

Natural useful therapeutic products from microbes

Abstract

Nature is an attractive source of new therapeutic candidates as a tremendous chemical diversity is found in millions of species of plants, animals, marine organisms and microorganisms. Natural products remain an important source of new drugs, new drug leads and new chemical entities. The exploitation of microorganisms to produce food ingredients has been going on since antiquity. Microorganisms are being employed, since several decades for the large scale production of a variety of bio-chemicals ranging from alcohol to antibiotics and in processing of foods and feeds. Microorganisms have great potential as natural sources of drugs for the treatment and prevention of diseases like cancer, anaemia, diarrhoea, obesity, diabetes, atopic dermatitis, Crohn's disease, etc. They are also potential sources of natural antioxidants, colours, immunosuppressants, enzyme inhibitors, hypocholesterolemic agents, vitamins, enzymes, and antibiotics.

Keywords: microbes, natural products, dietary supplements, therapeutics

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Charu Gupta,¹ Dhan Prakash,¹ Sneha Gupta²

¹Amity Institute for Herbal Research & Studies, Amity University, India

²Department of Zoology, R.G.P.G. College, India

Correspondence: Charu Gupta, Amity Institute for Herbal Research & Studies, Amity University, Sector-125, Noida-201 303, Uttar Pradesh, India, Email charumicro@gmail.com

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Introduction

The intestinal microbiota is a key component of both the metabolism and immunity of humans and animals. These can be helpful in healthcare, especially for the management of digestive diseases and food-borne illness.¹ Through genetic engineering it became possible to fully express biologically active copies of such powerful molecules from food and commensal bacteria. Genetically engineered probiotics can be used to treat inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, as well as other disorders resulting from an overactive immune system. Microorganisms are a prolific source of structurally diverse bioactive metabolites and have yielded some of the most important products of the pharmaceutical industry.² Microbial secondary metabolites are now being used for applications other than antibacterial, antifungal and antiviral infections. Bacteria, yeast and microalgae can act as producers or catalysts for the production of food ingredients, enzymes, proteins, vitamins, organic acids, antibiotics and nutraceuticals.³ With the current trend towards natural ingredients, there is renewed interest in microbial flavours and colours and bio-processing using enzymes.⁴ Lactic acid bacteria, in particular *Lactococcus lactis*, have been demonstrated to be ideal cell factories for the production of these important nutraceuticals. Developments in the genetic engineering of food-grade microorganisms means that the production of certain nutraceuticals can be enhanced or newly induced through over expression and/or disruption of relevant metabolic genes, e.g. in a recent research, scientists deleted a gene from the bacterium *Lactobacillus acidophilus* which is responsible for increasing inflammation, a defining characteristic of Crohn's disease and ulcerative colitis.² But the unaltered form of the bacterium also triggered production of a beneficial immune molecule, IL-10m, which helps to regulate the immune system. Thus, the goal of engineering the microbes is to deliver the beneficial effects without the harmful ones. Besides, the future of genetically modified probiotics is in food additives to control the release of human growth factors by the modified bacteria to fight against injury and inflammation in the gut e.g. the use of a plant sugar called xylan to stimulate the genetically modified human gut probiotic bacterium *Bacteroides ovatus* to produce specific proteins that can repair damaged cells and dampen down the immune system in the intestine that causes inflammation and disease.

Administration of xylan with the genetically engineered probiotic bacteria resulted in a significant improvement of colitis, reduced weight loss, improved stool consistency, reduced rectal bleeding and accelerated healing of damaged colonic cells. GM probiotics possess potential in clinical applications e.g. delivery of antigens for vaccines and thus are more readily accepted. This would provide a safer method of vaccination than the use of attenuated pathogens e.g. GM, *Lactococcus lactis*, produces IL-10 in the mouse intestine. This may provide new treatment strategies for inflammatory bowel disease, and similar applications may be useful for other diseases.⁵

