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Protective and curative effects of *Bacillus subtilis* SPB1 biosurfactant on high-fat-high-fructose diet induced hyperlipidemia, hypertriglyceridemia and deterioration of liver function in rats

Raida Zouari[a,b], Khaled Hamden[c], Abdelfattah El Feki[c], Khansa Chaabouni[d], Fatma Makni-Ayadi[d], Choumous Kallel[e], Fahima Sallemi[f], Semia Ellouze-Chaabouni[a], Dhouha Ghribi-Aydi[a,b,*]

[a] *Enzymes and Bioconversion Research Unit, National School of Engineers of Sfax (ENIS), Tunisia*
[b] *Higher Institute of Biotechnology of Sfax (ISBS), Tunisia*
[c] *Animal Ecophysiology Laboratory, Faculty of Sciences of Sfax (FSS), Tunisia*
[d] *Biochemistry Laboratory, CHU H. Bourguiba, Sfax, Tunisia*
[e] *Hematology Laboratory, CHU Habib Bourguiba, Sfax, Tunisia*
[f] *Anatomical Pathology and Cytology Laboratory, Ibn Nafis, Sfax, Tunisia*

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ABSTRACT

This study was aimed to assess the plausible anti-obesity effects of *Bacillus subtilis* SPB1 crude lipopeptide biosurfactant on high fat high fructose diet-fed rats (HFFD). Male Wistar rats were divided into five groups with the following treatment schedule: normal diet (CD), HFFD, HFFD supplemented with SPB1 biosurfactant from the first day of the experiment (HFFD + Bios1, 10 mg/kg/day), HFFD receiving standard drug (HFFD + Torva, 10 mg/kg/day) or SPB1 biosurfactant (HFFD + Bios2, 10 mg/kg/day) during the last 4 weeks of the study. The results showed an increase in body weight of HFFD by ~19% as compared to controls (CD). Moreover, serum lipase activity underwent a threefold increase which led to an increase in the levels of total cholesterol (T-Ch), triglycerides (TG) and LDL-cholesterol (LDL-Ch) in serum of untreated HFFD, as well as a rise in the calculated atherogenic index (AI). Furthermore, liver dysfunction indices such as AST, ALT, CPK, LDH, GGT, ALP and T-Bilirubins exhibited remarkable increases in serum of HFFD as compared to controls (CD). Whereas, the administration of *Bacillus subtilis* SPB1 biosurfactant to HFFD improved the body weight gain and serum lipids profile and reverted back near normal the activities of lipase and liver toxicity indicators. In addition, notable protective and curative effects were reported in liver tissues. Overall, these results suggest that the lipopeptides biosynthesized by *Bacillus subtilis* SPB1 achieved an anti-obesity effect through the inhibition of lipid digestive and liver dysfunction enzymes.

1. Introduction

Obesity is considered as an exceeding life style disorder notably in developing countries and it is prevailing at a frightful speed in new world countries as a result of fast food intake, including HFCS (*High Fructose Corn Syrup*) added products consumption, and lack of physical activity [1]. World Health Organization recent statistics indicate that more than 1.9 billion adults, 18 years and older, were overweight. Of these, over 600 millions were obese. Furthermore, an unsatisfactorily recorded fact reveals that 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese. Moreover, WHO reported that 41 million children under the age of 5 were overweight or obese in 2014 [2]. Obesity is associated with a large number of chronic diseases and defects like dyslipidemia, fatty liver disease, hypertension, coronary artery disease, type 2 diabetes, heart failure and stroke, osteoarthritis, obstructive sleep apnea, gallstones as well as reproductive and gastrointestinal cancers [3]. However, despite the obviously unavoidable progression of this disease and the reassuring results of some medications on lowering body weight and in alleviating numerous cardiometabolic complications, during the last few years, a large number of

* Corresponding author at: Enzymes and Bioconversion Research Unit, Department of biological engineering, National School of Engineers of Sfax, ENIS, BP W 3038 Sfax, Tunisia.
E-mail address: dhouhag@yahoo.fr (D. Ghribi-Aydi).

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the approved and wholesaled antiobesity drugs have been withdrawn from the market due to serious adverse effects [4]. Thus, there is an imperative need to identify natural products as antiobesity agents [5]. The use of safer lipase inhibitors is considered as a potent therapeutic approach to overcome obesity and hyperlipidemia by the delay of the digestion and absorption of fat. Pancreatic lipase inhibitory activity has been largely used for the exploration of potential effectiveness of natural products [6]. Most distinctively, wide arrays of bioactive metabolites namely the biosurfactants were described as efficient for preventing some highly prevalent chronic diseases [7]. In fact, it has been reported that microbial surfactants, which are surface active compounds produced extracellularly or as part of the cell membrane by several bacterial and fungal species, are endowed with interesting properties that are potentially useful for many therapeutic applications [8]. During a screening program on biosurfactant-producing strains, *Bacillus subtilis* SPB1 (HQ392822) was isolated [9]. Using mass spectra analyses, *Bacillus subtilis* SPB1 was found to produce surfactin isoforms with molecular weights of 1007, 1021, and 1035 Da; iturin isoforms with molecular weights of 1028, 1042, and 1056 Da; and fengycin isoforms with molecular weights of 1432 and 1446 Da. Two new clusters of lipopeptide isoforms with molecular weights of 1410 and 1424 Da and 973 and 987 Da, respectively, were also detected [10]. The *in vivo* toxicity of SPB1 crude lipopeptide biosurfactant was evaluated towards male mice in a previous study [11]. An LD_{50} value was determined to be about 475 mg/kg of body weight [11]. The obtained results showed that a daily administration of SPB1 biosurfactant did not show any death cases at any dose. Also, they did not notice any changes in the behavior of animals in a 28 day period of treatment. In a recent study we demonstrated for the first time that the hypoglycemic and the antilipidemic activities exhibited by *Bacillus subtilis* SPB1 crude lipopeptide biosurfactant were effective enough to alleviate induced diabetes in non obese experimental rats [12]. The present work was carried out to evaluate the antiobesity effects of *Bacillus subtilis* SPB1 crude lipopeptide biosurfactant on HFFD-fed rats.

