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Insights into the microbial production of 1-alkenes using *Jeotgalicoccus* sp. ATCC 8456 as model organism

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AFFIDAVIT

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Abbreviations

10:0	decanoic acid
12:0	dodecanoic acid
20:0	eicosanoic acid
16:0	hexadecanoic acid
6:0	hexanoic acid
9:0	nonanoic acid
8:0	octanoic acid
14:0	tetradecanoic acid
11:0	undecanoic acid
1-C10	1-decene
1-C12	1-dodecene
1-C20	1-eicosene
1-C17	1-heptadecene
1-C7	1-heptene
1-C16	1-hexadecene
1-C6	1-hexene
1-C19	1-nonadecene
1-C9	1-nonene
1-C18	1-octadecene
1-C8	1-octene
1-C15	1-pentadecene
1-C5	1-pentene
1-C14	1-tetradecene
1-C13	1-tridecene
1-C11	1-undecene
A	adenine (in the form of deoxyadenosine triphosphate)
aa	amino acid(s)
ACP	acyl carrier protein
ATCC	American Type Culture Collection
BASys	Bacterial Annotation System
BLASTn	Basic Local Alignment Search Tool for nucleotide sequences
BLASTp	Basic Local Alignment Search Tool for protein sequences
bp	base pair(s)
C	cytosine (in the form of deoxycytidine triphosphate)
C_x	carbon atom at a certain position

C=C	carbon-carbon double bond
CoA	coenzyme A
CPX	Czapek-Dox medium
ddH_2O	deionized and sterilized water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphate
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
e.g.	*exempli gratia* (for example)
EI	electron ionization
et al.	*et alii*, *et aliae* (and others)
G	guanine (in the form of deoxyguanosine triphosphate)
GC-MS	gas chromatography-mass spectrometry
H_2O	water
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HGAP	Hierarchical Genome Assembly Process
i.e.	*id est* (that is)
K_2HPO_4	dipotassium hydrogen phosphate
KAAS	KEGG Automatic Annotation Server
kbp	kilo base pairs
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	KEGG orthology identifier(s)
logP	partition coefficient
log-phase	exponential growth phase
MB	marine broth
MW	molecular weight
NaCl	sodium chloride
NB	nutrient broth
NCBI	National Center for Biotechnology Information
O_2	molecular oxygen
$O_2^{\bullet-}$	superoxide (anion)
OD_{600}	optical density measured at a wavelength of 600 nm
OF reaction	oxidative/fermentation test using Hugh-Leifson medium
$OH^{\bullet}$	hydroxyl radical
ORF(s)	open reading frame(s)

P. aeruginosa	*Pseudomonas aeruginosa*
PCR	polymerase chain reaction
PDA	potato dextrose agar
PDMS	polydimethylsiloxane
RI	retention index
rpm	rounds per minute
SD	standard deviation
sp.	species
SPME	solid phase microextraction
spp.	*species pluralis* (more than one species)
T	thymine (in the form of [deoxy]thymidine triphosphate)
TAE	TRIS acetate EDTA
TCVA(s)	two clamp volatile organic compounds assay(s)
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
TSBYE	tryptic soy broth yeast extract
UV/Vis	ultraviolet/visible
VOC(s)	volatile organic compound(s)
v/v	volume per volume
w/v	weight per volume

Abstract

1-Alkenes, also called terminal olefins or α-olefins, contain a carbon-carbon double bond at position C_1. They are of great industrial interest, as they serve as building blocks for polymers, drop-in biofuels, detergents, lubricants and are even applied in the manufacture of fine chemicals and pharmaceuticals. Naturally, 1-alkenes aid in survival and defense strategies of organisms due to their hydrophobic properties. Not only a broad variety of microorganisms, including *Jeotgalicoccus* sp. ATCC 8456, but also eukaryotes like insects or plants are able to produce terminal olefins.

Jeotgalicoccus sp. ATCC 8456 employs the cytochrome P450 monooxygenase of the family 152 (CYP152) $OleT_{JE}$, which requires either O_2 with additional redox mediator proteins or H_2O_2, to catalyze the decarboxylation of fatty acids to the corresponding C_{n-1} 1-alkenes. In this study this bacterium was used as a model organism to gain valuable insights into the formation of terminal olefins and their possible biological role.

Comprehensive knowledge about the growth behavior of *Jeotgalicoccus* sp. ATCC 8456 was obtained first. It was found that the bacterium is halotolerant, facultative-anaerobe, non-motile, and cannot utilize glucose as sole carbon source. Furthermore, strain ATCC 8456 displayed a very long generation time (> 2 h), even in the favored medium at 26 °C, where the highest cell densities were obtained. This might represent a challenge for optimized industrial applications. As the model organism is not able to survive at 37 °C, it is assumed to be not human-pathogenic.

Quantification of 1-alkenes under standard conditions using SPME GC-MS resulted in the identification of 1-C11, 1-C13, 1-C15, 1-C17, and 1-C19 in concentrations ranging from two to 350 nM, showing a preference for longer-chains.
Further, it was investigated if increased salinity or feeding with fatty acids could cause a shift in the production of 1-alkenes. Fatty acids are considered as precursors of α-olefin formation by *Jeotgalicoccus* sp. ATCC 8456. Whole genome sequencing allowed reconstruction of the fatty acid biosynthesis pathway. Whereas a correlation between salt concentration and terminal alkene formation could not be detected, feeding with fatty acids induced and enhanced the *in vivo* production of the expected C_{n-1} α-olefins.

Also, the probable redox chain of the 1-alkene synthesis was located within the genome, including $OleT_{JE}$, two ferredoxin reductases, a ferredoxin, as well as a paralogue monooxygenase of $OleT_{JE}$ that shares only 38% sequence similarity.

Additional genes responsible for detoxification of reactive oxygen species and osmotolerance could be deciphered, which might be involved in the regulation of the reaction mechanism of $OleT_{JE}$.

Due to the increasing importance of 1-alkenes, a SPME GC-MS based screening was conducted in order to expand the spectrum of terminal olefin producers.
Seven *Pseudomonas* spp. and one *Bacillus* sp. derived from the in-house strain collection were positively identified, with 1-C11 being the most predominant terminal olefin. 1-C11 as well as 1-C12 were found to impede fungal phytopathogens in previous research.

For evaluation of beneficial applications in environmental biotechnology, analytically pure 1-alkene standards (*in vitro*) and *Jeotgalicoccus* sp. ATCC 8456 (*in vivo*) were tested against a selection of bacterial and fungal plant pathogens using the two clamp volatile organic compounds assay. However, under the tested conditions, no inhibitory effect could be observed.

The obtained insights into the 1-alkene producer *Jeotgalicoccus* sp. ATCC 8456 are very valuable to understand and expand its application in biotechnology. Further evaluation of the 1-alkene forming enzymes concerning their catalytic mechanism and substrate scope is necessary to exploit their full potential for successful implementation in industrial large scale processes.

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1 Introduction

1.1 1-Alkenes

1-Alkenes, also called terminal (unsaturated) olefins or α-olefins [Fig 1] consist chemically of an aliphatic carbon-chain of various length, starting from ethylene, with a carbon-carbon double bond at position C_1 (C_α) [Hellwinkel 2006].

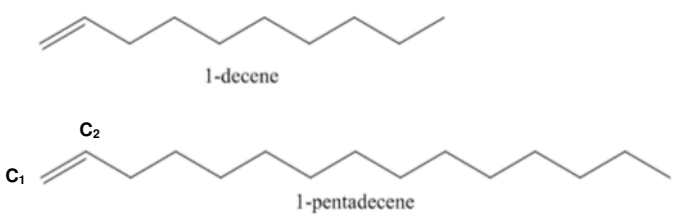

Fig 1. Two examples of 1-alkenes. 1-Decene (ten carbon atoms) and 1-pentadecene (15 carbon atoms) are shown, both containing a C=C between the carbon atoms 1 and 2.

1.1.1 Industrial importance of 1-alkenes

1-Alkenes, especially short-chained i.e. ethylene, propene and 1-butene [Dennig et al. 2016] are highly important primary building blocks for the industrial production of polymers e.g. plastic or drop-in biofuels [Schirmer et al. 2010]. Long-chain α-olefins serve as basis for lubricants or detergents like softeners [van der Klis et al. 2012]. Further, terminal olefins are also applied in the manufacture of fine chemicals, such as intermediates of pharmaceuticals or for fragrances [Sun et al. 2018]. In total, they are produced on a 100 000 t scale per year [Kourist 2015].

