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Biodegradation of 7-ketocholestrol (7-KC) by *Thermobifidafusca* IP1

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Abstract

7-ketocholesterol (7KC), an oxidized derivative of cholesterol, has been implicated in a variety of chronic diseases including atherosclerosis, Alzheimer's disease, Parkinson's disease, cancer and age-related macular degeneration. It is formed by the autooxidation of cholesterol and especially cholesterol-fatty acid esters found in lipoprotein deposits, its elevated concentrations are associated with disruption of cellular homeostasis, decreased cell viability, and increased cell death.Enzymatic cleavage of 7-KC can serve as a key solution for the cure of a number of chronic diseases directly associated with its accumulation. A bacterial strain isolated from manure piles was characterized taxonomically and identified as *Thermobifida fusca* IP1 on the basis of amplification and sequencing of 16s rDNA. This isolate was tested for its ability to degrade 7-ketocholesterol in M9 liquid medium and showed to utilize 7-KC as the sole carbon and energy source. The degradation of 7-KC was hundred percent when tested in liquid culture. High Performance Liquid Chromatography (HPLC) analysis also showed complete removal of the compound from the sample after twelve days. This bacteriacan effectively be used to remove 7-KC and can lead to the development of a single potential therapeutic enzyme preparation to target a number of above mentioned chronic diseases related to 7-KC.

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Introduction

7-Ketocholesterol (7-KC) is a naturally occurring oxysterol formed by the autooxidation of cholesterol and cholesterol-fatty acid esters (Dzeletovic *et al.*, 1995). It is commonly found in oxidized lipoprotein deposits associated with atheromatous plaques (Garcia-Cruset *et al.*, 2001; Ohtsuka *et al.*, 2006; van Reyk *et al.*, 2006) as well as in lipoprotein deposits in Bruch's membrane and choriocapillaris in the back of the retina (Moreira *et al.*, 2009). Cholesterol oxidation products, termed oxysterols, are increasingly considered of potential interest in the pathogenesis of atherosclerotic lesions, 7-Ketocholesterol is a major oxidation product of cholesterol found in human atherosclerotic plaque and is more atherogenic than cholesterol (Leonarduzzi *et al.*, 2002).

It has been shown to be the major cytotoxic component in oxidized LDL (Rodriguez *et al.*, 2004). This oxysterol is known to be highly inflammatory both *in vitro* (Brown and Jessup, 1999; Vejux and Lizard, 2009) and *in vivo* (Amaral *et al.*, 2013). Its inflammatory and cytotoxic properties have been implicated in the pathogenesis of numerous aging diseases (Vejux and Lizard, 2009), including atherosclerosis (Brown and Jessup, 1999; Reyk *et al.*, 2006), Alzheimer's disease (Vejux and Lizard, 2009; Poli *et al.*, 2013), cancer (Wang *et al.*, 2013), Parkinson's disease (Poli *et al.*, 2013) and age-related macular degeneration (Poli *et al.*, 2013; Rodrı´guez and Larrayoz, 2010).

In vitro experiments have shown that 7-ketocholesterol, a major oxysterol in plaques, induces vascular Smooth Muscle Cell's (SMC's) death with features of apoptosis, such as nuclear condensation and internucleosomal DNA fragmentation (Lizard *et al.*, 1999).It has been well established that exposure of cells to 7-KC elicit a variety of defense responses, including inflammation, apoptosis, and the stimulation of vascular endothelial growth factor (Vejux *et al.*, 2008). The ability of 7KC to disrupt cellular Ca^{2+} homeostasis is likely an integral part of its toxicity (Berthier *et al.*, 2004;Rimner *et al.*, 2005; Spyridopoulos *et al.*, 2001).

7-KC is a major intracellular oxysterol species. Sterols, including oxysterols, enter the cell via receptor mediated endocytosis of low density lipoproteins and traffic to the lysosomes, which are a major site of non-enzymatic oxysterol formation. Consequently, 7KC levels are the highest in the endosomal and lysosomal compartments (Brown *et al.*, 2000). 7KC is known to inhibit sphingomyelinase (Maor *et al.*, 1995) and facilitate the intralysosomal accumulation of both sphingomyelin and cholesterol, possibly leading to foam cell formation. Subsequent free cholesterol loading of lysosomes also promotes deacidification (Cox *et al.*, 2007), impairs organelle trafficking (Fraldi *et al.*, 2010), and inhibits chaperone mediated autophagy (Kaushik *et al.*, 2006). At micromolar concentrations, 7KC causes lysosomal membrane permeabilization (LMP). The cellular response to LMP depends on the degree of permeabilization, with mild LMP causing induction of apoptosis or apoptosis-like cell death and sustained LMP generally leading to necrosis (Mathieu *et al.*, 2012).The aim of the present study was to isolate and characterize bacteria from environmental sample(manure piles) capable of catabolizing 7-KC.

Materials and methods

Chemicals

The substrate 7KC was obtained from Sigma-Aldrich (97.1% pure). A modified M9 medium was used in all experiments as growth medium. The composition of M9 was, 6.0 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g HN_4Cl, 0.185 mg $(NH_4)_6Mo_7O_{24}.4H_2O$, 1.0 ml of 24.6% (w/v) $MgSO_4.7H_2O$ and 1.0 ml of 1.47% (w/v) $CaCl_2.2H_2O$ in 1000 ml of distilled water (final pH 7).

