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Xanthigen® reduces lipid deposition and improves stress resistance in *Caenorhabditis elegans*

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ABSTRACT

Xanthigen® is a nutraceutical combination of two well-known natural products, brown seaweed extract (rich in fucoxanthin) and pomegranate seed oil (rich in punicic acid), and it has been designed to use in weight management, in conjunction with a calorie restricted diet. In the nematode *Caenorhabditis elegans* Xanthigen® treatment caused a significant reduction in lipid deposition in wild-type N2 (WT-N2) animals but not in sirt-2.1-deficient strain, which raises the possibility that the prolipolytic or anti-lipogenic effect of Xanthigen® in these animals is mediated through Sirtuin 2.1 activation. This response has been well described for Xanthigen® in cell cultures and other animal models. In addition, Xanthigen® treatment conferred to both strains an increased resistance to thermal and oxidative stress, which opens the possibility that the effects of Xanthigen® are not mediated solely by Sirtuin 2.1 activation. We therefore explored whether Xanthigen® could activate diverse defence mechanisms such as DAF-16 activation, or GST induction in response to xenobiotics, by using the strains TJ356, CL2070 and CL2166, stably expressing Pdaf-16::GFP, Phsp-16.2::GFP and Pgst-4::GFP, respectively. Xanthigen® treatment provoked neither DAF-16 translocation to the nucleus nor increased expression of HSP16.2 and GST4, which opens the possibility that different mechanisms other than DAF-16 and those involved in xenobiotic responses, are activated by Xanthigen® and are capable of conferring to the nematode an increased resistance to thermal or oxidative stress.

Keywords: Xanthigen®, fucoxanthin, punicic acid, *Caenorhabditis elegans*, sirtuin 2.1, lipid deposition, thermal stress, oxidative stress

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INTRODUCTION

Epidemiological studies have shown that overweight and obesity lead to serious health problems such as cardiovascular disease, diabetes, musculoskeletal disorders, and even cancer [1,2]. In 2015, it was estimated that around 107 million children and 603 million adults were obese worldwide, with the prevalence being higher in women than in men of all ages[3]. Because overweight and obesity are the result of a sustained imbalance between caloric intake and caloric expenditure, lifestyle changes including calorie restriction and increasing energy expenditure are the two cornerstones in their treatment. Additionally, there are commercial formulations in the market that claimed as capable of enhancing the effects of lifestyle changes with a variable degree of scientific evidence [4, 5] .

Xanthigen® is a proprietary patented combination of fucoxanthin, a carotenoid from brown seaweed extract (*Undaria pinnatifida)*, and punicic acid, a conjugated fatty acid found in pomegranate (*Punica granatum)* seed oil, which helps to the weight management without toxic effects[6]. The effects of Xanthigen® are explained by the combination of properties of these two components. Many studies have shown anti-oxidant, anti-inflammatory, and anti-cancer activities of fucoxanthin[8,9]. In addition, it has been shown that fucoxanthin stimulated lipolysis and inhibited lipogenesis by increasing Uncoupling Protein 1 (UCP1) expression in both white and brown adipose tissue[10]. Moreover, in a clinical trial, this carotenoid has been shown to provoke a significant HbA1c (Glycated haemoglobin) reduction, in carriers of the G/G allele of the *UCP1* gene (-3826A/G, rs1800592), suggesting its utility in genetically obese high-risk populations[11]. Punicic acid is a conjugated linolenic acid with antidiabetic, hypolipidemic and anti-inflammatory properties[12]. The combination of both compounds results in a more pronounced effect than that observed with fucoxanthin or punicic acid alone[13,14]. In clinical trials performed on non-diabetic overweight and obese women, Xanthigen® promoted weight loss, reduced body and liver fat content and improved liver function tests. In addition, Xanthigen® treatment improved blood lipid profile, inflammatory markers, blood pressure, and waist circumference, suggesting a potential role in the metabolic syndrome[6]. In a more recent interventional study performed on two healthy premenopausal obese women, Xanthigen® consumption induced the proliferation of Brown Adipose Tissue (BAT, which produces heat by non-shivering thermogenesis) in cervical, supraclavicular and paravertebral spaces in one of them, although body weight remained without changes[15].

The molecular action of Xanthigen® has been studied both *in vitro* and *in vivo*. Lai et al. (2012)[16] reported that Xanthigen® suppressed accumulation of lipid droplets in differentiated 3T3-L1 adipocytes by modulating signaling pathways including Akt-dependent FoxOs signaling, as well as induction of Sirtuin-I (SIRT1). Similarly, Choi et al (2014)[17] reported that the anti-obesity activity of Xanthigen® in high-fat diet-fed mice was associated with a decreased expression of PPARɣ, and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and an increased activation of AMP-activated protein kinase (AMPK) α and β, and Acetyl-CoA carboxylase (ACC), with a reduction of leptin level and expression in adipose tissue and also the body weight. Sirtuins are NAD+-dependent protein deacetylases that are broadly conserved from bacteria to humans, and are involved in longevity and stress resistance. In the nematode *Caenorhabditis elegans (C. elegans*) these effects are mediated by the forkhead transcription factor DAF-16 (ortholog of the FOXO family of transcription factors encoded by a downstream gene in the insulin-like pathway). In fact, the effect of DAF-16 on life span extension in worms is dependent on a low insulin-like signalling and sir-2.1 overexpression[18].

Since Xanthigen® is capable of both reducing

body fat and modulating gene expression involved in processes related to lipid storage, aging, and stress resistance (SIR-2.1, FoxOs, AMPk), we considered of interest to study whether this compound was able to confer resistance to stress in the nematode *C. elegans*, which is of utility as a model to report the influence of plant extracts on the process of ageing or stress resistance due to its short life span and the feasibility of manipulating cell signalling pathways by simple biotechnological methods[19].