For this purpose, 1-alkenes are almost exclusively obtained from fossil based oil and gaseous resources. Together with the huge energy demands required (temperature and pressure) [Dennig et al. 2016], the complex multi-step processing, and thereby the need of rare or toxic metals e.g. nickel or rhenium [Keim 2013] severe environmental concerns appear. Besides, the selective terminal positioning of the alkene double bonds is challenging using chemical methods, as they tend to migrate within the molecule [Wang et al. 2013].

Biotechnological approaches provide a promising alternative for accessing α-olefins with respect to sustainability, e.g. using renewable feedstocks like fatty acids [Liu et al. 2014], or applying the generally milder production conditions for the selective formation of the double bond [Faber 2011].

1.1.2 Biological role of terminal unsaturated olefins

The biosynthesis of different hydrocarbons is found in nature among a broad variety of (micro)organisms employing diverse mechanisms and genes suggesting to be of ancient origin [Wackett and Wilmot 2015].
Although 1-alkenes are produced by numerous organisms [Gehlsen et al. 2009], the production level is typically relatively low, especially when compared to other hydrocarbons [Rui et al. 2015].

α-Olefins might be important substances that aid in survival of the producing organism e.g. providing protection against predators or an advantage for colonization of certain niches and habitats. Due to their hydrophobic properties, 1-alkenes are presumed to serve in very different ways: as surfactants assisting in the formation of a film of defensive secretes on the predator, as pheromones for marking of food sources or territory, and also as solvents for molecules like quinones [Gehlsen et al. 2009]. They have been not only found in insects like beetles [Dettner 1987] or bugs [Krall et al. 1999], but also in (higher) plants [Ney and Boland 1987].

The role of terminal olefins formed by prokaryotes is relatively unknown, despite there is evidence of similar functionalities as for other organisms. For example, 1-alkenes are present in the mixture of VOCs produced by *Pseudomonas* [Sheoran et al. 2015] or *Bacillus* [Munjal et al. 2016], which are used as biocontrol agents for plant protection against pathogens.
Other found bacterial 1-alkene producers are e.g. *Jeotgalicoccus* [Rude et al. 2011], *Micrococcus* [Beller et al. 2010], *Shewanella* and *Xanthomonas* [Sukovich et al. 2010] or cyanobacteria from the genus *Synechococcus* [Winters et al. 1969].

1.1.3 Metabolic routes of bacteria for the formation of (terminal) alkenes from fatty acid derivatives

Fatty acids are of great interest as precursors, for instance for advanced biofuels due to their high energy density as the carbon atoms are mainly present in low oxidation states [Dennig et al. 2016]. However, they cannot be applied directly [Torto-Alalibo et al. 2014]. It was demonstrated that fatty acids are taken by prokaryotes as one of the starting molecules for the formation of 1-alkenes [Wang et al. 2018].

To avoid competition with agricultural land needed for food and feed production, waste oil [Wang and Zhu 2018] or waste plant material, e.g. cellulose [Janßen and Steinbüchel 2014], can be used as feedstock for the (microbial) generation of (free) fatty acids.

There are three precursors for the biosynthesis of alkanes and alkenes, namely fatty acyl-acyl carrier protein (ACP)/acyl-coenzyme A (CoA), fatty acids, and in most cases fatty aldehydes [Wang and Zhu 2018]. Altogether, four possible routes using different enzymes for the production of 1-alkenes have been described [Du et al. 2017] [Fig 2].

The two step process derived from cyanobacteria implements fatty acyl starting molecules (acyl-acyl carrier protein reductase AAR, and aldehyde deformylating oxygenase ADO) to gain the corresponding C_{n-1} terminal alkenes [Herman and Zhang 2016].

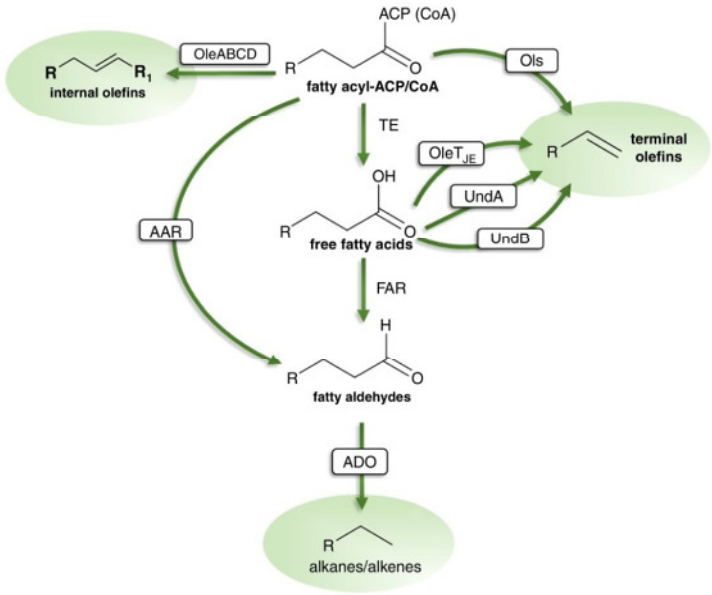

Fig 2. Simplified bacterial biosynthesis of hydrocarbons. Modified from [Herman and Zhang 2016]. 1-alkenes are synthesized e.g. by cyanobacterial AAR/ADO, UndA and UndB found in *Pseudomonas*, CurM/Ols from *Synechococcus* sp. PCC 7002 or $OleT_{JE}$ which was identified in *Jeotgalicoccus* sp. ATCC 8456. Thereby, the enzymes employ different catalytic mechanisms, also resulting in terminal olefins of different carbon chain length.

Other systems leading to α-olefins are found in *Pseudomonas* (fatty acid decarboxylase UndA, desaturase-like enzyme UndB) [Wang et al. 2018] or *Jeotgalicoccus* sp. ATCC 8456 (CYP152 family-belonging P450 fatty acid decarboxylase $OleT_{JE}$), both relying on the decarboxylation (UndA, $OleT_{JE}$) [Lee et al. 2018] or oxidation (UndB) [Rui et al. 2015] of free fatty acids.

In the cyanobacterium *Synechococcus* sp. PCC 7002 another pathway for 1-alkene formation was identified (type I polyketide synthase-like enzyme CurM/Ols) that uses fatty acyl substrates which are elongated and decarboxylated [Mendez-Perez et al. 2011].

All of the named enzymes favor the production of terminal olefins with different carbon chain lengths, presumably providing an advantage in tailor-made synthesis for certain application tasks. It was found that $OleT_{JE}$, CurM/Ols, as well as UndB are able to build 1-alkenes of medium-chain (C6 to C12) and long-chain length (≥ C12) [Lee et al. 2018], whereas the activity of UndA results in medium chain length (C9 to C13) [Rui et al. 2014].

1.2 Model organism *Jeotgalicoccus* sp. ATCC 8456

1.2.1 Discovery of *Jeotgalicoccus* species and their characteristics

Jeotgalicoccus species are gram positive, mostly facultative anaerobic bacteria belonging together with *Macrococcus*, *Staphylococcus* and *Salinicoccus* monophyletic to the family of Staphylococcaceae [Fig 3], therefore the cells are coccoid [Boone et al. 2001].

