

ENHANCED BIO-ENERGY STATE IN MICE LIVER AFTER ADMINISTRATION OF CORDYMAX



Wu ZM, Dai DW, Bao TT, Xu CF, Zhu J-S

Pharmanex Pharmacology Center Beijing, China; Institute of Materia Medica; Peking Union Medical University, Beijing, China; Pharmanex Provo, USA

Abstract

Natural *Cordyceps sinensis* and its mycelial fermentation product CordyMax have been advocated for centuries to enhance human vitality. This study was to evaluate the effect of CordyMax on tissue energetics of male C57-BL/6 mice using non-invasive ³¹P NMR spectroscopy. Mice were divided into 3 groups. Groups A and B (n=5 each) received an extract of CordyMax, 200 or 400 mg/kg/day, and Group C (n=6) received placebo. All treatments were given by gavage for 7 days and then discontinued. Hepatic β-ATP and inorganic phosphate were measured using a ³¹P NMR spectroscope [Bruker], after mice been anesthetized with pentobarbital (55 mg/kg, i.p.) and immobilized on shielding belt. An MDPA reference was placed on the back of the coil. Measurements were made at baseline, after 7 days of treatment, and 7 days after discontinuing treatment (washout phase). Tissue pH was calculated from chemical shift differences between α-ATP and Pi. At the end of the treatment phase, β-ATP was increased in relation to the MDPA reference in mice receiving CordyMax: Group A 3.81 ± 0.03 (+12.3% on average); Group B 4.00 ± 0.04 (+18.4%); compared with Group C 3.36 ± 0.04 (p<0.001). Inorganic phosphate was decreased in Groups A and B, but not in Group C (p<0.001). Consequently, the ratio β-ATP/Pi was also significantly increased in mice receiving CordyMax: Group A 4.81 ± 0.05 (+47.7% on average); Group B 4.50 ± 0.09 (+41.4%); compared with Group C 3.10 ± 0.04 (p<0.001). At the end of the washout phase, β-ATP had returned to baseline in Groups A and B. Hepatic tissue pH was unchanged throughout the study. We conclude that CordyMax increased steady state levels of hepatic bio-energy when administered to mice for 7 days. Our findings may explain the reported energizing effect of CordyMax in human subjects.



Cordyceps sinensis (Berk.) Sacc. 冬虫夏草
(Collected from Qinghai-Tibetan plateau of China)

Isolation & Purification

Cs-4 (A *Paecilomyces hepiali* Chen strain)

Industrial Fermentation

CordyMax

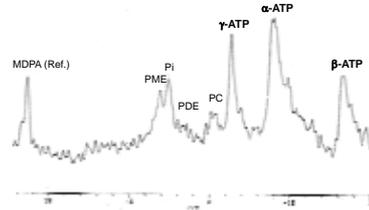
Introduction

- ◆ **CordyMax improves aerobic capability and enhances endurance in older humans** (*Chin J Integr Med* 2004; 10:187-192; *SH J Prevent Med* 2008, 20:367-369).

↑VO₂max, ↑Anaerobic Threshold,
↑Maximal Ventilation,
↓RER, ↓Lactic acid

- ◆ **CordyMax improves glucose and lipid metabolisms** (*J Alternat Compl Med* 2002; 8:309-314; *Chin J Clin Pharmacy* 2007; 16:214-217; *Proceedings 2008 Symposium Chin Asso Med Mycol.* 2008, pp157-164).

³¹P NMR Spectroscopy

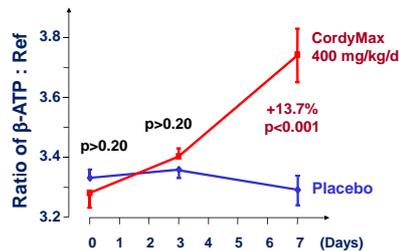


Note:

MDPA methylene diphosphonic acid
PME phosphomonoesters
Pi inorganic phosphate
PDE phosphodiester
PC phosphocreatine

³¹P NMR Spectroscopy on mouse liver:
α-, β-, γ-ATP, other triphosphorates compounds, and inorganic phosphate; calculate cellular pH.

Increases in Hepatic β-ATP In Response to Oral CordyMax



Experimental Design

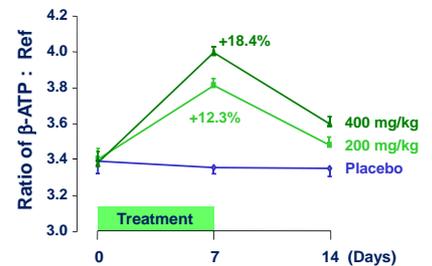
Group	Treatment	n
Placebo group	No treatment	6 mice
CordyMax 200 mg/kg	No treatment	5 mice
CordyMax 400 mg/kg	No treatment	5 mice

Timeline: 0 (days) → 7 → 14 (days). ³¹P NMR spectroscopy at 0, 7, and 14 days.

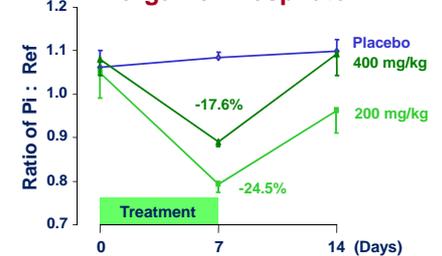
At the time indicated,

- Anaesthetized mice with Pentobarbital
- Preformed ³¹P NMR spectroscopy on mouse liver

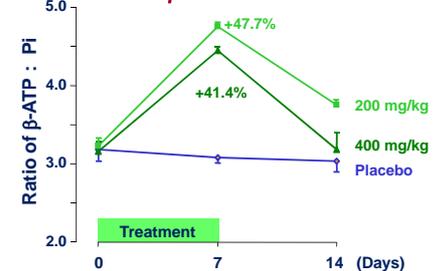
Changes in Hepatic β-ATP



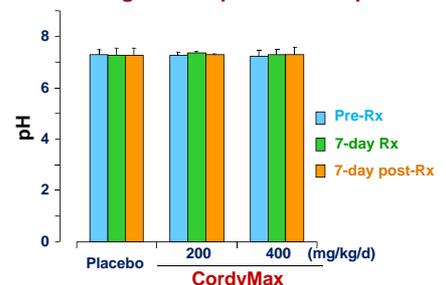
Decreases in Hepatic Inorganic Phosphate



Increases in the Ratio of β-ATP : Pi



Changes in Hepatic Tissue pH



Summary

7-day CordyMax Treatment:

- ↑ Hepatic ATP
- ↓ Hepatic inorganic phosphate
- ↑ Hepatic ratio of ATP:Pi

No Δ in hepatic tissue pH

Conclusion

- **CordyMax improved steady-state hepatic bio-energy status**

CordyMax Improves Glucose Metabolism in Animals and in Humans



Wu ZM, Nicodemus K, Zhao CS, Zhu J-S

Pharmanex Pharmacology Center, Beijing, China; Fit Stop Human Performance Lab, Encinitas, CA, USA, Pharmanex Provo, USA

Abstract

Preliminary reports demonstrated that supplementation with a mycelia fermentation product of *Cordyceps sinensis* (CordyMax) increased maximal O₂ uptake and anaerobic threshold in mid-age to elderly humans, and enhanced *in vivo* bio-energy metabolisms in animals (*J Alternat Complement Med* 7:231, 2001; *Chin J Integr Med* 10:187, 2004; *Shanghai J Prevent Med* 20:367, 2008). We further studied in a randomized, double-blind clinical trial the effect of CordyMax (4.5 g/day, 6 weeks) in highly-fit athletes on glucose metabolism. Male adventure racers and multi-sport endurance athletes (age 32 ± 4 yrs; VO_{2peak} 63 ± 8 ml/kg/min) were assigned to either a CordyMax or a control group (n=15 each). We found a 7% decrease in fast blood glucose within normal ranges after the CordyMax therapy (92 ± 1 to 87 ± 2 mg/dL; p<0.01), but no change in placebo controls. During prolonged sub-maximal exercise (70% VO_{2peak}, 60 min), reductions of respiratory exchange ratio were found in the CordyMax vs. control group (p=0.02). In mice given CordyMax for 4 weeks, responses of serum insulin and C-peptide to an oral glucose load were diminished and recovered to the pre-load levels quickly vs. control group (p<0.01 or 0.05) with no change in the glucose tolerant curve. The glucose-insulin index was lower in the CordyMax (7±1 x10⁵ units) vs. control group (10±1 x10⁵) (p<0.01). Our data suggest that CordyMax (1) safely lowers basal glucose in normal humans, (2) improves glucose metabolism by enhancing insulin receptor sensitivity, and (3) enhances fat mobilization and beta-oxidation thereby sparing glycogen expenditure during prolonged endurance exercise.



