JOURNAL OF MEDICINAL FOOD
J Med Food 17 (2) 2014, 179–188

DOI: 10.1089/jmf.2013.0031

Current Evidence on Physiological Activity and Expected Health Effects of Kombucha Fermented Beverage

Ilmāra Vīna, Pāvels Semjonovs, Raimonds Linde, and Ilze Deniņa

Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Microbiology and Biotechnology, University of Latvia, Rīga, Latvia.

ABSTRACT Consumption of kombucha fermented tea (KT) has always been associated with different health benefits. Many personal experiences and testimonials of KT drinkers are available throughout the world on the ability of KT to protect against a vast number of metabolic and infectious diseases, but very little scientific evidence is available that validates the beneficial effects of KT. The aim of this review is to give an overview of the recent studies in search of experimental confirmation of the numerous KT health-promoting aspects cited previously. Analysis of the literature data is carried out in correspondence to the recent concepts of health protection's requirements. Attention is given to the active compounds in KT, responsible for the particular effect, and to the mechanisms of their actions. It is shown that KT can efficiently act in health prophylaxis and recovery due to four main properties: detoxification, antioxidation, energizing potencies, and promotion of depressed immunity. The recent experimental studies on the consumption of KT suggest that it is suitable for prevention against broad-spectrum metabolic and infective disorders. This makes KT attractive as a fermented functional beverage for health prophylaxis.

KEY WORDS: • ***antioxidants*** • ***detoxification*** • ***functional beverages*** • ***health protection*** • ***kombucha fermented tea***

INTRODUCTION

SYSTEMATICALLY INCREASING INTOXICATION of organisms by xenobiotics, frequent insufficient work of fat-soluble essential endobiotics, oxidative stress, imbalance of energetic status, and depressed immunity cause many chronic multiorgan degenerative diseases and cancers.

Kombucha fermented tea (KT) is a popular beverage included among many traditional fermented foods across the world. Kombucha is a symbiotic culture of acetic acid bacteria (*Acetobacter aceti, Acetobacter pasteurianus, Gluconobacter oxydans*) and different yeasts (*Saccharomyces sp., Zygosaccharomyces kombuchaensis, Torulopsis* sp., *Pichia* sp., *Brettanomyces* sp.). Several lactic acid bacteria have been isolated from some kombucha associations as well.[1–4] The U.S. Food and Drug Administration and Kappa Laboratories, Miami, Florida, have carried out microbiological and biochemical tests and reported that KT is safe for human consumption. Afterward, that conclusion was confirmed repeatedly.[5–7] Curative effects on a number of human ailments have been attributed to KT consumption long ago, but those, mainly, have been based on personal observations and testimonials; only few effects have been demonstrated by scientific and experimental studies.[8] Therefore, over the last 10–15 years there has been much attention regarding the possible health benefits of KT.

KT, known to be associated with a number of health benefits, is often used for medicinal purposes.[9] For a long time, the beneficial properties of KT have been attributed primarily to the acidic composition of the beverage. It has been reported that many KT therapeutic effects may be due to substantial amounts of glucuronic acid (GlcUA), usnic acid, and lactic acid.[10]

Corresponding to this review, the recent studies are analyzed in search of firm experimental confirmation of KT's broad-spectrum health benefits; the main attention is on the active compounds discovered in KT and the mechanism of their action.

THE CURRENT VIEW ON CRUCIAL POINTS OF HEALTH MAINTENANCE

The role of KT in detoxication of the human organism and in activation of essential fat-soluble endobiotics

An important role in improvement of human health is detoxication of the organism and promotion of essential fat-soluble endobiotic metabolism, both due to the same active compound, that is, GlcUA and analogous or similar biochemical mechanisms.[11] GlcUA, present in KT, is well-known in the prophylaxis of human health as a significant detoxicant, which conjugates the toxic metabolites and

Manuscript received 3 February 2013. Revision accepted 2 September 2013.

Address correspondence to: Pāvels Semjonovs, Dr. Biol, Laboratory of Industrial Microbiology and Food Biotechnology, Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda Blvd. 4, LV-1010 Rīga, Latvia, E-mail: psem@latnet.lv

exogenous chemicals alien to normal human biochemistry, called xenobiotics (pharmaceuticals, environmental pollutants, toxins), and modifies them into compounds that have better solubility. Therefore, it becomes easier to release these xenobiotics from tissues and for them to be excreted from the body.[9,11,12] The biotransformation capability of xenobiotics, for example, glucuronidation, is reduced in elderly people. Insufficient and imbalanced nutrition can have a detrimental effect on detoxication ability, related to inadequate levels of proteins, vitamins, or essential minerals. These deficiencies can decrease the body's ability to synthesize the biotransforming enzymes. Utilization of KT helps to prevent tissues from absorbing the toxins found in an industrial environment.[13,14] The scientific studies overviewed have pointed on an important role of KT in the detoxication of the human organism.

Antioxidant properties of KT

KT's health benefits are attributed to its antioxidant activity.[9,15–19] Antioxidants are known to prevent many disorders and metabolic diseases caused by free radicals, such as disorders of eyes (retinal degeneration, cataract), skin (dermatitis, psoriasis, ageing), lungs (allergies and asthma), kidneys (nephritis and other chronic renal diseases), joints (gout), brain (migraine, neurodegenerative diseases, such as Alzheimers, Parkinsons, epilepsy or pre-epileptic syndrome in children), immune system (chronic inflammation and autoimmune disorders—rheumatoid arthritis etc.) and cancers.[13,20] Free radicals create oxidative stress—the state when the production of free radicals overwhelms the body's antioxidant defenses. An important approach to protect the human organism from oxidative stress is using antioxidant-rich products in everyday life. Sies and Stahl reported on vitamins E, C, beta-carotene, and other carotenoids present in KT as antioxidants. KT contains plenty of phenolic and other compounds that have potent antioxidant properties, decreasing lipid oxidation.[16,17] Because of the high content of phenolic compounds in traditional black tea (BT), KT significantly increases the free hydroxyl and superoxide radical scavenging activity.[18] The study confirms more potent antioxidant properties of KT when compared with unfermented BT.[18] KT contains more polyphenols, having an antioxidant activity 100 times higher than vitamin C and 25 times higher than vitamin E.[9] It has been reported that KT treatment potentiates the hepatic glutathione (GSH) antioxidant/detoxication system.[19] Thus, it has been shown that consumption of traditional KT, due to its antioxidant properties, may help to cure many chronic illnesses caused by oxidative stress.