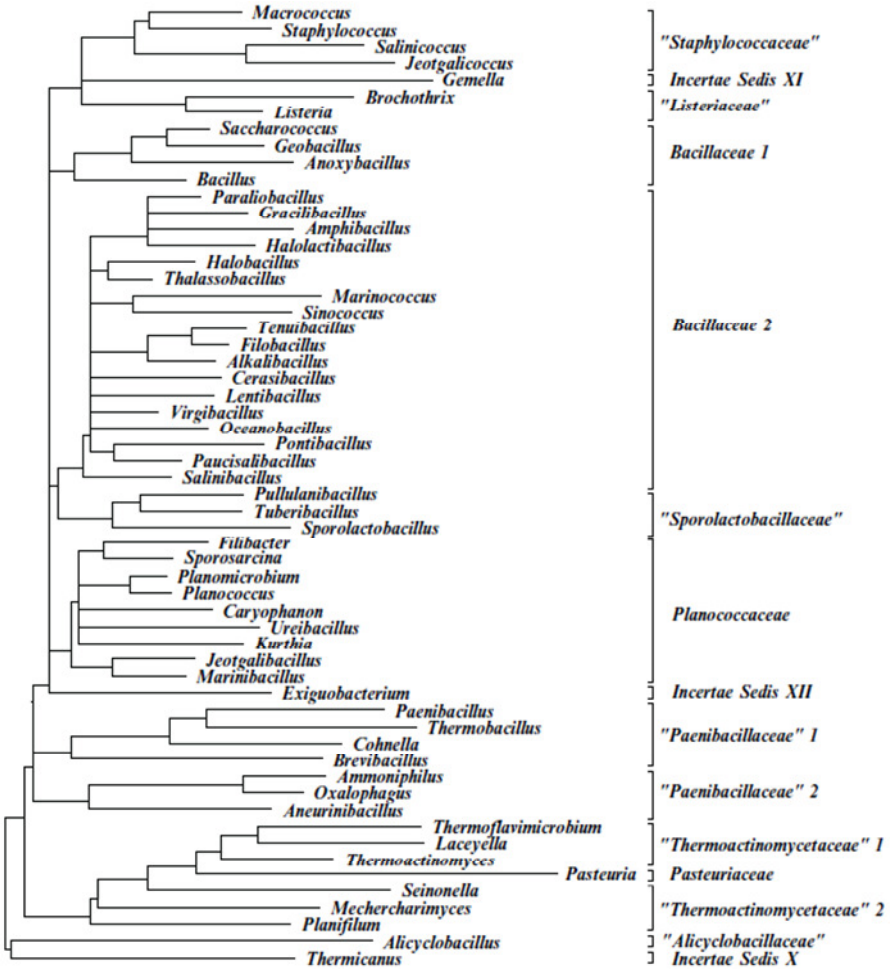

Fig 3. Phylogenetic relationship of the order of *Bacillales*. *Jeotgalicoccus* belongs to the family of Staphylococcaceae within the phylum of Firmicutes [Boone et al. 2001].

The genus was named after the source where they were first isolated: the Korean fermented fish sauce “jeotgal” (*Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus* [Yoon 2003]).
Many representatives are halotolerant to halophile [Boone et al. 2001]. Halotolerant microorganisms can grow at ionic concentrations higher than needed for growth (≥ 0.89% [w/v]), while halophilic prokaryotes require salt for survival (ca. 1 to 30% [w/v]) [Gargaud et al. 2015].

Afterwards, different *Jeotgalicoccus* spp. were found in various environmental habitats like seaside (*Jeotgalicoccus huakuii* [Guo et al. 2010]) and intertidal soil (*Jeotgalicoccus nanhaiensis* [Liu et al. 2011b]), salt lakes (*Jeotgalicoccus halophilus* [Liu et al. 2011a]), residential Arabian areas (*Jeotgalicoccus saudimassiliensis* [Papadioti et al. 2017] or in the exhaust air of a poultry house (*Jeotgalicoccus coquinae* and *Jeotgalicoccus aerolatus* [Martin et al. 2011]) and a pig barn (*Jeotgalicoccus schoeneichii* [Glaeser et al. 2016]. They were also isolated from (sea-related) animals e.g. a sea urchin (*Jeotgalicoccus marinus* [Chen et al. 2009]) or the southern sea elephant (*Jeotgalicoccus pinnipedialis* [Hoyles 2004]). Until now, this genus is relatively uncharacterized, also due to the fact that it has often been confused with *Staphylococci* and *Salinicocci* species [Schwaiger et al. 2010].

Today 12 strains of *Jeotgalicoccus* are known, including *Jeotgalicoccus* sp. ATCC 8456, whereby only the genome of *Jeotgalicoccus saudimassiliensis* was fully sequenced. For the strains *Jeotgalicoccus halophilus*, *Jeotgalicoccus halotolerans*, *Jeotgalicoccus marinus* as well as *Jeotgalicoccus psychrophilus* shotgun sequences are present in the database [Geer et al. 2010]. Besides, four *Jeotgalicocci* (*Jeotgalicoccus halotolerans* JCM5429, *Jeotgalicoccus huakuii* JCM 8176, *Jeotgalicoccus psychrophilus* JCM 5429 [Nusantara Putra et al. 2019], and also *Jeotgalicoccus* sp. ATCC 8456) are studied with respect to terminal unsaturated olefin production.

The strain *Jeotgalicoccus* sp. ATCC 8456 naturally synthesizes 1-alkenes employing the enzyme $OleT_{JE}$ [Rude et al. 2011]. Hence, it is perfectly suited to serve as model microorganism for further investigation of prokaryotic synthesis routes of terminal olefins.

At first, *Jeotgalicoccus* sp. ATCC 8456 was designated as *Micrococcus candicans*, but displayed a different alkene production pattern as well as a much lower GC-content compared to other *Micrococcus* spp. [Morrison et al. 1971]. Therefore, a misclassification was finally assumed [Rude et al. 2011].

1.2.2 Classification and basic chemical mechanism of $OleT_{JE}$

Non-heme iron (II)-dependent monooxygenases e.g. UndA [Rui et al. 2014] and to the CYP152 family-belonging heme-containing P450 monooxygenases e.g. $OleT_{JE}$ [Munro et al. 2018] are enzymes that have been identified to be responsible for 1-alkene production.

$OleT_{JE}$ is expressed in *Jeotgalicoccus* sp. ATCC 8456 and was the first enzyme reported to be able to catalyze the oxidative decarboxylation of fatty acids yielding C_{n-1} terminal unsaturated olefins [Rude et al. 2011] [Fig 4].
As a cytochrome P450 monooxygenase of the family 152 (CYP152) [Hammerer et al. 2018], $OleT_{JE}$ can use H_2O_2 as oxidant [Wise et al. 2017]. If the P450 reacts with H_2O_2 as sole electron and oxygen donor, the oxidation of the substrate through formation of the reactive iron-oxo species (compound 0) is directly induced. This mechanism is called the "peroxide shunt" [Belcher et al. 2014]. Therefore, electrons from NAD(P)H are not required. Peroxygenases of the bacterial CYP152 family use this pathway [McLean and Munro 2016].

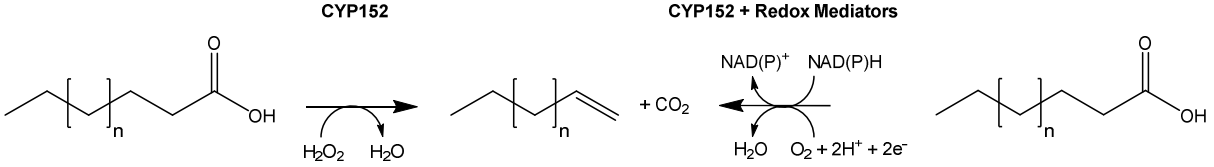

Fig 4. Catalytic reaction of the fatty acid decarboxylase from *Jeotgalicoccus* sp. ATCC 8456 ($OleT_{JE}$). $OleT_{JE}$ is a CYP152 family-belonging heme-containing P450 monooxygenase. Oxidative decarboxylation of (saturated) fatty acids to C_{n-1} terminal alkenes is shown. The catalysis is either performed with H_2O_2 or O_2 as oxidant. The latter requires additional redox mediator proteins (ferredoxin reductase and ferredoxin).

Although, $OleT_{JE}$ was classified as CYP152 peroxygenase [Rude et al. 2011], several doubts occurred as the enzyme shows poor stability at comparable low concentrations of H_2O_2 and is additionally more active using the milder oxidant O_2. Furthermore, $OleT_{JE}$ accepts various redox mediator proteins e.g. CamA (ferredoxin reductases) and CamB (ferredoxin) [Dennig et al. 2015].

The O_2-dependent oxidative decarboxylation of fatty acids follows the natural mechanisms of a cytochrome P450 monooxygenase, where redox partners (ferredoxin reductase and ferredoxin) transfer the electrons from NAD(P)H to the P450 protein [Faber 2011].

Despite that the natural reaction mechanism of $OleT_{JE}$ is not fully solved, enzymes employing fatty acids as substrate boast the shortest route for 1-alkene formation [Du et al. 2017].

Hence, together with UndA/UndB, $OleT_{JE}$ represents a promising tool for sustainable production of terminal olefins at industrial relevant scale [Wang and Zhu 2018].

1.3 Fatty acid synthesis and elongation

Since fatty acids are main precursors of 1-alkenes it is essential to understand the fatty acid synthesis in bacteria. The type II fatty acid synthase system (FAS II) is highly conserved among bacteria [Cronan and Thomas 2009]. It is responsible for the fatty acid synthesis in the cytoplasm.

Thereby, irreversible carboxylation of acetyl-CoA to the building block of fatty acids, malonyl-CoA via the acetyl-CoA carboxylase complex AccABCD [Fig 5] initiates the biosynthesis [Yao and Rock 2017b].