Various bacteria, mold, yeast and algae have been employed for the production of single cell proteins. Application of probiotic bacteria for the prevention of megaloblastic anaemia is a novel scientific approach that involves lactic acid-fermented foods, increases iron absorption by optimization of pH in the digestive tract, activates enzyme phytases, produces organic acids and other digestive enzymes. The probiotic bacteria are used for the manufacture of a natural remedy, for controlling weight gain and preventing obesity.⁶ Recently a protein has been isolated from probiotic bacteria that help to alleviate inflammatory bowel disorders (IBD). The bacteria are found in yoghurt and dairy products. Bacteria can be used to make anticancer agents and provide an extra source of lead compounds for the pharmaceutical industry, e.g. genetically engineered strains of the bacterium *Streptomyces parvulus* is used to produce compounds that selectively inhibit growth of human cancer cells. Similarly, the probiotic bacteria *Streptococcus thermophilus* has been shown to aid recovery from malnutrition due to short-term fasting and reduce the associated intestinal atrophy in animal studies. *S. thermophilus* is also known to have powerful antioxidant activity, protecting the body from dangerous free radicals which increase in the body due to ageing and stress. Similarly, *Eurotium* species have been found to produce several antioxidants.^{2,7} The utilization of microbes as the natural source of several therapeutic agents is being discussed in the present chapter.

Special features of microbial metabolites

The most important, inherent characteristics of the bioactive microbial metabolites are their microbial origin, their interaction with the environment and their unique chemical structures.

In general, natural products including the microbial metabolites may be practically utilized in three different ways:

- i. Applying the natural/fermentation product directly in the medicine, agriculture, or in any other fields.
- ii. Using as starting material for subsequent chemical or microbiological modification (derivatization).
- iii. They can be used as lead compounds for chemical synthesis of new analogs or as templates in the rational drug design (RDD) studies.

Bacteria as source of antimicrobial proteins (bacteriocins as biopreservatives)

Production of antimicrobial substances is often important trait in the context of bacterial fitness but also in terms of probiotic efficacy. Several probiotic bacteria produce a variety of antimicrobial compounds viz. short chain fatty acids, hydrogen peroxide, nitric oxide, bacteriocins etc.⁹ Bacteriocins are bacterially produced antimicrobial peptides with narrow or broad host ranges. Bacteriocins are ribosomal peptides that differ from other non-ribosomal peptides with antimicrobial activity in one critical feature that the former have a relatively narrow killing spectrum, as they are generally only toxic to bacteria closely related to the producing strain. These toxins have been found in all major lineages of bacteria and have even been found in some members of Archaea.⁶ Many bacteriocins are produced by food-grade lactic acid bacteria, a phenomenon which offers food scientists the possibility of directing or preventing the development of specific bacterial species in food. This can be particularly useful in preservation or food safety applications, but also has implications for the development of desirable flora in fermented food.¹⁰ Lactic acid bacteria and their antimicrobial metabolites have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. To date, nisin is the only bacteriocin that has found practical applications in some industrially processed foods. Its antibacterial activity and possible use as a biopreservative has been studied in a large number of food systems.¹¹ Its application for the control of some pathogens and food spoilage organisms has been approved in a number of countries. Limited studies have shown that pediocins from several *Pediococcus* strains can also be used effectively in food systems to control *Listeria monocytogenes*.

Lactobacilli continue to remain the most commonly used probiotic microorganisms. Currently available probiotic preparations contain *Lactobacillus delbreuckii* sp. *bulgaricus*, *L. acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. lactis* and *L. Reuteri*.^{2,12}

Microbes as source of antitumor drugs

Microbial metabolites are among the most important of the cancer chemotherapeutic agents. They started to appear around 1940 with the discovery of actinomycin and since then many compounds with anticancer properties have been isolated from natural sources. More than 60% of the current compounds with antineoplastic activity were originally isolated as natural products or are their derivatives. Among the approved products deserving special attention are actinomycin D, anthracyclines (daunorubicin, doxorubicin, epirubicin, pirarubicin and valrubicin), bleomycin, mitosanes (mitomycin C), anthracenones (mithramycin, streptozotocin and pentostatin), enediynes (calicheamicin), taxol and epothilones.²