Isolation of bacteria

Manure pile sample was collected from Rawalpindi, Pakistan in sterile falcon tubes. Samples were properly labelled and kept in refrigerator (4 °C) till the processing. Sample (10 g) was suspended into 100 ml of sterile distilled deionized water, which was further used for inoculation purpose.M9 medium (10

ml) amended with 7KC (1mg L^{-1} or 0.0025 M) as only carbon and energy source was inoculated with 10 drops of environmental sample. To enhance the solubility of 7KC, the medium was sonicated for 20 min prior to inoculation in a bath sonicator. After inoculation, culture was incubated in shaking incubatorat 30 °C and 250 rpm agitation speed. Aliquots (100 µL) were sampled after every 24 hours and analyzed for the decrease in the concentration of target compound by HPLC. Gowth was monitored by taking absorbance at 600 nm by spectrophotometer. After indication of decrease in concentration of 7-KC from the samples, cultures were streaked on M9 agar plates prepared by adding 5.0 mg of 7KC in methanol and evaporated to dryness on the surface.Plates were incubated at 30 °C for 72 hours. From M9 agar surface morphologically different colonies were pickedand streaked on the nutrient agar plates in order to get pure cultures.Pure cultures were further confirmed for 7KC biodegradation potential by inoculating in 1ml of M9 medium containing 1mg of 7KC as the only carbon and energy source.Onepure bacterial strain giving positive results for 7KC degradation from the environmental samples was selected for the molecular identification.

Molecular identification of bacteria

Genomic DNA extraction

According the method Adye and Mateles, (1969) bacterial culture of 48-72 hour washarvested by centrifugation at 7000 rpm for 20 minutes. The cells were frozen in liquid N_2. The frozen pellets were ground with a morter and pestle. The powder was suspended in STE (100mMTris-Cl, pH = 8.0, 150 mMNaCl and 100 mM EDTA) and incubated with 1% SDS at 37 °C for 3 hours. The solution was then centrifuged at 7000 rpm for 20 minutes to remove any cell debris.

The resulting solution was then extracted with a solution of phenol : chloroform : Isoamyl alcohol (25:24:1) saturated with 10mMtris, pH 8.0, 1mM EDTA two times. The genomic DNA was recovered by slowly adding cold ethanol to the aqueous solution such that the ethanol remained on top of the aqueous layer. At the interface a small glass rod was inserted and twirled around to spool the DNA onto the rod. The spooled DNA was resuspended in 1x, TE (pH = 7.6) (1ml-1M Tris-HCl, 0.2 ml-0.5M EDTA at pH 8.0, 988 ml-ddH_2O).

The genomic DNA was run at 100 volts for 90 minutes on a 1% (w/v) agarose gel in 1x TAE (Tris Acetate EDTA) buffer containing 5 µL ethidium bromide solution (10mg/ml) to determine its relative size and concentration.

PCR Amplification of the 16S Ribosomal DNA

The extracted DNA was subjected to PCR (Gradient Cycler, MJ Research, PTC-200, Peltier Thermal Cycler) to amplify the 16S ribosomal DNA segments. One Taq®2x master mix with GC buffer (New England BioLabs®Inc.) was used. It was an optimized blend of Taq and Deep Vent$_R$™DNA polymerases. Master mix formulation containedNTPs, $MgSO_4$ and other buffer components. Primers sequences used were 16S-27F (5'-AGAGTTTGATCMTGGCTCAG-3'), (T_m=53.2 °C) and 16S-1492R (5'-TACGGYTACCTTGTTACGACTT-3')(T_m=54.6 °C).The PCR mixture used had a final volume of 50 µL and contained 1 µL of each primer (100 µM), 25µL of one Taq 2x Master Mix with GC Buffer, 22 µL of distilled deionized water and 1 µL of template (800 ng). The temperature cycle for the PCR was 1 min of denaturation at 94 °C, 1 min of annealing at 48 °C, and 3 min of primer extension at 72 °C. 25 additional cycles were used and a final primer extension for 10 min. The amplified DNA product was run on 1.5% agarose gel (1.5g agarose/100 ml of 1X TAE) at 100 Volts for 30 minutes along with 1kb DNA ladder (New England BioLabs®Inc).The DNA fragment was excised from the agarose gel with a clean, sharp scalpel.

PCR product was purified by QIAquick® Gel Extraction Kit (250) (Qiagen Sciences Maryland, USA) according to the standard protocol recommended by the manufacturer.Automated Sequencing Reactions were performed with Perkin Elmer ABI Big Dye Reaction Mix.The sequences were matched against the NCBI GenBank Database

(http://ncbi.nlm.nih.gov/Blast) for phylogenetic identification of the bacteria.

Biotransformation experiment with purified culture

Purified culture was tested for its biodegradability for 7-KC. Three replicate batches (30 ml) were prepared in M9 medium, with 7-KC as only carbon source they were inoculated with bacterial culture of the isolate and incubated at 30 °C and 150 rpm. Samples (0.5 ml) were takenfrom each batch after 24 hours timeintervalsand were subjected to extraction.7KC was then extractedfrom the samples with a mixture of hexane/isopropanol (3:2), the solvent was evaporated using a rotary evaporator (BÜCHI rotavapor R-200), sample was redissolved in 50 µL of methanol, filtered andwasanalyzed by reverse phase HPLC (0.72 ml min^{-1}, 85:10:5 methanol/water/acetonitrile) using a Waters Delta 600 Separation Module with a Waters 2487 dual λ absorbance detector, photodiodearray (235 nm) and aPhenomenex LUNA C18 column (4.6by 150 mm).