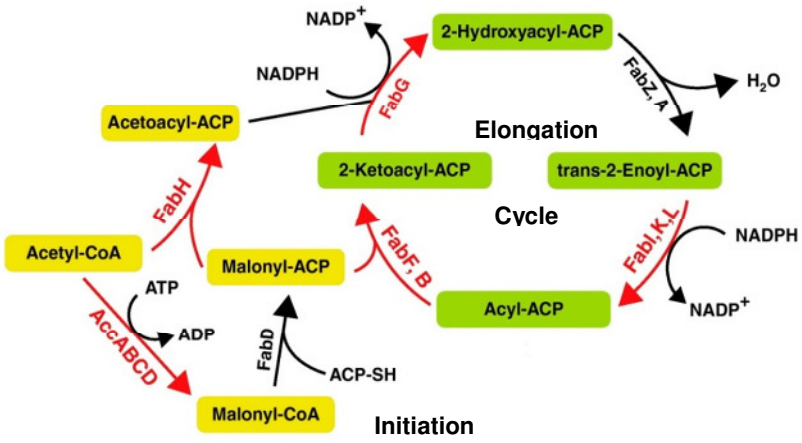

Fig 5. Bacterial fatty acid synthesis. Basic steps of the initiation pathway as well as the elongation cycle are shown [Parsons and Rock 2011]. Acetyl-CoA is carboxylated to malonyl-CoA (AccABCD). Malonyl-CoA is transferred to the ACP (FabD). Under emission of CO_2, malonyl-ACP condenses with another acetyl-CoA (FabH). Thereby, acetoacetyl-ACP (β-ketoacyl-ACP for further elongation cycles) is generated. Acetoacetyl-ACP (β-ketoacyl-ACP) is reduced to β-hydroxyacyl-ACP (FabG). Dehydration leads to *trans*-2-enoyl-ACP (FabA, FabZ) and subsequent reduction to acyl-ACP (FabI, FabK, FabL, FabV). For elongation, malonyl-ACP is added to acyl-ACP (FabB, FabF). There are rate limiting steps (highlighted in red) and equilibrium driven enzymatic conversions (highlighted in black).

Metabolically, acetyl-CoA derives mainly under aerobic conditions from pyruvate (pyruvate dehydrogenase PDH) through decarboxylation. Under anaerobic conditions acetyl-CoA is formed either non-oxidatively (pyruvate formate lyase PFL) or oxidatively (pyruvate synthase, pyruvate ferredoxin oxidoreductase PFOR) from pyruvate. Other routes are the activation of acetate to acetyl-CoA, or the degradation (β-oxidation) of fatty acids [Krivoruchko et al. 2015].

Next, malonyl-CoA is transferred to the malonyl-acyl carrier protein (malonyl-CoA:ACP transacylase FabD). Thereby, coenzyme A is cleaved off [Torto-Alalibo et al. 2014]. The acyl carrier protein is the key functionality of the fatty acid synthesis, as it binds all formed intermediates covalently via a thioester linkage [Cronan and Thomas 2009].

Subsequently, the malonyl-ACP is condensed with another acetyl-CoA (β-ketoacyl-ACP synthase III FabH) to form acetoacetyl-ACP under emission of CO_2 [Yao and Rock 2017a].

Under the consumption of one NADPH [Torto-Alalibo et al. 2014], the acetoacetyl-ACP (β-ketoacyl-ACP in all further elongation cycles) is reduced to β-hydroxyacyl-ACP (β-ketoacyl-ACP reductase FabG). The latter is then further dehydrated to *trans*-2-enoyl-ACP (β-hydroxyacyl-ACP dehydratase FabA, FabZ) [Yao and Rock 2017b].

Expending another NAD(P)H [Torto-Alalibo et al. 2014], *trans*-2-enoyl-ACP is reduced to acyl-ACP, therefore different isoenzymes exist (enoyl-ACP reductase FabI, FabK, FabL, FabV) [Rock and Jackowski 2002].

Successive elongation to the corresponding fatty acids is achieved, as acyl-ACP can be extended by two carbon atoms per cycle through condensation with malonyl-ACP (β-ketoacyl-ACP synthase II FabB, FabF), whereby β-ketoacyl-ACP is formed, leading to the next round of elongation [Yao and Rock 2017b].

1.4 Microbial defense strategies against reactive oxygen species

As $OleT_{JE}$ has been reported and characterized as a peroxygenase, employing hydrogen peroxide (H_2O_2) for the catalytic formation of 1-alkenes [Rude et al. 2011], it is of interest to understand which strategies are developed in bacteria to withstand oxidative stress.

Reactive oxygen species like hydrogen peroxide, superoxide ($O_2^{\bullet -}$), or hydroxyl radical ($OH^{\bullet}$) [Fu et al. 2015] are a consequence of aerobic life [Hillion and Antelmann 2015], but they are damaging to cells i.e. to nucleic acids, proteins, as well as membranes. Therefore, organisms (here bacteria) have evolved many different mechanisms to neutralize them [Ricci et al. 2002].
Reactive oxygen species occur endogenously during the autoxidation of redox enzymes and interfere not only with the ion metabolism, but also with ion-containing enzymes [Imlay 2015].

Ions are substantial for growth of almost all bacteria and also for the function of e.g. heme- or iron-sulfur cluster proteins [Fillat 2014].
The ion metabolism and uptake is controlled by the Fur family proteins that sense iron (Fur), zinc (Zur), manganese (Mur), nickel (Nur), heme (Irr) and peroxide (PerR).

Exogenously reactive oxygen species are also produced by lactic acid bacteria or plant as defense mechanism against microorganisms [Fu et al. 2015].

In Firmicutes, the main "peroxide-sensing" transcription factor is PerR which belongs to the ferric uptake regulator proteins (Fur) [Ruhland and Reniere 2019]. In its active state, it is bound to the DNA, thereby repressing the transcription of target genes involved in H_2O_2 resistance like those coding for peroxiredoxin (*ahpCF*), catalase (*katA*), miniferritin (*mrgA*), the heme biosynthesis operon (*hemAXCDBL*), the iron-uptake repressor (*fur*) or the zinc uptake system (*zosA*).
As PerR contains two metal-binding sites that define the activity of the transcription factor (structural Zn^{2+} and regulatory Fe^{2+}/Mn^{2+} site) [Hillion and Antelmann 2015], it shows the direct interconnection between oxidative stress response and metal homeostasis.

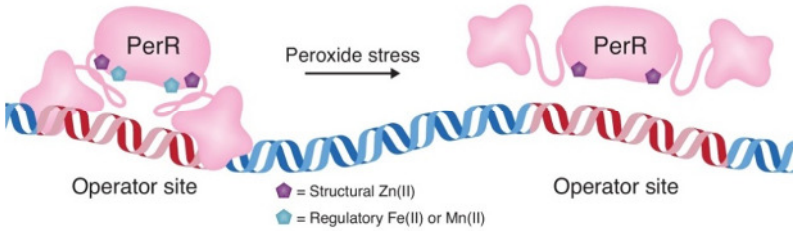

Fig 6. The transcriptional repressor PerR senses peroxide stress by metal-catalyzed histidine oxidation [Ruhland and Reniere 2019]. Peroxide inactivates PerR and induces the expression of the regulon.

If the peroxide concentration in the cytoplasm rises, an iron-mediated oxidation of a histidine residue of PerR occurs [Kim et al. 2017]. In consequence PerR dissociates, is degraded, and the expression of the regulon (independent genes) induced, activating the production of the peroxide resistance genes [Ruhland and Reniere 2019].

Superoxide dismutase (SOD) and catalase are other enzymes involved in peroxide resistance [Storz et al. 1990]. They are the first instance that eliminates $O_2^{\bullet-}$ species, which are typically generated endogenously as they cannot enter the cells due to their charge [Imlay 2015]. The $O_2^{\bullet-}$ is converted to H_2O_2 and molecular oxygen by the superoxide dismutase and hydrogen peroxide is further detoxified to H_2O and O_2 by the catalase [Lushchak 2011].

1.5 Aim of this thesis

The main goal of this study was the investigation of the microbial production of 1-alkenes and the screening for new producers. To gain a comprehensive insight, not only into the bacterial formation of α-olefins, but also into their ecological function, *Jeotgalicoccus* sp. ATCC 8456 was characterized as a model microorganism.

In order to receive comprehensive knowledge about the growth behavior of *Jeotgalicoccus* sp. ATCC 8456, the preference for certain growth conditions, regarding temperature, media composition, and salinity needed to be evaluated.

Since little information on *Jeotgalicoccus* sp. ATCC 8456 is available, a metabolic characterization was pursued. Proper assays for characterization were established to analyze oxygen tolerance, motility, as well as glucose utilization as sole carbohydrate source, and the existence of key enzymes like catalase and cytochrome c oxidase.