A successful non-actinomycete molecule is taxol (paclitaxel),

which was first isolated from the Pacific yew tree, *Taxus brevifolia*, but is also produced by the endophytic fungi *Taxomyces andreanae* and *Nodulisporium sylviforme*. This compound inhibits rapidly dividing mammalian cancer cells by promoting tubulin polymerization and interfering with normal microtubule breakdown during cell division. The drug also inhibits several fungi (*Pythium*, *Phytophthora* and *Aphanomyces*) by the same mechanism. In 1992, taxol was approved for refractory ovarian cancer, and today it is used against breast and advanced forms of Kaposi's sarcoma.³

Microbes as enzyme inhibitors

Enzyme inhibitors have received increasing attention as useful tools, not only for the study of enzyme structures and reaction mechanisms but also for potential utilization in medicine and agriculture. Several enzyme inhibitors with various industrial uses have been isolated from microbes.¹³ The most important are (1) clavulanic acid, the inhibitor of β -lactamases. Some of the common targets for other inhibitors are glucosidases, amylases, lipases, proteases and Xanthine Oxidase (XO).

Acarbose is a pseudotetrasaccharide made by *Actinoplanes* sp. SE. It contains an aminocyclitol moiety, valienamine, which inhibits intestinal α -glucosidase and sucrase. This results in a decrease in starch breakdown in the intestine, which is useful in combating diabetes in humans.¹⁴ Amylase inhibitors are useful for the control of carbohydrate dependent diseases, such as diabetes, obesity and hyperlipemia.¹⁵

Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. The inhibitors may also be useful for weight loss, as some versions of amylase inhibitors do show potential for reducing carbohydrate absorption in humans.¹⁶ The use of amylase inhibitors for the treatment of rumen acidosis has also been reported.¹⁷ Examples of microbial α -amylase inhibitors are paim, obtained from culture filtrates of *Streptomyces corchorushii*, and TAI-A, TAI-B, oligosaccharide compounds from *Streptomyces calvus* TM-521.¹⁸ Lipstatin is a pancreatic lipase inhibitor produced by *Streptomyces toxytricini* that is used to combat obesity and diabetes. It interferes with the gastrointestinal absorption of fat.¹⁹ The commercial product is tetrahydrolipstatin, which is also known as orlistat.

In the pathogenic processes of some diseases, such as emphysema, arthritis, pancreatitis, cancer and AIDS, protease inhibitors are potentially powerful tools for inactivating target proteases. Examples of microbial products include antipain, produced by *Streptomyces yokosukaensis*, leupeptin from *Streptomyces roseochromogenes* and chymostatin from *Streptomyces hygroscopicus*. Leupeptin is produced by more than 17 species of Actinomycetes.²⁰ Fungal products are also used as enzyme inhibitors against cancer, diabetes, poisonings, Alzheimer's disease, etc. The enzymes inhibited include acetylcholinesterase, protein kinase, tyrosine kinase, glycosidases and others.²¹

Microbes as immuno-suppressants

Suppression of the immune response either by drugs or by radiation, to prevent the rejection of grafts or transplants or to control autoimmune diseases, is called immunosuppression. A number of microbial compounds capable of suppressing the immune response have been discovered. Cyclosporin A was originally introduced as a narrow-spectrum antifungal peptide produced by the mold,

Tolypocladium nivenum (originally classified as *Trichoderma polysporum* and later as *Tolypocladium inflatum*), by aerobic fermentation. They are used in heart, liver and kidney transplants.²²

Other important transplant agents include sirolimus (rapamycin) and tacrolimus (FK506), which are produced by actinomycetes. Rapamycin is especially useful in kidney transplants as it lacks the nephrotoxicity seen with cyclosporin A and tacrolimus. It is a macrolide, first discovered in 1975 as a product of *S. hygroscopicus*.

The anti-proliferative effect of rapamycin has also been used in conjunction with coronary stents to prevent restenosis, which usually occurs after the treatment of coronary artery disease by balloon angioplasty. Rapamycin also shows promise in treating tuberous sclerosis complex (TSC), a congenital disorder that leaves sufferers prone to benign tumor growth in the brain, heart, kidneys, skin and other organs.