Results

Isolation and identification of 7KC degrading bacteria from environmental samples

Using enrichment technique several bacterial colonies were isolated for the sample of manure pile. These colinies were purified and tested for their growth on M9 media with 7-KC as sole carbon and energy source (unpublished data). One isolate having good positive activity on these plates was selected and purified. The bacterial isolate was growing in M9 media with 7-KC as sole carbon and energy source.

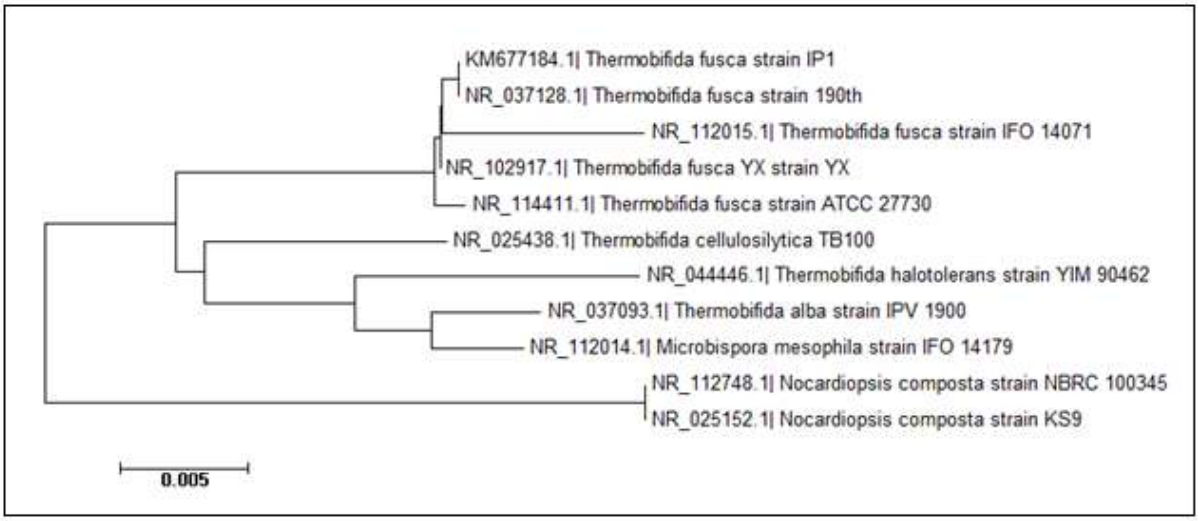

Fig. 1. Phylogenetic tree of *Thermobifida*fusca IP1 (accession number KM677184)isolated from manure piles.

Taxonomiccharachterization of the bacterial isolate IP1 was done. 16SrDNA genewas amplified and sequenced, sequences obtained were analysed by NCBI BLAST and phylogenetic tree was constructed (Fig. 1).The bacterial strain was identified as *Thermobifidafusca* (accession number KM677184).

Degradation of 7-KC by bacterial isolate ThermobifidafuscaIP1

The growth of the bacterial isolate *ThermobifidafuscaIP1* was detertmined by increase in OD_{600}, results are shown in Fig.2 there was a lag period of 3-6 hours after that gradual increase in growth (Absoption at 600nm) was observed. The OD_{600} reached to a value of 0.8 in 30 hours. Degradtion of 7-KC was monitored by HPLC. Within the set UV detection range of 190 to 400 nm, no accumulation of degradation metabolites was revealed.The absorption maximum for 7KC is 233 nm, allowed accurate determination of 7KC concentration.

On the basis of HPLC results, a rapid degradation was observed with *ThermobifidafuscaIP1* (Fig. 3, 4). There was gradual decrease in concentration of 7-KC from 1 $g.L^{-1}$ (initial concentration and reched to non

detectable concentration in 12 days). The strain IP1 cleared the 7KC 20% in 3 days, 85% in 6 days, 94% in 9 days and below detection levels in 12 days.

Discussion

Atherosclerosis, macular degeneration, and neurodegenerative diseases such as Alzheimer's disease, are associated with the intracellular accumulation of recalcitrant substances that impair cellular function and viability (Rittmann and Schloendorn, 2007). Reversing this accumulation may be a valuable therapy, but the accumulating substances are refractory to normal cellular catabolism so there is need of an exogenous enzyme supply to clear these accumulated compounds (de Grey *et al.*, 2005).