It was hypothesized, that certain abiotic factors like the salt concentration in the growth medium or the availability of fatty acids could shift the production of terminal unsaturated olefins in *Jeotgalicoccus* sp. ATCC 8456.

Therefore, the 1-alkene formation of the model organism was quantified not only under standard conditions, but also during induction with fatty acids using SPME GC-MS.

Furthermore*, Jeotgalicoccus* sp. ATCC 8456 was cultivated with increasing salinity and the α-olefin production measured with SPME GC-MS.

As the genome of the bacterium was not available, *de novo* whole genome sequencing was conducted. With the genomic information on hand, it was elucidated which pathways might be responsible for terminal olefin production, as well as which resistance mechanisms against osmotic stress and reactive oxygen species could be involved in the survival of the model microorganism in its habitat.

In order to expand the spectrum of terminal olefin producers, organisms from the strain collection were investigated for 1-alkene formation. Product detection via SPME GC-MS was done to clearly identify and quantify α-olefin formation.

Regarding aspects of beneficial applications of, on the one hand the model organism itself and on the other hand pure 1-alkenes in environmental biotechnology, their inhibitory effect on fungal and bacterial plant pathogens was tested.

2 Materials and Methods

All chemicals were of analytical grade and purchased from Sigma-Aldrich (St. Louis, Missouri, USA), Carl Roth (Karlsruhe, Germany), Merck (Darmstadt, Germany) or Thermo Fisher Scientific (Waltham, Massachusetts, USA) if not stated otherwise.

All liquids and/or materials were sterilized with autoclaving at 121 °C for 20 min. Non autoclavable additives were filter sterilized using a 0.2 µm disposable filter.

Further standard techniques such as sterile working, balancing or inoculation of media were performed according to general laboratory protocols operating with (micro-) organisms of the biohazard level 1.

2.1 Bacterial cultivation media and storage

Bacteria on solid media reused within a few weeks were kept at 4 °C or stored at -70 °C for long-term conservation. Liquid cultures were always prepared freshly, either starting from a colony on solid media or from other liquid cultures.

2.1.1 Liquid growth media

Jeotgalicoccus sp. ATCC 8456 was grown in ATCC® Medium 3 (three g/l beef extract and five g/l peptone from casein, pH 6.8 ± 0.2), NB (15.0 g/l nutrient broth II; Sifin diagnostics GmBH, Berlin, Germany), MB (40.1 g/l marine broth, solubilized with heat) as well as tryptic soy broth yeast extract (TSBYE, 30.0 g/l CASO broth and 0.5% [v/v] yeast extract [Rude et al. 2011]). Incubation was done for 24 h at 26 °C and 100 rpm if not stated otherwise and applied for pre- as well as for main cultures.

All bacteria used from the culture collection (Institute of Environmental Biotechnology, Graz University of Technology, Austria) were grown in NB or TSBYE overnight at 30 °C and 100 rpm if not stated otherwise.

2.1.2 Solid growth media

For every bacterial cultivation on solid medium, including *Jeotgalicoccus* sp. ATCC 8456, NB-agar (NB and 15.0 g/l agar) was chosen. Whereas strains of the culture collection required one to two days at 30 °C, it took *Jeotgalicoccus* sp. ATCC 8456, in dependence of the stage/age of the previous culture, three to five days at 26 °C to obtain substantial cell material if not stated otherwise.

2.1.3 Bacterial glycerol stocks

Long-term stored bacteria were prepared as glycerol stocks and kept at -70 °C.
Therefore, 500 µl of the bacterial overnight cultures, inoculated in three ml NB either from liquid or solid cultures, were carefully mixed to 500 µl of 50% (v/v) glycerol and stored at -70 °C.

2.2 Fungal cultivation

All fungi were grown on solid potato dextrose agar (PDA, 26.5 g/l potato extract glucose broth and 15.0 g/l agar) for approximately one week at room temperature. The plates were kept with the agar side on the bottom i.e. the fungi facing upwards. For further experiments, mycelial plugs (Ø five mm) were cut out.

For *Verticillium longisporum* V25, ten ml Czapek-Dox medium (33.4 g/l Czapek Dox Broth; Duchefa Biochemie B.V, Haarlem, Netherlands) needed to be distributed on a donor plate, grown for at least four days, using a drigalski spatula. The resulting solution was filtered through sterile mull to retain the mycelium. Afterwards, the spores were either used directly or 50-fold diluted and counted with a Thoma chamber (depth 0.100 mm, area of one small square 0.0025 mm^2; W. Schreck Hofheim, Germany).
For the latter, the chamber was cleaned and ten µl of the dilution pipetted under the cover glass. Under the microscope, the chamber consists of a large central square which is divided into 16 medium squares, each containing 25 small squares inside, nine of them divided in half. All cells within the 16 medium squares were counted and those that were located over the top and right sides of the square were included. Following this approach, the cells would not be counted twice.

The number of spores per ml was determined by Equation (1):

$$\text{spores/ml} = \frac{\Sigma_{\text{spores}}}{\text{N}_{\text{large squares}} * V_{\text{large square}} * \text{dilution}} \quad \textbf{(1)}$$

Σ . . . total amount of counted spores within the central large square

N . . . number of counted large squares (here: 1)

V . . . volume of the large square: square size [1 mm^2] · depth [0.1 mm] · 10^{-3} = [ml]

The spore solution was then spread, either undiluted or in the desired dilution with CPX medium, onto fresh PDA using a drigalski spatula. For large single plates 100 µl were applied, for one well of a 6-well plate 35 µl. The plates were grown with the agar side on the bottom i.e. the fungi facing upwards.

2.3 Whole genome sequencing of *Jeotgalicoccus* sp. ATCC 8456

2.3.1 Revitalization of *Jeotgalicoccus* sp. ATCC 8456

Bacterium *Jeotgalicoccus* sp. ATCC 8456 was obtained as freeze-dried culture from the American Type Culture Collection (ATCC, Manassas, Virginia, USA), deposited as *Micrococcus candicans* ATCC® 8456™. As recommended, one ml of ATCC® Medium 3 was taken to rehydrate the bacterium according to the company's protocol. For further revitalization, not only ATCC® Medium 3 but also NB and TSBYE were used. Three ml of each medium were inoculated with 30 µl rehydrated cells in 15 ml falcon tubes. Additional 30 µl were plated on NB-agar.

Glycerol stocks were prepared from the revitalized culture.

2.3.2 Isolation of genomic DNA

Subsequent to the resuspension of a single colony of *Jeotgalicoccus* sp. ATCC 8456 on NB-agar in five ml NB, the culture was grown overnight. The genomic DNA was isolated with the nexttec Genomic DNA Isolation Kit for Bacteria (nexttec™ Biotechnologie GmbH, Hilgertshausen, Germany). The kit required an OD_{600} of approximately 1.5.

Agarose gel electrophoresis was conducted to estimate the concentration of the isolated genomic DNA by comparing its intensity to that of the DNA standard.

To prepare the gel, 1% (w/v) agarose (peqGOLD Universal Agarose; VWR International GmBH, Erlangen, Germany) was melted in 1x TAE buffer (40 mM TRIS, 20 mM acetic acid and one mM EDTA). After filling it into the electrophoresis chamber the gel was completely covered with 1x TAE which was used as running buffer. Five µl of isolated genomic DNA were mixed with one µl of 6x loading dye (0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol and 60 mM EDTA in ten mM Tris-HCl, pH 7.6) before the total six µl were loaded onto the gel.

Two µl of 1 kb ready-to-use DNA ladder (GeneRuler™, 0.1 µg/µl, 50 µg) and six µl of λDNA/HindIII (0.5 µl of λDNA/HindIII 0.5 µg/µl, 50 µg + 4.5 µl of ddH_2O + one µl of 6x loading dye) were used as markers. A voltage of 90 V was applied for 30 min. Subsequent staining of the gel with ethidium bromide (0.0001% [v/v]) for 30 min visualized the DNA using UV transillumination.

The principle of UV/Vis spectrophotometry was another method used which allowed quantification of the genomic DNA.

One µl of nuclease-free H_2O was measured as blank value followed by applying one µl of the DNA solution onto the cleaned lens. The software calculates the concentration of the DNA and also the corresponding values for the ratios 260/280 and 260/230 [Desjardins and Conklin 2010].

2.3.3 Amplicon sequencing of the 16S rRNA gene from model organism *Jeotgalicoccus* sp. ATCC 8456

The 16S rRNA gene from *Jeotgalicoccus* sp. ATCC 8456 was amplified with PCR using primers specific for the highly conserved 16S rRNA gene region and verified by amplicon sequencing prior to the sequencing of the whole genome.