Tacrolimus (FK506) was discovered in 1987 in Japan. It is produced by *Streptomyces tsukubaensis*. It was approximately 100 times more active as an immunosuppressive than cyclosporin A. It was approved by the FDA for use as an immunosuppressant in liver transplantation. Furthermore, its use has been extended to include bone marrow, cornea, heart, intestines, kidney, lung, pancreas, trachea, small bowel, skin and limb transplants and for the prevention of graft-vs-host disease. Typically, it is also used against atopic dermatitis, a widespread skin disease. Recently, it has been reported that tacrolimus inhibits tumor growth factor- β -induced signaling and collagen synthesis in human lung fibroblastic cells. This factor plays a pivotal role in tissue fibrosis, including pulmonary fibrosis. Therefore, tacrolimus may be useful for the treatment of pulmonary fibrosis, although its use in the acute inflammatory phase may exacerbate lung injury.²³

Microbes as hypocholesterolemic drugs

Atherosclerosis is generally viewed as a chronic, progressive disease characterized by the continuous accumulation of atheromatous plaque within the arterial wall. The first member of the group (compactin; mevastatin) was isolated as an antibiotic product of *Penicillium brevicompactum* and later from *Penicillium citrinum*. An ethylated form, known as lovastatin (monacolin K; mevinolin), was isolated in the 1970s in the broths of *Monascus ruber* and *Aspergillus terreus*. Lovastatin, the first commercially marketed statin, was approved by the FDA in 1987. A semi-synthetic derivative of lovastatin is simvastatin,

a major hypocholesterolemic drug, selling for US\$7billion per year before becoming generic. Another statin, pravastatin (US\$3.6billion per year), is made through different biotransformation processes from compactin by *Streptomyces carbophilus* and *Actinomadura* sp. Other genera involved in the production of statins are *Doratomyces*, *Eupenicillium*, *Gymnoascus*, *Hypomyces*, *Paecilomyces*, *Phoma*, *Trichoderma* and *Pleurotus*.²⁴ A synthetic compound, modeled from the structure of the natural statins, is atorvastatin, which has been the leading drug of the entire pharmaceutical industry in terms of market share (approximately US\$14billion per year) for many years.²⁵⁻²⁷

Microbes as source of anti-fungals

There is an increased need for new drug leads to treat diseases in humans, animals and plants. A dramatic example is represented by the need for novel and more effective antibiotics to combat multidrug-resistant microbial pathogens. Natural products represent a major source of approved drugs and still play an important role in supplying chemical diversity, despite a decreased interest by large pharmaceutical companies. Novel approaches must be implemented to decrease the chances of rediscovering the tens of thousands of known natural products.²⁷ The vast number and variety of chemotherapeutic agents isolated from microbial natural products have greatly contributed to the improvement of human health. However, only a limited number of antifungal agents (polyenes and azoles, plus the recently introduced caspofungin acetate) are currently available for the treatment of life-threatening fungal infections.²⁸

The pneumocandins have been successfully used to develop an antifungal drug that has been recently approved by the FDA. This semi-synthetic pneumocandin, caspofungin acetate, is an aza-substituted derivative of pneumocandin B0. Pneumocandins are natural products derived from the fermentation of the fungus *Glarea lozoyensis*.²⁹ The introduction of additional amino groups in the peptide ring of pneumocandin B0 increased the solubility of the molecule and the potency against fungal pathogens by two orders of magnitude. The compound has been shown to be effective in animal models of disseminated candidiasis, aspergillosis, coccidiomycosis and pneumonia caused by *Pneumocystis carinii*. The clinical trials have demonstrated good tolerance of the compound and its efficacy in the treatment of oropharyngeal and oesophageal candidiasis, as well as in invasive aspergillosis. Cancidas has recently been approved by the FDA for use against invasive aspergillosis, refractory to, or intolerant of, other therapies.^{27,30,31}