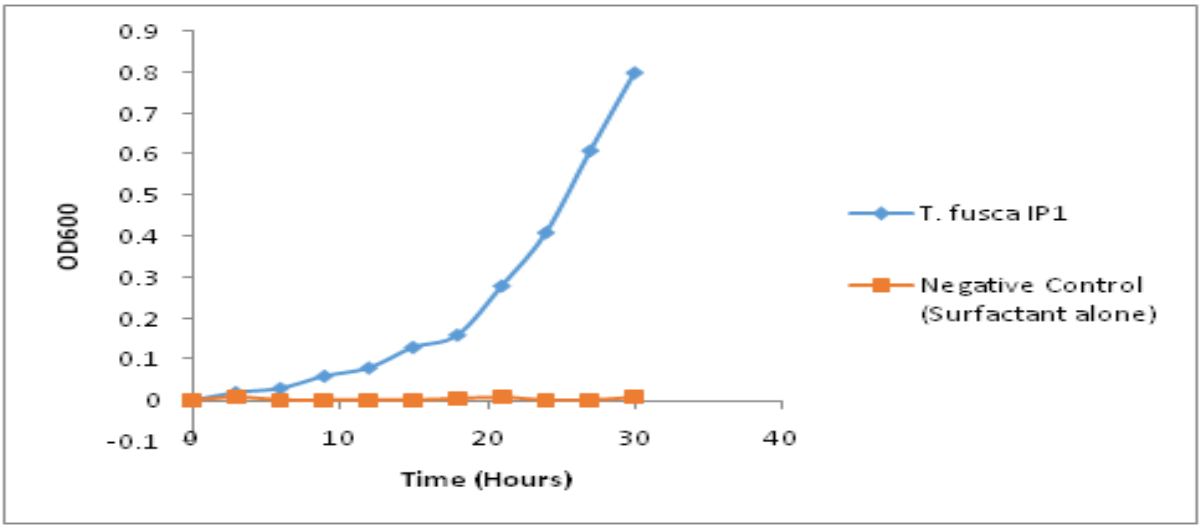

Fig. 2. Increase in OD600 of the isolate IP1 over a period of 30 hours. A negative control was run containing 7KC and surfactant only without IP1 inoculation.

On the other hand, these substances in ecosystem are naturally degraded in the soil and water by microorganisms. In environmental bioremediation, communities of microorganisms mineralize hydrophobic organics using a series of enzymes (Rittmann and Schloendorn, 2007). A radically new approach is augmenting humans' natural catabolic machinery with exogenous supply of microbial enzymes. Many recalcitrant organic molecules are naturally degraded in the soil. Since the soil in certain environments - graveyards, for example - is enriched in human remains but does not accumulate these substances, it presumably harbours microbes that successfully degrade and clear them up. The enzymes responsible could be identified and engineered to metabolise these substances *in vivo* (de Grey *et al.*, 2005).

Due to its high concentration in atherosclerotic plaques (Garcia-Cruset *et al.*, 2001; Dreizen *et al.*, 1978), cytotoxicity, and other pro-atherogenic properties, 7KC is a prominent target for medical bioremediation. Contributing to the rationale for its elimination is that 7KC has also been associated with Alzheimer's disease by several studies (Vaya and Schipper, 2007; Casserly and Topol, 2004; Carter, 2007). The etiology of atherosclerosis is clearly a complex process, and 7KC certainly contributes. Thus reducing levels of 7KC may subsequently reduce the rate of LDL uptake and apoptosis, slowing atherosclerotic progression (Mathieu *et al.*, 2009).

In medical bioremediation, we hope to utilize single or a combination of microbial enzymes to degrade the intracellular recalcitrant compounds and convert them to innocuous productsso that they can be cleared from the affected cells. Here, we present preliminary, but promising results for the bacterial biodegradation of 7-ketocholesterol, the main accumulator of foam cells associated with

atherosclerosis. In particular, we report on the isolation of bacterial strains found to effectively degrade 7-KC. All the isolates were capable of utilizing 7KC as the sole organic substrate, resulting in its mineralisation, a key step towards identifying the key enzymes that may lead to a therapy.

