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Bioactivities of some medicinal mushrooms : A modern perspective

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Medicinal mushrooms have a long tradition of use in China for their profound health promoting benefits. Recent studies are now confirming their medical efficacy and identifying many of the bioactive molecules. At least 700 species of mushrooms are known to have various therapeutic properties. Some currently isolated and identified sbustances of higher Basidiomycetes origin have expressed promising antitumour and immunomodulating effects.

Several antitumour polysaccharides such as β-D glucans with heterosaccharide chain of xylose,mannose, galactose, uronic acid or β-D glucan protein complexes as well as dietary fibres and terpenoids have been isolated from mushroom fruit bodies, submerged cultural biomass or liquid culture broth. The main medicinally important polysaccharides to have achieved clinical relevance are Lentinan, Schyzophyllan, Grifron-D, PSK (polysaccharide-K) and PSP (polysaccharide-peptide). In Japan, Russia, China and the USA different polysaccharide antitumour agents have been developed from *Lentinus edodes, Ganodarma lucidum, Schyzophyllum commune, Grifola frondosa, Inonotus obliquus, Flammulina velutipes.*. Mushrooms in general, and *Pleurotus, Lentinus, Grifola* in particular, because of their high fibre content, sterols, proteins, microelements and a low calorific value, are almost ideal for diets designed to prevent cardiovascular disease. In recent years Basidiomycetes and other higher fungi have been re-investigated as sources of novel antibiotics. Antioxidant and free radical scavenging activities of some mushrooms are reported have the potentiality to delay the ageing process.

The bioactivities of medicinal mushrooms in immunomodulalation and anti-cancer therapy represent the dominating theme of this review work. Only a brief resume of other quite significant medical properties, such as blood pressure-lowering, cholesterol lowering, liver protective, antifibrotic, anti-inflammatory, anti-diabetic, anti-viral and other anti-microbial activities will be presented here.

Key words : Mushrooms, metabolic products, medicinal properties, bioactivities

Fleshy mushrooms have long been valued worldwide as highly tasty and nutritional food (Chang and Miles, 1989). To the ancient Romans they are the 'food of the God', to the early Egyptian they are 'a gift from the God Osiris', while the Chinese appropriately consider them 'the elixir of life'. Historically, hot-water soluble fractions (decoctions and essences) from medicinal mushrooms are used in medicine in the Far East, where knowledge and practice of mushroom use primarity has originated (Hobbs, 1995, 2000). Mushrooms like *Ganodarma lucidum,* (Reishi), *Lentinus edodus* (Shiitake), *Inonotus obliquus* (Chaga) and many others have been collected and used for hundreds of years in Korea, China, Japan and eastern Russia and these practices form the basis of modern scientific studies of fungal medical activities (Ying *et al.,* 1987; Hobbs, 1995; 2000; Wasser and Weis, 1997 a, b; 1999; Stamets, 2000). By the term mushroom we generally mean "a macrofungus with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen in naked eye and to be picked by hand" (Chang and Miles, 1992). The number of mushroom species on earth is estimated to be 140 000, of which may be only 10% are known (Hawksworth, 2001). Meanwhile, of those approximately 14 000 species that we know today, about 50% are considered to possess varying degrees of edibility, more than 2000 are safe, and about 700 species are known to possess significant pharmacological properties (Chang and Bushwell, 1999 ; Wasser and Weis, 1999 ; Reshtnikov *et al.,* 2001). Significantly for modern medicine, they are the treasure trove of a large number of untapped new pharmaceutical products. Recent studies are now confirming their medical efficacy and identifying many of the bioactive molecules. The bioactivities of medicinal mushrooms in immunomodulation and anti-cancer therapy represent the dominating theme of this review work. Only a brief resume of other quite significant medical properties, such as blood pressure-lowering, cholesterol lowering, liver protective, antibiotic, anti-inflammatory, anti-diabetic, anti-viral and other anti-microbial activities (Ooi and Liu, 1999, 2000 ; Wasser and Weis, 1992a, b, Hobbs, 1995 ; Gunde-Cimerman, 1999) will be given here of the extensive additional medical properties of certain medicinal mushrooms which have been supported by recent scientific and medical studies.

