node and degree of involvement of a node in the participating clusters respectively. This tool showed the degree involvement of individual genes in the whole network as well as participating clusters. The functional annotations of the highest MCODE score with clustered candidate genes were determined by the integrative human gene database GeneCards (version 4.14). It provided gene-centric data of the annotated and predicted human genes with the functions and pathways associated with the candidate genes.

2.6. Assessment of overrepresentation of gene ontology categories by The Biological Networks Gene Ontology tool (BiNGO)

In order to identify enriched biological processes that are affected by COVID-19 immunopathology, a gene ontology (GO) functional enrichment analysis was performed using BiNGO (version 3.0.3) in Cytoscape 2.8.0 (http://www.cytoscape.org/), with a threshold of $p<0.001$. BiNGO is a tool to determine statistically overrepresented GO terms for a set of genes associated with

any biological processes as recorded in the Gene Ontology database. The BiNGO analysis was done with the MCODE derived gene clusters of the association network. Statistical test was set to “hypergeometric test”, multiple testing correction as “Benjamini & Hochberg False Discovery Rate (FDR) correction”, significance level was set to 0.05, the categories to be visualized was “overrepresented categories after correction”, reference set was “use whole annotation as reference set”, ontology file was “GO_Biological_Process”, and finally organism/annotation was selected as “*Homo sapiens*”.

3. Results

3.1. Overall effects of probiotics on URTI

URTI is one of the principal symptoms of SARS-CoV-2 infection. Therefore, in all the studies of meta-analysis, the duration and severity of URTI were compared between individual probiotic or probiotic formulation (or synbiotics) treatment and placebo-controlled groups including children and adult individuals. The forest plot showed the effect of probiotic treatment on URTI (**Fig. 2**)**.** There was no statistical heterogeneity as indicated by an I^2 value of 31.47 % and Q (chi-square) statistics of 42.32 ($p = 0.05$). The overall effect size of the study was 2.75 with $p = 0.006$. The effect of probiotic treatment on URTI was plotted according to specific strains of probiotics. The effects size (ES), confidence level (95% CI), weight percentage of clinical trials concerning the specific strains were also depicted. Thus, the forest plot of the meta-analysis study showed statistically significant effects of probiotic treatment on URTI.

3.2. Publication bias

Funnel plot showed the minimal evidence of publication bias among the selected studies associated with randomized double-blind placebo controlled human clinical trials of probiotic application on URTI (**Fig. 3**). Funnel plot with strain-specific probiotic treatment on URTI patients was symmetrical which indicated that no significant asymmetry was detected in any of the analyses.

3.3. Identification of the respective training genes associated with COVID-19 pathophysiology and anti-viral approaches with probiotic treatment

The data-mining exploratory technique found out about 36 data including clinical trials, *in-vitro* experiments, bioinformatic analysis, reviews, mini-reviews and editorial letters that are associated with COVID-19 pathophysiology and probiotic treatment from literature. The use of specific MeSH terms in EndNote and keywords used in the PubMed database excluded any non-specific search results. This confirmed the accuracy of the mining study performed with the reports exclusively on SARS-CoV-2 infection and application of probiotics. Manual analysis of the literature shortlisted 90 training genes of interest associated with COVID-19 pathogenesis (**Table 2**). These genes were used for the generation of association network and further analysis.

3.4. Generation of association network with the candidate genes of COVID-19 pathological mechanisms and anti-viral treatments with probiotics

All the 90 shortlisted training genes were used for the development of text-mining based association network. In the present pandemic situation, a detailed structured network interaction data related to COVID-19 pathophysiology and

treatment procedures are limited. So, the corresponding candidate gene of the text-mining based network, presented an alternative source of more general type of associations. The primary association network obtained through Cytoscape with training genes as query terms showed 453 nodes and 1273 edges. **Fig. 5** indicated the topology of the central association network highlighting the listed training genes involved in the SARS-CoV-2 infection. **Table 3** showed the network parameter statistics. According to the network topology, "the number of connected components" represented the network connectivity, which reflected a pairwise connection among all the nodes. Hence a lower number of connected components of 27, obtained by the network statistics, suggested a robust connectivity of the association network (**Table 3**). The clustering coefficient 0.633 designated the average clustering coefficient or degree involvement of respective nodes in the participating cluster of the network, with 5.620 average number of neighbours. The tendency to form high degree nodes or hub nodes was described by high "network heterogeneity" of 1.137 and the absence of any isolated nodes. The presence of hub nodes indicated the existence of a real functional network with multiple biologically relevant pathways associated with the novel coronavirus infection. The text-mining approach in conjunction with Cytoscape have been successfully adopted to illustrate actin dynamics during the post-ejaculatory life of spermatozoa [9], to demonstrate the synergistic mechanisms of therapeutic herbs for rheumatic arthritis [10] and to find out the effects of psychological stress on innate immunity and metabolism in human [11].

3.5. Cluster analysis of the association network identified the groups of promising genes related to COVID-19 clinical spectrum and anti-viral probiotic treatment

