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ORIGINAL CONTRIBUTION

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# The antioxidative effect of the bacteria Dienococcus radiophilus against LDL lipid peroxidation

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**Summary** *Background* Lipid peroxidation is an important process in the development of atherosclerosis. Thus agents with antioxidant properties may play an important role in the inhibition of atherosclerosis. *Objectives* In this study we aimed to show that the lipid extract of the bacteria Deinococcus radiophilus (leDR) has antioxidant properties against LDL oxidation. *Results* This antioxidant effect was shown in both transition metal ion and free radical generating systems. We also showed that leDR can protect LDL from UV light-induced oxidative damage. The antioxidative capacity of leDR is partly due to copper ion chelation. *Conclusion* We conclude that some specific bacteria constituent has the ability to inhibit LDL oxidation and, thus, to attenuate atherogenesis.

**Key words** antioxidants – lipid peroxidation – bacteria

## Introduction

Oxidative effect caused by free radicals is an initiating factor in many diseases, among which are inflammatory reactions, malignant diseases and atherosclerosis [1, 2]. The early atherosclerotic lesion is characterized by foam cells, which are derived from macrophages filled with cholesterol [3–5]. The major source of the cholesterol, which accumulates in these macrophages, is circulating low density lipoprotein (LDL), which had undergone oxidative modification. Thus, oxidized LDL is believed to play a key role in the development of atherosclerosis, and inhibition of oxidative modification of LDL may attenuate the development of atherosclerosis. There is an extensive investigation on the role of antioxidants against LDL oxidation and especially those of natural biological sources [6–8].

Dienococcus radiophilus (DR) is a gram positive aerobic bacteria, which is resistant to ultraviolet radiation. The bacteria is characterized by specific lipid components, including polar lipids, mainly phosphoglycolipids. The bacteria lacks phosphatidyl glycerol and its deviates, and palmitoleic acid (16:1) is the dominant acid among the fatty acids [9–11].

In this study we aimed to examine the ability of DR lipid extract to affect LDL lipid peroxidation in vitro. Our results show that leDR could protect LDL against lipid peroxidation.

## Methods

### Dienococcus radiophilus (DR)

DR is an aerobic gram positive bacteria containing red pigments. These bacteria are very stable to UV light up to 500K rad. DR was first isolated from food conserva-

tives which were sterilized by UV light [12]. The high stability is due to the specific external membrane of the bacteria and possibly a very effective excision repair system which can remove thymidine dimers efficiently [10, 13].

DR was cultivated in a fermentor at 30 °C, pH = 7, on TGY medium (tryptone-glucose yeast extract, agar medium). At the end of the cultivation, the bacteria were pelleted by centrifugation and freeze dried [14].

The lipids were extracted from the dried material in methanol:chloroform (1:2,V:V) and were separated by vacuum liquid chromatography (VLC).

### Lipid oxidation

LDL was separated from plasma by discontinuous density gradient ultracentrifugation and dialyzed against saline with EDTA (1 mmol/l) [15]. LDL was diluted in phosphated buffered saline (PBS) to 200 mg protein/l and dialyzed overnight against PBS at 4 °C to remove EDTA. Oxidation of LDL was carried out in a shaking water bath at 37 °C, under air, in plastic tubes.

For metal ion dependent oxidation, LDL was incubated for four hours at 37 °C with freshly prepared copper sulfate (5 µmol/l). For metal ion independent oxidation, LDL was incubated for four hours at 37 °C with AAPH (1 mmol/l), which is an azo compound that thermally decomposes to produce peroxyl radicals at a constant rate [16].

LDL oxidation was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS) [17] and of lipid peroxides [18]. Formation of conjugated dienes was continuously monitored at 234 nm [19].

The free radical scavenging capacity of leDR was analyzed by the DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay [20].

## Results

The addition of 50 µg/ml of the bacteria DR extract to an LDL solution (100 µg protein/ml) inhibited "copper ions" or AAPH-induced LDL lipid peroxidation, as measured by a 90 % and 70 % inhibition in lipid peroxides formation, respectively (Fig. 1A), or by 90 % and 86 % inhibition in the formation of thiobarbituric acid reactive substances (TBARS), respectively (Fig. 1B).

Kinetic monitoring of copper ion-induced LDL oxidation revealed that in the presence of DR lipid extract (20 µg/ml) a prolongation of the lag phase (the time needed for LDL lipids to undergo oxidation) by 30 minutes was obtained, whereas 40 µg/ml of the DR extract retarded the onset of LDL oxidation by more than 200 minutes (Fig. 2).