MATERIALS AND METHODS

Test Materials

The wild-type *C. elegans* strain N2 (variety Bristol) (WT-N2) and the sirt-2.1-deficient strain VC199 sir-2.1 (ok434) IV were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, USA), The *C. elegans* transgenic strains TJ356, zIs356 (Pdaf-16: GFP); CL2070 drIs70 (Phsp-16.2: GFP); CL2166 davIs 19 (Pgst-4: GFP) were kindly donated by the laboratory of A. Miranda (Biomedical Institute of Seville, IBIS, Spain), and were maintained on 6 or 10 cm with nematode growth medium (NGM). NGM plates seeded with OP50 bacteria, according to standard methods[20]. All worms were cultured at 20°C. The strains were routinely propagated on NGM with *E. coli* strain OP50 as food and maintained at 20 °C. A volume of 100 µL of OP50 (overnight culture) was dropped on the midpoint of 60-mm NGM plates, which were allowed to dry overnight before worms were transferred. All experiments were repeated at least three times

Xanthigen®, a nutritional supplement composed of brown seaweed extract containing 0.42 % of fucoxanthin and pomegranate seed oil, having 35% of punicic acid, was provided by Nektium Pharma (Canary Islands, Spain). To perform all assays, 1 mg powder of the product was added to the surface of NGM plates containing *E. coli* OP50.

Reagents used in this work were provided by Sigma Aldrich (Spain) and Panreac Quimica (Spain).

Oil Red O Staining

C. elegans is widely used as a model for studying conserved pathways for fat storage and metabolism; we used for stained fat the Oil-Red-O Staining method (ORO)[21].

Both wild-type (WT-N2) and sir-2.1-defficient worms were incubated with either 1 mg/plate of Xanthigen® or vehicle (control group). After 5 days, synchronized worms were washed two times with 1x PBS pH 7.4 and allowed to settle by gravity, and resuspended in 120 µl of PBS to which an equal volume of 2x MRWB buffer (160 mM KCl, 40 mM NaCl, 14 mM Na2 EGTA, 1 mM Spermidine HCL, 0.4 mM Spermine, 30 mM NaPIPES, 0.2% β- mercaptoethanol) containing 2% paraformaldehyde (PFA). Worms were then incubated one hour at room temperature; buffer was aspirated, and were washed with 1x PBS to remove PFA and were the resuspended in 60% isopropanol and incubated for 15 minutes at room temperature to dehydrate. Oil-Red-O stain solution 0.2% w/v in isopropanol was added, and animals were incubated overnight with rocking.

The nematodes were washed tree times with 1x PBS 0.01% TritonX-100 and observed with an Olympus MVX10 microscope. Pixel 174 measurement from ORO stained worms was performed with Image J software.

Stress condition evaluation

All assays were carried out at 20 °C. Synchronized populations were obtained from larval stage (L1) to adults (L4 stage) in 60mm dishes. Plates with *E. coli* OP50, which serves as nematode food, were pre-treated with doses of 1 mg of Xanthigen® and dispersed as a thin layer. A control group exposed to the same solvent, dimethyl sulfoxide (DMSO) and Tween-80 at 2% was used in parallel. Worms were judged to be dead when they ceased pharyngeal pumping and did not respond to prodding with a platinum wire.

Sensitivity to oxidative stress was measured by quantifying survival following exposure to a reactive oxygen species (ROS)-generating compound such as juglone[22]. Age-synchronized

nematodes (WT-N2 and sir-2.1-defficient worms) were incubated with 1mg of Xanthigen® or vehicle for 3 days in liquid NGM at 20ºC. The oxidative stress was induced with 240 µM juglone (5-hidroxi-1,4-naftoquinone) and worms were maintained at room temperature. The oxidative stress was evaluated as percentage of survival of the worms, and evaluated at 1, 2, and 3-hour using touch-provoked movement and ceased pharyngeal pumping.

For thermal stress evaluation, synchronized 1-day-adult WT-N2 and -sir-2.1-defficient worms, pre-treated with 1 mg of Xanthigen® or vehicle (control) since L1, were exposed to 36.5°C for 8 h, thermal stress conditions[23]. Since 20ºC is considered the standard temperature for growing C. elegans in laboratory, after the procedure the plates were returned to this temperature for 24 additional hours.

The thermal stress was evaluated as percentage (%) of survival of the worms at 6-hour using touch-provoked movement and ceased pharyngeal pumping.

Overexpression of Pdaf-16::GFP, Phsp-16.2::GFP and Pgst-4::GFP

After synchronization, nematodes of the transgenic strains TJ356, CL2070 and CL2166, stably expressing Pdaf-16::GFP (FOXO transcription factor DAF-16), Phsp-16.2::GFP (heat shock protein 16.2), and Pgst-4::GFP (glutathionine-S-transferase-4) respectively, were transferred to NGM agar plates containing the respective compound or solvent control for additional 24 h. Subsequently, around 40 L3 larvae of each group were placed on microscope slides coated with 3% agarose, anaesthetized with 10 mM sodium azide, and covered with coverslips. As a positive control both of DAF16 and Hsp16.2, transgenic worms were grown at 20ºC plated with E. coli OP50 and exposed at 37ºC on the 3rd day of adulthood were used. As a positive control of GST4, transgenic worms were exposed to acrylamide at dose of 500 mg/L on the 3rd day of adulthood[24]. Cellular localization of DAF-16, Hsp16.2 and GST4 was analysed by fluorescence microscopy on an Axio Observer D1 Fluorescence Microscope (Zeiss, Göttingen, Germany) using appropriate filters (ex. 472±30 nm, em. 520±35 nm). All experiments were done in triplicate.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of the mean. Statistical significance of differences between groups was analysed by one-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test to allow for multiple comparisons. Statistical significance was reported if $P<0.05$ was achieved.