The text-mining based association network concerning SARS-CoV-2 infection and anti-viral probiotic treatment was applied for the identification of small clusters with densely connected nodes by MCODE plug-in in Cytoscape. MCODE tool followed an algorithm called "top overlap" by which it made groups of "genes of interest" according to their correlation coefficient and highest scoring edge among all other candidate genes of the network. This method was advantageous since it had directed mode that allows fine-tuning of relevant cluster interconnectivity among rest of the network. The algorithm also minimized the chances of false-positive results, increasing the robustness and accuracy of the analysis [12]. Therefore, MCODE tool provided highly interconnected nodes with similar functions among the whole network. MCODE derived a total of 38 densely bridged modules from the first network (**Fig. 6**). The individual clusters were further selected based on their individual scores for functional studies with removal of lower confidence data points. The details of the modules were given in **Table 4**. Among the 38 derived modules, 11 resultant clusters with MCODE score ≥ 3, nodes ≥ 4, edges ≥ 6 were chosen for functional annotation. Module 1 with MCODE score 16, 16 nodes and 120 edges, was the highest-scoring module, whereas module 2 with MCODE score 14, 14 nodes and 91 edges, was the second-highest and module 3 with score 8.457, 36 nodes and 147 edges was the third highest. The network matrices like node degree, clustering coefficient of the primary network and MCODE derived modules provided the topological parameters to understand the importance of nodes for pathway analysis. The highest-scoring module 1 (MCODE score 16) consisted of 16 nodes (*src, limk1, rps6ka3, aak1, mapk1, mknk2, map3k1, gak, map2k1, fgfr1, mapkapk5, map3k7, bmp2k, zak, gsk3, yes1*) (**Fig. 5**) and the highest number of nodes or edges containing module 3 (*cxcl5, cxcl12, tnc, has2, cxcl6, mmrn1, mmp13, smox, rela,*

ccl2, chi3l1, csf2, il5, il25, klk15, lcn2, il18, vegfa, eng, plau, pgf, prl, angpt2, igfbp1, hgf, erbb2, fgf2, stat3, stat5, ifna1, soat1, cd68, il9, itgam, ptprc, cd14) was preferred as the most significant functionally relevant clusters with respect to COVID-19 infection. Cluster analysis by MCODE algorithm had been adopted by several research groups for biological interpretation and elucidation of complex molecular networks (see discussion) [13].

3.6. Determination of functional annotation profile of the sub-clusters to elucidate the protective avenues of probiotics against SARS-CoV-2 infection

The MCODE plug-in decided the module score which signified the density and size of the individual module reflecting their functional importance in the probiotic-SARS-CoV-2 interaction. A higher scoring MCODE module implied a tighter connection of the genes with more than one associative gene and a possible multi-functional role. Therefore, the modules with higher score and nodes and edges could be assigned the key regulatory functions in the disease pathogenesis that could also be targeted for the probiotic treatment. The functional annotations and the pathways associated with candidate genes of the selected MCODE clusters were determined by GeneCards and NCBI.

The genomic information of the nodes showed that MCODE cluster 1 candidate genes *src, limk1, rps6ka3, aak1, mapk1, mknk2, map3k1, gak, map2k1, fgfr1, mapkapk5, map3k7, bmp2k, zak, gsk3, yes1* were involved in the receptor mediated endocytosis and phagocytosis, stress-mediated cellular metabolic pathways (**Fig. S1**). Therefore, probiotic supplementation could heal the disrupted intestinal barrier and prevent the virus entry.

MCODE cluster 2 consisting of 14 nodes (*tnf, il6, map1lc3b, ros1, cd38, bax, fas, sqstm1, il10, fcgr3a, cybb, il32, bcl2, icam1*)(**Fig. S2**) had functions in the activation of immune cells (B cells, T cells, natural killer cells) in response to stress stimuli, induction of the NF-κb mediated inflammatory pathways and subsequent apoptosis as well as production of cytokines. Several probiotic strains are reported to balance the immune activations and inflammatory cytokines which could have immense implications in the SARS-CoV-2 induced immunological complications.

Although MCODE cluster 3 was the third in rank based on score, it contained the highest number of node and edges that might indicate the interconnections of multiple cellular pathways (**Fig. S3**). The GeneCards derived functional profile of nodes expectedly showed the interconnections of four cellular events upon the viral infection: a) activation of principal anti-viral interferons (IFNs) and innate immune cells (*stat3, stat5, ifna1, soat1, cd68, il9, itgam, ptprc, cd14*); b) activation of pro-inflammatory cytokines and their downstream signaling through JAK-STAT pathway (*ccl2, chi3l1, csf2, il5, il25, klk15, lcn2, il18*), c) activation of pro-inflammatory cytokines and chemokines resulting in immune cell (neutrophil, monocyte) migration and infiltration in lung tissues and d) synthesis of lung damaging hyaluronan by *has2* or hyaluronan synthase-2 (*cxcl5, cxcl12, tnc, has2, cxcl6, mmrn1, mmp13, smox, rela*), a pro-inflammatory cytokine induced growth factors, responsible for vascular permeability and pulmonary dysfunction (*vegfa, eng, plau, pgf, prl, angpt2, igfbp1, hgf, erbb2, fgf2*). Although limited studies are available on the ability of probiotics to degrade glycosaminoglycan molecules, probiotics could have effects in degradation of hyaluronan substances, synthesized by SARS-CoV-2 induced pro-inflammatory cytokines.