Anticancer and Immunomodulating bioactive molecules from mushrooms

Medicinal mushrooms represent an unlimited source of anti-tumor and immunostimulating polysaccharides. In a recent review Reshetnikov *et al,* (2001) have listed 650 species and 7 intraspeific taxa from 182 genera of higher Hetero and Homo-Basidiomycetes that contain pharmacologically active polysaccharides that can be derived from fruit-bodies, culture mycelium and culture broths.

Polysaccharides belong to a structurally diverse class of macromolecules, polymers of monosaccharide residues joined to each other by glycosidic linkages. In comparison with other biopolymers such as proteins and nucleic acid ; they present the highest capacity carrying for biological information because they have the greatest potential for structural variability (Wasser, 2002). The nucleotides in nucleic acids and the amino acids in proteins can interconnect in only one way whereas the monosaccharide residues in polysaccharides can interconnect in several points to form a variety of branched and unbranched structures (Sharon and Lis, 1993). Mushroom polysaccharides are present mostly as glucans with different types of glycosidic linkages, such as (1-3), (1-6)-β-glucans and (1-3)-α-glucans, but some are true heteroglycans. The others are mostly bound to protein residues as PSP complexes (Gorin and Barreto-Berger, 1983). The main source of antitumour polysaccharides appear to be fungal cell walls that consists of polysaccharides. However, chitin and chitosan have no antitumour activity (Mizuno, 1995).

A large number of antitumour and immunostimulating polysaccharides of different chemical structures from higher Basidiomycetes mushrooms have been investigated. The main types are presented in Table 1.

Most often there is a main chain, which is either β(1-3), β(1-4) or mixed β(1-3), β(1-4) with β(1-6) side chains. Hetero-β-D-glucans, which are linear polymers of glucose with other D-monosaccharides, can have anticancer activity but α-D-glucans from mushroom usually lack anticancer activity (Wasser, 2002). Heteroglucan side chains contain glucuronic acid, galactose, mannose, arabinose or xylose as a main component or in different combinations. Glycans are polysaccharides containing units other than glucose in their backbone. Some correlation has been drawn between the chemical structure and antitumour activities of mushroom polysaccharides. A wide range of glycans extending from homopolymers to highly complex heteropolymers (Ooi and Liu, 1999) exhibits antitumour activity. Differences in activity can all be correlated with ability of the polysaccharide molecule to solubilize in water, size of the molecules, branching rate and form. Such structural features as β-(1-3) linkages in the backbone (main chain) of the glucan and additional β-(1-6) branch points are needed for antitumor activity (Wasser, 2002). β-glucans with only (1-6) glycosidic linkages have little or no activity. Higher molecular weight glucans have been reported by Mizuno *et al.* (1996) and Mizuno (1999) to be more effective than those of low molecular weight against tumours. Different approaches exist to improve the antitumour activity of mushroom polysaccharides by chemical modification, which is also necessary to improve their chemical qualities, water solubility and ability to permeate stomach walls after oral digestion (Wasser, 2002).

These medicinal polysaccharides are primarily modifiers of biological response where they interact with immune system to up-regulate or down regulate specific aspects of the response of the host and this may result in various therapeutic effects (Bohn and BeMillar, 1995). Wasser and Weis (1999) have reported that mushroom polysaccharides are regarded as biological response modifiers (BRM). This basically means that : (i) they cause no harm and place no additional stress on the body; (ii) they help the body to adapt to various environmental and biological stresses ; and (iii) they exert a non-specific action on the body, supporting some or all of the major systems, including nervous, hormonal and immune systems, as well as regulatory functions (Brekhman, 1980). The polysaccharides from mushrooms do not attack cancer cells directly, but produce their antitumour effects by activating different immune response in the host (Wasser, 2002).

The main medicinally important polysaccharides that have undergone extensive anti-cancer clinical trials include Lentinan (*Lentinula edodes*), Schizophyllan (*Schizophyllum commune*), PSK (polysaccharide-K, commercially sold as Krestin), PSP (polysaccharopeptide) (*Trametes versicolor*), and Griforn-D (*Grifola frondosa*) (Kidd, 2001).