RESULTS

Effect of Xanthigen® on lipid deposition in *C. elegans*

To study whether Xanthigen® was able to reduce lipid deposition in *C. elegans*, both WT-N2 (WT) and sir-2.1-defficient worms (sir-2.1) were treated with Xanthigen® or vehicle for five days and the extension of lipid deposition was then measured by the ORO method. Results are shown in Figure 1. WT animals treated with vehicle served as controls and their lipid deposition was considered to have a relative value of 1.000 ± 0.03. Treatment with Xanthigen® to WT animals caused a significant 13% decrease (0.871 ± 0.02) in lipid deposition as compared to controls (Bonferroni test: $P<0.001$). Sir-2.1-defficient worms exhibited a modest and non-significant 2.4% (0.976 ± 0.02) decrease in lipid deposition when compared to WT control (Bonferroni test: $P=0.447$). In these animals, Xanthigen® treatment caused a non-significant 3% decrement, in lipid deposition compared with sir-2.1 Cont (0.946 ± 0.02; Bonferroni test: $P= 0.447$). These results indicate that Xanthigen® is capable of reducing lipid deposition through a mechanism mediated by SIRT-2.1, although the involvement of other possible mechanisms cannot be ruled out.

C. elegans converts excess energy into triglycerides that are stored in hypodermic cells of the intestine distinct droplet-like structures (Figure 2). WT worms exposed to Xanthigen®

presented a lower intensity of colour detected by ORO method, which corresponds to a poorer content of triglycerides. This effect was reduced in the mutated sir-2.1, with no significant differences between Xanthigen® treated or non-treated worms.

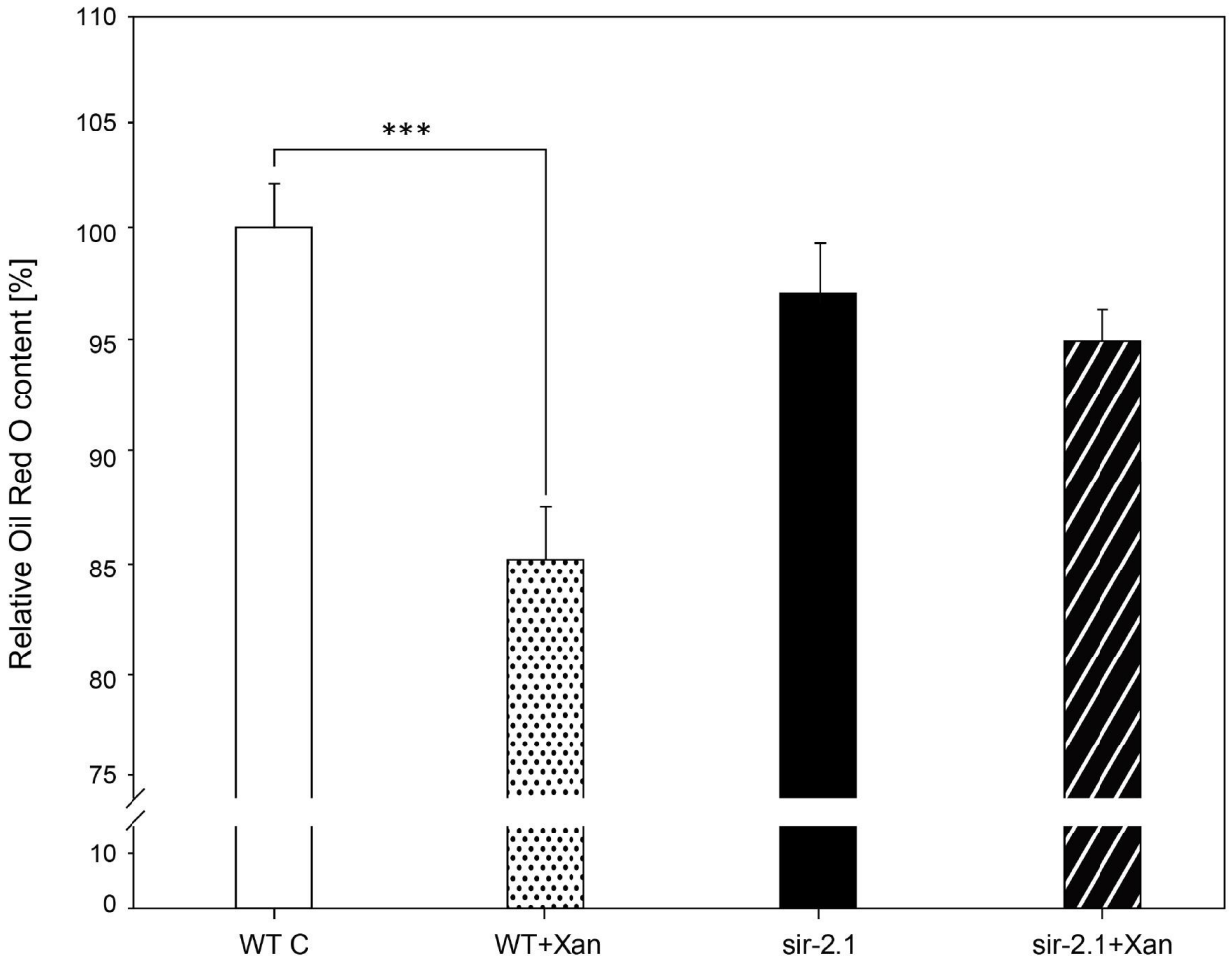

Figure 1. Effect of Xanthigen® (Xan) on fat store in *C. elegans* wild-type-N2 (WT) and sir-2.1 deficient worms (Sir-2.1) by Oil Red O (ORO) staining. Results are expressed as percentage of values obtained from WT Control (WT-C) group set as 100%. Values are means with standard errors represented by vertical bars. Statistical was analysed by one-way ANOVA followed by the Bonferroni post hoc-test. *** indicates statistical difference ($p<0.001$) from the WT-C group.