Energizing properties of KT

Particular attention is given to KT due to its energizing properties.[20] Corresponding to knowledge on health prophylaxis biochemistry, KT's energizing ability can be explained by formation of iron (set free from tea) and gluconic acid synthesized chelating complex, which increases the level of blood hemoglobin, improves a consumer's tissues' supply of oxygen, and stimulates ATP synthesis. Weak organic acids and vitamin C, synthesized by kombucha, can enhance an effect on nonheme (plant-derived) iron absorption, and potency to increase the vitality; vitamin B group can promote energy regeneration by enzymatic activation of lipid and protein metabolism.[9]

Influence of KT on immunity

Potential impact on the host have KT's ability to modulate an immune system. The role of free radicals in inflammatory reactions is the theme of intensive studies because an oxidative stress raises chronic multiorgan inflammations and the following degenerative changes.[21,22] It has been confirmed experimentally, that KT decreases oxidative stress and its provoked immuno-suppression.[19] It has also been shown that vitamin C, present in KT, has a potency to support the immune system.[9,20] The antioxidant ability of KT provides protection against cell damage, development of inflammatory diseases, depression of an immunity, and origin of tumors (Table 1).[9,10,12,13,17–19,23–41]

METABOLIC DISEASES (NONCOMMUNICABLE CHRONIC DEGENERATIVE DISEASES)

These diseases are multifactor disorders associated with chronic troublesome disruptions in carbohydrate, fat, and protein metabolism, emanating from defects in reactive oxygen species scavenging enzymes, defects in action of digestive enzymes, etc.

Gastric illnesses

The gastro-toxicity of nonsteroidal anti-inflammatory pharmaceuticals, often leading to gastric ulceration and delayed healing, remains a crucial problem. The currently available synthetic antiulcer pharmaceuticals are expensive, show side effects, and cannot prevent ulcer recurrence, because oxidative stress and acid secretion contribute to stomach ulceration. Particular attention to KT is due in connection with its beneficial influence on the digestive system.[20] It is mentioned that KT intake regulates gastric functions, mainly intestinal activities.[6] KT stimulates such physiological actions important for digestion, such as contractions of the stomach and intestines.[13] Oxygen free radicals are known to induce gastro-intestinal injury, mediated by various agents, including indomethacin, a nonsteroidal anti-inflammatory pharmaceutical that can cause disorders of stomach mucous membrane's blood circulation.[42] Extensive study on KT's ability to prevent gastric mucosal injury and to inhibit progression of gastric ulcers has been carried out.[23] The healing activity of BT comparable to KT against indomethacin-induced stomach ulceration has been studied in a mouse model. The KT sample produced by fermenting BT for 4 days showed the best 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and phenolics content.[23] Both BT and KT extracts (15 mg/kg) effectively heal the gastric ulceration with relative efficacy as KT > BT. The healing capacities of the KT extracts could

TABLE 1. EXPERIMENTALLY PROVEN KOMBUCHA FERMENTED TEA HEALTH OR PHYSIOLOGICAL EFFECTS

Health or/and physiological status	*Proposed mechanism of action*	*Experimental model*	*Literature source*
Gastric diseases			
Indomethacin-induced gastric ulceration	Healing gastric ulceration	Mice	23
Obesity			
Hypercholesterolaemic diet	Weight-losing effect Reduces retroperitoneal adipose tissue:body weight ratio Reduces lipid deposits within the cytoplasm of liver cells	Mice	18
Diabetes			
Alloxan-induced	Inhibition of α-amylase and lipase activities	Rats	26
	Inhibition of porcine pancreatic α-amylase	*In vitro*	24
Alloxan-induced	Reduces blood sugar level	Mice	25
Hypercholesterolemia	Returns increased blood cholesterol to normal levels	Rats, rabbits, cats, dogs, humans	13
Hypercholesterolaemic diet	Lowers total cholesterol and LDL-Ch	Mice	18
Hypercholesterolaemic diet	Reduces levels of total cholesterol and LDL-Ch, increases level of HDL-Ch	Ducks	9
		Rats	12
	Delays absorption of LDL-Ch and triglycerides, increases level of HDL-Ch	Rats	26
Oxidative stress caused illnesses			
Hypercholesterolaemic diet	Increases total antioxidant capacity and superoxide dismutase, decreases level of MDA	Mice	18
Induced by hypothermia, hypoxia and acetaminophen	Decreases levels of SGOT, SGPT, MDA, LDH, increases level of GSH	Rats	10
LDL oxidation *in vitro* by $CuSO_4$	Protects LDL from oxidation	*In vitro*	18
Aflatoxin B_1-induced	Increases level/activity of antioxidant enzymes	Rats	28
Lead-induced	Increases levels of GSH and antioxidant enzymes	Rats	17
Trichloroethylene-induced	Restores GSH, LDH, and NO levels to normal	Rats	27
Chromate(IV)-induced	Decreases levels of MDA and reverses other chromate-induced changes	Rats	19
Hepatic disturbances/hepatotoxicity	Decreases ASAT, ALAT, γ-glutamyl transpeptidases activities, decreases creatinine and urea contents	Rats	26
Carbon tetrachloride-induced toxicity	Decreases levels of ASAT, ALAT, ALPs in plasma, MDA content in plasma and liver tissues; reduces the macro and micro vesicular zonal necrosis in liver tissues	Rats	30
Acetominophen-induced	Decreases levels of ASAT, ALAT, ALPs, LDH, bilirubin in serum, returns to normal levels total protein and albumin, decreases acetaminophen-induced histopathological changes	Mice	33
Aflatoxin B_1-induced	Decreases levels of liver marker enzymes and reduces necrosis of liver tissue	Rats	28
	Decreases levels of liver marker enzymes	Mice	29
Cytotoxicity			
Phenol-induced	Reduces frequency of micronucleus and account of lesions in tissues	Mice	32
Tertiary butyl hydroperoxide-inuced	Maintains membrane integrity and prevents the alterations in the cellular antioxidant status	*In vitro*	31
Cancer	Inhibits angiogenesis of prostate cancer cells	*In vitro*, androgen-independent prostate cancer cell line	34
	Exhibits cytotoxic effect on cancer cells, reduces activities of matrix metalloproteinases and cell motility of cancer cells, reduces host cell invasion by cancer cells	*In vitro*, cell lines of human lung carcinoma, human osteosarcoma and human renal carcinoma	35