冬虫夏草

Cordyceps sinensis (Berk.) Sacc.
(Collected from Qinghai-Tibetan plateau)

Isolation & Purification

Cs-4 (A *Paecilomyces hepiali* Chen strain)

Industrial Fermentation

CordyMax

Introduction

- ◆ CordyMax improves aerobic capability and enhances endurance in older humans (*Chin J Integr Med* 2004; 10:187-192; *SH J Prevent Med* 2008, 20:367-369).

↑VO_{2max}, ↑Anaerobic Threshold,
↑Maximal Ventilation,
↓RER, ↓Lactic acid

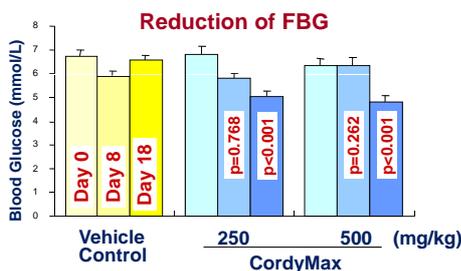
- ◆ CordyMax improves lipid metabolism (*Proceedings 2008 Symposium Chin Asso Med Mycol.* 2008, pp157-164).

- ◆ CordyMax improves steady-state hepatic bio-energy status (*J Alternat Compl Med* 2001; 7:231-240).

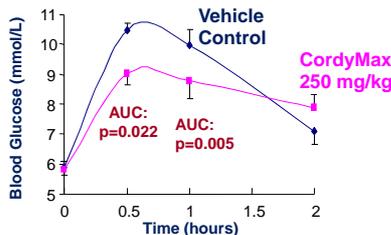
Experimental Design (1):

Placebo	(n=12 rats)
CordyMax 250 mg/kg	(n=12 rats)
CordyMax 500 mg/kg	(n=12 rats)

0 (days) 18



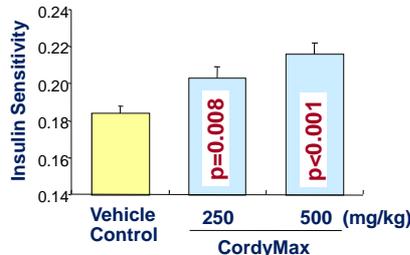
Improvement of oral Glu tolerance



Reduction of fasting serum insulin



Increase in insulin sensitivity



Experimental Design (2):

Randomized, Double-Blind, Placebo Controlled

Placebo	(n=15)	(4.5 g/day)
CordyMax	(n=15)	(4.5 g/day)

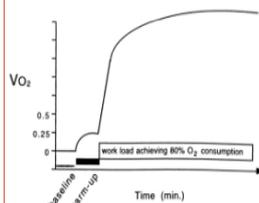
0 (weeks) 6
↑ Sub-maximal exercise-metabolism testing ↑

Inclusion Criteria:

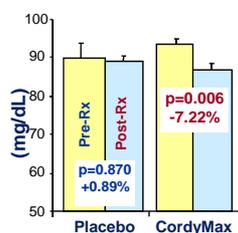
Healthy, highly fit, male endurance athletes under the age of 40 years

Training Background	Training vol. Hrs./wk.	Number of Subjects
Adventure Racers	11 ± 7	9
Pro/Competitive Triathletes	15 ± 7	10
Active Military (Reco/SeALs)	11 ± 1	3
Olympic Rower/Kayak	10	1
Ultra-distance runner	14	1
High Fit Multi-sport	9 ± 4	6

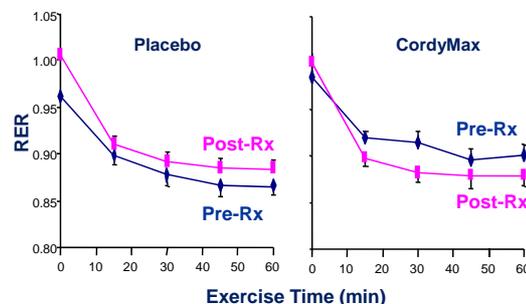
Constant work-rate, sub-maximal exercise



Decrease in FBG



Reduction of RER during sub-maximal exercise



Summary and Conclusion

CordyMax Treatment:

- ◆ Improves oral glucose tolerance, and reduces fasting blood glucose within normal ranges;
- ◆ Increases insulin sensitivity, and reduces fasting serum insulin;
- ◆ Enhances fat mobilization and beta-oxidation thereby sparing glycogen expenditure during prolonged submaximal exercise in highly-fit humans.

CORDYMAX INCREASES SERUM HDL-CHOLESTEROL AND REDUCES OXIDIZED LDL-CHOLESTROL IN HUMANS WITH REDUCED SERUM HDL-CHOLESTEROL



Wu ZM, Wang BE, Pei Y, Liu CZ, Li SQ, Zhang W, Xu ZB, Rippe J, Zhu JS

Pharmanex Clinical Center, Beijing; Beijing Friendship Hospital and Beijing Association for Medico-Pharmaceutical Research & Development; Rippe Lifestyle Institute, Shrewsbury, MA, USA; Pharmanex Provo, UT, USA

Abstract

Literature reported CordyMax, a mycelial fermentation product of *Cordyceps sinensis*, regulated the blood lipids in hyperlipidemic patients and prevented the formation of atherosclerosis in animals and humans. (*Administ Tradit Chin Med* 1995;5:14-18). We tested the effect of CordyMax (3.0 g/day) in 133 dyslipidemic subjects on increasing HDL cholesterol (HDL-c) and reducing oxidized LDL cholesterol (ox-LDL). Subjects with reduced serum HDL-c (<40 mg/dL for males or <45 mg/dL for females) were randomized to a CordyMax or placebo group (double-blind). Eight weeks of CordyMax did not change total cholesterol and triglycerides significantly. At Week 8, LDL-c was reduced with CordyMax significantly by 5.4% (p=0.002). Most dramatically, ox-LDL was reduced by 22.9% in CordyMax group (p=0.001). Atherosclerosis Index [= (TC - HDL-c)/HDL-c] reduced with CordyMax by 29% (p<0.001). HDL-c was increased with CordyMax by 31.1% (p<0.001) in females and by 12.3% (p=0.003) in males. Apolipoprotein A1 was increased with CordyMax by 34.7% (p=0.005). The ratio of ox-LDL/HDL-c was reduced by 10.5% (p=0.003). In conclusion, CordyMax significantly lowers LDL-c, ox-LDL and Atherosclerosis Index, and increases HDL-c and ApoA1, reducing the risk of atherosclerosis and cardio- and cerebro-vascular diseases.

Introduction

- ◆ CordyMax increases activities of scavenging oxygen free radicals in elderly senescent patients (*Administ Tradit Chin. Med.* 1995;5:14-18)
 - ↑ Superoxide Dismutase (SOD) activity
 - ↓ Plasma malondialdehyde (MDA)
- ◆ CordyMax interrupts the formation of atheromatous plaque in the aorta and inhibits thrombosis on the surface of experimentally injured endothelia of arteries in rabbits (*Administ Tradit Chin. Med.* 1995;5:6; Chapter 4, in: *Advanced Study for TCM Herbs*, Vol. 1. IMM, ed., 1995:91-113)
- ◆ CordyMax decreases total and LDL-cholesterol in hyperlipidemic patients (*Intl J Orient Med* 1990; 15:77-80)
- ◆ CordyMax improves lipid-glucose metabolisms in elite athletes (*Chin J Clin Pharmacy* 2007; 16:274-277)



Cordyceps sinensis (Berk.) Sacc. 冬虫夏草
(Collected from Qinghai-Tibetan plateau of China)

Isolation & Purification

Cs-4 (A *Paecilomyces hepiali* Chen strain)

Industrial Fermentation

CordyMax

Study Aims

1. To examine the effect of CordyMax on serum HDL-cholesterol
2. To examine the effect of CordyMax on serum oxidized LDL-cholesterol

Experimental Design

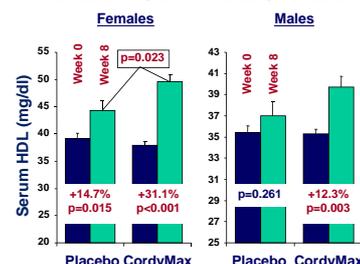
Randomized; Double-blind;
Placebo Controlled