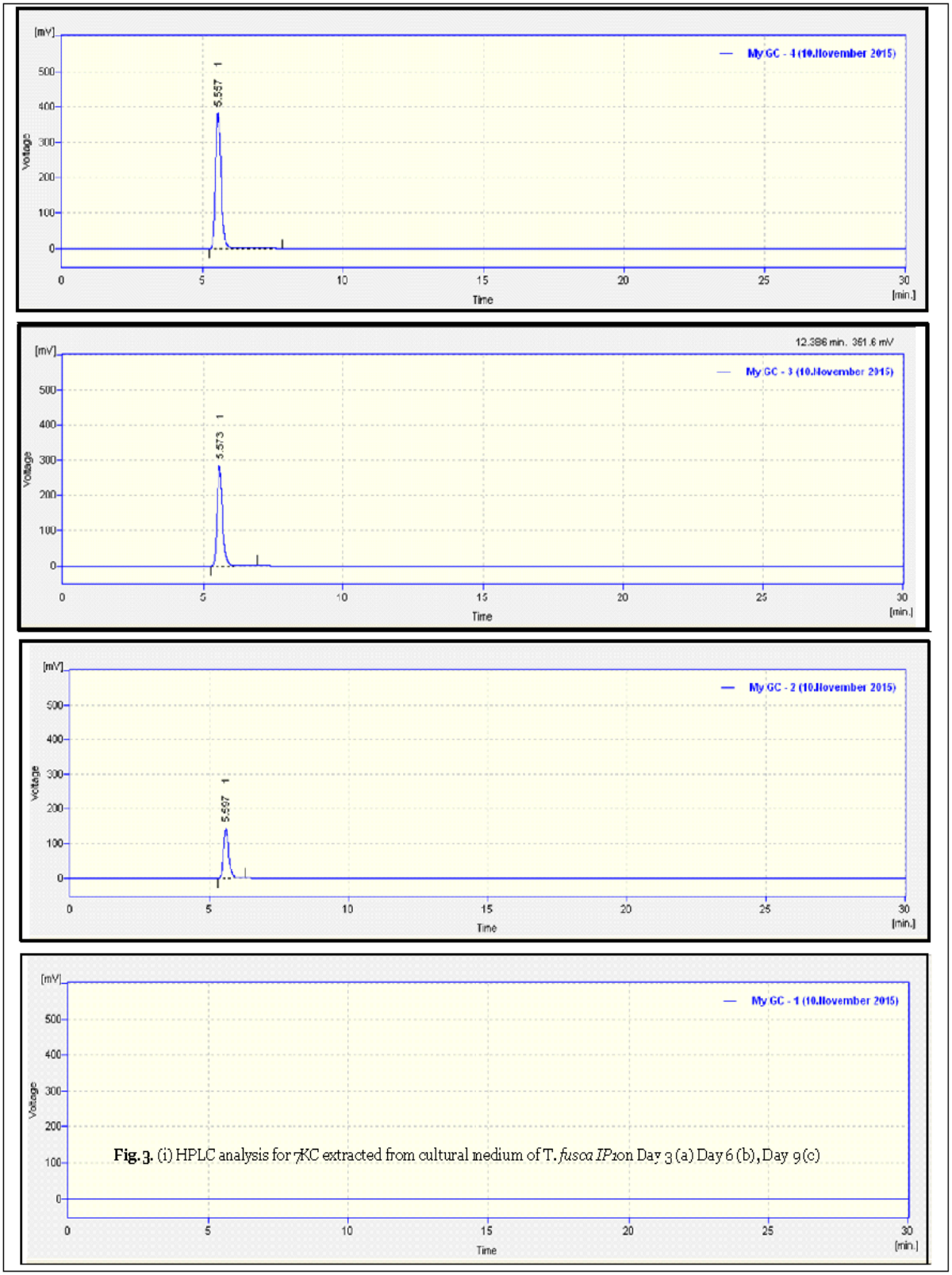

Fig. 3. (i) HPLC analysis for 7KC extracted from cultural medium of T. *fusca IP1*on Day 3 (a) Day 6 (b), Day 9 (c) and and Day 12 (d).

We got several positive hits for 7KC biodegradation from environmental samples. One of isolate IP1 was further investigated for its 7KC biodegradation activity, and was identified as Thermobifidafusca on the basis of 16srDNA sequencing results.Several steroid degrading bacteria have been isolated from soil samples previously (Merino *et al.*, 2013).On the basis of HPLC results, complete degradation was observed with isolate*Thermobifidafusca IP1*. The isolateremoved the 7KC below detection levels within 12 days.

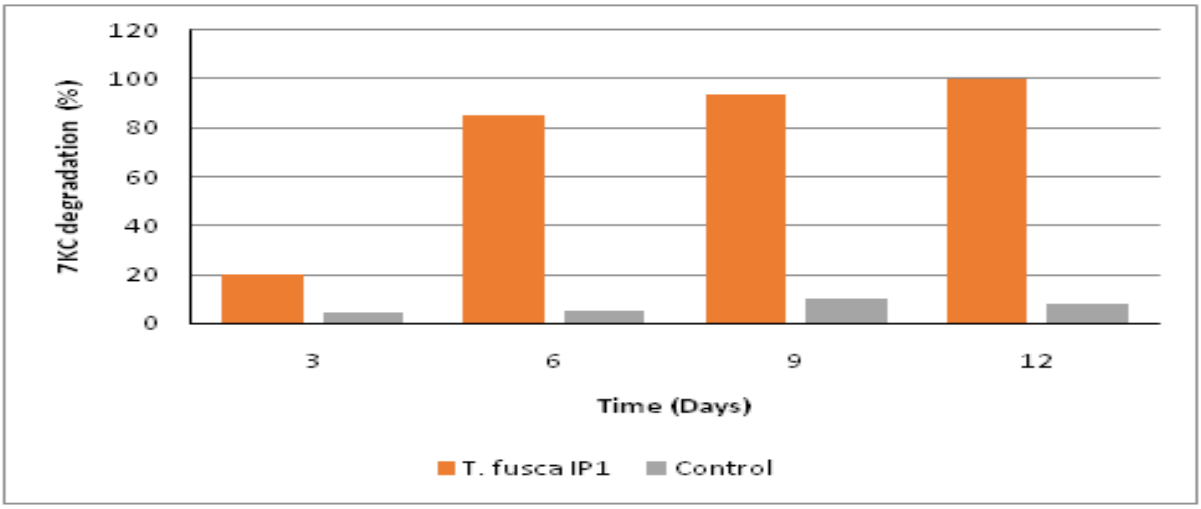

Fig. 4. HPLC analysis of 7KC degradation (experiment conducted at 30 °C, pH 7, 150 RPM) by environmental isolate, Thermobifidafusca IP1 in the 12-days incubation period. Three samples were run for isolate IP1 and the control.