(*continued*)

Table 1. (Continued)

Health or/and physiological status	*Proposed mechanism of action*	*Experimental model*	*Literature source*
Bacterial infections/Infectious diseases	Exhibits antibacterial activity against *Helicobacter pylori, Salmonella typhimurium, Staphylococcus aureus, Bacillus cereus*, *Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli, L. monocytogenes. P. aeruginosa,* and other gram-positive and gram-negative and pathogenic yeasts, *e.g.*, *Candida sp.*	*In vitro*	2,8,17,19, 36–41

GSH, glutathione; ASAT, aspartate transaminase; ALAT, alanine transaminase; MDA, malondialdehyde; NO, nitric oxide; LDL-Ch, low-density lipoprotein-cholesterol; SGOT, serum glutamic oxaloacetate transferase; SGPT, serum glutamic pyruvate transferase; ALP, alkaline phosphatase; HDL-Ch, high-density lipoprotein-cholesterol.

be attributed to their ability to protect the mucin content (the first guarding line-mucin and bicarbonates barrier); to its antioxidant activity, that is, prevention of lipid oxidation in epithelial cell membranes (the second guarding line), the ability of KT to reduce gastric acid secretion, which causes mucous membrane (the third guarding line) damage and gastric ulceration. KT extract preparation (15 mg/kg) is as effective in ulcer healing as the positive control omeprazole (3 mg/kg).[23]

Obesity

Many personal reports reviewed by the scientists inform about the antiobesity effects of KT, but little scientific information has been available for a long time.[43] As it is reported by Danielian, 2005, KT harmonizes and balances metabolism in general and abolishes or limits fat accumulation.[13]

It has been mentioned that regular KT ingestion contributes significantly to weight gain inhibition. Weight-loss effects caused by traditional KT have been found in studies of the hypolipidemic effect, which has been connected with lipase inhibition, and as a result, calorie intake restriction.[18] Obesity often causes diabetes mellitus type 2 and hypertension. The mechanism of porcine pancreatic α-amylase inhibition by flavanols and the possible impact of consumption KT on starch digestion, in line with weight management and diabetes type 2, are currently under study.[24]

Diabetes mellitus (hypoglicemic effect of KT)

Diabetes mellitus is a chronic metabolic disorder that constitutes a major public health problem throughout the world, connected to dysfunction of endogenous insulin secretion (type 1) or high stable blood glucose content as a result of different metabolic mistakes (type 2). The prevalence of type 2 diabetes mellitus is increasing worldwide at alarming rates.

Oxidative stress could be proposed as a primary reason for diabetes.[10] Several therapeutic strategies are currently available for the treatment of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, enhancement of insulin action at the target tissues, inhibition of dietary starch and lipid degradation, and treatment with oral hypoglycemic agents. The inadequacies associated with conventional medicines have led to a targeted search for alternative natural therapeutic agents among functional foods like KT. Even as early as 1929, Arauner reported that KT has an antidiabetic property—decreasing blood sugar levels.[13] It has been reported recently that repeated administration of 1.71 mL/kg for 3 days in alloxan-induced diabetic albino mice demonstrated a significant fall in blood sugar level, and the hypoglycemic effect was persistent.[25]

KT samples have been tested on starch digestion with porcine pancreatic α-amylase.[24] KT strongly inhibits starch hydrolysis; the active compounds have been suspected to be monomeric and/or oligomeric phenolic compounds. As a result of KT intake, the concentration of phenolics becomes sufficient for inhibition of pancreatic α-amylase in the small intestine, with an impact on blood glucose level. The inhibition potency increases during kombucha fermentation.[24] To describe the antidiabetic properties of KT, the activities of pancreatic lipase and α-amylase have been studied.[26] Pancreatic lipase has often been employed in studies of natural products for potential application as antidiabetic agents, which play a key role in lipid metabolism. The inhibition of this enzyme significantly decreases the uptake and digestion of lipids, and as a result, decreasing the level of blood glucose. Pancreatic α-amylase catalyzes the initial step in the hydrolysis of starch to oligoglucans, further being degraded to glucose that enters the bloodstream and leads to hyperglycemia. The findings revealed that compared to BT, KT is a better inhibitor of pancreatic lipase and α-amylase activities in the plasma and a better suppressor of increased blood glucose levels. Histological analyses show efficient protection of the liver-kidney functions of diabetic rats, evidenced by significant decreases in aspartate transaminase (ASAT), alanine transaminase (ALAT), and γ-GTP activities, creatinine, and urea contents being increased by hyperglycemia. The conclusion is that KT induces curative effects on diabetic rats. KT can, therefore, be considered as a potential candidate for future application as a functional supplement for the treatment and prevention of diabetes.[26]

Renal (nephritic) pathologies

KT effects on oxidative stress induced nephrotoxicity this has been studied in rats.[27] Trichloroethylene (TCE) has been

used to induce oxidative stress, which generates free radicals and alters antioxidants or oxygen-free radical scavenging enzymes. TCE increased the malondiahyde (MDA) and nitric oxide content in kidneys; urea and creatinine concentrations in serum; total free radical levels in blood and γ-glutamyl transferase and lactate dehydrogenase (LDHE) activities in serum, whereas it decreased the GSH level in kidney homogenate. The present study indicates that KT may eliminate damage caused by environmental pollutants, for example, TCE and may be beneficial to patients suffering from renal impairment.[27]