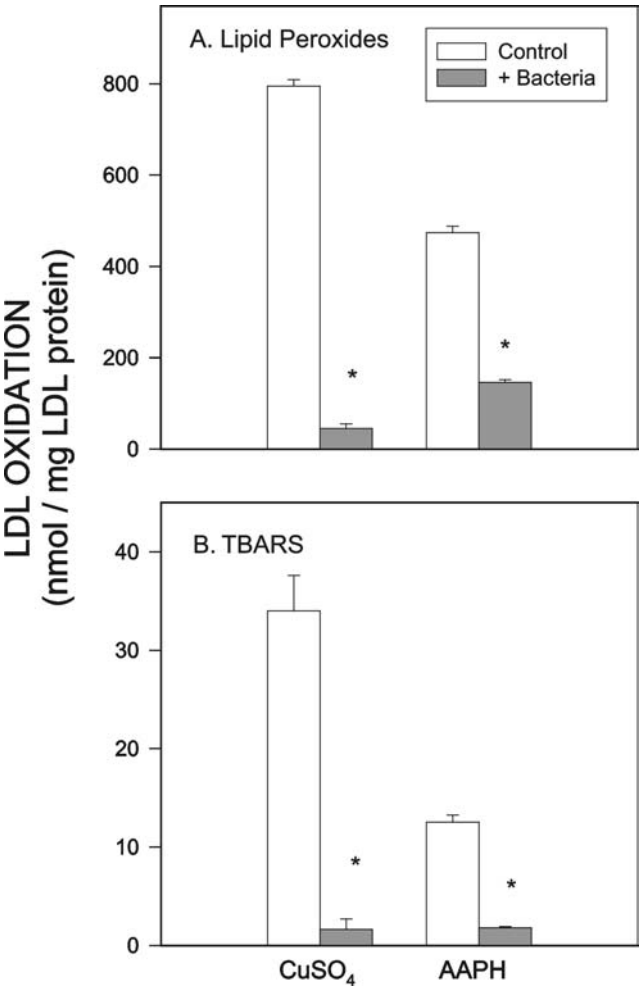


**Fig. 1** The effect of the bacteria Dienococcus radiophilus (DR) lipid extract on LDL oxidation induced by copper ion (**A**) or the free radical generator AAPH (**B**)

In order to study the mechanism by which the DR bacteria lipid extract inhibits lipid peroxidation, we analyzed the free radical scavenging capacity of the bacteria extract by using the DPPH assay. This compound shows a stable strong absorption at 517 nm, which decreases if this compound accepts a free radical to form a new substance. The addition of bacteria extract to DPPH did not induce a significant decrease in the optical density at 517 nm, suggesting that it possesses only a limited capacity to scavenge free radicals (Fig. 3A).

Since antioxidants may act as such also due to chelation of metal ions, we tested the possibility that the bacteria extract possesses a copper ion binding capacity. For this purpose we incubated LDL with increasing concentrations of $CuSO_4$ in the absence (control) or presence of 50 µg/ml of the bacteria extract. The inhibitory effect of the bacteria extract on LDL oxidation could be overcome by high concentrations of copper ions, suggesting that the bacteria extract inhibits LDL oxidation

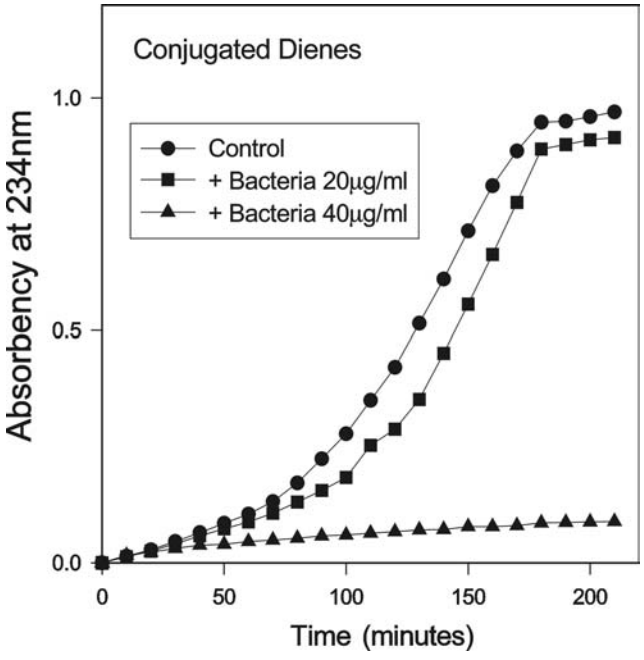


**Fig. 2** Effect of bacteria DR lipid extract concentration on the kinetics of LDL oxidation

at least in part due to its capacity to chelate copper ions (Fig. 3B).