The candidate genes of cluster 5 (*tlr2, tlr4, nlrp3, mydd88, hmgb1, mapk14, creb1, rps6ka5, jun, ephb2, mapk8, atf2, fos, maa*) represented the genes responsible for TLR-mediated innate immune responses and subsequent activation of inflammasome complex, with stress-induced MAPK signaling pathway (**Fig. S4**). The virus entry might activate the host innate immune system through TLR-mydd88 mediated pathways and eventually activate the cellular inflammasome complex. The functional annotations of cluster 6 candidate genes (*dgat2, adipor2, dgat1, pgc, adipoq*) indicated glucose and lipid metabolic pathways and innate immune responses (**Fig. S5**). Host defence mechanisms by proliferation of Th1 cell suppressing Th2 and Th17 cells, isotype switching and anti-inflammatory cytokine production, induction of growth regulatory pathways were represented by MCODE cluster 9 candidate genes (*il27ra, il27, cxcl10, il17d, il6st, il6r, jak2, ptpn18, mapk3, epo, akt1, egfr, reg1a, ptpn1, cat, frap1, malat1, acan, eif4ebp1, sma4, smad6, smad2, smad7*) (**Fig. S6**). The genomic functional annotations of cluster 10 (*ace2, mas1, ace, ang, agtr1, il22, il2, gli2, cd4, cd40lg, fus, th1l, pdgfb*) and cluster 11 (*rab18, rab13, mtg1, rce1*) candidate genes showed the reported ACE2-ADAM17 mediated entry of SARS-CoV-2 in the host cell with the resulting dysregulation of the renin-angiotensin system (RAS) involving *ace2, ace, mas1, ang* genes (**Fig. S7**). Moreover, cluster 13 candidate genes (*hif1a, dlk1, adam17, epas1*) indicated the receptor mediated endocytosis of the virus entry with the consequent activation of oxidative stress-responsive pathways mediated by *hif1α* (**Fig. S8**). Probiotic supplementation could alter the adverse conditions of the infection by regulating host metabolic pathways through secretion of short chain fatty acids (SCFAs). Additionally, probiotics could balance the inflammatory cytokines and chemokine levels in the body by regulating oxidative stress which could alter the SARS-CoV-2

infection condition. The effects of probiotics in controlling blood pressure may play significant role in altering *ace2* mediated dysregulation of RAS. Finally, the candidate genes of MCODE cluster 35 (*pparg, twist1, lpa, slc12a, mbtps1, srebf1, mbtps2*) and cluster 38 (*hk2, nr1i2, foxa2, pik3ca, f10, inpp5d, hspb1, mcl1*) designated the involvement of glucose and lipid metabolic pathways, adipocyte differentiation, VEGF signaling pathway and induction of atherosclerosis (**Fig. S9**). Although the MCODE score of cluster 38 was < 3, the module was selected for analysis due to presence of 8 nodes and 10 edged that might reflect significant function and pathways in the disease. Treatment with probiotic formulations might reverse any metabolic abnormality upon SARS-CoV-2 infections by releasing SCFAs. The molecular events and pathways indicated by MCODE clusters was supported by the published scientific evidences available for SARS-CoV-2 infection and pathogenesis, which elucidated the promising yet unexplored functional pathways of these genes.

3.7. GO enrichment analysis to assess the overrepresentation of gene ontology categories by the Biological Networks Gene Ontology tool (BiNGO)

The BiNGO of Cytoscape analyzed the enrichment of the GO terms assigned to the candidate genes of the MCODE derived clusters. The p-values of each GO term showed significantly overrepresentation of the GO ontology associated with the candidate genes. The enrichment analysis of the MCODE clusters enabled us to understand the enrichment of the molecular processes in terms of overrepresentation of GO ontology. Several research groups have used similar BINGO enrichment analysis to understand the breast cancer susceptibility or to detect key pathways responsible for gastric cancer or to analyze protein-protein network.

The enrichment or overrepresented GO terms associated with the MCODE cluster candidate genes are detailed in **Table 5.** The analysis showed that cluster 1, cluster 2, cluster 3 and cluster 5 involved the pathways related to viral infection, PAPMS mediated immune response to extracellular signals, stress, leukocyte migration, activation of cellular oxidative stress and inflammatory processes through phosphate metabolic processes, regulation of MAPKKK cascade and jun kinase activity. Therefore, these physiological events associated with SARS-CoV-2 infections could be modulated by probiotic supplementation. Probiotics could heal the intestinal barriers and minimize the viral entry as well as can balance the immune responses and inflammatory conditions. Cluster 6, cluster 9, cluster 10, cluster 11 and cluster 13 were involved in regulation of innate immune cell differentiation, regulation of glucose and lipid transport, cholesterol efflux, glycerol, glycerolipid, acylglycerol, triglyceride, TNF and cytokine signaling by JAK-STAT pathway in addition to regulation of renin-angiotensin mediated blood pressure. Probiotic supplementation could play immense role in immune cell development and differentiation, regulation of inflammatory processes, metabolic activity as well as in regulation of blood pressure through renin-angiotensin system. Cluster 35 and cluster 38 genes were involved in lipid, cholesterol, steroid, glucose and monosaccharide metabolic processes, xenobiotic drug transport, regulation of monocyte neutrophil differentiation, response to cytokine IL6. This enrichment analysis of the GO terms of candidate genes enlighten us with the major biological processes associated with SARS-CoV-2 pathogenesis and provided the cellular domains for anti-viral probiotic mechanisms to defeat the viral infection [8].

4. Discussion

The successful application of probiotics in respiratory infections of human clinical trials encourages us to study on the probiotics application to alleviate SARS-CoV-2 infection. The meta-analysis is an epidemiological study design that quantitatively examine the available outcomes and estimate the effect of treatment much precisely than a single study contributing to the pooled analysis. In this study, by using systematic network and meta-analysis, we provide a promising probiotic mechanism that can reinforce the immunity as well as mitigate the SARS-CoV-2 infection.

Probiotics could confine the virus entry and reduce the adverse effects caused by anti-viral immune activation and dysregulation of renin-angiotensin system (RAS)

ACE2 receptor is a master regulator of RAS and blood pressure. However, SARS-CoV-2 enter into the host enterocytes and lung tissues through ADAM17 mediated proteolytic cleavage of ACE2 receptor [14]. The disruption of the protective “gut-lung -axis” increases the propensity of the infection in the underlying ACE2 containing epithelial cells. SARS-CoV-2 infection may downregulate cellular ACE2 expression with resulting accumulation of angiotensin II (Ang II). Therefore, infected lung alveolar and small intestinal epithelial cells, heart, kidney, vascular endothelial and smooth muscle cells (highly expressing ACE2) cause vasoconstriction, tissue inflammation, oxidative stress which worsen the health conditions of COVID-19 patients [14].