Table 1 Approximate number of bioactive microbial natural products according to their producers.⁸

Source	Antibiotics	"Other bioactive" metabolites	Total bioactive metabolites	Practically used in 'human therapy'	Inactive metabolites
Bacteria	2900	900	3800	10~12 (8~10)	3000 to 5000
Actinomycetes	8700	1400	10100	100~120 (70~75)	5000 to 10000
Fungi	4900	3700	8600	30~35 (13~15)	2000 to 15000
Total	16500	6000	22500	140~160 (~100)	20000 to 25000

Table 2 Microorganism derived anticancer agents

Compound	Microorganism	Use in cancer
Actinomycin	<i>Streptomyces</i> spp.	Sarcoma and germ cell tumors
Bleomycin	<i>Streptomyces verticillus</i>	Germ-cell, cervix and head and neck cancer
Daunomycin	<i>Streptomyces coeruleorubidus</i>	Leukemia
Doxorubicin	<i>Streptomyces pneuceticus</i>	Lymphoma, breast, ovary, lung and sarcomas
Epirubicin	<i>Streptomyces pneuceticus</i>	Breast cancer
Idarubicin	<i>Streptomyces pneuceticus</i>	Breast cancer and leukemia
Mitomycin C	<i>Streptomyces caespitosus</i>	Gastric, colorectal, anal and lung cancer
Geldanamycin	<i>Streptomyces hygroscopicus</i>	Experimental
Rapamycin	<i>Streptomyces hygroscopicus</i>	Experimental
Wortmannin	<i>Talaromyces wortmanni</i>	Experimental

Microbes for treatment and prevention of anaemia

The conventional method for producing folic acid through chemical synthesis is known, in which the raw materials used are expensive and the yield of the end product is low. There are some folic acid producing bacteria and yeast that accumulates high concentrations of folic acid in the medium and can even be cultured in whey or milk plasma. The various identified bacteria that produce and enhance the uptake of folic acid are *Lactococcus lactis* sub sp. *cremoris*, *L. lactis* sub sp. *lactis*, *Bifidobacterium adolescentis*, *B. pseudocatenulatum*; yeasts like *Candida famata*, *C. guilliermondii*, *C. glabrata*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*, *Pichia glucozyma*, and *Yarrowia lipolytica*. The vitamin B12 producing bacteria are *Pseudomonas denitrificans* and *Propionibacterium shermanii*.³²

Thus the probiotic bacteria can be used for the prevention of megaloblastic anaemia. It involves lactic acid-fermented foods that increases iron absorption by optimization of pH in the digestive tract, activates enzyme phytases, and produces organic acids and other digestive enzymes.³³ In a study, a strain of probiotic bacteria developed by Swedish firm Probi doubled the absorption of iron from food in women. *Lactobacillus plantarum* 299v or in short, Lp299v not only helps digestive system, but also improves immune system. It is also good for the heart and helpful in reducing the unexpected gas and bloating conditions. It improves bowel moments by making it more normal and regular. The probiotic bacteria *Lactobacillus plantarum* 299v reduces the negative effects of an antibiotic drug on colonic fermentation.³⁴ Recently, many medicines are produced using genetically engineered bacteria or fungi that synthesize the medicine in giant bioreactors. Erythropoietin can be man-made in bioreactors by bacteria and is used to treat anaemia but the treatment requires frequent, even daily injections. Researchers have purified specialized cells from human blood that normally repair the lining of blood vessels. They genetically engineered these cells to express erythropoietin. The cells were then mixed with mesenchymal stem cells, which are able

to form blood vessels. This mixture was then injected underneath the skin of mice that had been made anaemic either by radiation (as often occurs in chemotherapy patients) or by loss of kidney tissue. The cell mixture spontaneously formed networks of blood vessels underneath the skin. The vessel lining secreted erythropoietin and cured anaemia in both types of mice.³²