Since it was documented that the bacteria DR are resistant to ultraviolet radiation, we studied whether the bacteria extract can protect LDL from ultraviolet radiation-induced oxidation. Thus LDL (100 μg of protein/ml) was exposed for two hours to ultraviolet radiation at a wavelength of 235 nm in the absence (control) or presence of 50 μg/ml bacteria extract, after which the LDL was subjected to copper ion-induced oxidation. Our results demonstrate that the addition of bacteria extract to the LDL suspension during radiation reduced the susceptibility of LDL to oxidation by 65% (from $14 \pm 3$ to $9 \pm 2$).

These results suggest that the bacteria extract can protect LDL from lipid peroxidation induced by ultraviolet radiation.

## Discussion

The present study demonstrated, for the first time, that a specific bacteria (DR) can act as an antioxidant against LDL oxidation. This effect was shown to be bacteria dose-dependent and could be related to metal ion chelation, but not to free radical scavenging.

However the bacteria was an efficient antioxidant against LDL oxidation not only in the copper ion-induced LDL oxidation but also in lipoprotein oxidation by free radicals such as AAPH [20]. These results suggest

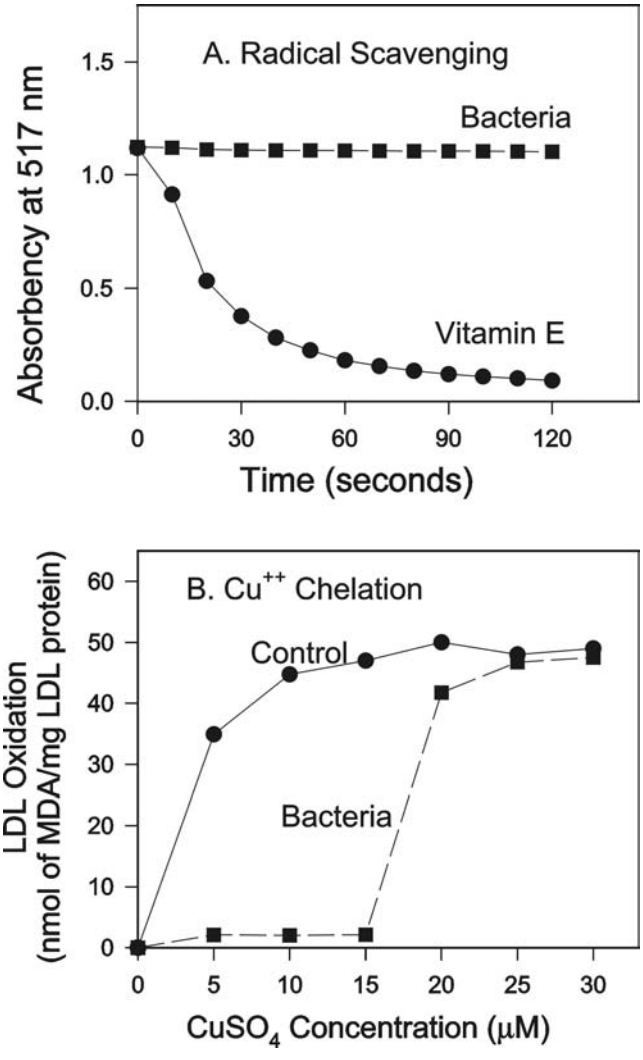


**Fig. 3** The ability of the bacteria DR lipid extract to scavenge free radicals (**A**) and to chelate copper ions (**B**)

that the DR bacteria can affect LDL oxidation also by means other than copper ion chelation and free radical scavenging [21]. Such an antioxidant effect of DR bacteria lipid extract on LDL oxidation may be related to some interaction of the bacteria lipid extract with LDL which may cause conformational changes in the lipoprotein which could possibly prevent a direct interaction of LDL with reactive oxygen species produced during LDL oxidation.

The inhibitory effect of the bacteria DR on LDL oxidation is not an obvious observation as we expect that most types of bacteria will react to increase free radical formation [22]. The antioxidant property of the bacteria DR against LDL oxidation may be the result of some specific constituents in this bacteria with lipophylic properties which can bind to LDL, chelate transition metal ions and hence reduces LDL oxidation.

As LDL oxidation is the hallmark of early atherosclerosis, the inhibition of this process can attenuate atherogenesis as a result of reduced uptake of the lipoprotein by arterial wall cells including monocyte-derived macrophage and smooth muscle cells [23]. We are now in the process of isolating, characterizing and analyzing DR bacteria constituents that possess an antioxidant activity against LDL oxidation. These analyses may allow us to identify appropriate potent antioxidants from a natural source, in order to advance our management of LDL oxidation and atherosclerosis [24].

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