2. Materials and methods

2.1. Microorganism strain and biosurfactant production

The strain used in the present work is *Bacillus subtilis* SPB1 (GenBank accession HQ392822), a wide type isolated in our laboratory from a Tunisian soil contaminated by hydrocarbons, as reported by Ghribi et al. [13]. *Bacillus subtilis* SPB1 was shown to produce a highly effective biosurfactant that belongs to the class of lipopeptides. It was selected on the basis of the high haemolytic and emulsification activities of its biosurfactant which could reduce surface tension of the water from 70 to 34 mN m^{-1} [14]. *Bacillus subtilis* SPB1 strain was streaked on a nutrient agar slant and incubated at 37 °C. After 24 h, one loop of cells was dispensed in 3 ml of LB medium and incubated overnight at 37 °C. Aliquots (0.2 ml) were used to inoculate 250 ml Erlenmeyer flasks containing 50 ml LB medium and incubated in a rotatory shaker at 150 rpm and 37 °C for six hours. The obtained culture was used to inoculate the production medium. *Bacillus subtilis* SPB1 biosurfactant production was performed in 250-ml Erlenmeyer flasks containing 50 ml of the liquid mineral optimized medium, as described in a previous work by Mnif et al. [15]. At the end of the cultivation, the culture was centrifuged at 10.000 rpm and 4 °C for 20 min to remove bacterial cells.

2.2. Preparation of the crude lipopeptide powder

The supernatant free cells served for biosurfactant extraction during three consecutive cycles of acid precipitation-dissolution [15]. In fact, each time, the pellet formed by acid precipitation was suspended in alkaline water and the pH was readjusted to 8 with NaOH 1N. The supernatant was collected by centrifugation at 10.000 rpm and 4 °C for 20 min followed by a second acid precipitation. The final pellet formed was washed three times with acid water (pH = 2), dissolved in distilled water at a concentration of 10 mg/ml, the pH was adjusted to 8 with NaOH 1 N and lyophilized. This served as a crude lipopeptide preparation to perform this study [15].

2.3. Animals, diets and experimental design

Thirty adult male Wistar rats, weighing 283 ± 5.51 g were obtained from the Tunisian Pharmaceutical Industries (SIPHAT, Tunisia). Animals were kept in an environmentally controlled room (40% humidity, 22 °C temperature and 12-h light-dark cycle) in the laboratory of Animal Ecophysiology of Sfax City, Tunisia. All the animal studies conducted in this work followed the International Guidelines for Animal Care Directive 86/609/EEC [16], and were approved by the Tunisian Ethics Committee of the University of Sfax (Sfax, Tunisia) for the care and use of laboratory animals. All rats were kept to acclimate for one week before the onset of the experiment and they were fed on a standard diet composed of corn, soya and vitamins (Table 1), supplied by the Company of Animals Nutrition, Sfax, Tunisia. The hypercaloric high-fat-high-fructose diet (HFFD) was composed of 79.9% normal diet, 10% sheep fats, 10% fructose and 0.1% cholic acid (Table 1).

A total of 30 male Wistar rats were randomly divided into five groups, with six rats in each group. They received the following treatment schedule:

CD group: Control rats fed on a standard diet (control diet group, CD);

HFFD group: Rats fed on high-fat-high-fructose diet (HFFD);

HFFD + Torva group: Rats fed on HFFD and received additional 10 mg/kg of body weight of a commercial drug Torva™ (atorvastatin) in a volume of 1 ml water during 4-weeks daily before the end of the experiment (curative effect);

Table 1
Composition of rats' administered food: standard (CD) and high-fat-high-fructose diet (HFFD). Basic food contains corn, soya, vitamins and minerals as the subsequent amounts (Nasri et al. [31] with modification).

		CD	HFFD
Nutritional properties (%)	Moisture	14	11.84
	Fibers	3.4	2.83
	Proteins	22	18.75
	Lipids	3.5	11.69
	Ash	6.7	5.92
	Carbohydrate	50.4	38.97
	Fructose	0	10
	Caloric value (kcal/kg)	2850	3860
Amino acids (%)	Methionine	60	60
	Cysteine	0.38	0.38
	Threonine	0.80	0.80
	Tryptophane	0.30	0.30
Mineral mix (mg/kg)	Manganese	80	80
	Iron	48	48
	Copper	18.75	18.75
	Zinc	65	65
	Selenium	0.30	0.30
	Cobalt	0.20	0.20
	Iode	1.20	1.20
Vitamins and Antioxidants (mg/kg)	Vitamin A	13000	13000
	Vitamin D3	4375	4375
	Vitamin H	62.5	62.5
	Antioxidants (BHA-BHT)	125	125

BHA: Butylated hydroxyanisole; BHT: Butylated hydroxytoluene.