Effect of Xanthigen® on Oxidative stress

To study the protective role of Xanthigen® on oxidative stress, juglone test, at a concentration of 240 µM, was added to wild type and sir-2.1-defficient worms, either in the presence or absence of Xanthigen®. Survival rate was assessed at 1, 2, and 3 hours.

As shown in Figure 3, in WT worms, survival rate decreased with increasing times of juglone exposure (survival rate after 1 hour: 83.4 ± 4.3%; after 2 hours: 64.0 ± 4.5%; after three hours: 41.7 ± 3.9%). The addition of Xanthigen® to the incubation media significantly increased survival rate of the worms at each of the three times of incubation tested: 93.3 ± 2.5% after 1 hour (112% increase; Bonferroni test: P=0.012); 75.7 ± 3.2% after 2 hours (118% increase; Bonferroni test: P=0.006); 56.1 ± 2.4% after three hours (135% increase; Bonferroni test: P<0.001).

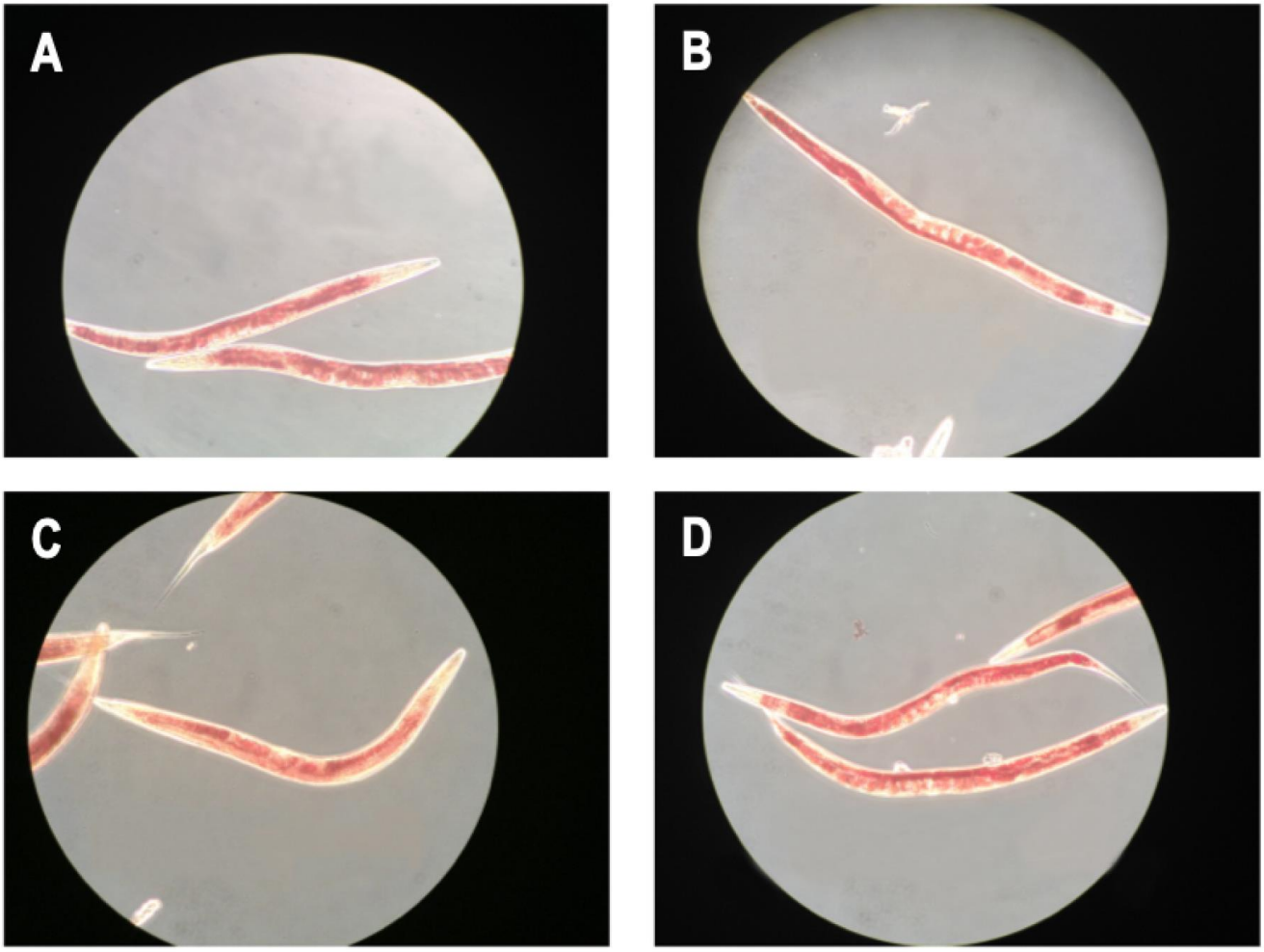

Figure 2. Representative pictures showing the level of triglycerides in hypodermic cells of the intestines of the nematodes by ORO staining method. Wild-type-N2 (WT) and Sir-2.1 deficient worms (sir-2.1) were placed both in the presence (C: WT-XAN; D: sir-2.1-XAN) or absence of Xanthigen® (A: WT-Control; B: sir-2.1-Control), at a dose of 1 mg per plate during 5 days. WT worms exposed to Xanthigen® presented a lower intensity of colour, which corresponds to a poorer content of triglycerides. This effect was reduced in the mutated sir-2.1, with no significant changes between Xanthigen® treated/non-treated worms.