Our functional annotations and enrichment analysis of candidate genes of MCODE cluster 10 (***ace2***, *mas1*, ***ace***, ***ang***, *agtr1*, *il22*, *il2*, *gli2*, *cd4*, *cd40lg*, *fus*, *th1l*,

pdgfb) and MCODE cluster 13 (***hif1a***, *dlk1*, ***adam17***, *epas1*) (**Fig. S7 and S8; Supplementary table S1**) indicate that probiotics could have potential roles in limiting viral entry through ADAM17-ACE2 mediated receptor endocytosis. Further enrichment analysis shows that application of probiotics could mitigate the adverse effects of dysregulated RAS system, *hif1a* mediated oxidative stress [15], activated immune cells (monocytes and NK cells) and elevated pro-inflammatory cytokines.

Indeed probiotics are known to heal the damaged epithelial barrier which could protect the underlying ACE2 expressing cells [16]. Probiotics have been proved to induce anti-oxidative nitric oxide (NO) production, reduce hypertension [17] and oxidative stress [18], secrete ACE-inhibitory peptides [19], SCFAs (acetate, propionate and butyrate) to induce anti-inflammation and control the blood pressure [20]. Recombinant probiotic *Lactobacillus paracasei* in conjugation with ACE2 has been applied for cardiovascular protective role of ACE2 in mice [21].

Probiotics could play a protective role in cytokine storm and lung injury caused by SARS-CoV-2 infection

After entry, the viral RNAs activate cellular innate immune system (TLRs) [22] and inflammatory pathways (NLRP3 and NF-κB) as a protective mechanism. Activated TLRs promote first-line anti-viral response through MYD88 and IRF3/7 mediated type-I IFN production [23,24]. The NLRP3 inflammasome complex assists in the secretion of pro-inflammatory IL1b and IL18 which activates T-cells or macrophages to secrete IL6 and TNFα. The released pro-inflammatory cytokines (IL1B, IL18, IL6, TNFα) further converts naive T-cells to Th1/ CTLs/ CD8+ or Th17 and triggers the secretion of pro-inflammatory IFNγ and IL17. However, NF-κB pathway can be turned on by either of two ways: activated NLRP3 or TLR4 and

stress-induced MAPK signaling (ERK1/2, JNKs and p38/ MAPK14) pathway [25,26]. The activated NF-κB pathway contributes to pro-inflammatory cytokine secretion and apoptosis in enterocytes and lung tissues. The resulting tissue injury activates local circulatory innate immune cells and establishes a proinflammatory feedforward loop of cytokines termed as cytokine storm [25]. The surge of cytokines and chemokines induces VEGF, IL8, additional IL6, and reduces E-cadherin expression on endothelial cells ensuing vascular permeability. While it also elicits leukocyte trafficking and migration of monocytes, neutrophils, NK cells, macrophages, dendritic cells in the lung cells. The resulting immune cells and cytokines induces hyaluronan synthesis which participate in the pathophysiology of ARDS, the hallmark of SARS-CoV-2 infection [27].

Our functional annotations and enrichment analysis of MCODE cluster 5 candidate genes (***tlr2, tlr4, nlrp3, mydd88***, *hmgb1,* ***mapk14****, creb1, rps6ka5,* ***jun,*** *ephb2,* ***mapk8****, atf2,* ***fos****, maa*) indicate that probiotics could be associated with TLR mediated innate immune response during SARS-CoV-2 infection. TLR-3, 7/8, reported to recognize the SARS-CoV-2 RNA, was also present in association network but not in MCODE cluster (**Fig. S4; Supplementary table S2**). The presence of *nlrp3* in cluster 5 hints the activation of inflammatory and antiviral IFNs which is further supported by cluster 2 (***tnf, il6****, map1lc3b,* ***ros1****, cd38,* ***bax,*** *fas, sqstm1,* ***il10****, fcgr3a,* ***cybb****,* ***il32****,* ***bcl2,*** *icam1*) and cluster 3 (*cxcl5, cxcl12, tnc,* ***has2****, cxcl6, mmrn1, mmp13, smoxlus, rela, ccl2, chi3l1, csf2,* ***il5, il25****, klk15, lcn2,* ***il18,*** ***vegfa****, eng, plau, pgf, prl, angpt2, igfbp1, hgf, erbb2, fgf2, stat3, stat5,* ***ifna1****, soat1, cd68,* ***il9****, itgam, ptprc, cd14*) candidate genes (**Fig. S2 and S3; Supplementary table S3**). The highest node degree of IL6 and all the cytokine and chemokine genes confirm their significant participation indicating reported elevation in the

immunopathology of COVID-19. Additionally, the presence of apoptotic genes like *bax, bcl2, fas, cybb* may indicate towards TNFα, IL6 mediated programmed cell death of T-cells depicting the observed global lymphocytopenia in the COVID-19 patients [28]. However, receptors like *cd68* (NK cells), *cd14* (monocytes) and *has2* gene may depict the rapid migration and infiltration of innate immune cells in the lung tissue with injury in response to chemoattractant chemokines. The candidate genes of cluster 9 (***il27ra**, **il27**, cxcl10, **il17d, il6st, il6r, jak2,** ptpn18, **mapk3**, epo, akt1, **egfr**, reg1a, ptpn1, cat, frap1, malat1, acan, eif4ebp1, sma4, smad6, smad2, smad7*) and cluster 1 (*src, limk1, rps6ka3, aak1, **mapk1, mknk2, map3k1**, gak, **map2k1**, fgfr1, mapkapk5, map3k7, bmp2k, zak, gsk3, yes1*) show the involvement of MAPK signaling and pro-inflammatory cytokine induced growth regulatory pathways (**Fig. S6 and S1; Supplementary table S4)**. Therefore, our analysis indicates that probiotics could play a protective role in above mentioned signaling pathways.