Microbes for treatment and prevention of obesity

Obesity is a significant risk factor for major diseases including Type II diabetes, coronary heart disease, hypertension and certain forms of cancer.³⁵ Obesity arises when energy intake, principally stored as triglycerides, exceeds energy expenditure.³⁶ Obesity is a complex trait influenced by diet, developmental stage, age, physical activity and genes. The probiotic bacteria are used for the manufacture of a natural remedy, for controlling weight gain, preventing obesity, increasing satiety, prolonging satiation, reducing food intake, reducing fat deposition, improving energy metabolism, treating & enhancing insulin sensitivity and treating obesity. Recent studies suggested that manipulation of the composition of the microbial ecosystem in the gut might be a novel approach in the treatment of obesity. Such treatment might consist of altering the composition of the microbial communities of an obese individual by administration of beneficial microorganisms, commonly known as probiotics.³⁷ Animal studies have demonstrated the efficacy of some strains of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its re-absorption (which enters the blood as cholesterol).³⁷ A meta-analysis that included five double blind trials examining the short term (2-8weeks) effects of a yoghurt with probiotic strains on serum cholesterol levels found a minor change of 8.5mg/dL (0.22mmol/L) (~4% decrease) in total cholesterol concentration, and a decrease of 7.7mg/dL (0.2mmol/L) (~5% decrease) in serum LDL concentration.³⁸

A slightly longer study evaluating the effect of yoghurt with probiotic strains on twenty-nine subjects over six months found no statistically significant differences in total serum cholesterol or LDL values. However, the study did note a significant increase in serum HDL from, 50mg/dL (1.28mmol/L) to 62mg/dL (1.6mmol/L) following treatment. This corresponds to a possible improvement of LDL/HDL ratio.³⁸ It has also been reported that the *Lactobacillus* (*Lb. sporogenes* and *Lb. acidophilus* NCFB 1748) and *Bifidobacterium* genus representatives may have a critical role in weight regulation as an anti-obesity effect in experimental models and humans, or as a growth-promoter effect in agriculture depending on the strains.³⁸

Microbes for irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder that results in abdominal pain, altered bowel habits and irregular stool characteristics.³⁹ *Lactobacillus salivarius* or *Bifidobacterium infantis* found significant improvements in typical IBS symptoms with the administration of probiotics. Commonly reported improvements were reductions in bloating, flatulence, speed of colonic transit and abdominal pain. Intestinal micro-organisms play various roles in human health such as complex food digestion, metabolizing drugs, detoxifying toxic compounds, producing essential vitamins and preventing colonization of pathogens. Most of the micro-organisms found in the GI tract are anaerobic bacteria, which are uncultivable under standard laboratory conditions. The type and number of bacteria in the GI tract varies depending on age, gender, geographical origin and environmental factors, such as diet

and dietary supplements.^{40,41} *Firmicutes* and *Bacteroidetes* are the dominant beneficial bacteria present in the normal human GI tract, and the latter was reported in lower numbers in constipation-predominant IBS patients.^{42,43}

Microbes for treatment and prevention of atopic dermatitis

Atopic dermatitis (AD) is the most common chronic skin condition in infants and children, with a prevalence of 10% to 20%. Geographic location affects the prevalence of this disease, with the highest prevalence in the United States and Europe. Important factors in the susceptibility to develop AD include a genetic basis (82%) and environmental factors (18%).⁴⁴ In addition, AD has been linked to food hypersensitivity, especially milk and egg proteins. The term *eczema* has been recently proposed, but for practical purposes, both AD and eczema will be used. Allergic diseases are associated with an imbalance in the TH1/TH2 cytokine activation of TH2 cells and with stimulation of IgE and IgA synthesis, leading to allergic reactions.⁴⁵ Probiotics can inhibit the TH2 response while stimulating the production of TH1 and TH1 cytokines, such as interferon.^{45,46} In children with atopic disease, the use of probiotics was associated with an increase in interferon γ production and inhibition of allergen-induced tumor necrosis factor α , IgE and several allergy-induced cytokines.^{46,47}

According to a report, two probiotic strains significantly improved atopic dermatitis, which affects 17.2 percent of the U.S. population. The clinical study evaluated the impact of a mixture of *Lactobacillus acidophilus* DDS-1 and *Bifidobacterium lactis* UABLA-12 (from UAS Labs) on 90 preschool children (ages 1 to 3 years) with moderate to severe atopic dermatitis (AD). Probiotic supplementation stabilizes the intestinal barrier function and decrease gastrointestinal symptoms in children with atopic dermatitis.² Therefore, probiotics may present attractive alternatives given the low probability for the development of adverse effects.