Sir-2.1-defficient worms had a decreased survival rate as compared to wild-type animals at the three periods of exposure to juglone tested: 60.4 ± 4.8% after 1 hour (72% of the wild-type control; Bonferroni test: P<0.001); 37.3 ± 5.2% after two hours (58% of the wild-type control; Bonferroni test: P<0.001); and 16.8 ± 4.7% after three hours (40% of the wild-type control; Bonferroni test: P<0.001). In these animals, treatment with Xanthigen® provoked a significant increase in the survival rate at the three times examined: 75.0 ± 3.2% after 1 hour (124% increase; Bonferroni test: P<0.001); 51.6 ± 3.5% after 2 hours (138% increase; Bonferroni test: P<0.001); and 32.4 ± 3.3% after 3 hours (193% increase; Bonferroni test: P<0.001). These results indicate that sir-2.1 activation is able to confer worm resistance to oxidative stress. The fact that Xanthigen® was capable of increasing the survival rate after oxidative stress in both wild type and sir-2.1-defficient worms suggests that the effect may be independent of sir-2.1 activation.

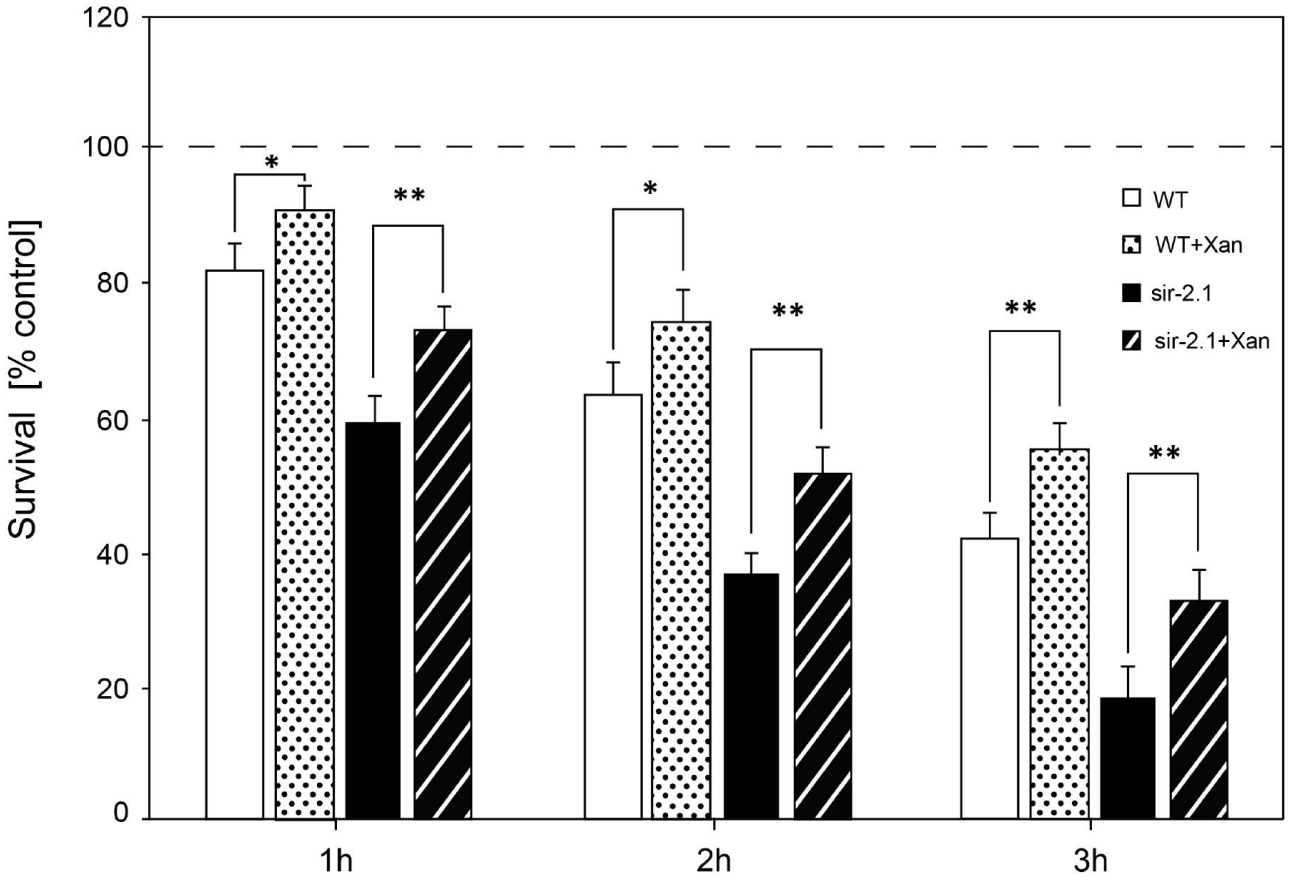

Figure 3. Effect of Xanthigen® on survival at different time, 1, 2 and 3 hours after inducing oxidative stress by juglone (240 µM) in wild-type (WT) and sir-2.1-worm (sir-2.1). Values are means with standard errors represented by vertical bars. Results are expressed as percentage of survival obtained from WT or sir-2.1 w/o juglone treatment set as 100% (dashed line). * $p<0.05$, ** $p<0.005$ by the Bonferroni post hoc test.

Effect of Xanthigen® on survival rate of worms exposed to thermal stress

To study whether Xanthigen® was able to confer protection against thermal stress, adult, hermaphrodite worms of the two varieties, WT and sir-2.1-deficient worms, were cultured in NGM and exposed to thermal stress. As shown in Figure 4, survival rate after thermal stress was about 50% in WT worms (47.2 ± 5.8%). Xanthigen® treatment increased survival rate by 50% (72.0 ± 7.1%; $P<0.001$). In sir-2.1-defficient worms, survival rate was only 29.2 ± 5.6%, significantly lower than that exhibited by wild-type animals ($P<0.001$); treatment with Xanthigen® caused a significant 42% increase in survival rate (41.6 ± 6.1%; $P=0.014$). In fact, survival rate in sir-2.1-defficient animals treated with Xanthigen® was not significantly different than that exhibited by WT treated with vehicle (Bonferroni test: $P=0.789$), although was significantly lower than that exhibited by WT-N2 animals treated with Xanthigen® (Bonferroni test: $P<0.001$).