The master mix for one reaction included two µl genomic DNA (20 ng/µl) as template, 2.5 µl reverse primer 1492r (5'-TACGG**Y**TACGTTGTTACGACTT-3', **Y** = C or T, ten µM; Institute of Environmental Biotechnology, Graz University of Technology, Austria), 2.5 µl forward primer 27F (5'-AGAGTTTGATC**M**TGGCTCAG-3', **M** = A or C, ten µM; Institute of Environmental Biotechnology, Graz University of Technology, Austria), ten µl 5x Taq&Go™ Ready-to-use PCR Mix (MP Biomedicals, Santa Ana, California, USA) and 33 µl nuclease-free H_2O to achieve a total volume of 50 µl per PCR. The reaction was set twice. As negative control two µl of nuclease-free H_2O were added instead of genomic DNA.

The PCR started with initial denaturation at 98 °C for four min followed by 25 cycles consisting of denaturation at 98 °C for 30 s, annealing at 48 °C for 30 s and elongation at 72 °C for 90 s and finished with a final elongation at 72 °C for five min.

Agarose gel electrophoresis was used in order to estimate the size of the amplified DNA fragment by comparing it to a DNA standard.
Thus, two times five µl of PCR product were mixed with one µl of 6x loading dye before being loaded onto the gel. Two µl of 1 kb ready-to-use DNA ladder (GeneRuler™, 0.1 µg/µl, 50 µg) were taken as marker. Additionally, this step was needed to purify the 16S rRNA gene for further sequencing. Purification was performed using the Wizard® SV Gel and PCR Clean-Up System (Madison, Wisconsin, USA).

Sequencing was realized at LGC Genomics GmbH (Berlin, Germany). The amplicon was sequenced in two runs using the forward and reverse primer sequences, respectively.
Afterwards, the sequencing result was compared by the Basic Local Alignment Search Tool for nucleotides (BLASTn) to the already published 16S rRNA gene under the accession number HQ709267 [Rude et al. 2011].

2.3.4 Whole genome sequencing of *Jeotgalicoccus* sp. ATCC 8456

De novo sequencing at GATC Biotech GmBH (Konstanz, Germany) made harvest of at least 600 mg wet cell material from the exponential growth phase necessary.
Therefore, four 300 ml flasks containing each 50 ml NB were inoculated with 2% (v/v) of a preculture of *Jeotgalicoccus* sp. ATCC 8456 in NB. After 24 h the OD_{600} was determined and the cell mass harvested with centrifugation at 4000 rpm and 4 °C for 6 min. The pellets were flash-frozen with liquid nitrogen and stored at -70 °C until they were sent for sequencing on dry ice.

The genome of *Jeotgalicoccus* sp. ATCC 8456 was sequenced with the pacific biosciences single molecule real-time (SMRT) sequencing technology, short PacBio RS [Buermans and den Dunnen 2014].

2.3.5 Genome assembly and annotation

As soon as the whole genome was sequenced, the reads were assembled to reconstruct the complete circular bacterial genome.
For this purpose, *de novo* Hierarchical Genome Assembly Process (RS_HGAP Assembly.2) implemented within the analysis pipeline SMRT Analysis 2.2 (Pacific Biosiences, Menlo Park, California, USA) was chosen. HGAP is optimized for quality and consists of pre-assembly, *de novo* assembly and assembly polishing of a single library type [PacBio 2015].

Using the Bacterial Annotation System (BASys) for the annotation of the circularized genome of *Jeotgalicoccus* sp. ATCC 8456 allowed identification of its genes.
BASys is an automated web tool that accepts raw DNA sequence data resulting in textual and image outputs [Van Domselaar et al. 2005]. The assembled genome needed to be uploaded as FASTA formatted file.

2.4 Physiological, genotypic and phenotypic characterization of *Jeotgalicoccus* sp. ATCC 8456

2.4.1 Analysis of the genome and phylogeny

Visualization and reconstruction of the fatty acid metabolism and further genomic pathway information was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) [Kanehisa and Goto 2000; Kanehisa et al. 2017; Kanehisa et al. 2019] and the annotation result derived from BASys.
Therefore, the annotated genomic DNA sequence of the *Jeotgalicoccus* sp. ATCC 8456 was saved in FASTA format and uploaded as query onto the KEGG Automatic Annotation Server (KAAS) for complete or draft genome [Moriya et al. 2007] and set to nucleotide. The search against KEGG GENES Database was computed using BLAST and the GENES data set for prokaryotes with default selection, as well as the bi-directional best hit assignment method.

Further, genes were in general classified applying comparative searches in the annotated genome (BASys), the resulting pathway maps (KAAS) and BLASTp in databases like the National Center for Biotechnology Information (NCBI) with default settings [Geer et al. 2010].

Maximum likelihood phylogeny analysis was performed with MAB (Méthodes et algorithmes pour la bio informatique) in advanced mode [Dereeper et al. 2008]. Therefore, the 16S rRNA gene of *Jeotgalicoccus* sp. ATCC 8456 was compared to all deposited ones in the NCBI database. All strains of *Jeotgalicoccus* as well as of the closest neighbor of all other bacterial species (18 in total) were selected for the subsequent phylogeny analysis.
Process steps were run as follows: multiple alignment using MUSCLE [Edgar 2004], curation with Gblocks, construction of the phylogenetic tree with PhyML, and for the visualization of the phylogenetic tree TreeDyn was chosen. The workflow was run step by step.
The MUSCLE alignment was initiated in full mode with default 16 iterations. A stringent selection which does not allow many contiguous nonconserved positions was defined using curation with Gblocks. Phylogeny PhyML applied 100 resamplings (bootstraps) on the curated alignment and GTR model was chosen as substitution model. The tree was displayed as phylogram.

AntiSMASH bacterial version [Blin et al. 2017] was applied in order to identify gene clusters for secondary metabolite synthesis in the genome of *Jeotgalicoccus* sp. ATCC 8456. The GenBank file downloaded from BASys served as nucleotide input.

2.4.2 Determination of the bacterial growth phases

The preference of *Jeotgalicoccus* sp. ATCC 8456 for certain liquid cultivation media (i.e. ATCC® Medium 3, NB, MB, as well as TSBYE) was evaluated identifying the bacterial growth phases and thereof calculating the doubling time from the log-phase.
Thus, a single colony was resuspended in one ml 0.89% (w/v) NaCl. For each medium, two 100 ml flasks were filled with 20 ml thereof and afterwards inoculated with 2% (v/v) of the bacterial resuspension. Samples were taken one to three times per day for six days and the OD_{600} was measured taking the corresponding media as blank. The standard deviation was calculated using n - 1.

As the exponential growth phase follows a 1st order reaction kinetics, the doubling time could be computed from the exponential growth phase of *Jeotgalicoccus* sp. ATCC 8456 in the corresponding liquid media using Equation (2) and solving the exponential term for the generation time as indicated in Equation (3):

$$A_f = A_i * e^{k\Delta t} \quad \textbf{(2)}$$

A_i . . . initial absorption at the beginning of the log-phase
A_f . . . final absorption at the end of the log-phase
k . . . reaction rate coefficient [min^{-1}; h^{-1}]
Δt . . . time difference between start and end of the log-phase [min; h]

$$T_d = \frac{\ln(2)}{k} \quad \textbf{(3)}$$

T_d . . . doubling time of the cells during the log-phase [min; h]

The doubling time was achieved using the mean value of the OD_{600} for each medium at a certain time point.

2.4.3 Analysis of the salt tolerance towards increasing NaCl concentrations

The effect of increasing salt concentrations (i.e. osmotic pressure) on the model organism was also investigated.
For this purpose, *Jeotgalicoccus* sp. ATCC 8456 was cultivated with 1 to 10% (w/v) NaCl, added in whole-number steps to TSBYE prior to autoclaving. TSBYE naturally contains 0.5% (w/v) NaCl and was used as the standard condition. For each concentration two 15 ml falcon tubes containing three ml medium were inoculated with 1% (v/v) of a resuspension of a single colony in one ml 0.89% (w/v) salt. Samples were taken in duplicates from each tube and their OD_{600} was measured. The standard deviation was calculated using n - 1.

2.4.4 Effects of the temperature on the growth

To find the optimal growth temperature for *Jeotgalicoccus* sp. ATCC 8456, single colonies were streaked onto fresh NB-agar and kept as duplicates for six days at 26 °C, 30 °C and 37 °C, respectively. The difference between the number of appearing colonies was documented.