Lentinan (a cell wall constituent extracted from fruiting bodies or mycelium) is a highly purified, high molecular weight polysaccharide in a triple helix structure containing only glucose molecules (Hobbs, 2000). It is known to be able to restore the suppressed activity of helper T-cells in the tumour-bearing host to their normal state, leading to complete restoration of humoral immune responses (Ooi and Liu, 1999). There is an immense literature related to the anticancer effect of lentinan on animals and human carcinomas. Hobbs (2000) has reported that *L. edodes* produces two bioactive preparations, which are efficient immune modulators, mycelium extract and Lentinan. These two bioactive polymers appear to act as host defence potentiators restoring and enhancing the responsiveness of host cells to lymphocytokines, hormone and other biologically active substances. The immunopotentiation has been shown to occur by stimulating the maturation, differentiation or proliferation of cells involved in host defence mechanism. Chihara *et al.* (1987) and Chihara (1992) have reported that Lentinan increases host's resistance against various kinds of cancer and has the potential to restore the immune function of affected subjects. Lentinan has been satisfactorily proven to potentiate human immunity (Chihara *et al.*, 1987, Borchers *et al.*, 1999, Wasser, 1999).

Schizophyllan, from *Schizophyllum commune*, is relatively similar to Lentinan in composition and biological activity, and its mechanism of immunomoduation and anti-tumour action appears to be quite similar (Jong *et al.,* 1991). Various clinical trials have been carried out in Japan and schizophyllan has been approved for clinical use in Japan. Recently schizophyllan has also been shown to increase overall survival of patients with head and neck cancers (Kimura *et al.,* 1994). In a randomized controlled study of schizophyllan in combination with radiotherapy, schizophyllan has significantly prolonged the overall survival of stage II cervical cancer patients but not stage III (Okamura *et al.,* 1986, 1989). In a prospective, randomized clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil) and schizophyllan in various combinations, patients treated with schizophyllan has a better overall survival than patients who has not received the polysaccharides (Miyazaki *et al.,* 1995). Human clinical trials have proved the beneficial activity of treatment with Schizophyllan for patients with recurrent and inoperable gastric cancer, stage II cervical cancer, and advanced cervical carcinoma (Hobbs, 1995). Schizophyllan has been found rather ineffective against gastric cancer, but has extended survival time in patients with head and neck cancer. (Borchers, 1999 ; Kimura *et al.* 1994). Several Japanese pharmaceutical companies currently produce schizophyllan commercially.

Protein bound polysaccharides PSK (Krestin) and PSP have been isolated from the mushroom *Trametes versicolor.* Both PSK and PSK are potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. Numerous reports have documented the ability of PSK and PSP to activate cellular and humoral components of the host immune system. In addition, these polysaccharides have been shown to inhibit the growth of tumour cell lines and to have *in vivo* anti-tumour activity (Tzianabos, 2000). PSK has remarkable immune enhancing activity and a broad antineoplastic scope. With regard to its antitumour property, it acts directly on tumour cells, as well as indirectly in the host to stimulate cellular immunity (Hobbs, 1995 ; Stamets, 2000). An intriguing feature of PSK is that after injection of PSK at one tumour site it has been shown to inhibit tumour growth at other sites, thus helping to prevent metastasis. PSK has been used both orally and intravenously in clinical medicine. It has been shown to be effective against many types of cancer such as stomach, oesophageal, colorectal and breast cancer (Hobbs, 1995 ; Stamets, 2000) but seldom with satisfactory results if administered alone (Wasser, 2002).