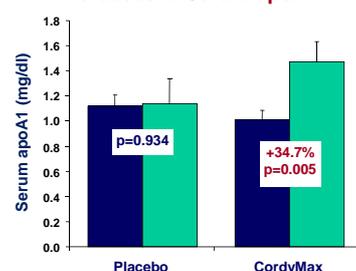
Inclusion Criteria:

- Serum HDL-cholesterol
- Males ≤ 40 mg/dl
- females ≤ 45 mg/dl

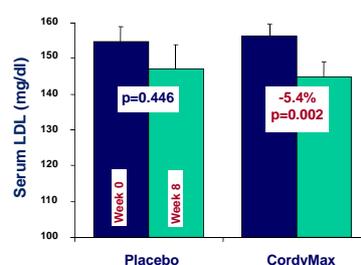
Increases in Serum HDL-Cholesterol



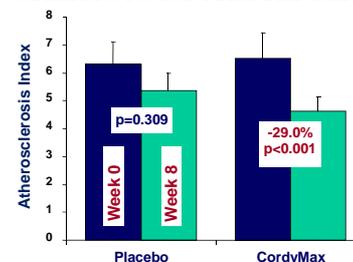
Increases in Serum apoA1



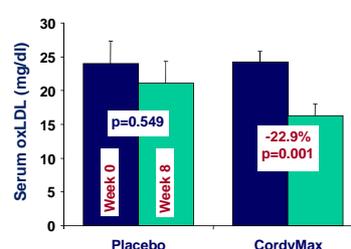
Reduction of Serum LDL-Cholesterol



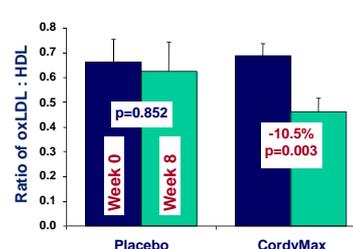
Reduction of Atherosclerosis Index



Reduction of Oxidized LDL-Cholesterol



Reduction of Ratio of OxLDL:HDL



Summary

CordyMax supplementation for 8 weeks

In all patients

- ↑ Serum HDL-cholesterol
- ↓ Serum oxLDL
- ↓ Ratio of oxLDL:HDL

In patients with baseline serum LDL-c >130 mg/dL

- ↓ Serum LDL-cholesterol
- ↑ Serum apoA1
- ↓ Atherosclerosis Index
- No Δ serum triglycerides

Conclusion

CordyMax treatment increases serum HDL-c and decreases oxidized LDL-c in patients with reduced baseline serum HDL-c, indicating reduced risks of atherosclerosis, and ischemic cardio- and cerebro-vascular diseases.

CordyMax Extends the Lifespan of Mice

— A preliminary report

Wu ZM, Zhang Y, Tan NZ, Zhao CS, Yang JY, Zhu J-S
 Pharmanex Beijing Pharmacology Center, Beijing China;
 Pharmanex Research Institute, Provo, USA



Abstract

Cordyceps sinensis and its mycelia fermentation product CordyMax have been used for Centuries for anti-fatigue and endurance enhancement. We reported anti-fatigue and endurance enhancement properties, and improvement of glucose, lipid and energy metabolisms by CordyMax in animals and humans in previous studies. In this study, we explored possible anti-aging effects of CordyMax in mice. A total of 192 healthy ICR mice (12 months of age, half males and half females) were randomized into 4 groups, receiving either vehicle or CordyMax at a dose of 500, 1000, or 1500 mg/kg.bw. All mice were fed with regular forage or forages contained CordyMax in different concentrations. Body weight was monitored once a week. Calorie intake was monitored twice per week and adjusted carefully to match the average calorie intake levels for vehicle controls, males and females respectively. CordyMax administration continues until all mice die. Mice have been treated for 64 weeks thus far. The preliminary results show: (1) no significant differences in body weight and calorie intake were observed amongst 4 groups. (2) Compared to controls, the survival time of 75% animals in the CordyMax groups (500, 1000, and 1500 mg/kg.bw) extends over 14, 14, and 16 weeks respectively, and the survival time of 50% animals in the CordyMax groups extends over 9, 1, and 5 weeks respectively. Analysis with use of Kaplan-Meier Cumuli Survivor Plot showed significantly extended lifespan of the mice and reduced death risks by CordyMax: $p=0.049$ (Week 36); $p=0.036$ (Week 40); $p=0.059$ (Week 48); $p=0.027$ (Week 64). The low dose CordyMax treatment (equivalent to the human dose) appeared to show the best survivor curve. This study demonstrates the lifespan-extending effects of CordyMax in mice, while the experiment is still ongoing. At the meanwhile, additional studies demonstrated that CordyMax has anti-oxidation activity, and improves glucose, lipid and energy metabolisms and aerobic exercise capacity in animals and in humans (reported separately), all of which support the general anti-aging function of CordyMax.

Introduction

- Cordyceps sinensis* is traditionally believed as a medicinal herb with anti-aging activities and promoting longevity.
- Literature indicates the therapeutic functions of *Cordyceps sinensis* and its mycelia fermentation product CordyMax in improving energy, glucose, and lipid metabolisms and benefiting to cardiovascular, the liver, the lungs and kidneys health. (Zhu et al. J. Altern Compl Med. 1998; 4: 289-303, 429-457)
- We have reported anti-fatigue and vitality-endurance enhancement properties of CordyMax, and improvement of energy, glucose, and lipid metabolisms by CordyMax in animals and humans in previous studies.
- The aim of this study is to test the lifespan extension effect of CordyMax in mice.

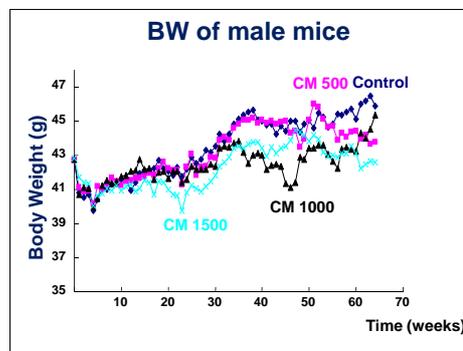
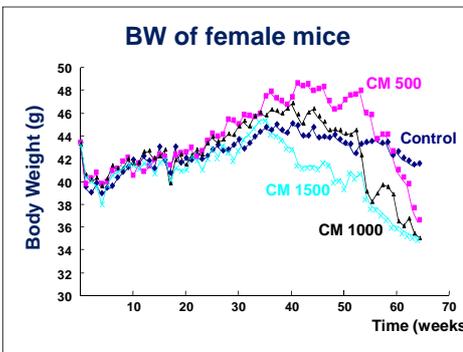
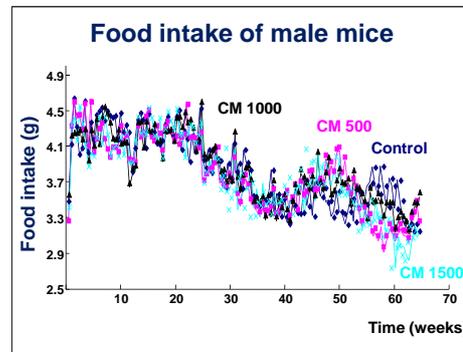
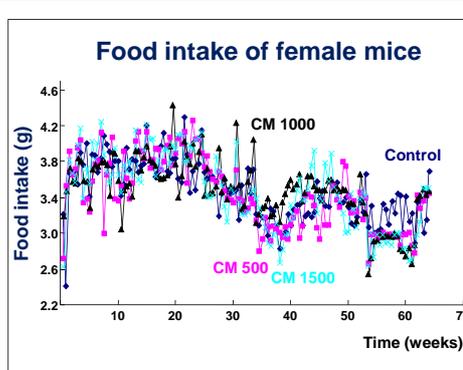
Cordyceps sinensis (Berk.) Sacc. 冬虫夏草
 (Collected from Qinghai-Tibetan plateau of China)

Isolation & Purification

Cs-4 (A *Paecilomyces hepiali* Chen strain)

Industrial Fermentation

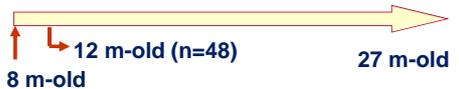
CordyMax



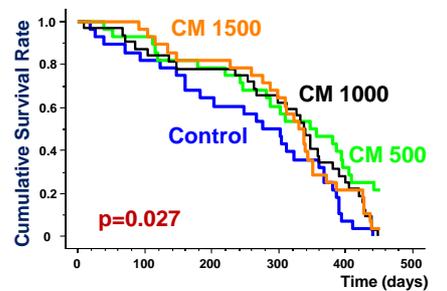
Deaths in Each Group

Vehicle control	28 dead	20 Live
CM 500 mg/kg	22 dead	26 Live
CM 1000 mg/kg	32 dead	16 Live
CM 1500 mg/kg	27 dead	21 Live