These results suggest that sir-2.1, upon activation after thermal stress participates in the mechanisms that confer protection against thermal damage. Since Xanthigen® is capable of protecting worms against thermal stress both in the presence or absence of SIR-2.1, it opens the possibility that Xanthigen® exerts its protective effect on the animals through mechanisms independent of sir-2.1 activation.

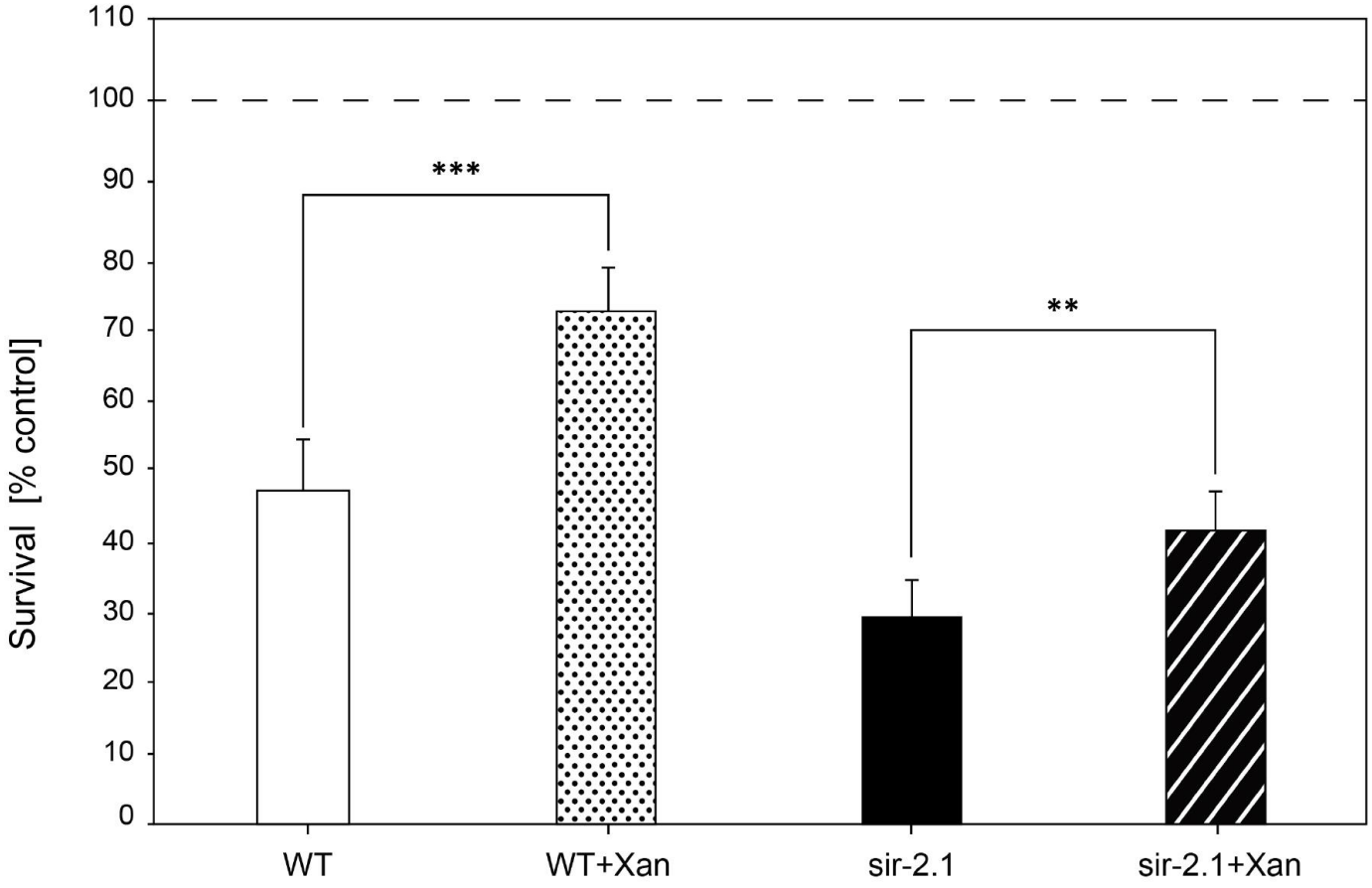

Figure 4. Effect of Xanthigen® on survival after thermal stress (36.5 ºC) in *C. elegans* wild-type (WT) and Sir-2.1-worm (Sir-2.1). The dashed line indicates the non-thermal treated WT and Sir-2.1 control groups (100%). Values are means with standard errors represented by vertical bars. ** P<0.005, ***P<0.001, by the Bonferroni post hoc test.

Effect of Xanthigen® on DAF-16 translocation, HSP-16.2, and GST overexpression

In order to evaluate other possible mechanisms implicated in the protective effect of Xanthigen® on stress resistance, we used specifics worm strains to study the expression of proteins with a well-known role in response to stress.

DAF-16 plays a role in both oxidative and heat stress as well as starvation. It is the sole orthologous of the FOXO family of transcription factors in *C. elegans* and DAF-16/FOXO proteins are a family of transcription factors that integrate different signals by phosphorylation of proteins including insulin/IGF-1, TOR, AMPK, or JNK pathways to modulate important processes, via shuttling from cytoplasm to nucleus, and the expression of genes that influence in cell growth, proliferation, differentiation, or longevity[25]. Under these circumstances, cytosolic DAF-16 protein is phosphorylated and then it relocates to the nuclei and promotes both the expression of genes that confer resistance to stress and longevity. The product of one of these genes is HSP-16.2, which can be considered as a marker of DAF-16 activation. In the absence of thermal stress, DAF-16 is located in the cytoplasm and Hsp-16.2 expression is very low. Under heat shock DAF-16 is translocated to the nuclei and promotes Hsp16.2 expression.