Another (1-3)-β-glucan, Grifolan, from *Grifola frondosa* is similar to schizophyllan in primary structure (Adachi *et al.,* 1990). When D-fraction plus Maintake has been combined with chemotherapy, the overall response rates are increased by 12-28% when results from cancer types are combined. The Food and Drug Administration (USA) has approved Grifon-D (GD) for trial under an Investigational New Drug Application (IND) for patients with advanced cancer and some US-based clinical trials are currently underway at various Institutions (Nanba, 1997b). Several studies have shown that β-D-glucan derived from *Grifola frondosa* (also known as Maintake) have strong antitumour activity and recently, a highly purified extract, β-glucan β1,6 glucan branched with a β 1-3-linkage Grifron-D, GD) has become available. GD has considerable immunomodulating and antitumour activities in animal models, and is orally bioavailable (Nishida *et al.,* 1988). Maintake D-fraction and crude Maitake powder have demonstrated remarkable inhibition of metastasis in a mouse model, especially in the prevention of hepatic metastases, which in one series of experiments is reduced by 81% (Maitake powder) to 91% (D-fraction) (Nanba, 1995). GD has been shown to have a cytotoxic affect on human prostate cancer cells (PC9) *in vitro*, possibly acting through oxidative stress, and causing 95% cell death by an apoptosis (Fullerton *et al.,* 2000).

Immunomodulating effects of *Ganoderma lucidum* has been used extensively as "mushrooms of immortality" in China and other Asian countries for 2000 years (Shiao *et al,* 1994). Several major substances with potent immuno-modulating action have been isolated from this mushroom, including polysaccharides (in particular β-D-glucan), proteins (e.g., Ling Zhi-8) and triterperoids (Gao and Zhou, 2001). The major immuno-modulating effects of these active substances derived from *G. lucidum* include mitogenicity and activation of immune effector cells such as macrophages, NK and T cells (Gao and Zhou, 2001). Ganoderma is the premier medicinal mushroom and the annual value of extracts of it alone sold worldwide is estimated at over 1.6 billion US Dollar (Chang and Bushwell, 1999), and mostly used as a tonic for the immune system.

Three polysaccharide based carcinostatic (immunotherapeutic) agents, Krestin, Lentinan and Sonifilan, have already been developed from mushroom (Mizuno, 1999). These are used currently in the treatment of cancer of the digestive organs, lung and breast, as well as cancer of the stomach and cervical cancer respectively. Several mushroom species belonging to the polyporaceae family are now regarded as the next drug producers. Mushroom polysaccharides are also expected to be developed into multipurpose medicines that are not only carcinostatic but also anti-inflammatory, antiviral (against AIDS), hypoglycaemic and antithrombotic.

Blood pressure-lowering and cholesterol lowering effects

A highly significant cause of death in most developed countries is coronary artery disease. Mushrooms in general, and *Pleurotus, Lentinus* and *Grifola* in particular, because of their high fibre content, sterols, proteins, microelements and a low calorific value, are almost ideal for diets designed to prevent cardiovascular diseases as first suggested by Traditional Chinese Medicine (Hobbs, 1995). Mevinolin (lovastatin) produced commercially from the filamentous fungus *Aspergillus terreus* was the first specific inhibitor of HMG-CoA reductase to receive approval for the treatment of hypocholesteremia (Alberts *et al.,* 1980). The genus *Pleurotus* of the medicinal mushrooms has several species that produce mevinolin (Gunde-Cimerman 1999). *P. ostreatus* has been shown to produce the highest amount of lovastatin in the fruit-body, especially in the lamellae or gills. Mevinolin has been detected in submerged fermentation broth of *P. saca* and in the surface fermentation broth of *P. sapidus* (Gunde-Cimerman *et al.,* 1999). It has been suggested that *Pleurotus* mushrooms could be recommended as a natural cholesterol lowering substance within the human diet (Gunde-Cimerman, 1999). Antilipemic effects of polysaccharides from *Tremella fuciformis* and *T. aurantia* have been shown to lower plasma cholesterol levels (Kiho *et al.,* 1992, 1994). Several small studies with *Lentinus* extracts in Japan have shown positive decreases in serum cholesterol in young women and people older than 60 years of age (Hobbs, 1995). Nucleic acids from *L. edodes* also have significant platelet agglutinating inhibitory effects (antithrombotic activity). PSK also causes decreases in LDL cholesterol in hyperlipidemia patients (Tsukagoshi *et al.,* 1984).