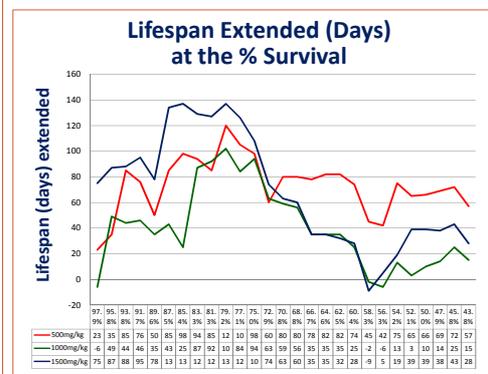
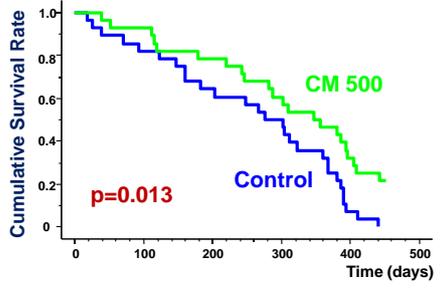
After 64 weeks of CM Treatment



Kaplan-Meier Cumulative Survival Plot



Kaplan-Meier Cumulative Survival Plot



Experimental Design: Lifespan studies in mice

Vehicle control	(n=48 mice)
CM 500 mg/kg	(n=48 mice)
CM 1000 mg/kg	(n=48 mice)
CM 1500 mg/kg	(n=48 mice)



Summary

- Analysis with use of Kaplan-Meier Cumuli Survivor Plot showed significant extension of mouse lifespan and reduced death risks by CordyMax: $p=0.049$ (Week 36); $p=0.036$ (Week 40); $p=0.059$ (Week 48); $p=0.027$ (Week 64).
- The low dose CordyMax treatment (equivalent to the human dose) appeared to show the best survivor curve.
- This study demonstrates the lifespan-extending effects of CordyMax in mice, while the experiment is still ongoing.

Anti-oxidation activities of CordyMax: a mechanism of its anti-aging property



Wu ZM, Yang JY, Tan NZ, Zhang Y, Zhao C, Zhu J-S
Pharmanex Beijing, China; Pharmanex Research Institute,
Provo Utah, USA

Abstract

Cordyceps sinensis and its mycelia fermentation product CordyMax have been used for Centuries for anti-fatigue and endurance enhancement. We reported anti-fatigue and endurance enhancement properties, and improvement of glucose, lipid and energy metabolisms by CordyMax in animals and humans in previous studies. We also demonstrated the anti-aging effect of CordyMax in mice. To explore the mechanism of the anti-aging actions, we tested anti-oxidation activity of CordyMax in mouse models with oxidative damage. Mice were randomized into 5 groups, receiving vehicle or CordyMax at a dose of 500, 1000, or 1500 mg/kg.bw for 60 days. Mice in vehicle and 3 CordyMax groups were given a single dose of 11 Gy ^{60}Co γ -rays radiation, and sacrificed in Day 4 after radiation. We found that plasma glutathione (GSH) and the thiol groups, and liver superoxide dismutase (SOD) and catalase (CAT) were significantly reduced by 29.0%, 22.6%, 6.0% and 24.7%, and liver protein carbonyl groups was significantly increased by 37.3% in vehicle controls, compared to normal mice. As compared to vehicle controls, CordyMax therapy at a dose of 500, 1000, or 1500 mg/kg.bw increased plasma thiol groups by 22.9%, 20.8%, and 25% respectively ($p=0.001$, 0.001 , and <0.001), and liver CAT by 16%, 14.8%, and 16.4% respectively ($p=0.001$, 0.002 , and <0.001). CordyMax at a dose of 1000 or 1500 mg/kg.bw reduced liver protein carbonyl groups by 8.7% and 13.5% respectively ($p=0.035$ and 0.001), and increased plasma GSH by 26.3% and 26.5% ($p=0.023$ and 0.026). Liver SOD was increased with CordyMax (1000 and 1500 mg/kg.bw) by 9.4% and 5.7% (both $p<0.05$). Liver GSH-reductase was increased with CordyMax (500mg/kg) by 10.5% ($p=0.039$). The results indicate that CordyMax improves antioxidant capacity in mice with radiation-induced oxidative injury, representing one of the mechanisms of anti-aging functions of CordyMax.

Introduction

- ◆ *Cordyceps sinensis* is believed as an "Yin-Yang" double invigorating and anti-aging TCM herb
- ◆ CordyMax, the mycelia fermentation product of *Cordyceps sinensis*, has the following functions:
 - Anti-fatigue and endurance enhancement
 - Improvement of glucose, lipid & energy metabolisms
- ◆ Oxidative stress and free radical damages are generally believed as the most important cause of aging and induce aging-related biochemical/molecular changes
- ◆ Isotope radiation induces production of large amount of free radicals and oxidative injury, causing acute aging and death

Cordyceps sinensis (Berk.) Sacc. 冬虫夏草
(Collected from Qinghai-Tibetan plateau of China)

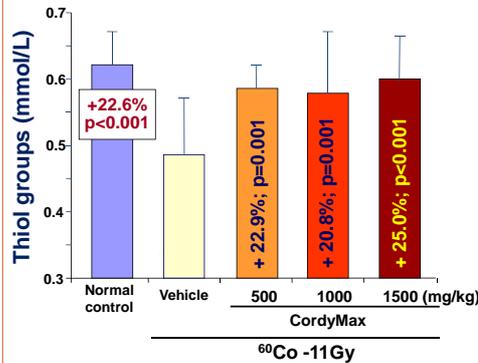
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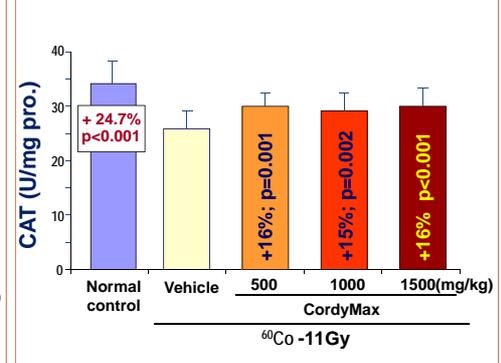
Industrial Fermentation

CordyMax

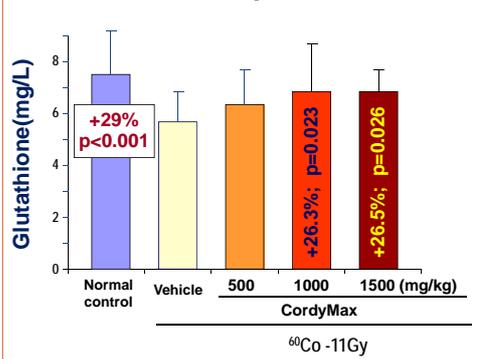
Increases in plasma thiol groups



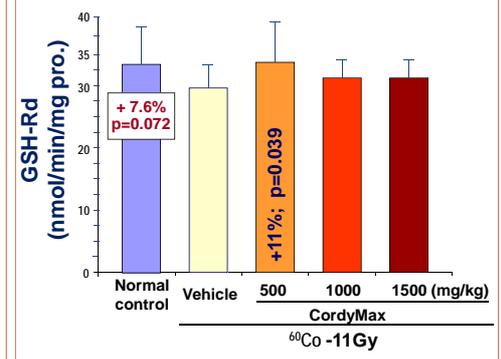
Increases in liver CAT



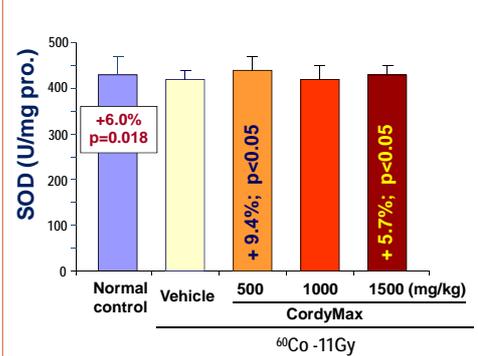
Increases in plasma GSH



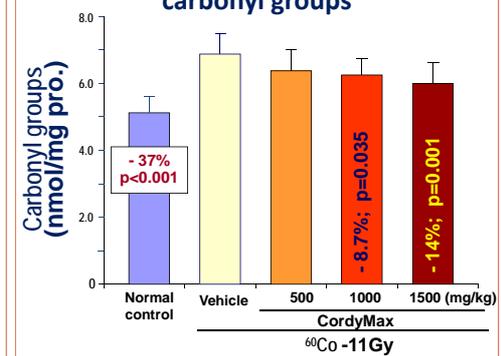
Increases in liver GSH-Rd



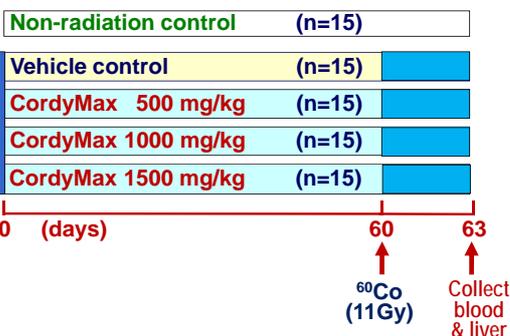
Increases in liver SOD



Decreases in liver protein carbonyl groups



Experimental Design:



Summary

CordyMax Treatment for 60 days in radiation-induced oxidative injured mice:

- ↑ Plasma thiol groups
- ↑ Plasma glutathione
- ↑ Liver SOD
- ↑ Liver CAT
- ↑ Liver GSH-Rd
- ↓ Liver protein carbonyl groups

Conclusion

- ◆ CordyMax improves antioxidant capacity in mice with radiation-induced oxidative injury.
- ◆ The results from this study supports the concept of CordyMax as an anti-aging product that has been demonstrated by the lifespan-extending study.

ANTI-FATIGUE AND ENDURANCE ENHANCEMENT PROPERTIES OF CORDYMAX IN HUMANS



Zhu JS, Xiao Y, Li CL, Huang XZ, Hagan RD

Pharmanex Research Institute, Provo UT, USA; Shihezi University, Xinjiang; Pharmanex Clinical Center, Beijing; Peking Union University, Beijing; Peking University, Beijing; The Fit Stop Human Performance & Health Enhancement Laboratory, Encinitas, CA, USA

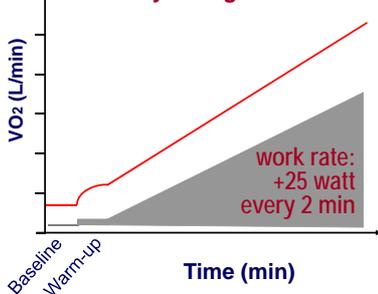
Abstract

Cordyceps sinensis and a standardized mycelial fermentation product of *C. sinensis*, CordyMax, are known as medicinal herbal products for invigoration, health preservation, anti-aging, and anti-fatigue. Their anti-fatigue and endurance enhancement functions have been reported. By use of a traditional symptom-analysis method in a double-blind clinical trial, elderly patients with senescence-related symptoms reported improvement of fatigue, dizziness, intolerance to cold temperature, sexual dysfunction, etc. by CordyMax supplementation in majority of patients (J Appl Tradit Chin Med 1993;1:32). Animal studies demonstrated that CordyMax improved steady state bio-energy ATP levels in mouse liver by use of *in vivo* serial ^{31}P NMR spectroscopy (J Alternat Compl Med 2001, 7:231), and promoted efficient use of limited oxygen supply to support body's essential physiological activities and greater tolerance to hypoxia-induced acidosis (Chin Tradit Herbal Drugs 1986;17: 209). We examined the anti-fatigue and endurance enhancement properties of CordyMax with use of sports physiology methods. In double-blind clinical trials with use of an incremental work rate protocol on a cycle ergometer and/or treadmill, we found that CordyMax increased VO_2max by 7.0%, anaerobic threshold by 12.6%, maximal ventilation by 10.4% and maximal work rate by 5.9%, indicating improvement of aerobic exercise capacity in healthy sedentary adults of advance ages. In healthy young athletes and by use of a constant work rate protocol, CordyMax therapy increased O₂ pulse by 7.6% and reduced heart rate (HR) by 2.2%, RER by 2.5%, and lactic acid by 10.5% during endurance exercise, indicating improvement of cardiovascular and metabolism functions during endurance exercise. CordyMax also accelerated HR recovery 3 min post maximal exercise by 6.3%. In summary, CordyMax supplementation influences favorably aerobic capacity and cardiovascular, pulmonary, and metabolic functions during maximal and endurance exercise, improves fatigue and endurance performance, and facilitates recovery from exercise.

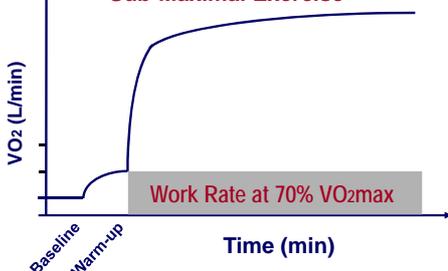
Introduction

- ◆ *Cordyceps sinensis* is traditionally believed as an anti-aging tonic herb
- ◆ CordyMax, the mycelia fermentation product of *C. sinensis*, improves senescence-related symptoms, including fatigue, and increases activities of scavenging oxygen free radicals in elderly senescent patients (Administ Tradit Chin Med 1995; 5:14-18)
- ◆ CordyMax extends lifespan and enhances body's anti-oxidant activities
- ◆ CordyMax improves energy, glucose and lipids metabolisms in humans and animals (J Alternat Compl Med 2001; 7:231-240; J Alternat Compl Med 2002; 8:309-314; Chin J Clin Pharmacy 2007; 16:274-277; Proceedings 2008 Symposium Chin Asso Med Mycol. 2008, pp157-164)

Incremental work-rate (IWR) exercise on cycle ergometer

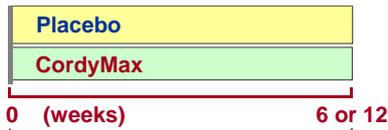


Constant Work Rate (CWR) Sub-Maximal Exercise

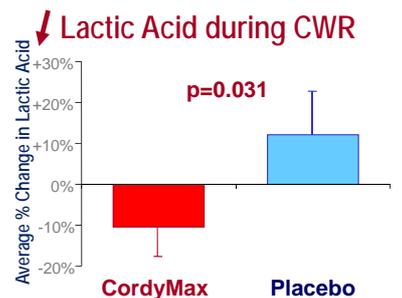
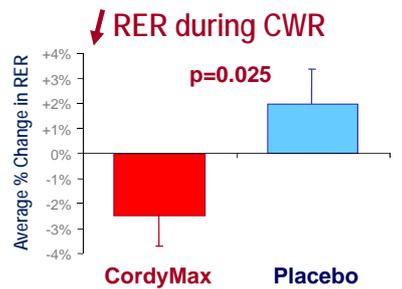
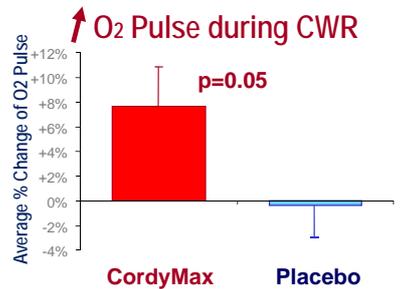
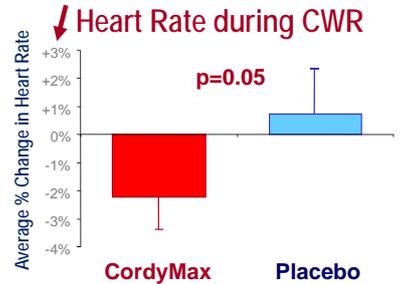
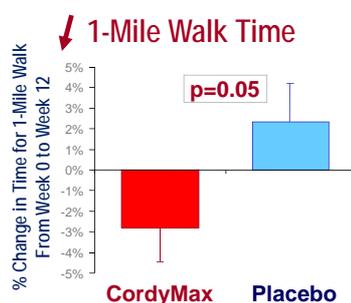
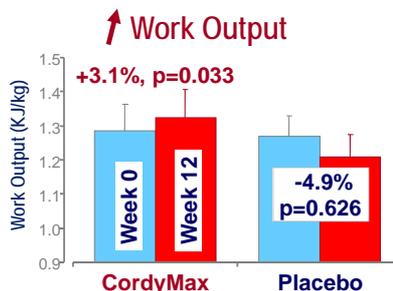
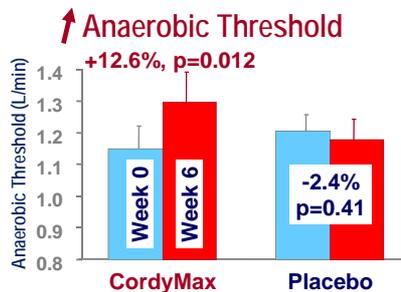
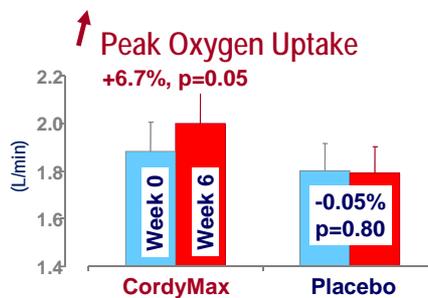