7KC like Cholesterol is a steroid, i.e. a class of terpenoid lipids, an imbalance in their blood level causes serious diseases in humans.Bacterial degradation of steroids is widespread, many bacteria are capable of transforming steroids, and this property is used for the biotechnological production of steroid drugs (Bortolini*et al.*, 1997; Donova and Egorova, 2012; Mahato and Garai, 1997). In addition, bacteria from diverse phylogenetic groups can degrade naturally occurring steroids completely and use them as sources of carbon and energy (Holert *et al.*, 2013).

Bacteria belonging to the genera *Nocardia, Arthrobacter, Bacillus, Brevibacterium, Corynebacterium, Streptomyces, Microbacterium, Serratia, Achromobacter, Pseudomonas* or*Protaminobacter,* were reported to accomplish partial or complete cholesterol degradation (Whitmarsh, 1964; Brown and Peterson, 1966; Arima *et al.*, 1969; Ferreira and Tracey, 1984; Drzyzga *et al.*, 2009; 2011; Fernández de Las Heras*et al.*, 2009; Ge *et al.*, 2011).

Conclusion

In summary, we have found thatisolated bacterial strain can degrade the oxysterol 7KC. The isolate demonstrated ability to utilize 7KC as sole carbon source. Overall, these results support the notion that oxysterol levels might be controlled by biodegradation processes, and further investigation of specific microbial enzymes involved in catabolism as well as the specific pathways involved in microbial 7KC degradation can be the next goals leading to come up with identifying enzymes capable of transforming oxysterols for potential environmental, industrial, pharmaceutical, and medical applications.This attempt at harnessing an exogenous enzyme to achieve the catabolic function of cells is in some ways similar to enzyme replacement therapies aimed at reversing lysosomal storage disease.7-KC has been associated with numerous age-related and neurodegenerative diseases, and thus is an important therapeutic target. The biodegradation of 7KC and its

ultimate removal from body can serve as a mean to reverse disease conditions associated with its accumulation in body.

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References

Amaral J, Lee JW, Chou J, Campos MM, Rodrıguez IR. 2013. 7-Ketocholesterol induces inflammation and angiogenesis in vivo: a novel rat model. PLoS One **8(2),** e56099. http://dx.doi.org/10.1371/journal.pone.0056099.

Arima K, Nagasawa M, Bae M, Tamura G. 1969. Microbial transformation of sterols. Part I. Decomposition of cholesterol by microorganisms. Agricultural and Biological Chemistry **33,** 1636–1643. http://dx.doi.org/10.1080/00021369.1969.10859516

Berthier A, Lemaire-Ewing S, Prunet C, MonierS, Athias A, Bessede G, Pais de Barros JP, Laubriet A, Gambert P, Lizard G, Neel D. 2004.Involvement of a calcium-dependent dephosphorylation of BAD associated with the localization of Trpc-1 within lipid rafts in 7-ketocholesterol-induced THP-1 cell apoptosis. Cell Death and Differentiation 11**(8)**, 897-905. http://dx.doi.org/10.1038/sj.cdd.4401434

Bjo¨rkhem I, Cedazo-Minguez A, Leoni V, Meaney S. 2009. Oxysterols and neurodegenerative diseases. Molecular Aspects of Medicine **30,** 171–179. http://dx.doi.org/10.1016/j.mam.2009.02.001

Bortolini O, Medici A, Poli S. 1997. Biotransform ations on steroid nucleus of bile acids. Steroids **62,** 564–577. http://dx.doi.org/10.1016/S0039-128X(97)00043-3

Brown RL, Peterson GE. 1966. Cholesterol oxidation by soil Actinomycetes. Journal of General Microbiology **45,** 441–450. http://dx.doi.org/10.1099/00221287-45-3-441

Brown AJ, Jessup W. 1999. Oxysterols and atherosclerosis. Atherosclerosis **142(1),** 1–28. Review. http://dx.doi.org/10.1016/S0021-9150(98)00196-8

Brown AJ, Mander EL, Gelissen IC, Kritharides L, Dean RT, Jessup W. 2000. Cholesterol and oxysterol metabolism and subcellular distribution in macrophage foam cells. Accumulation of oxidized esters in lysosomes. The Journal of Lipid Research **41(2),** 226–237.

Carter CJ. 2007. Convergence of genes implicated in Alzheimer's disease on the cerebral cholesterol shuttle: APP, cholesterol, lipoproteins, and atherosclerosis. Neurochemistry International **50,** 12–38. http://dx.doi.org/10.1016/j.neuint.2006.07.007

Casserly I, Topol E. 2004. Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. Lancet **363,** 1139–1146. http://dx.doi.org/10.1016/S0140-6736(04)15900-X

Cox BE, Griffin EE, Ullery JC, Jerome WG. 2007. Effects of cellular cholesterol loading on macrophage foam cell lysosome acidification. The Journal of Lipid Research **48(5),** 1012–1021. http://dx.doi.org/10.1194/jlr.M600390-JLR200

De Grey AD. Alvarez PJ, Brady RO, Cuervo AM, Jerome WG, McCarty PL, Nixon RA, Rittmann BE, Sparrow JR. 2005. Medical bioremediation: prospects for the application of microbial catabolic diversity to aging and several major age-related diseases. Ageing Research Reviews **4(3),** 315-338. http://dx.doi.org/10.1016/j.arr.2005.03.008

Donova MV, Egorova OV. 2012. Microbial steroid transformations: current state and prospects. Applied Microbiology and Biotechnology **94,** 1423–1447.