Hypoglycaemic effects of mushrooms

Due to their high content of fibre and proteins and low fat content, extracts of edible mushrooms have been considered to be ideal foods for dietetic prevention of hyperglycemia (Gunde-Cimerman, 1999). Extracts of several medicinal mushrooms, including *Tremella aurantia, Cordyceps sinensis, Ganoderma lucidum* and *Auricularia auricula-judae* have been shown to lower blood glucose (Kiho *et al.,* 1995 ; Yang *et al.,* 2000). The blood glucose and triglyceride (TG) lowering effects of water soluble extracts from *Lentinus edodes, Pleurotus ostreatus* and *Phellinus linteus* in the streptozotocin-induced diabetic model have been clearly demonstrated (Kim *et al.,* 1997 ; Kim *et al.,* 2001). Such results strongly suggest that these mushrooms have potential preventive and therapeutic action in diabetes mellitus (Type I and II). The hypoglycemic polysaccharide from *Phellinus lintues* has been successfully achieved by submerged culture (Kim *et al.,* 2002). Recently the limited availability of *Cordyceps milliarus,* used in Chinese traditional medicine for hypoglycemic activity, prompted the development of mycellial fermentations in liquid culture (Yang *et al.,* 2000).

Hepatoprotective activity of mushroom

Ganoderma lucidum, highly ranked medicinal mushroom in Oriental traditional medicine, has been widely used for the treatment of chronic hepatopathy of various etiologies and recent studies (Gao and Zhou, 2003) suggest several possible mechanisms. These include antioxidant and radical-scavenging activity, modulation of hepatic Phase I and II enzymes, inhibition of β-glucuronidase, antifibrotic and antiviral activity, modulation of nitric oxide production, maintenance of hepatocellular calcium homeostasis, and immunomodulating effects. A polysaccharide fraction from *L. edodes* has showen liver protective action in animals together with improved liver function and an enhance production of antibodies to hepatitis B (Mizuno, 1995).

Antimicrobial activity

Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Consequently, the antimicrobial activity of various antitumour polysaccharides from medicinal mushrooms are being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Researchers have shown that a water extract of *L. edodes* demonstrated growth-enhancing effects on colon-inhabitating beneficial lactic acid bacteria, *Lactobacillus brevis* and *Bifidobacteria breve.* The effective factor in the extract is considered to be the disaccharide sugar, trehalose. The author suggest that the *L. edodes* extracts can improve the beneficial intestinal flora of the gut and reduce the harmful effects of certain bacterial enzymes such as β-glucosidase, β-glucuronidase and tryptophanase as well as reducing colon cancer formation (Bae *et. al.,* 1997).

Antioxidant, anti-inflammatory, free radical scavenging activities of mushrooms

A wide variety of pathological damage, such as DNA, carcinogenesis and cellular degeneration, related to the ageing process and ageing itself can be caused by reactive oxygen species (ROS) produced by sunlight, ultraviolet and ionising radiation, chemical reactions and metabolic processes. Furthermore, there is a vast accumulation of studies that implicate oxygen derived free radicals such as superoxide, hydroxyl radicals and high energy oxidants such as peroxy nitrite as mediators of inflammation, shock and ischemia reperfusion injury (Cuzzocrea *et al.,* 2001). Several mushroom species have been studied for anti-inflammatory and antioxidant activities (Ukai *et al.,* 1983). Extracts of *G. lucidum* can apparently remove the hyperoxide radical believed to be a main factor in the human ageing process (Liu *et al.,* 1997).

The term 'medicinal mushroom' is now increasingly gaining worldwide recognition as a rapidly developing area of biotechnology for cancer therapy and other therapeutic activities. Higher Basidiomycetes mushrooms are yet to be thoroughly studied ; even the inventory of the known species is incomplete, comprising may be only 10% of the true number of species existing (Hawksworth, 2001 ; Kirk *et al.,* 2001). Of the 651 species and 7 intraspecific taxa from 182 genera of higher Hetero and Homobasidiomycetes, the great majority have been reported to possess pharmacologically active polysaccharides in their fruit bodies, culture media or culture broth (Reshetnikov *et al.,* 2001). The antitumour polysaccharides from various mushrooms are characterized by their molecular weight, degree of branching and higher structure (Wasser *et al.,* 2000). A wide range of innovative

Table 1 : Chemical structure of atitumour and immunostimulating polysaccharides from higher Basidiomycetes (Wasser, 2002)