To Examine the Effects of CordyMax on Aerobic Capacity, Endurance Enhancement, and Exercise metabolism in Humans

Randomized, Double-blind, Placebo-controlled



Exercise tests
◆ Healthy older subjects & athletes



Summary & Conclusion

CordyMax Treatment:

- ↑ Peak O₂ uptake
- ↑ Anaerobic threshold
- ↑ Work output
- ↑ O₂ pulse
- ↓ Peak heart rate
- ↓ RER
- ↓ Blood lactate

Time for 1-mile walk and for recovery

CordyMax Treatment:

In healthy subjects, improved aerobic exercise capacity, endurance, glucose-lipid metabolisms during endurance exercises

Maturation of *Cordyceps sinensis* associates with co-existence of *Hirsutella sinensis* and *Paecilomyces hepiali* DNA



Zhu J-S, Guo Y, Yao YS, Zhou YJ, Zheng TY, Chen W

Pharmanex Research Institute, Provo UT, USA; Shihezi University, Xinjiang; Pharmanex Clinical Center, Beijing; Institute of Microbiology, Chinese Academy of Sciences, Beijing

Abstract

A decades-long debate has not given a consensus on the anamorph-teleomorph connection for *Cordyceps sinensis* (Cs). Literature reported isolations of *Paecilomyces hepiali* and *Hirsutella sinensis* from natural *Cordyceps sinensis*, and evidence of molecular existence of *H. sinensis* in *C. sinensis*. We tested a hypothesis in this study that *P. hepiali* and *H. sinensis* co-exist in natural *C. sinensis*, and their proliferation predominance changes during maturation of *C. sinensis*. Mycological and molecular approaches were employed to examine the growth of *P. hepiali* and *H. sinensis* and their genes in freshly collected *C. sinensis* after thorough prior cleaning and surface sterilization. A nested PCR method with use of a touch-down program was used to identify the genes of *P. hepiali* and *H. sinensis* in the caterpillar body and stroma of natural *C. sinensis*. We found that *P. hepiali* and *H. sinensis* were detected simultaneously in freshly collected *C. sinensis* by mycological and molecular examinations. Maturation of *C. sinensis* after it is visible above ground associates with a large decrease in the ability of competitive growth of *H. sinensis* ($p < 0.001$). *P. hepiali* and *H. sinensis* genes were found in both caterpillar body and stroma of natural *C. sinensis*. We conclude that *C. sinensis* is a complex traditional Chinese herb with multiple fungi living in its caterpillar and stroma. Its maturation from early May to late June associates with dynamic changes in proliferation predominance of the fungi.

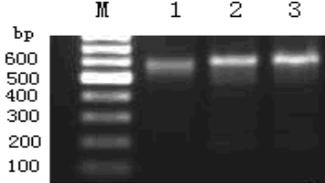
Scientific Background

- ◆ *Cordyceps sinensis* (Berk.) Sacc. is a precious tonic herb used in China for centuries, having broad nutritional and health benefits (Zhu et al., *J Alternat Compli Med* 4:289-303 & 429-457, 1998). Its life cycle is complex and its natural supply is scarce.
- ◆ Although on the basis of DNA sequence *Hirsutella sinensis* is hypothesized as the anamorph (asexual stage) of *C. sinensis*, >10 fungi were isolated from natural *C. sinensis*, possibly suggesting a multi-fungi symbiosis in natural *C. sinensis*.
- ◆ Through competitive proliferation, live *Paecilomyces hepiali* and *Hirsutella sinensis* were identified simultaneously (Guo and Zhu, *FASEB J*. 19: A1033, 2005).

Experimental Methods

- ◆ 100 pieces of fresh *C. sinensis* were collected from Qinghai-Tibetan Plateau in early May, following by immediate on-site clean, surface sterilized and freeze in liquid nitrogen for transportation and further process.
- ◆ Caterpillars and stromata of natural *C. sinensis* were separately grounded into powder in liquid nitrogen. Ribosomal DNA was extracted.
- ◆ A nested, dual-step PCR technique was used (Guo and Zhu, *FASEB J*. 20: A431, 2006).
 1. A pair of primers, ITS4/5, were used for the 1st PCR: annealing at 40 °C for 30 cycles.
 2. A pair of *Paecilomyces hepiali* primers, Phsp3 and Phsp5, and a touch-down PCR protocol were used for the 2nd PCR: initially annealing at 70 °C with 0.3 °C decrement for each cycle, for 35 cycles.
 3. DNA sequencing and restrictive endonuclease digestions with *Bam*HI and *Acc*I specific for *Paecilomyces hepiali* and *Ava*I and *Alu*I for *Hirsutella sinensis*.

ITS PCR Products

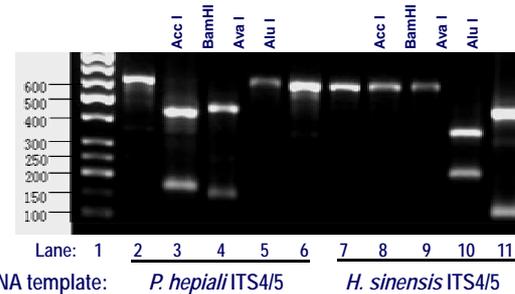


ITS4/5 DNA segments by single-step PCR. rDNA was extracted from *P. hepiali* (Lane 1), *H. sinensis* (Lane 2), and *C. sinensis* (Lane 3) as the templates, and ITS4 & ITS5 primers were used.

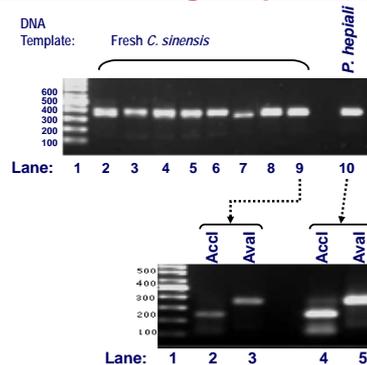
DNA Sequences



Endonuclease Digestions of *P. hepiali* & *H. sinensis* ITS PCR Products

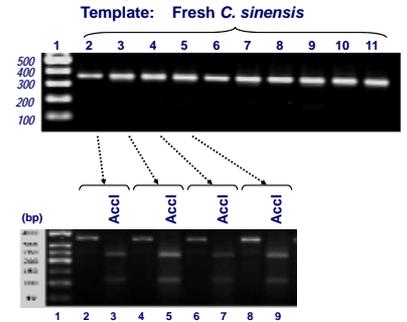


PCR Products Using Phsp3/5 Primers



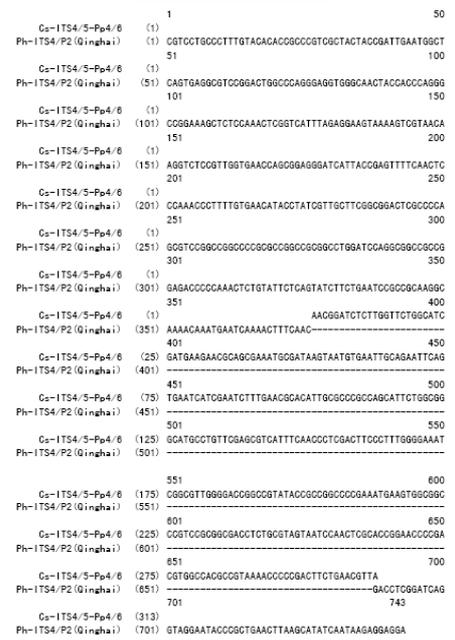
PCR products were amplified from rDNA templates extracted from *C. sinensis* and *P. hepiali* with use of *P. hepiali* "specific" primers Phsp3-Phsp5 (upper panel), and digested with *Acc*I and *Ava*I (lower panel).