Kidney stones could result from metabolic poisoning, insufficient regulation of calcium metabolism by vitamin D_3, and levels of electrolytes by cortisol, other cortico-tropic steroid hormones, as well as deficiency of cobalamin—vitamin B_{12} and folic acid vitamin B_9. Consumption of KT, containing GlcUA and other organic acids, which increases water-solubility and improves transport and bioavailability of steroid hormones and fat-soluble vitamins, prevents an accumulation of heavy metals, unsoluble oxalates, inorganic salts of calcium, phosphorus, and uric acid.[11] Use of KT for reduced kidney calcification has been reported and a potential of KT to prevent kidney stone formation has been proven repeatedly.[13,20] High levels of glucuronides are found in the urine after KT intake, which suggests increased intake of GlcUA or attributes to the presence of a potent beta-glucuronidase inhibitor, D-saccharic acid 1,4- lactone, which can be found in KT as well.[20,44]

Cardiovascular diseases

Endothelial dysfunction. Endothelial dysfunction is a result of oxidative stress–free radicals which cause lipid peroxidation in endothelial cell walls. That damage is an advance factor of atherosclerosis, so endothelial dysfunction creates a threat to blood vessels and the heart. KT, due to its antioxidant activity, promotes a regeneration of cellular walls.[13,20]

Atherosclerosis (KT as antihypercholesterolemic agent). Hypercholesterolemia is a malfunction of metabolic systems and acts as secondary cause of other diseases. Free radicals and low-density lipoprotein-cholesterol (LDL-Ch) changed into oxidized form can cause damage to arterial walls and increase probability of atherosclerosis, leading to the blockage of blood vessels. Hypercholesterolemia can promote heart attack, stroke, asthma, and cataracts.[45]

KT as an antihypercholesterolemic agent has been extensively studied on animals and in clinical trials with humans. Danielian reported in her review that already in 1927 Herman for the first time achieved a return of increased blood cholesterol to normal levels in animal experiments (rats, rabbits, cats, dogs) after KT treatment.[13] They performed clinical trials in 52 atherosclerotic patients with high levels of plasma cholesterol. Traditional KT, fermented for 7–8 days, provided a good therapeutical effect; serum total cholesterol levels decreased to the normal one. It was reported that treatment of atherosclerotic patients by KT, resulted in significant reduction in atherosclerosis symptoms.[13] The effect of feeding KT on the level of total cholesterol, LDL-Ch and high-density lipoprotein-cholesterol (HDL-Ch) has also been studied in duck blood; KT intake (up to 25% of drinking water substituted by KT) significantly reduced levels of total cholesterol and LDL-Ch, and simultaneously increased level of HDL-Ch.[9] The best results have been obtained after 10 days KT treatment.[9] It has been reported that cholesterol deposits may be neutralized by GlcUA present in KT aiding the liver and kidneys in excreting the excess sterols from the body.[9]

Different mechanisms of KT antihypercholesterolemic action have been suggested by researchers, antioxidants are known to be a major factor for antihyperlipidemia. Suppression of oxidative stress as a mechanism of hyperholesterolemic atherosclerosis reduction has been shown.[18,20] KT contains more poliphenols than its unfermented tea substrate, as well vitamins E and C, known as high-level antioxidants.[18,24,36] Therefore, traditional KT has been proven experimentally *in vivo* to protect from atherosclerotic disorders, contributing to lipoprotein-bound antioxidant activity. It has been mentioned that KT reduces atherosclerosis through the regeneration of cellular walls.[20] The relationship of coronary heart disease to cholesterol lowering has been mentioned.[18,20]

KT that has a high level of GlcUA has been used to evaluate a decline in blood cholesterol in rats The results obtained establish the ability of KT to decrease blood plasma total cholesterol as much as 45–52%; triglyceride values decline to 18–27%, LDL-Ch 75–91%, with HDL-Ch values increasing to 18–27%.[12]

KT could reduce cholesterol levels in the blood through the inhibition of cholesterol synthesis enzyme HMG (3-hydroxy 3-metilglutaril CoA reductase) activity in liver and/or through the mechanism of increased excretion of cholesterol. KT has been noted to induce a marked delay in the absorption of LDL-Ch and triglycerides and a significant increase in HDL-Ch in rats.[26]

Hypertension. It is often a result of obesity, progression of coronary, and renal diseases. Simultaneously, it is a risk factor of myocardial stroke. It has been mentioned that KT reduces blood pressure and prolongs lifespan.[20,46] The experimental studies on cats and dogs have shown the strengthened myocardial contractions after using KT becoming more frequent and with amplitude increases. KT has been offered as a prophylactic functional beverage against headaches and dizziness caused by hypertension, and KT consumption is recommended for hypertension treatment.[13]

Anemia. The main risk factor for development of anemia is iron deficiency. The World Health Organization considers iron deficiency to be the largest international nutritional disorder.

The absorption of iron is enhanced by hydrochloric acid containing gastric juice: soluble iron salts are formed, that is, ionized form of iron. Hypochlorhydria (stomach acid deficiency) often results in iron deficiency anemia. Divalent iron

compounds found in animal source foods are absorbed better than trivalent iron compounds from plant sources; therefore, vegetarians sometimes have an iron deficiency. Organic acids found in KT, that is, gluconic acid and ascorbic acid, reduce trivalent iron compounds from plant sources to divalent iron ions, and form with them chelate compounds that dissolve well in an acidic medium. Such an acidic medium is temporarily formed by consumption of KT, providing body with iron. It has been proven that vitamin C, present in KT, also enhances iron absorption and is important in preventing megaloblastic anemia of infants.[47–49] Consumption of KT is particularly recommended for elderly people and vegetarians because it enhances the absorption of plant source iron and helps prevent iron deficiency.