Worms were assigned to three groups: the negative control was neither treated with

Xanthigen® nor exposed to thermal shock at 37 ºC for 1 hour; the positive control was exposed to thermal stress but did not receive Xanthigen, and finally, the third group did not undergo thermal stress but was treated with Xanthigen®.

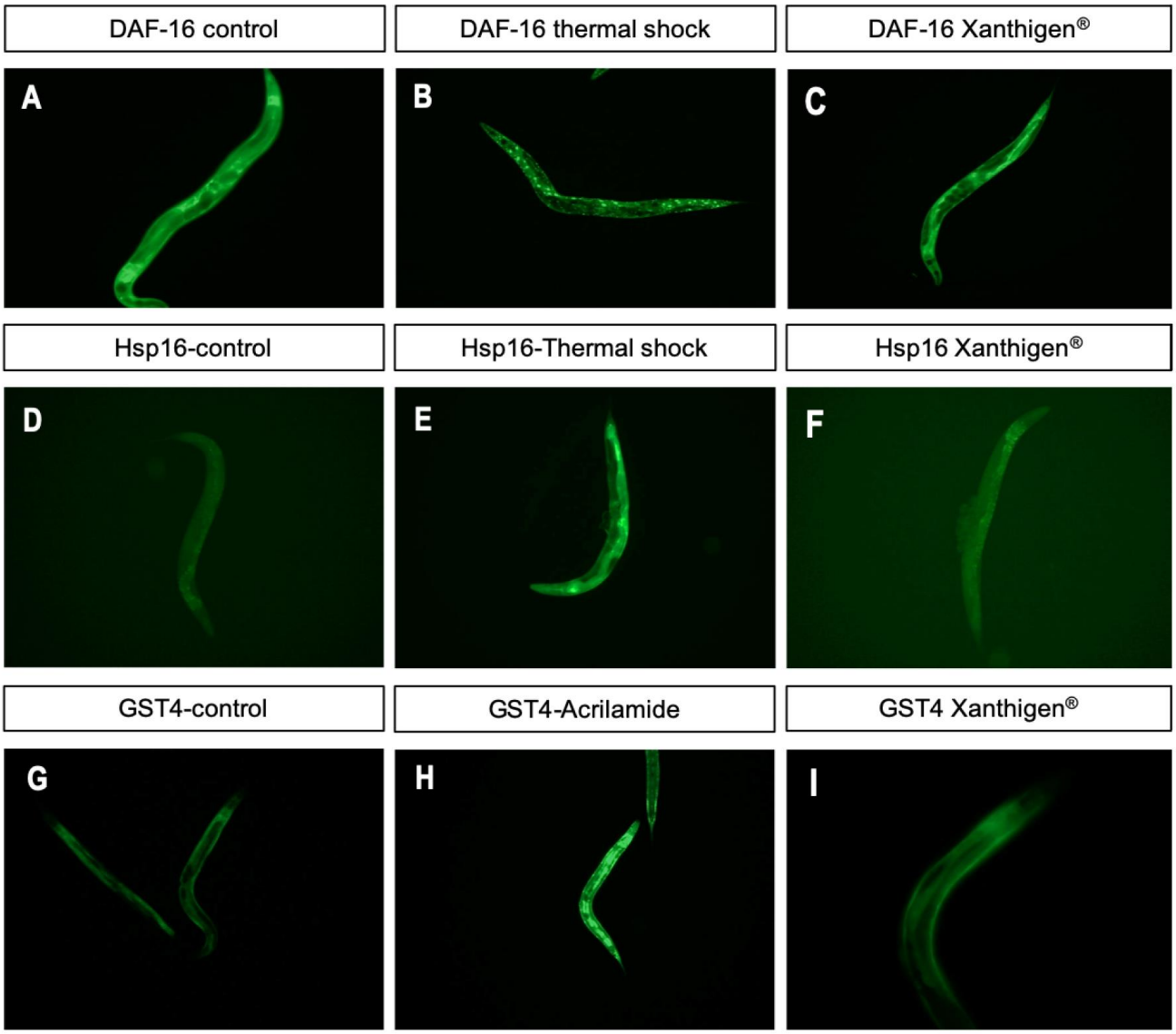

Figure 5. Green fluorescence protein (GFP) images of DAF16 expression (A-C) in adults Daf16::GFP transgenic strain (TJ356), Hsp16 expression (D-F) in adults Phsp16.2::GFP transgenic strain (CL2070) and GST4 expression (G-I) in adult Pgst-4::GFP transgenic strain (CL2166) shown at 10x. Daf16 and Hsp16 transgenic worms were heat stressed for 1 hour at 37°C as positive control. A) DAF16 localization in the cytoplasm in absence of stressor (negative control). B) DAF-16 localization in the nuclei is increased after stress (positive control). C) DAF16 localization in the cytoplasm after treatment with 1mg/plate of Xanthigen®. D) Hsp16 is not expressed in absence of stressor (negative control). E) Hsp16 is expressed after heat stress (positive control). F) Hsp16 is slight expressed after 1 mg/plate Xanthigen® treatment. G) GST4 expression in absence of stressor as negative control, H) GST4 expression after induction stress with acrylamide as positive control I) GST4 expression after treatment with 1mg/plate of Xanthigen®.

Xanthigen® treatment did not increase the translocation of cytosolic *daf-16*::GFP into nuclei, which suggests that the protective effect of Xanthigen® on thermal stress is not mediated by DAF-*16.*

The treatment of Phsp16.2::GFP strain with Xanthigen® increased slightly the expression of Hsp16.2 with respect to negative control, although Hsp16.2 expression in the positive control was greater. This result suggests that Xanthigen® may exert a positive effect against thermal stress mediated by Hsp16.2, although its contribution is small.