Probiotics protect the intestinal barrier by inhibiting cytokine-induced intestinal epithelial cell apoptosis [29]. A probiotics mixture consisting of *L. acidophilus, L. casei, L. reuteri, Bifidobacterium bifidium*, and *Streptococcus thermophilus* is reported to induce both T-cell and B-cell hypo-responsiveness and downregulate T helper (Th) 1, Th2, and Th17 cytokines without inducing apoptosis [30]. Moreover, probiotic formulations can maintain the balance between pro and anti-inflammatory cytokine secretion which is key factor for the development of a robust adaptive and innate immune system. Several clinical trials on URTI individuals have reported the decreased pro-inflammatory and increased anti-inflammatory cytokine upon probiotic application. Limited search has done which reveals the ability of probiotics to degrade glycosaminoglycan molecules [31] that may result in reduced expression of *has2* and hyaluronan breakdown. Probiotics are also proven to downregulate NF-κB

signaling pathway by regulating MAPK and ERK pathways reducing systemic inflammation [32].

Probiotics could improve cardiovascular complications and lipidomic abnormalities

Upregulation of the ACE/Ang II/AT1R axis in RAS shifts the regulation toward inflammation, vasoconstriction, hypertrophy, proliferation, and fibrosis, all factors that contribute to the development and progression of cardiopulmonary diseases. Conversely, stimulation of the vasoprotective ACE2/Ang-(1–7)/MasR axis produces a counter-regulatory response that promotes cardiovascular health [33]. Dysbiosis of the gut and lung microbiomes is associated with cardiopulmonary disease. Chronic elevation of IL6 and cytokine storm promotes macrophages to release MCP-1 which aids atherogenesis, expression of cell adhesion molecules, and proliferation and migration of vascular smooth muscle cells resulting in cardiovascular diseases (e.g. coronary atherosclerosis, inflammation in the vascular system and thrombosis) [34]. Enhanced angiotensin II also causes EGFR transactivation induced vascular remodeling [35].

The candidate genes encoding growth factors of MCODE cluster 3 (***vegfa****, eng, plau, pgf, prl, angpt2, igfbp1, hgf, erbb2, fgf2, stat3, stat5*) along with overrepresentation GO terms indicates the role of probiotics in the mitigation of elevated angiotensin II induced cardiovascular complications (**Fig. S3; Supplementary table S3**). The functional annotations and enrichment analysis of the candidate genes of MCODE cluster 6 (*dgat2, adipor2, dgat1, pgc, adipoq*), cluster 35 (*pparg, twist1, lpa, slc12a, mbtps1, srebf1, mbtps2*) and cluster 38 (*hk2, nr1i2, foxa2, pik3ca, f10, inpp5d, hspb1, mcl1*) indicates that probiotics could have

potential roles in glucose and lipid metabolism (**Fig. S5 and S9; Supplementary table S5**). The lipidomic and cholesterol metabolic abnormalities due to SARS-CoV-2 infection are poorly documented. The limited reports indicate that there is an enhanced glucose and lipid need for the viral replication and metabolism since viruses hijack the host's metabolic processes. The overrepresentation of GO terms associated with lipid and cholesterol metabolism reflect the lipid demanding processes (viral replication, endocytosis and exocytosis) involved in SARS-CoV-2 infection [36].

Probiotic supplementation has been proved to reduce total cholesterol, LDL, triglycerides and increase HDL count. The antihypertension capability of the probiotics makes them an affordable and adjunctive treatment option in hypertension, diabetes, and cardiovascular diseases and other dyslipidaemia associated health issues [37].

5. Conclusion

Our systematic network and meta-analysis study aid us to propose a mechanistic model of probiotic actions in the alleviation of COVID-19 (**Fig. 7**). Probiotic consumption could reduce the propensity of viral entry by healing the ACE2 containing epithelial barrier. The SCFAs and ACE inhibitory peptides released by beneficial bacteria could balance the dysregulated RAS. Hence the blood pressure or cardiovascular complications can be mitigated. The induced NO production could decrease the cellular oxidative stress. This can lead to downregulation of inflammatory (NLRP3 and NF-κB) pathways and eventually secretion of pro-inflammatory cytokines or chemokines. Probiotics might balance pro and anti-inflammatory cytokine level and increase the T-cell count in the SARS-CoV-2

infected patients. Finally, probiotics might also degrade the hyaluronan and hence could improve ARDS. Therefore, probiotics could be considered as a potential preventive and alternative treatment strategy for both mild and severe stages of COVID-19. However, further research is required for elucidation.

Competing interests

The author(s) state that there is no conflict of interest.

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Figure legends

Fig. 1 Systematic literature search selection process. The PRIMSA diagram details show our applied search and selection process during the study.

Fig. 2 Forest plot showing the patients with upper respiratory tract infection (URTI) treated with probiotics-based therapy versus placebo controls (after adjustment for heterogeneity)

Fig. 3 Funnel plot of the standard error plotted against the effect size to examine publication bias shows the effect of probiotics on upper respiratory tract infection (URTI) in different clinical trials

Fig. 4 Methodological schematic: The step-by-step representation of the approaches developed to identify the functional modules and enriched pathways to understand the mode of action of the probiotics in the mitigation of SARS-CoV-2 infection

Fig. 5 Association network topologies concerning SARS-CoV-2 pathogenesis and probiotic treatment. The training genes associated with SARS-CoV-2 infection and subsequent probiotic application are highlighted in yellow.