Hepatic disturbances (increase of liver cell potency by KT)

Many hepatic diseases can impair an individual's capacity to biotransform xenobiotics, such as pharmaceuticals and toxins. There have been some experimental studies during the last decade on increasing liver cells potency by KT.[10,28–30]

Pharmaceutical paracetamol or acetaminophen, chemically named N-acetyl-p-aminophen, is one of the mostly used analgesic and antipyretic medicament around the world. In overdose it causes hepatotoxicity leading to liver failure in humans. Paracetamol is metabolized in the liver. Glucuronidation is believed to account for 40–60% of the metabolism of that.[50] It has been shown that KT prevents hepatotoxicity in animals, induced by paracetamol in an acute dose (1 mg/kg) orally.[10] Experimental hepatotoxicity can be characterized by enhanced plasma levels of serum glutamic oxaloacetate transferase, serum glutamic pyruvate transferase, LDHE, and MDA. The results obtained by KT treatment show a noteworthy decrease of the markers tested (paracetamol-induced hepatotoxicity). The researchers stated that the hepatoprotective effect is connected with the antioxidant activity of KT; the role of detoxication due to GlcUA presence in KT might be considered as well.[10] KT protection against acetaminophen-induced hepatotoxicity in mice was also studied by use of other markers. In case of hepatic toxicozes, significant increases in the levels of bilirubin and liver enzymes ASAT, ALAT, alkaline phosphatase (ALP), and LDHE are observed, while blood total protein and albumin levels have been reduced significantly; glycogen storage in hepatocytes, hepatocellular degeneration and necrosis, mononuclear cell infiltration in portal area take place. The researchers concluded that KT prevents acetaminophen-induced hepatotoxicity in mice and might provide a useful therapy for intoxicated patients.[29]

KT, rich in strong antioxidants, is expected to ameliorate liver damage induced by aflatoxin. The mechanism of hepatoprotection, observed during kombucha fermented black tea (BTK) treatment, may be due to BTK high antioxidant activity and the presence of GlcUA.[28]

KT hepatoprotective and curative properties against toxicity, induced by carbon tetrachloride (CCl_4) (xenobiotic, that induces lipid peroxidation and causes liver cell damage), have been studied.[30,51] CCl_4 significantly increases the levels of liver marker enzymes ALAT, ASAT, ALP, and MDA. KT tends to decrease them; treatment period up-growth intensified decreasing of marker enzyme levels. The regeneration/reverting rate of liver necrotic tissues to normal condition has been more pronounced in BTK-fed animals when compared to animals fed by unfermented BT. The scientists have assumed that KT's antioxidant properties can be the main reason for the efficient hepatoprotective and curative properties against CCl_4-induced hepatotoxicity.[52]

Obesity can provoke a steatosis—fatty degeneration of hepatic cells, fatty droplets appearing within the cytoplasm of liver cells, and the breakdown of fats in liver can be disrupted by poisoning. Steatosis is usually reversible, if the cause of the problem is corrected. Corresponding to the data reviewed in this article, hepatocites intoxication, its outer and inner membrane lipid peroxidation, and hepatic enzyme destruction have been identified as playing a pathogenic role in liver diseases. The KT intake markedly reduces the number of fatty droplets and significantly increases hepatic cells' productivity due to the presence of GlcUA as a detoxifier, as well as the presence of antibiotics- polyphenolics, and vitamins E and C. Treatment with KT inhibits accumulation of triglycerides and total cholesterol within the hepatocytes.[18]

Pathologies of the nervous system

Unbalanced oxidative stress is known as an inducer of neurodegenerative diseases; for example, Parkinson's disease.[53] Kleene and Schachner, have drawn our attention to specific GlcUA containing glycans in the context of the nervous system, providing insights into their possible functional roles.[54] KT contains several amino acids and methylxanthine alkaloids (caffeine, theophylline, and theobromine), ascorbic acid (vitamin C), and the B group vitamins (including folic acid-B9), necessary for normal metabolism in the nervous system.[9,20,24,55] KT can help for headaches, nervousness, and epilepsy prevention.[6] It has been advised that KT intake may improve temper, and prevent depression in the elderly.[13]

Pulmonary diseases

The causes of chronic pulmonary degenerative diseases often leading to lung cancers on the significant conditions of health maintenance, that is, growing intoxication and oxidative stress that result in suppressed immunity and often in different lung infections. The study of physiologically active components in fermented BTK has shown significantly increased content of theophyline, a xanthine bronchodilator, rapidly and widely distributed in the tissues after absorption.[56] The treatment dose is 0.18–1.0 g daily.[57] A cup of BT contains 0.014 mg of theophylline, whereas BTK contains 1.44 mg of theophylline per cup, which is helpful as it becomes a part of the therapeutic dose for patients on theophylline treatment.[56] So, daily BTK consumption can help asthma patients.

Joint problems

GlcUA, synthesized by kombucha symbiosis, in the human body can be converted into glucosamine, chondroitin-sulphate, other acidic mucopolysaccharides and glucoproteins, is associated with cartilage, collagen, and the fluid that lubricates joints; that's why it is used as nutraceutical in osteoarthritis.[58–60] The possibility of KT consumption to relieve arthritis has been successfully used for clinical tests in the USA, Russia, Sweden, and Germany.[13] It has been reported that KT has curative properties against rheumatism and gout, explained by its detoxifying ability.[13] Gout, rheumatism, and arthritis, raised partially by the accumulation of toxins in the joints, may be relieved by glucuronidation.[20,59]

Cancers

It has been reported that the daily consumption of KT was correlated with an extremely rare incidence of cancer in the population.[13] It has also been reported that carcinoma has been cured successfully by KT.[13] Subsequently, other researches proposed that long-term KT consumption increases immune system's anticancer defensive properties.[13,20,22] It was assumed that KT modulates immunity and prevents cancer proliferation in particular, at early stages of tumor growth, due to synergetic action of such kombucha symbiosis products as GlcUA, lactic and acetic acid, and antibiotic compounds.[13] KT can regulate cell proliferation, has anticarcinogenic effects, especially for hormone-dependent tumors.[18] It is mentioned that polyphenolic molecules exert numerous effects on tumorigenic cell transformation, and on tumor cells *in vitro* and *in vivo*.[61] During the few last years, some potential anticancer compounds and the mechanisms of their actions have been discovered in KT.[34] KT's influence on development of an angiogenesis-dependent prostate cancer demonstrates that KT significantly reduces prostate cancer cells development and metastasis by downregulating the expression of angiogenesis stimulators, such as hypoxia-inducible factor 1α, interleukin-8, vascular endothelial growth factor, cyclooxygenase-2, matrix metalloproteinase-2 (MMP-2). These findings suggest that KT may be useful for prostate cancer treatment/prevention.[34] The effects of KT on viability and invasiveness of various cancer cell lines, such as human lung carcinoma, human osteosarcoma, and human renal carcinoma has been studied.[35] It has been confirmed that MMPs play an important role in these processes and is linked to tumor metastasing. KT inhibits MMPs in a concentration-dependent manner. Malonate and vitexin have been purified from an ethyl acetate fraction of KT and the researchers have proven experimentally that these two compounds may be among the active compounds responsible for cytotoxic and anti-invasive effects of KT.[35] It has been mentioned that folic acid (vitamin B_9), present in KT modulates cellular cycle and cell proliferation, and can play a role in cancer development.[62]