Nested PCR Products Using Phsp3/5 Primers



PCR products were amplified from rDNA templates extracted from caterpillars and stromata of *C. sinensis* with use of a nested PCR protocol (upper panel), and digested with *Acc*I (lower panel).

Sequences of the Nested PCR Products



Summary

- ◆ *P. hepiali* genes were amplified from caterpillar and stroma of natural *C. sinensis* (GenBank #EF55097), in addition to the detections of *H. sinensis* genes.

Discussions

- ◆ We found simultaneous existence of live *P. hepiali* and *H. sinensis* and their genes in nature *C. sinensis*.
- ◆ The multi-fungi existence in natural *C. sinensis* may suggest complex anamorph-teleomorph connections to *C. sinensis*.

Maturational alterations of differential expressions of GC:AT-biased *Cordyceps sinensis* mutants and *Paecilomyces hepiali* in natural *Cordyceps sinensis*



Zhu J-S, Gao L, Yao YS, Zhou YJ, Zheng TY, Chen W
Pharmanex Research Institute, Provo, UT, USA; Shihezi University, Xinjiang; Pharmanex Clinical Center, Beijing

Abstract

A decades-long debate has not given a consensus on the anamorph-teleomorph connection for *Cordyceps sinensis* (Cs). Literature reported simultaneous detections of *Paecilomyces hepiali* (Ph) and *Hirsutiella sinensis* (Hs) and their DNA in freshly collected natural Cs and a >50% decline of colony-forming ability of caterpillar Hs along with Cs maturation under competitive proliferation conditions. In this study, we tested the expressions of Ph and multiple Hs-related Cs genes during Cs maturation. Southern blotting analysis revealed dramatic increases in expressions of Ph and Hs genes with Cs maturation, with use of Ph (ITS₅₋₁₇₉) and Hs (ITS₅₋₁₆₂) specific probes. Two Hs-related species on Hs blot were seen after the genomic Cs DNA was digested with *EcoRI*, probably representing the GC:AT-biased mutants. They expressed differentially in stroma and caterpillar body at different stages of Cs maturation. The AT bias does not express in premature Cs caterpillar, but highly predominately in premature stroma; while the GC bias expresses oppositely in the premature Cs compartments. The differential expressions altered non-proportionally in the caterpillar body and stroma when Cs matures. The expression pattern of the AT-bias species is highly similar to that for Ph. *EcoRI* digestions of PCR products in ITS1/5.8S rDNA regions when Cs stroma genomic DNA used as the template showed increased quantity of digestible species in proportion to the increased length of stroma during Cs maturation. In conclusion, Cs maturation associates with augmented expressions of both Ph and Hs genes and altered differential expressions of GC:AT-biased Cs mutants. These maturation-related changes in the expressions of Cs-associated fungi represent the important elements in Cs life cycle, opening an avenue to unmask the anamorph-teleomorph connection of this precious Chinese herb.

Scientific Background

- ◆ *Cordyceps sinensis* (Berk.) Sacc. has been used as a precious medicinal herb in china for centuries
- ◆ It has broad nutritional and health benefits (Zhu et al., *J Alternat Compli Med* 4:289-303 & 429-457, 1998)
- ◆ Co-existence of fungi *P. hepiali* and *H. sinensis* was found in fresh *C. sinensis* (Zhu et al., *J Mycol Res* 5:214-224, 2007)

Maturation of *C. sinensis* and the Temperature on Qinghai-Tibet Plateau

	Less Matured	Matured
Harvest Season	Early May	Late June
Temperature on the Plateau	0-10 °C	10-20 °C
Stroma	Short	Long
	A: "Type 1"	B: "Type 2"



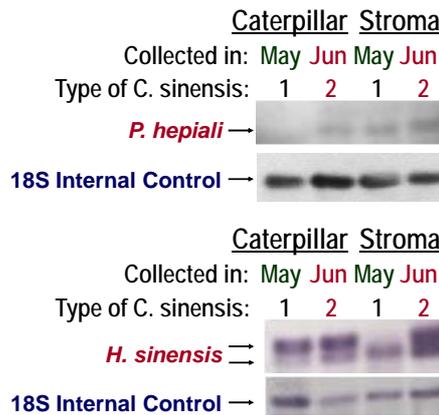
Collection of fresh *C. sinensis*

- ◆ Type 1 *C. sinensis*: 50 pieces
- ◆ Type 2 *C. sinensis*: 50 pieces
- ◆ *C. sinensis* were collected from Qinghai-Tibet Plateau, followed by on-site cleaning, surface sterilizing and freezing in liquid nitrogen.

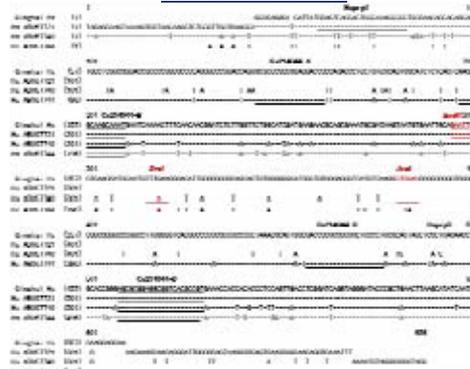
rDNA preparation, Southern blotting and PCR analysis

- ◆ Caterpillar and stroma of the sterilized *C. sinensis* were grounded separately into powder in liquid nitrogen for genomic DNA extraction with DNeasy Plant Mini Kit (Qiagen).
- ◆ *AvaI*, *DraI*, & *EcoRI* were used for the genomic DNA and PCR product digestions
- ◆ Probes:
 - *P. hepiali* probe: 175 bp (ITS5-179)
 - *H. sinensis* probe: 158 bp (ITS5-162)
 - Internal control probes: a 18S fragment
- ◆ Probes were labeled with DIG High Prime DNA Labeling and Detection Kit (Roche)

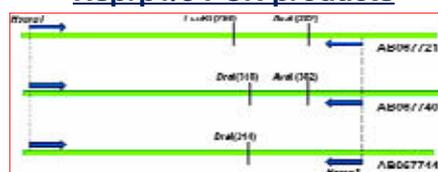
Southern blots: *P. hepiali* (upper) & *H. sinensis* (lower) rDNA in Type 1 or 2 *C. sinensis*



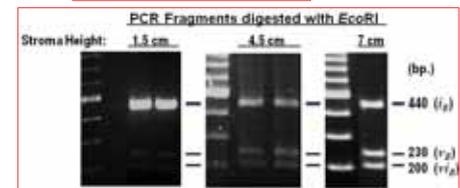
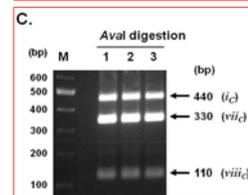
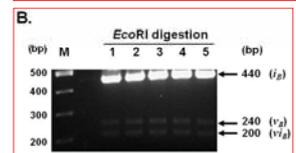
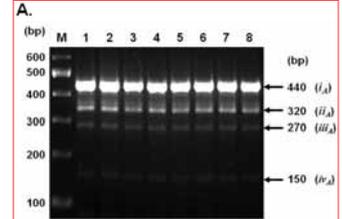
GC- and AT-biased mutants of *C. sinensis*



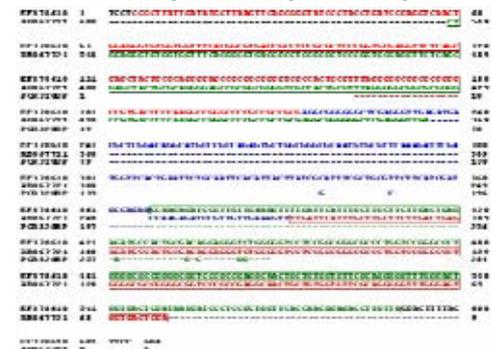
Restrictive digestions of Hsprp1/3 PCR products



Restrictive digestions of Hsprp1/3 PCR products



Reversely orientated rDNA sequences (GC-bias)



Summary & Conclusions

- ↑ Biomass of *P. hepiali* and *C. sinensis* mutants rDNA with *C. sinensis* maturation
- ◆ Differential expressions of *C. sinensis* mutants:
 - Premature caterpillar: only AB067721 (GC-bias)
 - Premature stroma: predominated with AT-biased mutants (AB067740 or AB067744)
 - Mature caterpillar: still predominated by AT-biased mutants (AB067740 or AB067744), but more GC-bias expresses (AB067721)
 - Mature stroma: equally express with AT-bias (AB067740 or AB067744) and GC-bias (AB067721)
 - With *C. sinensis* Maturation:
 - In stroma: expression of GC-bias increases
 - In caterpillar: expression of AT-biases increases
- ◆ *C. sinensis* exists reversely orientated GC-bias rDNA, indicating reverse chromosome exchanges
- ◆ Expressions of *P. hepiali* and AT-biased mutants are synchronized with *C. sinensis* maturation

MATURATION OF *CORDYCEPS SINENSIS* ASSOCIATES WITH CHANGES IN PROFILES OF PROTEINS AND SMALL MOLECULAR WEIGHT ORGANIC CHEMICALS



Zhu J-S, Zhang, LJ, Qi Y, Liu XJ, Zhang L, Wu ZM, Zheng TY, Chen W

Pharmanex Research Institute, Provo UT, USA; Shihezi University, Xinjiang; Pharmanex Clinical Center, Beijing; Pharmanex R&D Center, Shanghai; Fudan University, Shanghai

Abstract

Background: Literature reported differential expressions of *Paecilomyces hepiali* and *Hirsutella sinensis* DNA in the caterpillar body and stroma of natural *Cordyceps sinensis* during *C. sinensis* maturation.