2.4.5 Oxygen tolerance and motility of *Jeotgalicoccus* sp. ATCC 8456

Furthermore, the requirement to grow with or without oxygen and the ability of self-dependent movement was determined.
Therefore, sterile glass test tubes were filled with five ml TSBYE-agar of semi-solid consistency (TSBYE and 0.3% [w/v] agar). Two test tubes were inoculated with a single colony of *Jeotgalicoccus* sp. ATCC 8456 by stabbing approximately two third to the bottom using an inoculation loop. *Escherichia coli* BL21(DE3) (Institute of Environmental Biotechnology, Graz University of Technology) was used as reference strain.

2.4.6 Detection of expressed enzymatic activities

Catalase is an enzyme that converts H_2O_2, a potent oxidizing agent, into H_2O and O_2 under excessive formation of foam [Steinbüchel et al. 2013].
A small amount of cell material of *Jeotgalicoccus* sp. ATCC 8456, cultivated on NB-agar, was placed onto a clean microscope slide. Then, a few drops of 3% (v/v) H_2O_2 were pipetted onto the smear. If mixing is needed, metal loops must be avoided as they give false positive results due to degradation of the metal. Positive results are indicated by the rapid evolution of bubbles. For negative results there are no or only a few scattered bubbles [Hemraj et al. 2013]. *Pseudomonas aeruginosa* (Institute of Environmental Biotechnology, Graz University of Technology, [Appendix; Tab 12]), also cultivated on NB-agar, was chosen as positive control.

The detection of oxidase activity is a physiological test based on the cytochrome c oxidase dependent oxidation of a detection reagent resulting in a deep purple-bluish color change of the affected bacterial colonies. Cytochrome c oxidase allows microorganisms to grow aerobically [Steinbüchel et al. 2013].
Thus, two drops of a 1.5% (w/v) solution of *N,N*-dimethyl-*p*-phenylenediamine hydrochloride were placed on a sterile filter paper and a sufficient amount of cell material of *Jeotgalicoccus* sp. ATCC 8456, cultivated on NB-agar, was smeared onto it.
If the colonies do not appear deep purple-bluish after ten s, a negative result is given [Sigma-Aldrich 2018]. *Escherichia coli* BL21(DE3) was taken as the negative control, whereas *Pseudomonas aeruginosa* was used as the positive reference. Both control strains were also cultivated on NB-agar.

2.4.7 Evaluation of the utilization of glucose

Hugh-Leifson medium (two g/l peptone, five g/l NaCl, 0.3 g/l K_2HPO_4, 0.03 g/l bromthymol blue, pH 6.0 - 7.6; Merck KGaA, Darmstadt, Germany, 1% [w/v] glucose, and 0.3% [w/v] agar) is used to distinguish between aerobic (oxidative) and anaerobic (fermentative) carbohydrate metabolism e.g. for glucose utilization. Therefore, the pH indicator bromthymol blue is present, which turns yellow when acidic, green when neutral, and blue when alkaline [Hugh and Leifson 1953].

First, the medium was prepared and autoclaved containing all additives except of glucose. Afterwards, the latter was filter sterilized as initial solution of 10% (w/v) glucose monohydrate and added aseptically to the still hot liquid to a final concentration of 1% (w/v).

Then, five ml of the Hugh-Leifson medium were semi-solidified per sterile glass test tube. Two tubes were inoculated with a single colony of the same strain by stabbing approximately two third to the bottom using an inoculation loop. *Escherichia coli* BL21(DE3) was used as reference of a fermentative microorganism and *Pseudomonas aeruginosa* as oxidative bacterium, besides of *Jeotgalicoccus* sp. ATCC 8456, to identify its way of carbohydrate breakdown.

For each bacterium one test tube was kept under aerobic and the other one under anaerobic conditions. To sustain anaerobic conditions, the agar was covered with a layer of one cm of sterile mineral oil (high purity grade mineral oil light, white; Amresco Inc., Solon, Ohio, USA). Incubation took place for six days at 30 °C for *Escherichia coli* BL21(DE3) and *Pseudomonas aeruginosa* or at 26 °C for *Jeotgalicoccus* sp. ATCC 8456.

2.4.8 Influence of volatile formation on the growth of fungal plant pathogens

It should be evaluated if VOCs, produced by *Jeotgalicoccus* sp. ATCC 8456, would be able to inhibit the growth of plant pathogenic fungi.

Therefore, three ml TSBYE were inoculated with a colony of *Jeotgalicoccus* sp. ATCC 8456, taken from NB-agar. After 24 h, the OD_{600} was measured and diluted to 0.1 using a 0.89% (w/v) solution of NaCl. 50 µl thereof were streaked onto NB-agar in 6-well plates followed by pre-incubation for 24 h.

The next day, the fungi [Tab 3] were prepared on the opposing 6-well plates containing ca. three ml PDA. Whereas mycelium plugs of *Botrytis cinerea*, *Fusarium culmorum*, and also *Rhizoctonia solani* were cut from the corresponding donor plate, 35 µl of an undiluted ($1.4 \cdot 10^6$), a 1:1 diluted ($0.7 \cdot 10^6$), as well as a 1:3 diluted ($0.47 \cdot 10^6$) spore suspension of *Verticillium longisporum* V25 were streaked onto the PDA solidified within the 6-well plates.

The concentration of the spores was determined beforehand using Equation (1). Incubation as TCVA-setup was done at room temperature for one week.
As positive control, a filter paper soaked with bacillol (Bacillol® AF; Paul Hartmann AG, Heidenheim, Germany) was included and as negative control, the opposing NB-agar against one of the fungi was kept uninoculated.

2.5 Gas chromatography-mass spectrometry for quantification of bacterial 1-alkene production

If not stated otherwise, all GC-MS related devices were purchased from Agilent Technologies (Santa Clara, California, USA).

1-C16 and 1-alkenes ≥ 1-C20 were not relevant for the experiments and thus not ordered.

Mean values and standard deviations (with n - 1) were calculated using only representative measurements. The representative measurements were selected based on the overall trend of the product formation observed for all replicates.

2.5.1 General method of solid phase microextraction gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry is widely applied to identify volatile analytes [Babushok 2015]. Therefore, bacterial terminal unsaturated olefin formation was quantified by GC-MS (Agilent 7890B/5977A Series Gas Chromatograph/Mass Selective Detector; and PAL RSI 85; CTC Analytics AG, Zwingen Switzerland) together with linear calibration graphs of the corresponding 1-alkene standards [Appendix; Tab 14] (Sigma-Aldrich, St. Louis, Missouri, USA and Tokyo Chemical Industry Co. Ltd., Tokyo, Japan).
Headspace sampling was applied, where a SPME fiber adsorbs the volatiles that are present in the gas phase of a closed vial. The SPME principle relies on the formation of an equilibrium between the analyte in the gaseous (adsorbed on the fiber) and the aqueous/solid phase [Pawliszyn et al. 1997].

Previous to the extraction, the fiber was conditioned once for eight min at 40 °C in the GC injector. All standards/samples were extracted with a PDMS-coated fiber (30 µm, for non-polar semi-volatiles [MW 80-500 g/mol]; Supelco, Bellefonte, Pennsylvania, USA) for 15 min at 35 °C. A splitless thermal desorption for 30 min at 270 °C, 9.1473 psi, and a septum purge flow of three ml/min in the heated injection port of the gas chromatograph followed [Tuduri et al. 2001]. Then, the analytes were separated using a HP-5MS column ([5%-phenyl]-methylpolysiloxane, 30 m x 250 µm x 0.25 µm, nonpolar and utilizable from -60 °C to 350 °C) with a flow of 1.2 ml/min under 9.1473 psi. The parameters of the oven were set as there was no equilibration time, starting at 40 °C (hold for two min), with a subsequent increase of 5 °C/min up to 110 °C and furthermore 10 °C/min up to 280 °C (hold for three min). The eluents were electron ionized, their masses identified with quadrupole mass spectrometry, the mass range of 50 to 350 g/mol detected, the output analyzed with the associated software (Agilent MSD Productivity), and the obtained mass spectra assigned to the corresponding 1-alkene using the reference library (NIST EI Mass Spectral Library, NIST MS Search Version 2.2, 2014) and the analytically pure reference substances.

2.5.2 Determination of the retention times and indices of 1-alkene standards

For confirming the bacterial formation of 1-alkenes, it is essential to know their time of elution from the GC-column (retention time), as well as their individual dimensionless retention index (RI), which serves for device-independent normalization. The latter is expressed as a combination of two important GC properties: the specific retention volume of a compound and its relative retention [Babushok 2015].