Microbes for treatment of crohn's disease

Crohn's disease is a common chronic disorder that affects the gastrointestinal tract and is believed to develop as a result of an aberrant immune response to intestinal microbes in a genetically susceptible host.

There are different types of Crohn's disease, depending on the part of the gastrointestinal tract that is affected. Crohn's disease may involve the small intestine, the large intestine, the rectum, or the mouth.

The infectious pathogens implicated in Crohn's disease are mainly *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Mycobacterium avium* subsp. *Paratuberculosis*. *Lactobacillus* GG is a safe probiotic bacterium known to transiently colonize the human intestine. It has been found to be useful in treatment of several gastrointestinal conditions characterized by increased gut permeability. A study showed that *Lactobacillus* GG may improve gut barrier function and clinical status in children with mildly to moderately active, stable Crohn's disease.⁴⁸

Microbes as source of anti-oxidants

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular

diseases, autoimmune disorders, neurodegenerative diseases, aging etc. The isolation of microbial antioxidants came into focus of research in the early 1980s, although various studies have established a relationship between antioxidants and microorganisms.^{49,50} The probiotic bacteria *Streptococcus thermophilus* has been shown to aid recovery from malnutrition due to short-term fasting and reduce the associated intestinal atrophy in animal studies. *S. thermophilus* is also known to have powerful antioxidant activity, protecting the body from dangerous free radicals which increase in the body due to ageing, stress, sugar, antibiotics and other chemicals and toxins. In a study, the anti-oxidant activity (AOA) of ethyl acetate extracts of several *Penicillium* and *Aspergillus* species, including *Rhizopus oryzae*, were evaluated and it was found that the extracts of two *Penicillium* and four *Aspergillus* species protected linoleic acid better than the control.^{7,51} One species, *Aspergillus candidus* CCRC 31543, protected the oil as well as BHA. In a subsequent study, it was found that sucrose or lactose and ammonium sulphate in the culture media enhanced the *A. candidus* CCRC 31543 production of antioxidants. Ethyl acetate extraction of the broth and of the mycelium produced extracts with similar activity. Gallic acid is a phenolic acid found in many natural sources, including microbial products. Gallic acid has been isolated from cultures of *Penicillium* and *Aspergillus*. *Streptomyces* sp. USF-319 produces three radical scavenging antioxidants, of which one inhibits 5-lipoxygenase. The antioxidants include mycotrienin II, trienomycin A, and trienomycin B, which are ansamycin antibiotics. Mycotrienin II was the most active compound of the three, but was considered a moderate antioxidant compared to BHT (butylated hydroxytoluene). However, mycotrienin II was found to inhibit 5-lipoxygenase.^{51,52}

Carotenoids are the final group of antioxidants that can be synthesized by microorganisms. Research has proved that β -carotene from *Blakeslea trispora* and *Duniella salina*, and lycopene from *B. trispora* and *Streptomyces chrestomyceticus*, subsp. *rubescens* were approved for human foods as colourants. Astaxanthin from microbial sources, e.g. *Xanthophyllomyces dendrorhous*, has been approved for use in fish foods. Astaxanthin and lycopene were found to have excellent singlet oxygen quenching activity. A study has shown that the AOA of astaxanthin is 10 times greater than that of lutein, β -carotene, zeaxanthin, and canthaxanthin.^{50,51}

Microbes as source of natural colours

Extraction of colours from the microbial source is an upcoming field. Various types of microorganisms like bacteria, fungi, yeasts and algae are coloured. Natural colours can be extracted from these sources using simple and effective protocols. The various advantages of producing pigments from microorganisms include independence from weather conditions, easy and fast growth and colours of different shades can be obtained by growing on cheap substrates. One major advantage of using microbes as source of natural colours is that because of their high growth rate they can be mass multiplied. The major pigments produced by microbes are red, yellow and blue. Most research has been focused on yellow and red pigment production, such as monascue produced by *Monascus* sp., carotenoid from *Phaffia rhodozyma*, *Micrococcus roseus*, *Brevibacterium linens* and *Bradyrhizobium* sp., and xanthomonadin from *Xanthomonas campestris* pv.⁵³

However, study of blue bacterial pigments is limited, because many bacteria are not capable of producing blue pigment.