Fig. 6 MCODE derived association network topologies of SARS-CoV-2 pathogenesis and probiotic treatment. Network topologies with a minimum MCODE score of 3 are shown for the derived networks (38) after applying the clustering algorithms of MCODE. The most significant eleven clusters (yellow, with minimum score of 3 with 4 nodes and 6 edges) are further selected for pathway analysis.

Fig. 7 The mechanistic model representing the potential role of probiotics in COVID-19. **a** Probiotics heal epithelial barrier that could reduce the virus entry. **b** Probiotics are the source of SCFAs that are involved in regulating blood pressure. **c** Probiotics

release ACE inhibitory peptides that could reduce angiotensin II (Ang II) level. **d** Probiotics induce anti-oxidative Nitric oxide (NO) production that could reduce oxidative-stress. **e** Probiotics could reduce NF-KB, IL1β, IL18 levels. **f** Probiotics could maintain balance between pro and anti-inflammatory cytokines. **g** Probiotics could attenuate inflammation, cytokine and chemokine production. **h** Probiotics could improve cardiovascular complications. **i** Probiotics could reduce apoptosis, increase T-cell count. **j** Probiotics could degrade hyaluronan

Table 1 The probiotic products with formulations applied in human clinical trials in prevention of upper respiratory tract infection (URTI)

Probiotic strain	Formulation	Age group	Dosage	Mode of administration	Function	References
Bifidobacterium animalis subsp *lactis* (BB-12)	*B. animalis* subsp *lactis* (BB-12) and *L. rhamnosus* (LGG)	Infants 8 –14 months (Healthy)	1 g of maltodextrin powder with 1×10^9 cfu each of BB-12 and LGG for 6 months	Oral	No significant effect	[38]
Bifidobacterium animalis subsp *lactis* Bi-07 ATCC PTA-4802	*B. animalis* subsp *lactis* Bi-07 ATCC PTA-4802, *L. acidophilus* NCFM (*N*_110), or *L. acidophilus* NCFM	Children 3–5 years (Healthy)	1 g sachet with 1×10^{10} cfu of each bacterium with 120 ml 1% fat milk twice daily for 6 months	Oral	Reduced fever, rhinorrhoea, cough incidence and antibiotic requirement	[39]

Bifidobacterium lactis (Probial BS 01-LMG P-21384)	*L. plantarum* (Probial LP 02-LMG P-21020), *L. rhamnosus* (Probial LR 04-DSM 16605) and *B. lactis* (Probial BS 01-LMG P-21384)	Person 15–56 years (Healthy)	1 sachet (0.1 g) with 10×10^9 cfu of each bacterium daily for 90 days	Oral	Significantly decreased URTI	[40]
Bifidobacterium animalis subsp. lactis BB-12	Individual	Children 1–18 years (Hospitalized)	1 g maltodextrin powder with 1×10^9 cfu cells for the entire duration of the hospital stay	Oral	No significant effect	[41]
Bifidobacterium animalis subsp. *lactis* (BB-12)	Individual	Children 1.43–7.48 years (Healthy)	1 g maltodextrin powder with 1×10^9 cfu cells for 90 days	Oral	No significant effect	[42]
Bifidobacterium longum	Individual	Children >11 years	*Bifidobaeterium* tetravaccine tablets	Oral	Reduced the frequency of	[43]

		(Recurrent respiratory tract infected)	(Live) for 2 months		URTI	
Bifidobacterium bifidum R0071	*L. acidophilus helveticus* R0052, *B.longum* subsp. *infantis* R0033, *B. bifidum* R0071	Children 3–7 years (Healthy)	1.5 g sachet with 5×10^9 cfu live cells daily for 3 months	Oral	Prevented usual acute infectious illnesses, decreased the risk of occurrence of common infectious viral diseases including common cold, flu, respiratory problems	[44]
Lactobacillus acidophilus CUL60 [NCIMB	*L. acidophilus* CUL21 (NCIMB 30156), *L.*	Children 3–6 years (healthy)	1 tablet with 1×10^{10} cfu of *lactobacillus* spp. and 0.25 $\times10^{10}$	Oral	Potential preventives for URTI	[45]

30157], *Lactobacillus acidophilus* CUL21 [NCIMB 30156]	*acidophilus* CUL60 (NCIMB 30157), *B. bifidum* CUL20 (NCIMB 30153) and *B. animalis* subsp. lactis CUL34 (NCIMB 30172)		cfu of *bifidobacterium* spp. and 50 mg vitamin C daily for 6 months			
Lactobacillus acidophilus DDS-1 (NCIMB 30333)	*L. acidophilus* DDS-1 and *B. lactis* UABLA-12	Children 3–12 years (Healthy)	~1 g of powder with 5×10^9 cfu of *Lactobacillus* spp. and *Bifidobacterium* spp. in 1:4 ratio and 50 mg FOS for 7 months	Oral	Reduced acute respiratory infection (ARI)	[46]
Lactobacillus acidophilus W22	*B. bifidum* W23, *B. lactis* W51, *E. faecium* W54, *L. acidophilus* W22, *L. brevis* W63, and *L. lactis* W58	Adults 20–35 years (healthy)	4 g sachet with 1×10^{10} cfu of each bacterium daily for 12 weeks	Oral	Reduced the incidence of URTI	[47]