Dreizen S, Stern MH, Levy BM. 1978. Diet-induced arteriopathies in the rabbit aorta and oral vasculature. Journal of Dental Research **57,** 412–417. http://dx.doi.org/10.1177/00220345780570024501

Drzyzga O, Navarro Llorens JM, Fernández de Las Heras L, GarcíaFernández E, Perera J. 2009. Gordoniacholesterolivorans sp. nov., a cholesterol-degrading actinomycete isolated from sewage sludge. International Journal of Systematic and Evolutionary Microbiology **59,** 1011–1015. http://dx.doi.org/10.1128/AEM.05149-11

Drzyzga O, Fernández de lasHeras L, Morales V, Navarro Llorens JM, Perera J. 2011. Cholesterol degradation by *Gordonia cholesterolivorans*. Applied and Environmental Microbiology **77,** 4802–4810. http://dx.doi.org/10.1128/AEM.05149-11

Dzeletovic S, Babiker A, Lund E, Diczfalusy U. 1995. Time course of oxysterol formation during in vitro oxidation of low density lipoprotein. Chemistry and Physics of Lipids **78,** 119–128. http://dx.doi.org/10.1016/0009-3084(95)02489-6

Fernández de Las Heras L, GarcíaFernández E, María Navarro Llorens J, Perera J, Drzyzga O. 2009. Morphological, physiological, and molecular characterization of a newly isolated steroid-degrading actinomycete, identified as *Rhodococcus*ruber strain Chol-4. Current Microbiology **59,** 548–553. http://dx.doi.org/10.1007/s00284-009-9474-z

Ferreira NP, Tracey RP. 1984. Numerical taxonomy of cholesterol-degrading soil bacteria. Journal of Applied Microbiology **57,** 429–446. http://dx.doi.org/10.1111/j.1365.2672.1984.tb01409.x

Fraldi A, Annunziata F, Lombardi A, Kaiser HJ, Medina DL, Spampanato C, Fedele AO, Polishchuk R, SorrentinoNC, Simons K, Ballabio A. 2010. Lysosomal fusion and SNARE function are impaired by cholesterol accumulation in lysosomal storage disorders. The EMBO Journal **29(21),** 3607–3620. http://dx.doi.org/10.1038/emboj.2010.237

Garcia-Cruset S, Carpenter KL, Guardiola F, Stein BK, Mitchinson MJ. 2001. Oxysterol profiles of normal human arteries, fatty streaks and advanced lesions. Free Radical Research **35,** 31–41. http://dx.doi.org/10.1080/10715760100300571

Ge F, Li W, Chen G, Liu Y, Zhang G, Yong B, Wang Q, Wang N, Huang Z, Li W, Wang J, Wu C, Xie Q, Liu G. 2011. Draft genome sequence of *Gordonianeofelifaecis* NRRL B-59395, a cholesterol-degrading actinomycete. Journal of Bacteriology **193,** 5045–5046. http://dx.doi.org/10.1128/JB.05531-11.

Holert J, Kulic Z, Yucel O, Suvekbala V, Suter MJ, Moller HM, Philipp B. 2013. Degradation of the acyl side chain of the steroid compound cholate in *Pseudomonas* sp. strain Chol1 proceeds via an aldehyde intermediate. Journal of Bacteriology **195,** 585–595. http://dx.doi.org/10.1128/JB.01961-12

Kaushik S, Massey AC, Cuervo AM. 2006. Lysosome membrane lipid microdomains: Novel regulators of chaperone-mediated autophagy. The EMBO Journal **25(17),** 3921–3933. http://dx.doi.org/10.1038/sj.emboj.7601283

Kostka JE, Prakash O, Overholt WA, Green SJ, Freyer G, Canion A, Delgardio J, Norton N, Hazen TC, Huettel M. 2011. Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the deepwater horizon oil spill. Applied and Environmental Microbiology **77(22),** 7962–7974. http://dx.doi.org/10.1128/AEM.05402-11

Leonarduzzi G, Sottero B, Poli G. 2002. Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review). The Journal of nutritional biochemistry **13(12),** 700-710.

http://dx.doi.org/10.1016/S0955-2863(02)00222-X

Lizard G, Monier S, Cordelet C, Gesquiere L, Deckert V, Gueldry S, Lagrost L, Gambert P. 1999. Characterization and comparison of the mode of cell death, apoptosis versus necrosis, induced by 7beta-hydroxycholesterol and 7-ketocholesterol in the cells of the vascular wall. Arteriosclerosis Thrombosis, and Vascular Biology **19,** 1190– 1200.
http://dx.doi.org/10.1161/01.ATV.19.5.1190

Lordan S, Mackrill JJ, O'Brien NM. 2009. Oxysterols and mechanisms ofapoptoticsignaling: implications in the pathology of degenerative diseases. The Journal of Nutritional Biochemistry **20,** 321–336.
http://dx.doi.org/10.1016/j.jnutbio.2009.01.001

Mahato SB, Garai S. 1997. Advances in microbial steroid biotransformation. Steroids **62,** 332–345.
http://dx.doi.org/10.1016/S0039-128X(96)00251-6