The *skn-1* transcription factor has been predicted to turn on a variety of antioxidant response genes and has been shown to regulate the induction of glutathionine-S-transferase-4 (*gst-4*) in response to oxidative stress. The CL2166 is a strain, which carries a transcriptional fusion of GFP to the *gst-4* promoter[10]. In animals not treated with acrylamide, gst-4 expression was very weak. Treatment of these animals with Xanthigen® increased slightly gst-4 expression, although in a much lower extent than when animals were exposed to acrylamide. These results suggest that Xanthigen® by itself is only capable of inducing a slight xenobiotic response in *C. elegans* (Figure 5).

DISCUSSION

C. elegans is a free-living nematode widely used in research involving moving behaviour, neurodegenerative disorders, aging, or obesity. This model possesses many advantages in research, with more than 65% of the human diseases-related genes conserved[26]. Among the characteristic components of Xanthigen® , there are previous studies of the role of fucoxanthin increasing lifespan of *C. elegans*[27], and also others recent about the beneficial effects of pomegranate extracts in different organisms including worms[28].

In our study in transgenic *C. elegans*, we move forward investigating the molecular mechanisms underlying the antilipogenic effect of Xanthigen® and we confirmed that Xanthigen® reduces lipid deposition through sir-2.1 pathway regulation. Choi et al. (2014)[17] reported that Xanthigen® treatment significantly decreased body weight and adipose tissue in mice subjected to a high fat diet through down-regulation of PPARɣ and upregulation of AMPK pathway. Similarly, in a preadipocyte differentiation model it was also demonstrated that Xanthigen® reduces lipid accumulation and suppresses adipocyte differentiation by mechanisms that involve an increase of intracellular levels of AMPK and SIRT1 levels, and a concomitant decrease in PPARɣ[16]. AMPK, a key pathway mediating glucose uptake and fatty acid oxidation, is activated under energy reduction and inhibits mTOR pathway, thus facilitating catabolism and reducing fat accumulation. SIRT1 belongs to Sirtuins family and plays a critical role in metabolic health. Among its versatile functions, it has been described the regulation of fat and glucose metabolism in response to physiological changes in energy levels, the aging process, or stress response[29]. Both AMPK and SIRT1 are two fuel-sensing molecules that have coexisted in cells throughout evolution. During low energy status, like exercise or caloric restriction, SIRT1 is induced to restore energy balance regulating fat and glucose metabolism while in energy excess, like overfeeding or sedentary lifestyle, SIRT1 is repressed. Sirt1/AMPK pathway activation can reduce lipid accumulation of liver in obese mice and improve insulin resistance in diabetic mice. In a recent report, performed on FL83B hepatocytes, fucoxanthin increased Sirt1 expression and enhanced phosphorylation of ACC and AMPK, reducing hepatic lipid accumulation[30]. In this study we have shown the role of Xanthigen® as a caloric restriction mimetic and confirmed “in vivo” the results showed in 3T3-L1 adipocytes by Lai et al[16].

We have also shown that Xanthigen® treatment confers resistance to stress. In thermal and oxidative stress conditions Xanthigen® treatment increased survival rate independently of sir-2.1 or DAF-16 translocation. In addition,

Xanthigen® did not cause the induction of GST, suggesting that its beneficial effect on stress resistance is not mediated by a response to xenobiotics. A mechanism that could help to explain the protective effect of Xanthigen® on stress resistance could be given by Small Heat Phock proteins (sHSPs). These proteins are biomarkers of the exposure to thermal and oxidative stressors[31]. Treatment with Xanthigen® partly increased the expression of Hsp16.2 with respect to the negative control, although the effect was not greater than the positive control. The expression of small heat shock protein HSP-16 is regulated by Heat Shock Factor-1 (HSF-1), a key nuclear protein that regulate the heat-shock-inducible gene expression[32]. Hsp-16.1 and hsp-16.2 genes have been shown to act by counteracting the presence of misfolded proteins in the cytoplasm and nucleus due to oxidation or other types of protein. This effect has been also shown by others polyphenols like catechins. A green tea extract standardized to contain 30% of catechins reduces the oxidative stress and elevates the stress resistance by attenuating the heat shock protein (hsp-16.2) response[33]. Further studies demonstrated that the increased stress tolerance of 6-gingerol-mediated worms could result from the promotion of stress resistance proteins such as heat shock protein (HSP-16.2)[34].

Stress resistance, defined as the ability to appropriately manage stressors, is thought that slows the aging process and improve health span[35]. Our results lead to include Xanthigen® in the list of plant extracts capable of improving resistance to different types of stressors, and although Xanthigen® does not seem to increase lifespan, its ability to improve stress resistance, under energetic, thermal or oxidative stress conditions make it a hopeful aid to increase the health span of cells.

CONCLUSIONS

Two conclusions can be highlighted from the results presented above: 1) Xanthigen® is capable of inhibiting lipid deposition in *C. elegans*; and, 2) Xanthigen® treatment confers to worms an increased resistance to thermal and oxidative stress.

Abbreviations

UCP1: uncoupling protein 1; HbA1c: Glycated haemoglobin; BAT: Brown adipocyte; SIRT-1: NAD-dependent deacetylase sirtuin-1; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A reductase); PPARγ: Peroxisome proliferator-activated receptor gamma; AMPK: AMP-activated protein kinase; ACC: Acetyl-CoA carboxylase; C. elegans: Caenorhabditis elegans; WT-N2: Wild type C. elegans strain N2; NGM: Nematode growth medium; ORO: Oil red O; PBS: Phosphate buffered saline; PFA: Paraformaldehyde; DMSO (dimethyl sulfoxide): ROS: Reactive oxygen species: SD: Standar desviation; ANOVA: Analysis of variance; Pdaf-16: forkhead box O transcription factor DAF-16; Phsp-16.2: heat shock protein 16.2; Pgst-4: glutathione-S-transferase-4); GFP: green fluorescent protein fusion;

Acknowledgement

Not applicable

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Conflict of interest

Laura López-Ríos and Rubén Pérez-Machín are employed by the sponsor of the reported studies, Nektium Pharma SL.

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