Hypothesis: Differential expressions of *P. hepiali* and *H. sinensis* in natural *C. sinensis* may alter the profiles of proteins and other organic chemicals during maturation of *C. sinensis*.

Design and Methods: SELDI-TOF MS and HPLC analyses were used to profile proteins and small molecular weight organic chemicals in caterpillar body and stroma of natural *C. sinensis* and mycelia of *P. hepiali* and *H. sinensis*.

Results: Maturation of *C. sinensis* after it is visible above ground associates with altered profiles of proteins and small organic chemicals. The profiles for the mycelia of fungi do not completely match to those for the stroma or caterpillar body of natural *C. sinensis* during its maturation. The profiles for *H. sinensis* mycelia appear to be more distinct from those for natural *C. sinensis*, than the profiles for *P. hepiali* mycelia.

Conclusions: The maturation of *C. sinensis* associates with significant changes in the component organic chemicals. The compounds from all component fungi may contribute jointly to the overall pharmacological functions of natural *C. sinensis*.

Scientific Background

- ◆ *Cordyceps sinensis* (Berk.) Sacc. has been used in China as a precious tonic herb for centuries
- ◆ It has broad nutritional and health benefits (Zhu et al., *J Alternat Compli Med* 4:289-303 & 429-457, 1998).
- ◆ Co-existence of *P. hepiali* and *H. sinensis* was found in fresh *C. sinensis* (Zhu et al., *J Mycol Res* 5:214-224, 2007)
- ◆ Competitive proliferation abilities are altered associated with unparallel alterations of gene copies of the fungi (EB2008 Program #704.4, Apr. 6 Poster Board #C303)

Maturation of *C. sinensis* and the Temperature on Qinghai-Tibet Plateau

	Less Matured	Matured
Harvest Season	Early May	Late June
Temperature on the Plateau	0-10 °C	10-20 °C
Stroma	Short	Long
	A: "Type 1"	B: "Type 2"



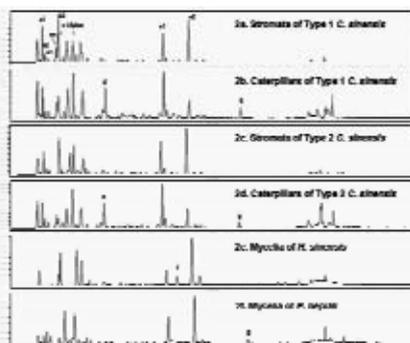
Collection of fresh *C. sinensis*

- ◆ Type 1 *C. sinensis*: 90 pieces
- ◆ Type 2 *C. sinensis*: 90 pieces
- ◆ Fresh *C. sinensis* were collected from Qinghai-Tibet Plateau in early May for Type 1 *C. sinensis* and in late June for Type 2, followed by on-site cleaning, surface sterilizing and freezing in liquid nitrogen for further process.

HPLC profiling of small molecule chemicals

◆ Caterpillars and stromata of *C. sinensis*, and freeze-dried *P. hepiali* and *H. sinensis* mycelia were individually grinded into powder in liquid nitrogen. Small molecules were extracted in either methanol or water, and analyzed for total quality profiling on HPLC with an ODS-C18 (262 nm).

◆ The mobile phase was a linear gradient of Acetonitrile (A) and 0.1% H3PO4 with 5mM Sodium salt of 1-Hexanesulfonic Acid (B).



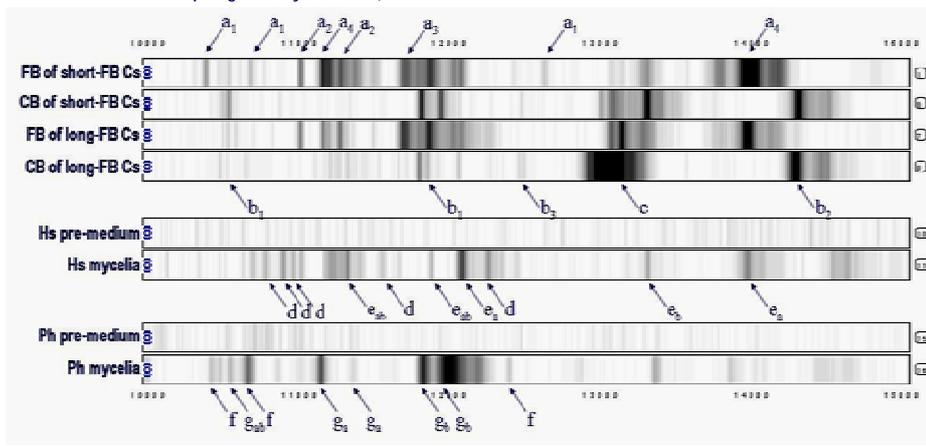
Characteristics of water soluble chemicals

Peaks (Elution Time, min)	a (11.62, 13)		b (14.2, 16)		c (11.6, 12.8)		d (11 vs. 12)		e (11 vs. 12)	
	Triplex 4' - 5'	Height ratio: 5.5' vs. 5.5'	Triplex 6.5' - 8.2'	Height ratio: 12'	Triplex 12'	Height ratio: 19.5' vs. 22.8'	21.4'	21.4'	21.4'	21.4'
2a: T1, E	H, L, L	< 1.0	H, H, H	—	H	> 1.0	—	—	—	—
2b: T1, E	H, L, L	< 1.0	H, H, H	H	H	> 2.0	—	—	—	—
2c: T2, C	H, L, L	> 1.0	H, H, H	H	H	> 1.0	—	—	—	—
2d: H.s.m.	L, L, L	< 1.0	—	—	—	> 0.5	H	—	—	—
2e: P.h.m.	L, L, L	Single 12'	H, H, H	—	—	> 1.0	—	—	—	—

Note: The lines 2a through 2f outline the chemical profiles corresponding to the panels in Fig. 2. "T1" stands for Type 1 *C. sinensis*, "T2" for Type 2 *C. sinensis*, "S" for Stroma, "C" for Caterpillar, "H.s.m." for *H. sinensis* mycelia, "P.h.m." for *P. hepiali* mycelia, "H" for taller peak, "L" for shorter peaks, "+" for presence, and "-" for absence.

SELDI-TOF MS profiling of proteins

- ◆ Caterpillars and stromata of *C. sinensis* and freeze-dried *P. hepiali* & *H. sinensis* mycelia were individually grinded into powder in liquid nitrogen. The powder (0.5 g each) was dissolved in 600 ml Tris-Glycine (pH 8.3) & centrifuged at 14,000 rpm for 5 min at 4 °C.
- ◆ Supernatants were diluted with PBS to about 200-300 nM, before being subjected to a normal-phase chip and analyzed on a PBS-II protein chip reader (Surface-enhanced laser desorption ionization - time of flight mass spectra, or SELDI-TOF MS; Ciphergen Biosystems Inc.).



Summary & Conclusions

- ◆ Distinct profiles of proteins and small molecule chemicals were noted for caterpillars and stromata during *C. sinensis* maturation. The maturation associates with dynamic changes in profiles of component chemicals, supporting its superior function profiles.
- ◆ The profiles for *P. hepiali* and *H. sinensis* mycelia do not completely match to those for *C. sinensis*.
- ◆ The profiles for *P. hepiali* mycelia appear to be closer to those for *C. sinensis*, than those for *H. sinensis*.