Infectious diseases

Since resistance to antibiotics has become an increasingly important challenge to the global health problem, the finding of KT's antibacterial properties would be very promising and could be useful to find out an alternative to current synthetic antimicrobial pharmaceuticals. The kombucha fermentation process can eventually lead to an innovative production of natural antimicrobials.[63] The bacteria and yeasts present in kombucha form a symbiosis able to inhibit the growth of potential contaminating bacteria.[1,64] In a few unconfirmed cases, usnic acid has been found in KT.[65–67] Later the researchers mentioned that the beneficial health effects of KT are due to antibacterial properties of usnic acid, but these data haven't been confirmed repeatedly.[68]

Many studies proved that KT with high total acidity exert antibacterial activity against a broad range of bacteria, in particular, against *Helicobacter pylori*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus*, etc.[2,8,17,19,36–41] The antimicrobial properties of KT are supposed to be largely caused by organic acids, synthesized by kombucha symbiosis. Steinkraus reported that antibiotic activity of KT against *H. pylori*, *Escherichia coli*, *S. aureus,* and *Agrobacterium tumefaciens* is primarily related to the acetic acid produced during the fermentation; unfermented tea extracts used at the same concentration don't have any effect.[37] The antimicrobial effect was observed in the fermented samples of KT, containing 33 g/L total acid (7 g/L acetic acid).[8] It is confirmed, that weak organic acids have optimal inhibitory activity at a low pH, because they favor the uncharged, undissociated state of the molecule, that is freely permeable across the plasma membrane and thus, enters into the cell. Inhibition of pathogenic bacteria growth by weak acids has been proposed to be due to a multiple factors, including (1) membrane disruption; (2) inhibition of essential metabolic reactions; (3) stress on intracellular pH homeostasis; (4) the accumulation of toxic anions.[69–75]

It was pointed on antibacterial effect by combined action of gluconic and acetic acid.[39] On the other hand, the active water-soluble antimicrobial components that are formed during kombucha fermentation and inhibit the growth of both gram-positive and gram-negative pathogenic microorganisms are substances other than organic acids or ethanol, proteins and tannins originally present in tea or KT.[39] They are very likely bacteriocine-like substances produced by bacteria and yeasts during kombucha fermentation. It has also been found that KT contains strong water-insoluble bacteriocine with activity against broad-spectrum of infective diseases, such as diptheria, scarlet fever, influenza, typhoid, paratyphoid fever, and dysentery.[13]

CONCLUSIONS

It has been proven experimentally that KT has the four main potencies necessary for numerous biological activities: a detoxifying property, protection against free radical damage, energizing capabilities, and promotion of immunity.

KT health prophylaxis and curative effects on a number of metabolic and infective diseases are confirmed often by proving the particular bioactive compounds, present in KT; and the possible biochemical mechanisms of their actions have also been suggested.