Polysaccharide	Species	References
Glucans		
α-(1-3)-glucan	*Armillariella tabescens*	Kiho *et al.*, 1992a
Linear α-(1-3)-glucan	*Amanita muscaria*	Kiho *et al.*, 1994
	Agrocybe aegerita	Yoshida *et al., 1996*
α(1-4)- ; β-(1-6)-glucan	*Agaricus blazei*	Fujimaya *et al.*, 1998b
α(1-6)-; α-(1-4)-glucan	*Agarcus blazei*	Mizuno *et al.*, 1999a
β-(1-6)-glucan	*Lyophyllum dacestes*	Ukawa *et al.*, 2000
	Armillariella tabescens	Kiho *et al.*, 1992a
β-(1-6)- ; β-(1-3)-glucan	*Agaricus blazei*	Mizuno *et al.*, 1990a
	Grifola fronodosa	Nanba *et al.*, 1987
β-(1-6)-; α-(1-6)-glucan	*Agaricus blazei*	Mizuno *et al.*, 1990a
β-(1-3)-glucuronoglucan	*Ganoderma lucidum*	Saito *et al.*, 1989
Manoxyloglucan	*Grifola frondosa*	Mizuno *et al.*, 1986
Galactoxyloglucan	*Hericium erinaceus*	Mizuno, 1999b
Xyloglucan	*Grifola frondosa*	Mizuno *et al.*, 1986
	Polyporus confluense	Mizuno *et al.*, 1992
	Pleurotus palmonurius	Zhuang *et al.*, 1993
Xylogalactoglucan	*Inonotus obliquus*	Mizuno *et al.*, 1999a
Mannogalactoglucan	*Pleurotus palmonurious*	Gutierrez *et al.*, 1996
	Pleurotus cornucopiae	Kim *et al.*, 1994
	Ganoderma lucidum	Cho *et al.*, 1999
	Agaricus blazei	
Galactomannoglucan	*Flammulina velutipes*	Ikekawa *et al.*, 1982
	Hohen buehelia serotina	Mizuno *et al.*, 1994
	Leucopaxillus giantess	Mizuno *et al.*, 1995
Glycans		
Arabinogalactan	*Pleurotus citrinopileatus*	Zhuang *et al.*, 1994a
Glucogalactan	*Ganoderma tsugae*	Wang *et al.*, 1993
Fucogalactan	*Sarcodon aspratus*	Mizuno *et al.*, 2000
α-(1-6) mannofucogalactan	*Fomitella fraxinea*	Cho *et al.*, 1998
Fucomannogalactan	*Dictyophora indusiatica*	Hara *et al.*, 1998
Mannogalactan	Pleurotus palmonurius	Zhuang *et al.*, 1993
Mannogalactofuran	*Grifola frondosa*	Zhuang *et al.*, 1994a
Xylan	*Hericium erinaceus*	Mizuno, 1999b
Glucoxylan	*Hericium erinaceus*	Mizuno, 1999b
	Pleurotus palmonurius	Zhuang *et al.*, 1993
Mannoglucoxylan	*Hericium erinaceus*	Mizuno, 1999b
α-(1-3)-mannan	*Dictyophora indusiatica*	Ukai *et al.*, 2001
Gluconomannan	*Agaricus blazei*	Tsuchida *et al.*, 2001
β-(1-2) ; β-(1-3)-glucomannan	*Agaricus blazei*	Mizuno *et al.*, 1999b
Galactoglucomannan	*Lentinus edodes*	Fuji *et al.*, 1979

biologically active polysaccharides has been detected among higher Basidiomycetes fungi and their practical application is dependent on not only their unique properties but also on biotechnological availability (Wasser, 2000). Asian pharmaceutical companies currently produce most of the medicinally important compounds. Many of the anti-cancer polysaccharides are orally bioavailable, while others are effective only by intraperitonial injection. In China about 20 Mycomedicines are commercially prepared and sold. They are sedatives, anticancerous, antiradiation drugs, liver protectants, recuperating agents for stomach and intestine and medicines for stimulating bile secretion and for dizziness and headache. Searching for new bioactive substances from mushrooms and studying their medicinal value have recently become a great matter of significance.

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