Lactococcus lactis W58	*B. bifidum* W23, *B. lactis* W51, *E. faecium* W54, *L. acidophilus* W22, *L. brevis* W63, and *L. lactis* W58	Adults 20–35 years (healthy)	4 g sachet with 1×10^{10} cfu of each bacterium daily for 12 weeks	Oral	Reduced the incidence of URTI	[47]
Lactobacillus paracasei subsp. paracasei, *L. casei* 431	Individual	Person 18–60 years (healthy)	100 ml milk with 1×10^{9} cfu live cells once daily for 6 weeks	Oral	Reduced the duration of common cold and influenza like illness (ILI) episodes in healthy adults	[48]
Lactobacillus paracasei N1115 (N1115)	Individual	Elderly person ≥45 (Healthy)	3.6×10^{7} cfu/mL live cells for 12 weeks	Oral	Reduced the risk of acute upper tract infections in the elderly. Enhanced T-cell-mediated	[49]

					natural immune defense	
Lactobacillus paracasei CBA L74 (FM-CBAL74)	Individual	Children 12–48 months (Healthy)	150 ml of milk or water with 5.9×10^{9} cfu/g live cells for 3 months	Oral	Reduced the risk of acute upper tract infections in the elderly. Enhanced T-cell-mediated natural immune defense	[50]
Lactobacillus paracasei CBA L74 (FM-CBAL74)	Individual	Children 12–48 months (Healthy)	150 ml of milk or water with 5.9×10^{9} cfu/g live cells for 3 months	Oral	Reduced the risk of acute upper tract infections in the elderly. Enhanced T-cell-mediated natural immune defense	[51]
Lactobacillus	*L. paracasei* 8700:2	Person	1 g maltodextrin and	Oral	Reduced	[52]

paracasei 8700:2 (DSM 13434)	(DSM 13434) and *L. plantarum* HEAL 9 (DSM 15312)	18–65 years (Healthy)	lyophilised bacteria with 1×10^9 cfu/day live cells for 12 weeks		frequency and duration of common cold, URTI	
Lactobacillus plantarum PL02	*B. longum* PL03 (33%), *L. rhamnosus* KL53A (33%) and *L. plantarum* PL02 (34%)	Children 5 months to16 years (with respiratory tract infection)	1 tablet with 1×10^8 cfu cells twice daily for 4 weeks	Oral	No significant function was observed	[53]
Lactobacillus plantarum L-137	heat-killed *L. plantarum* L-137	Elderly person 40–64 years (healthy)	1 tablet with 50 mg of bacteria daily for 12 weeks	Oral	Decreased URTI incidence in healthy subjects through augmentation of immune functions	[54]
Lactobacillus reuteri	Individual	Person 18–65	100 ml liquid with 1×10^8 cfu live cells	Oral	Shortened duration of	[55]

ATCC55730		years (healthy)	for 80 days		respiratory diseases	
Lactobacillus rhamnosus (Probial LR 04-DSM 16605)	*L. plantarum* (Probial LP 02-LMG P-21020), *L. rhamnosus* (Probial LR 04-DSM 16605) and *B. lactis* (Probial BS 01-LMG P-21384)	Person 15– 62 years (Healthy)	1 capsule (5 g) with 0.1 g=10×10^9 cfu *L. plantarum*; 0.1 g=10×10^9 cfu *L. rhamnosus*; 0.1 g=10×10^9 cfu *B. lactis*; 3 g FOS and 1.7 g glucose / maltodextrin daily for 3 months	Oral	Improved health by reducing the incidence and severity of respiratory diseases	[40]
Lactobacillus rhamnosus GG	*L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* Bb99 and *P. freudenreichii* ssp shermanii JS	newborn infants (Healthy)	1 capsule with 8-9×10^9 cfu of each bacterium for 6 weeks	Oral	Increased resistance to respiratory infections	[56]
Lactobacillus	Individual	Children 13–86	100 ml of fermented milk with 1×10^9 cfu	Oral	Reduced the	[57]

rhamnosus GG		months (healthy)	cells daily for 3 months		risk of URTI	
Lactobacillus rhamnosus GG	Individual	Children >12 months (non-healthy)	100 ml of fermented milk with 1×10^9 cfu cells daily for 3 months	Oral	Reduced the risk of URTI	[58]
Lactobacillus rhamnosus GG	Individual	Children 2–6 years (healthy)	Milk with 6.7×10^5 to 1.9×10^6 cfu/ml cells for 28 weeks (amount of milk consumed by each child was recorded)	Oral	Reduced the risk of URTI	[59]
Lactobacillus rhamnosus LGG	*L. rhamnosus* LGG and *B. animalis ssp. lactis* BB-12	Adults 18–24 years (Susceptible to upper respiratory	5 g powder (stick) with 1×10^9 cfu cells each of LGG and BB-12 daily for 12 weeks	oral	Mitigated decrements in health-related quality of life (HRQL) during upper respiratory	[60]

		infections)			infections (URI)	
Lactobacillus rhamnosus GG	Individual	Elderly person ≥65 years (hospital residents)	2 capsules with 1×10^{10} cfu cells daily for 6 months	Oral	Reduced the risk of URTI	[61]
Lactobacillus casei Shirota (LcS, YIT 9029)	Individual	Person 30–49 years (Healthy)	1 drink with1×10^{11} cfu live cells daily for 12 weeks	Oral	Reduced risk of URTI and common infectious diseases (CID)	[62]
Lactobacillus casei strain Shirota (LcS)	Individual	Person 18–67 years (Healthy)	80 ml fermented milk with 4×10^{10} cfu cell of LcS per day	oral	Reduced the duration of acute URTIs	[63]
Lactobacillus casei DN-114 001	*L. casei* DN-114 001, *S. thermophilus* and *L. delbrueckii subsp.*	Elderly person ≥70 years (Healthy)	2 bottles of 100 g/d with 1×10^{10} cfu/100 g live cells for 112 days	Oral	Reduced risk of URTI and common infectious	[64]

	bulgaricus				diseases (CID)	
Lactobacillus casei DN-114 001	*L. casei* DN-114 001, *S. thermophilus* and *L. delbrueckii subsp. bulgaricus*	Person 18–65 years (Healthy)	2 bottles of 100 g/d with 1×10^{10} cfu/100 g live cells for 112 days	Oral	Reduced risk of URTI and common infectious diseases (CID)	[65]