ACKNOWLEDGMENT

This work was supported by ERDF project No. 2010/0322/2DP/2.1.1.1.0/10/APIA/VIAA/108.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Liu CH, Hsu WH, Lee FL, Liao CC: The isolation and identification of microbes from a fermented tea beverage, Haipao, and their interactions during Haipao fermentation. *Food Microbiol* 1996;13:407–415.
2. Greenwalt CJ, Steinkraus KH, Ledford RA: Kombucha, the fermented tea: microbiology, composition, and claimed health effects. *J Food Prot* 2000;63:976–981.
3. Kurtzman CP, Robnett CJ, Basehoar-Powers E: *Zigosaccharomyces kombuchaensis*, a new ascosporogeneous yeast from 'kombucha tea'. *FEMS Yeast Res* 2001;1:133–138.
4. Yang Z, Zhou F, Ji B, Li B, Luo Y, Yang L, Li T: Symbiosis between microorganisms from kombucha and kefir: potential significance to the enhancement of kombucha function. *Appl Biochem Biotechnol* 2010;2:446–455.
5. U.S. Food and Drug Administration (1995). FDA Talk Paper: FDA cautions consumers on ''kombucha mushroom tea.'' National Press Office, Rockville, MD, USA, *Talk Paper*, T95–T15.
6. Allen CM: Kombucha unveiled. Kombucha FAQ. Part six—Research and Test Results. http://users.bestweb.net/∼om/FAQ/part06.html (accessed Jan 2013).
7. Vijayaraghavan R, Singh M, Rao PV, Bhattacharya R, Kumar P, Sugendran K, Kumar O, Pant SC, Singh R: Subacute (90 days) oral toxicity studies of kombucha tea. *Biomed Environ Sci* 2000;13:293–299.
8. Greenwalt CJ, Ledford RA, Steinkraus KH: Determination and characterization of the antimicrobial activity of the fermented tea kombucha. *Lebenson Wiss Technol* 1998;31:291–296.
9. Adriani L, Mayasari N, Kartasudjana RA: The effect of feeding fermented kombucha tea on HLD, LDL and total cholesterol levels in the duck bloods. *Biotechnol Anim Husb* 2011;27:1749–1755.
10. Pauline T, Dipti P, Anju B, Kavimani S, Sharma SK, Kain AK, Sarada SKS, Sai Ram M, Ilavazhagan G, Kumar D, Selvamurthy W: Studies on toxicity; anti-stress and hepatoprotective properties of kombucha tea. *Biomed Environ Sci* 2001;14:207–213.
11. Vīna I, Semjonovs P, Linde R, Patetko A: Glucuronic acid containing fermented functional beverages produced by natural yeasts and bacteria associations. *Int J Res Rev Appl Sci* 2013;14:17–25.
12. Suhartatik N, Karyantina M, Marsono Y, Rahayu ES, Kuswanto KR: Kombucha as anti hypercholesterolemic agent (*in vitro* study using SD rats). *Proceedings of the 3rd International Conference of Indonesian Society for Lactic Acid Bacteria (3rd IC- ISLAB): Better Life with Lactic Acid Bacteria: Exploring Novel Functions of Lactic Acid Bacteria*, 2011, Yogyakarta, Indonesia.
13. Danielian LT: *Kombucha (Kombucha) and Its Biological Features*. Meditsina, Moscow, 2005. (In Russian.)
14. Naland H: *Kombucha: Teh dengan Seribu Khasiat. Agromedia Pustaka*. Jakarta, 2008. (In Indonesian.)
15. Sies H, Stahl W: Vitamins E and C, B-carotene and other carotenoids as antioxidants. *Am J Clin Nutr* 1995;62:1315S–1321S.
16. Dufresne CJ, Farnworth ER: A review of latest research findings on the healthpromotion properties of tea. *J Nutr Biochem* 2001;12:404–421.
17. Dipti P, Yogesh B, Kain AK, Pauline T, Anju B, Sai Ram M, Singh B, Mongia SS, Kumar GI, Selvamurthy W: Lead induced oxidative stress: beneficial effects of kombucha tea. *Biomed Environ Sci* 2003;16:276–282.
18. Yang Z-W, Ji B-P, Zhou F, Li B, Luo Y, Yang L, Li T: Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. *J Sci Food Agric* 2009;89:150–156.
19. Sai Ram M, Anju B, Pauline T, Dipti P, Kain AK, Mongia SS, Sharma SK, Singh B, Singh R, Ilavazhagan G, Kumar D, Selvamurthy W: Effect of kombucha tea on chromate(VI)-induced oxidative stress in albino rats. *J Ethnopharmacol* 2010;71:235–240.
20. Dufresne C, Farnworth E: Tea, kombucha and health: a review. *Food Res Int* 2000;33:409–421.
21. Meydani SN, Wu D, Santos MS, Hayek MG: Antioxidants and immune status in aged persons: overview of present evidence. *Am J Clin Nutr* 1995;62:1462S–1476S.
22. McCord JM: The evolution of free radicals and oxidative stress. *Am J Med* 2000;108:652–659.
23. Banerjee D, Hassarajani SA, Maity B, Narayan G, Bandyopadhyay SK, Chattopadhyay S: Comparative healing property of kombucha tea and black tea against indomethacin-induced gastric ulceration in mice: possible mechanism of action. *Food Funct* 2010;1:284–293.
24. Kallel L, Desseaux V, Hamdi M, Stocker P, Ajandouz EH: Insights into the fermentation biochemistry of kombucha teas and potential impacts of kombucha drinking on starch digestion. *Food Res Int* 2012;49:226–232.
25. Shenoy C: Hypoglycemic activity of bio-tea in mice. *Indian J Exp Biol* 2000;38:278–279.
26. Aloulou A, Hamden K, Elloumi D, Ali MB, Hargafi K, Jaouadi B, Ayadi F, Elfeki A, Ammar E: Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. *BMC Complement Altern Med* 2012;12:63.
27. Gharib OA: Effects of kombucha on oxidative stress induced nephrotoxicity in rats. *Chin Med* 2009;4:23.
28. Jayabalan R, Malini K, Sathishkumar M, Swaminathan K, Yun S: Biochemical characteristics of tea fungus produced during kombucha fermentation. *Food Sci Biotechnol* 2010;3:843–847.
29. Jalil A, Amin D, Mohammad HF, Saeid H: Protective effect of kombucha tea against acetaminophen-induced hepatotoxicity in mice: a biochemical and histopathological study. *Comp Clin Path* 2012;21:1243–1248.
30. Murugesan GS, Sathishkumar M, Jayabalan R, Binupriya AR, Swaminathan K, Yun SE: Hepatoprotective and curative properties of kombucha tea against carbon tetrachloride-induced toxicity. *J Microbiol Biotechnol* 2009;19:397–402.
31. Bhattacharya S, Manna P, Gachhui R, Sil PC: Protective effect of kombucha tea against tertiary butyl hydroperoxide induced cytotoxicity and cell death in murine hepatocytes. *Indian J Exp Biol* 2011;49:511–524.
32. Yapar K, Cavusoglu K, Oruc E, Yalcin E: Protective effect of kombucha mushroom (KM) tea on phenol-induced cytotoxicity in albino mice. *J Environ Biol* 2010;31:615–621.
33. Abshenas J, Derakhshanfar A, Ferdosi MH, Hasanzadeh S: Protective effect of kombucha tea against acetaminophen-