Maor I, Mandel H, Aviram M. 1995. Macrophage uptake of oxidized LDL inhibits lysosomalsphingomyelinase, thus causing the accumulation of unesterified cholesterol-sphingomyelin-rich particles in the lysosomes. A possible role for 7-Ketocholesterol. Arteriosclerosis Thrombosis, and Vascular Biology **15(9),** 1378–1387.
http://dx.doi.org/10.1161/01.ATV.15.9.1378

Mathieu JM, Schloendorn J, Rittmann BE, Alvarez PJ. 2009. Medical bioremediation of age-related diseases. Microbial Cell Factories **8,** 21.
http://dx.doi.org/10.1186/1475-2859-8-21

Mathieu JM, Wang F, Segatori L, Alvarez PJ. 2012. Increased resistance to oxysterol cytotoxicity in fibroblasts transfected with a lysosomally targeted *Chromobacterium* oxidase. Biotechnology and Bioengineering **109,** 2409–2415.
http://dx.doi.org/10.1002/bit.24506

Merino E, Barrientos A, Rodríguez J, Naharro G, Luengo JM, Olivera ER. 2013. Isolation of cholesterol- and deoxycholate-degrading bacteria from soil samples: evidence of a common catabolic steroid-degrading pathway involved in steroid assimilation. Applied Microbiology and Biotechnology **97,** 891–904.
http://dx.doi.org/10.1007/s00253-012-3966-7

Moreira EF, Larrayoz IM, Lee JW, Rodrı´guez IR. 2009. 7-Ketocholesterol is present in lipid deposits in the primate retina: potential implication in the induction of VEGF and CNV formation. Investigative Ophthalmology and Visual Science **50,** 523–532.
http://dx.doi.org/10.1167/iovs.08-2373

induce apoptosis of human vascular smooth muscle cells. Journal of Atherosclerosis and Thrombosis **13,** 256–262.
http://dx.doi.org/10.5551/jat.13.256

Poli G, Biasi F, Leonarduzzi G. 2013. Oxysterols in the pathogenesis of major chronic diseases. Redox Biology **31,** 125–130. Review.
http://dx.doi.org/10.1016/j.redox.2012.12.001

Rimner A, Al Makdessi S, Sweidan H, Wischhusen J, Rabenstein B, Shatat K, Mayer P, Spyridopoulos I. 2005. Relevance and mechanism of oxysterolstereospecifity in coronary artery disease. Free Radical Biology and Medicine **38(4),** 535-544.

Rittmann BE, Schloendorn J. 2007. Engineering away lysosomal junk: medical bioremediation. Rejuvenation Research **10,** 359–365.
http://dx.doi.org/10.1089/rej.2007.0594

Rodriguez IR, Alam S, Lee JW. 2004. Cytotoxicity of oxidized low-density lipoprotein in cultured RPE cells is dependent on the formation of 7-ketocholesterol. Investigative Ophthalmology and Visual Science **45,** 2830–2837.
http://dx.doi.org/10.1167/iovs.04-0075

Rodrı´guez IR, Larrayoz IM. 2010. Cholesterol

oxidation in the retina: implications of 7KCh formation in chronic inflammation and age-related macular degeneration. The Journal of Lipid Research **51,** 2847–2862. Review.
http://dx.doi.org/10.1194/jlr.R004820

Spyridopoulos I, Wischhusen J, Rabenstein B, Mayer P, Axel DI, Frohlich KU, KarschKR. 2001. Alcohol enhances oxysterol-induced apoptosis in human endothelial cells by a calcium-dependent mechanism. Arteriosclerosis, Thrombosis, and Vascular Biology **21(3),** 439-444.
http://dx.doi.org/10.1161/01.ATV.21.3.439

vanReyk DM, Brown AJ, Hult'en LM, Dean RT, Jessup W. 2006. Oxysterols in biological systems: sources, metabolism and pathophysiological relevance. Redox Report **11,** 255–262. Review.
http://dx.doi.org/10.1179/135100006X155003

Vaya J, Schipper HM. 2007. Oxysterols, cholesterol homeostasis, and Alzheimer disease. Journal of Neurochemistry **102,** 1727–1737.
http://dx.doi.org/10.1111/j.1471-4159.2007.04689.x.

Vejux A, Malvitte L, Lizard G. 2008. Side effects of oxysterols: cytotoxicity, oxidation, inflammation, and phospholipidosis. Brazilian Journal of Medical and Biological Research **41,** 545–556.
http://dx.doi.org/10.1590/S0100879X2008000700001

Vejux A, Lizard G. 2009. Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. Molecular Aspects of Medicine **30,** 153–170. Review.
http://dx.doi.org/10.1016/j.mam.2009.02.006

Wang SF, Chou YC, Mazumder N, Kao FJ, Nagy LD, Guengerich FP, Huang C, Lee HC, Lai PS, Ueng YF. 2013. 7-Ketocholesterol induces P-glycoprotein through PI3K/mTORsignaling in hepatoma cells. Biochemical Pharmacology **86,** 548–560.
http://dx.doi.org/10.1016/j.bcp.2013.06.006

Whitmarsh JM. 1964. Intermediates of microbiological metabolism of cholesterol. Biochemical Journal **90,** 23-24.