Actinohodine-related blue pigments are produced by *Streptomyces coelicolor* A3 (2), mixture of violacein and deoxyviolacein by *Chromobacterium violaceum* and *Janthinobacterium lividum*.⁴ In addition to its application in dyeing fabrics, violacein also exhibits cytotoxic activity in human colon cancer cells, anti-leishmanial, anti-ulcerogenic, antiviral, antibiotic, anti-tumoral and anti-*Trypanosoma cruzi* activities.^{54,55}

Microbes for the treatment of diabetes

Diabetes is a common and sometimes fatal disease that occurs when the supply of insulin is insufficient for the body to break down sugar properly. The majority of insulin used by people to manage diabetes is produced using biotechnology. Bacterial cells are genetically modified to produce large quantities of human insulin, which is then purified for therapeutic use. Millions of people worldwide now use Humuline, which is a major brand name for 'human' insulin produced using GM bacteria.⁵⁶

Recently friendly gut microbes have been engineered to make a specific protein that can help regulate blood sugar in diabetic mice. Although the research is still in the very early stages, the microbes can be grown in yogurt, and may provide an alternative treatment for people with diabetes.^{57,58}

The researchers created a strain of non-pathogenic *E. coli* bacteria that produce a protein called GLP-1. In healthy people, this protein triggers cells in the pancreas to make insulin. Recently some scientists showed that engineered bacterial cells secreting the protein could trigger human intestinal cells in a dish to produce insulin in response to glucose. In a new research, researchers fed the engineered bacteria to diabetic mice. After 80 days, the mice went from being diabetic to having normal glucose blood levels. Diabetic mice that were not fed the engineered bacteria still had high blood sugar levels. The promise is that a diabetic could eat yogurt or drink a smoothie as glucose-responsive insulin therapy rather than relying on insulin injections. Creating bacteria that produce the protein has a number of advantages over using the protein itself as the treatment. The bacteria can secrete just the right amount of the protein in response to conditions in the host that could ultimately minimize the need for self-monitoring and allow the patient's own cells (or the cells of the commensal *E. coli*) to provide the appropriate amount of insulin when needed.^{1,2,59,58} In addition, producing the protein where it's needed overcomes some of the problems with protein-based drugs, which can be expensive to make and often degrade during digestion.

Microbes for treatment of allergies

An increased prevalence of atopic diseases, atopic dermatitis, allergic rhinitis and asthma has been reported.⁶⁰ Evidence suggests that specific strains of probiotics have an effect on inflammatory processes as demonstrated by the reduction of certain local and systemic immune markers and these actions may be mediated via the GALT, one of the three intestinal lines of defense. Probiotics may affect the production of inflammation-producing cells and accessibility of allergens, normalize the intestinal microbiota, impacting on the intestinal barrier function and help regulate the secretion of inflammatory mediators.^{2,61,62}

Conclusion

Over viewing our present knowledge and the future perspectives it may be stated that there is continuous growth of the number of new microbial metabolites, however, the qualitative improvement

is much more important. This represents significant practical results both in the human therapy and agriculture. The greatest part of the world's biodiversity still remains unexplored and the new high-speed approaches allow its successful exploitation. Cloning and genetic engineering offer alternative approaches and the chance of incorporating the suitable biosynthetic pathways from un-culturable strains into appropriate hosts. Natural products, in general, from microbes can be expected to play an important role in the ongoing transition from the empirical screening to the really rational drug design.

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Conflict of interest

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