induced hepatotoxicity in mice: a biochemical and histopathological study. *Comp Clin Path* 2012;21:1243–1248.
34. Sriharia T, Arunkumarb R, Arunakaranb J, Satyanarayanac U: Downregulation of signalling molecules involved in angiogenesis of prostate cancer cell line (PC-3) by kombucha (lyophilized). *Biomed Prev Nutr* 2012;3:53–58.
35. Jayabalan R, Chen P-N, Hsieh Y-S, Prabhakaran K, Pitchai P, Marimuthu S, Thangaraj P, Swaminathan K, Yun SE: Effect of solvent fractions of kombucha tea on viability and invasiveness of cancer cells—characterization of dimethyl 2-(2-hydroxy-2-methoxypropylidine) malonate and vitexin. *Indian J Biotechnol* 2011;10:75–82.
36. Jayabalan R, Subathradevi P, Marimuthu S, Sathishkumar M, Swaminathan K: Changes in free-radical scavenging ability of kombucha tea during fermentation. *Anal Methods* 2008;109:227–234.
37. Steinkraus KH, Shapiro KB, Hotchkiss JH, Mortlock RP: Investigations into the antibiotic activity of tea fungus/kombucha beverage. *Acta Biotechnol* 1996;16:199–205.
38. Battikh H, Bakhrouf A, Ammar E: Antimicrobial effect of kombucha analogues. *LWT Food Sci Technol* 2012;47:71–77.
39. Sreeramulu G, Zhu Y, Knol W: Characterization of antimicrobial activity in kombucha fermentation. *Acta Biotechnol* 2001;21: 49–56.
40. Chu SC, Chen CS: Effects of origins and fermentation time on the antioxidant activities of kombucha. *Food Chem* 2006;98: 502–507.
41. Talawat S, Ahantharik P, Laohawiwattanakul S, Premsuk A, Ratanapo S: Efficacy of fermented teas in antibacterial activity. *Kasetsart J Nat Sci* 2006;40:925–933.
42. Skuja N, Danilāns A, Geldnere G: *Praktiskā gastroenterolog̀ija un hepatoloǵ*. Zvaigzne, Rīga, Latvija, 1999. (In Latvian.)
43. Frank GW: Fascination of kombucha. *Am Raum Zeit* 1991;2: 51–56.
44. Roussin, M: Kombucha Consumer Research Group. www.kombucha-research.com/kcrg/aboutus.htm (accessed January 2013).
45. Grundy SM, Balady GJ, Criqui MH, Fletcher G, Greenland P, Hiratzka LF, Houston-Miller N, Kris-Etherton P, Krumholz HM, LaRosa J, Ockene IS, Pearson TA, Reed J, Washington R, Smith SC, Jr.: Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction. *Circulation* 1998;97: 1876–1887.
46. Hartmann AM, Burleson LE, Holmes AK, Geist CR: Effects of chronic kombucha ingestion on open-field behaviors, longevity, appetitive behaviors, and organs in C57-BL/6 mice: a pilot study. *Nutrition* 2000;16:755–761.
47. Cook JD, Redd MB: Effect of ascorbic acid intake on newborn iron absorption from a complete diet. *Am J Clin Nutr* 2001;73:93–98.
48. Halberg L, Hulthen L: Prediction of dietary iron absorption: an algorithm for calculating absorption and bio-availability of dietary iron. *Am J Clin Nutr* 2000;71:1147–1160.
49. Jacob RA: Vitamin C. In: *Modern Nutrition in Health and Disease* (Shils ME, Olson JA, Shike M, eds.) Lea & Febiger, Philadelphia, PA, USA, 1994; 432–448.
50. Nelson LH, Neal AL, Howland MA, Hoffman RS, Goldfrank LR, Flomenbaum NE: *Goldfrank's Toxicologic Emergencies*. McGraw-Hill, New York, USA, 2010.
51. Jeon TI, Hwang SG, Park NG, Jung YR, Shin SI, Choi SD, Park DK: Antioxidative effects of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. *Toxicology* 2003;187:67–73.
52. Kumaravelu P, Dakshinamoorthy DP, Subramaniam S, Devaraj H, Devaraj NS: Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. *Biochem Pharm* 1995;49:1703–1707.
53. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L: Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005;45:287–306.
54. Kleene R, Schachner M: Glycans and neural cell interactions. *Nat Rev Neurosci* 2004;5:195–208.
55. Roche J: The History and Spread of Kombucha. http://w3.trib.com/~kombu/roche.html (accessed Jan 2013).
56. Pasha C, Reddy G: Nutritional and medicinal improvement of black tea by yeast fermentation. *Food Chem* 2005;89:449–453.
57. Motaff AC: *Clarkes Isolation and Identification of Drugs*. The Pharmaceutical Press, London, United Kingdom, 1992.
58. Jayabalan R, Marimuthu S, Swaminathan K: Changes in content of organic acids and tea polyphenols during kombucha tea fermentation. *Food Chem* 2007;102:392–398.
59. Yavari N, Assadi MM, Larijani K, Moghadam MB: Response surface methodology for optimization of glucuronic acid production using kombucha layer on sour cherry juice. *Aust J Basic Appl Sci* 2010;4:3250–3256.
60. Deal CL, Moskowitz RW: Nutraceuticals as therapeutic agents in osteoarthritis. The role of glucosamine, chondroitin sulfate, and collagen hydrolysate. *Rheum Dis Clin North Am* 1999;25:379–395.
61. Korkina LG, De Luca C, Kostyuk VA, Pastore S: Plant polyphenols and tumors: from mechanisms to therapies, prevention, and protection against toxicity of anti-cancer treatments. *Curr Med Chem* 2009;16:3943–3965.
62. Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS, Burke CA, McKeown-Eyssen GE, Baron JA: Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst* 2009;101: 432–435.
63. Mo H, Zhu Y, Chen Z: Microbial fermented tea—a potential source of natural food preservatives. *Trends Food Sci Technol* 2008;19:124–130.
64. Balentine DA: Special issue: tea and health. *Crit Rev Food Sci Nutr* 1997;8:691–692.
65. Steiger KE, Steinegger E: On the tea fungus. *Pharm Acta Helv* 1957;32:88–93.
66. Stadelman E: Der Teepilz und seine antibiotische Wirkung. In: *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Erste Abteilung/Referate*. Allgemeine Medizinische Mikrobiologie, Chemotherapie und Chemotherapeutica, 1961, pp. 401–435. (In German.)
67. Hauser SP: Dr. Sklenar's kombucha mushroom infusion—a biological cancer therapy. *Schweiz Rundsch Med Prax* 1990;79: 243–246.
68. Blanc PhJ: Characterization of the tea fungus metabolites. *Biotechnol Lett* 1996;18:139–142.
69. Freese E, Sheu CW, Galliers E: Function of lipophilic acids as antimicrobial food additives. *Nature* 1973;241:321–325.
70. Stratford M, Anslow PA: Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. *Lett Appl Microbiol* 1998;27:203–206.
71. Bracey D, Holyoak CD, Coote PJ: Comparison of the inhibitory effect of sorbic acid and amphotericin B on *Saccharomyces cerevisiae*: is growth inhibition dependent on reduced intracellular pH? *J Appl Microbiol* 1998;85:1056–1066.

72. Krebs HA, Wiggins D, Stubs M, Sols A, Bedoya F: Studies on the mechanism of the antifungal action of benzoate. *Biochem J* 1983;214:657–663.
73. Salmond CV, Kroll RG, Booth IR: The effects of food preservatives on pH homeostasis in *Escherichia coli. J Gen Microbiol* 1984;130:2845–2850.
74. Cole MB, Keenan MHJ: A quantitative method for predicting shelf life of soft drinks using a model system. *J Ind Microbiol* 1987;2:59–62.
75. Eklund T: The effect of sorbic acid and esters of para-hydroxybenzoic acid on the proton motive force in *Escherichia coli* membrane vesicles. *J Gen Microbiol* 1985;131:73–76.