Table 2 Training gene set entangled with SARS-CoV-2 infection and probiotic treatment

Training gene	References
ifnγ, il4, cd11c, cd80, cd86	[66]
il1β, il2, il6, il8, il10, ifnα, tnfα, tlr3, tgev	[67]
il1, il12, il15, il13, il17, nf-kb, stat3	[68]
ace2, tmprss2, il6, il1β, il2, il8, il17, g-csf, gmcsf, ip-10, mcp-1, ccl3, and tnfα, hs-crp, ighv3–23, ighv3–7, ighv3–15, ighv3–30, and igkv3–11, ighv3–23-ighj4, il1β, il1ra, il7, il8, il9, il10, fgf, g-csf, gm-csf, ifnγ, ip-10, mcp-1, mip-1α, mip-1β, pdgf, tnfα, vegf, il2, il7, il10, g-csf, ip- 10, mcp-1, mip-1α, tnfα, ifn-α2, ifnγ, il1ra, il2, 4, 7, 10, 12, 17, ip-10, g-csf, m-csf, tlr3, tlr7, tlr8, rig-i, mda5, cxcl2, mcp-1, jak, ccl3, myd88, inos, cd86, gp130, adam17, il-6rα, sil-6rα, egfr, aak1, gak, s1p, s1prs, ras/erk, pi3k/akt/enos, plc/ca2+, trif, at_1 , ccl2, $s1p_1$, ifnβ	[1]
crp, hmgcoa, app, scfa, gpr43, hdac	[69]
g-csf, ip10/cxcl10, (mcp-1, thp-1, il22, il8, tnfα, mpo, inos, cox-2, nf-κb, il6	[70]
ace2, il10, il17, mtor	[71]
hla, has2	[72]

*** Table 3 Network statistics of the association network of probiotics-COVID-19 axis.**

	Topology feature details						
Network	**Network heterogeneity**	**Number of Nodes**	**Nodes Edges**	**No. of isolated nodes**	**Avg. num. of neighbours**	**Clustering coefficient**	**Number of connected components**
Training gene set	1.137	453	1273	0	5.620	0.633	27

* The table contains details of the primary association network obtained by text-mining results using an Agilent Literature search (ALS) plugin. ‘‘Network clustering coefficient’ is the average of the clustering coefficients for all nodes in the network, and the ‘average number of neighbours’ indicates the average connectivity of a node in the network.

Table 4 Details of MCODE clusters derived from association network of COVID-19 pathogenesis with probiotic treatment

MCODE cluster	Score	Number of nodes	Number of edges
*1	16	16	120
*2	14	14	91
*3	8.457	36	148
4	8	8	28
*5	6.769	14	44
*6	5	5	10
7	5	5	10
8	5	5	10
*9	4.818	23	53
*10	4.167	13	25
*11	4	4	6
12	4	4	6
*13	4	4	6
14	3	3	3
15	3	3	3
16	3	3	3
17	3	3	3
18	3	3	3
19	3	3	3
20	3	3	3
21	3	3	3
22	3	3	3
23	3	3	3
24	3	3	3
25	3	3	3
26	3	3	3
27	3	3	3
28	3	3	3
29	3	3	3
30	3	3	3
31	3	3	3
32	3	3	3
33	3	3	3
34	3	3	3
*35	3	7	9
36	3	3	3
37	3	3	3
*38	2.857	8	10

* The modules which are selected for functional annotation and BiNGO enrichment analysis

Table 5 GO enrichment analysis of the MCODE clusters

MCODE derived cluster	BINGO derived overrepresented GO terms
Cluster 1	Phosphate metabolic processes, stress-activated protein kinase signaling, protein phosphorylation and modification (MAPKKK and JNK pathways)
Cluster 2	Regulation of immune response and cytokine production in response to stress and hormonal stimulus, regulation of apoptosis in response to external signals and NO biosynthesis, cell locomotion and leukocyte migration, chemotaxis
Cluster 3	Response to stimulus, hypoxia, regulation and activation of immune system, lymphocyte chemotaxis, blood vessel differentiation and morphogenesis
Cluster 5	Cellular metabolic pathways (MAPK), PAMPs dependent symbiotic interactions, regulation of RNA metabolism and DNA dependent transcription (RNA polymerase II)
Cluster 6	Regulation of macrophage, granulocyte, myeloid leukocyte differentiation, glucose, lipid transport and metabolism, response to nutrient, hormones, external stimulus, regulation of TNF and cytokine signaling pathway
Cluster 9	Regulation of cellular phosphorous metabolic processes and proteins phosphorylation, regulation of cytokine production, regulation of TGFβ production, SMAD signaling pathway, cell proliferation and differentiation
Cluster 10	Viral entry and reproduction, regulation of cellular metabolic processes and systemic arterial blood pressure by RAS, T-cell, B-cell proliferation and migration in response to hypoxia, stress, chemical stimulus, somatic

	diversification
Cluster 11	Vesicle mediated transport
Cluster 13	Response to hypoxia, regulation of transcription with RNA polymerase II in response to stress, monooxygenase, oxidoreductase, nitric oxide synthase pathway, production and regulation of cytokines and chemokines, TGFβ, VEGF signaling pathway, regulation of, catecholamines and norepinephrine, phenol signaling pathway
Cluster 35	Regulation of lipid, cholesterol, steroid metabolic processes, LDL metabolic pathway, lipid and cholesterol transport
Cluster 38	Regulation glucose, hexose, monosaccharide metabolic pathways, xenobiotic drug transport, monocyte neutrophil differentiation, response to cytokine IL-6

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