The terminal olefins were acquired in analytical purity (Sigma-Aldrich, St. Louis, Missouri, USA and Tokyo Chemical Industry Co. Ltd., Tokyo, Japan) and injected into air-tight sealed 20 ml GC-MS vials (20 ml ND18 Headspace Screw Vial [clear], 75.5 x 22.5 mm, rounded bottom and ND18 Magnetic Screw Cap [eight mm hole] with Silicone/PTFE Septa [white/blue], 1.5 mm 55° shore A; BGB Analytik Vertrieb GmbH, Rheinfelden, Germany).

The references were applied either as pure gas for the terminal olefins 1-C5, 1-C6, 1-C7, and 1-C8 or as a dilution in five ml ddH_2O for 1-C9, 1-C10, 1-C11, 1-C12, 1-C13, 1-C14, 1-C15, 1-C17, 1-C18, as well as 1-C19 [Tab 1].

Tab 1. Sample preparation of analytically pure 1-alkene standards. A-Olefins 1-C5 to 1-C8 were taken as pure gas. The less volatile 1-alkenes 1-C9 to 1-C19 needed to be diluted with ddH_2O in order to define their retention times and retention indices.

Standard	Sample preparation
1-Pentene	200 µl from the gas phase of the standard
1-Hexene	150 µl from the gas phase of the standard
1-Heptene	150 µl from the gas phase of the standard
1-Octene	150 µl from the gas phase of the standard
1-Nonene	1:2 million of the standard with ddH_2O
1-Decene	1:2 million of the standard with ddH_2O
1-Undecene	1:4 million of the standard with ddH_2O
1-Dodecene	1:4 million of the standard with ddH_2O
1-Tridecene	1:4 million of the standard with ddH_2O
1-Tetradecene	1:4 million of the standard with ddH_2O
1-Pentadecene	1:4 million of the standard with ddH_2O
1-Heptadecene	1:4 million of the standard with ddH_2O
1-Octadecene	1:4 million of the standard with ddH_2O
1-Nonadecene	1:4 million of the standard with ddH_2O

The retention times and the mass spectra were determined using SPME GC-MS.
Afterwards, the non-isothermal retention indices were calculated according to the following Equation (4) [Van den Dool and Kratz 1963]:

$$I_x = 100n + \frac{100(t_x - t_n)}{(t_{n+1} - t_n)} \quad \textbf{(4)}$$

I_x . . . retention index of compound x

n . . . integer of the amount of carbon atoms of the *n*-alkane eluting immediately before compound x

t_x . . . retention time of compound x [min]

t_n . . . retention time of the reference *n*-alkane eluting immediately before compound x [min]

t_{n+1} . . . retention time of the reference *n*-alkane eluting immediately after compound x [min]

The obtained mass spectra together with the RI and retention time values of the individual α-olefin standards allowed identifying them within the bacterial samples.

2.5.3 Linear calibration graphs with terminal unsaturated olefin standards

As the extraction process was applied for *in vivo* measurements, an internal standard could not be used [Chen et al. 2004]. For quantification, external standards were prepared in the form of linear calibration graphs of the corresponding 1-alkenes 1-C10, 1-C11, 1-C12, 1-C13, 1-C14, 1-C15, 1-C17, 1-C18, as well as 1-C19. Due to the increasing volatility it was not feasible to obtain satisfying linear calibration graphs for terminal alkenes ≤ 1-C9.

Dilutions in DMSO were prepared to receive final 1-alkene concentrations of 2000, 1000, 100, ten, five, as well as one nM in three ml of TSBYE [Appendix; Tab 13]. The medium was pipetted in 20 ml GC-MS vials before adding 30 µl of the corresponding DMSO dilutions.
Linear calibrations were calculated after measuring the dilution series using SPME GC-MS.

2.5.4 Cultivation of *Jeotgalicoccus* sp. ATCC 8456 with increasing salinity

The effect of increasing NaCl concentrations on the quantitative 1-alkene formation of the model microorganism was tested.
Starting from a preculture of *Jeotgalicoccus* sp. ATCC 8456, three ml TSBYE containing 1 to 10% (w/v) NaCl were inoculated with 1% (v/v) and cultivated for 24 h. For these main cultures, TSBYE was always provided in 20 ml GC-MS vials beforehand. A culture in three ml TSBYE without addition of NaCl was used as the standard condition. Then, SPME GC-MS was applied.
This experiment was conducted twice. For the first run, all samples, including the control, were measured once. For the second run, the vials were surveyed as duplicates.

2.5.5 Effect of fatty acid feeding on the production of α-olefins by the model organism

It was studied, if feeding *Jeotgalicoccus* sp. ATCC 8456 fatty acids would cause a shift in the corresponding terminal olefin production.
For this purpose, three ml TSBYE were inoculated with a single colony of this bacterium, sampled from NB-agar. This preculture was grown for 24 h.
As fatty acid substrates, saturated hexanoic (6:0), octanoic (8:0), nonanoic (9:0), decanoic (10:0), undecanoic (11:0), dodecanoic (12:0), tetradecanoic (14:0), hexadecanoic (16:0), and octadecanoic acid (18:0) were used. The acids were prepared as 100-fold concentrated stock in DMSO or ethanol and added to three ml TSBYE, which was pipetted into 20 ml GC-MS vials first, for a final concentration of 0.5% (either v/v or w/v in dependence of the carbon chain length). Prior to SPME GC-MS, the medium was inoculated with 1% (v/v) of the preculture and *Jeotgalicoccus* sp. ATCC 8456 grown for 24 h. The volume change of the medium trough addition of the fatty acid was neglected for the calculation of the inoculum. Furthermore, a culture without fatty acids was used as control under standard conditions.

2.5.6 Screening for other bacterial 1-alkene producers

Bacteria from the culture collection (Institute of Environmental Biotechnology, Graz University of Technology, Austria, [Appendix; Tab 12]) were primarily selected according to their taxonomic relationship to microorganisms capable of 1-alkene biosynthesis [Rude et al. 2011; Beller et al. 2010; Sukovich et al. 2010; Winters et al. 1969]. Whereby, the choice was expanded to the phylum level.
Precultures were inoculated from the corresponding glycerol stocks in three ml TSBYE. Strains which did not grow in this medium were cultivated in three ml NB instead.
Before SPME GC-MS measurements were conducted, three ml TSBYE or three ml NB were prepared in 20 ml GC-MS vials, inoculated with 1% (v/v) of the precultures and cultivated overnight.
All chosen bacteria were screened in two rounds.

2.6 Evaluating inhibitory activity of 1-alkenes against bacterial and fungal plant pathogens

Biogenic volatile organic compounds play an important role, not only for plant growth promotion, but also as biological control agents against (soil-borne) pathogenic organisms like bacteria or fungi [Weller 1988]. Therefore, it was evaluated *in vitro* if 1-alkenes are capable of inhibiting plant pathogens. In order to do so, the two clamp volatile organic compounds assay (TCVA) was employed.

2.6.1 General procedure of the two clamp volatile organic compounds assay

Depending whether fungi or bacteria were taken as test organisms, disposable 6-well or 12-well plates (Sarstedt Ag & Co. KG, Nümbrecht, Germany) were used, respectively. A one mm sterile silicone foil (six or 12 holes à Ø five mm) was placed between two opposing plates for tightening the connection. One plate contained the bacteria or fungi, the other the pure 1-alkene standards. Two clamps kept the setup in place [Cernava et al. 2015]. For the 6-well plates approximately three ml agar were poured per well. 12-well plates needed ca. one ml agar per well.
On the plate side of the 1-alkene standards, 25 µl DMSO, pipetted onto a sterile filter paper, were taken as negative control and a paper towel soaked with bacillol (Bacillol® AF; Paul Hartmann AG, Heidenheim, Germany) as positive control. In this way, the microbial growth inhibition by terminal olefins was tested.

2.6.2 *In vitro* assay with a selection of bacteria causing plant diseases

Seven important plant pathogenic bacteria were chosen from the strain collection (Institute of Environmental Biotechnology, Graz University of Technology, Austria, [Tab 2]).

Tab 2. Phytopathogenic bacteria chosen for *in vitro* TCVAs with α-olefins.

Species	Strain	Target plant/plant part
Bacillus pumilus	DSM 492	Infection of mango leaves, apple fruits
Burkholderia cepacia	DSM 7288	Rot of onion skin
Clavibacter michiganensis	DSM 20134	Infection of tomatoes
Pectobacterium carotovorum	ECC16A1	Pectolytic soft rot of diverse hosts e.g. beets
Pseudomonas viridiflava	2d1	Infection of unrelated hosts e.g. pumpkin leaves, kiwi
Xanthomonas campestris	DSM XC3586	Infection of crucifers
Xanthomonas cucurbitae	6g5	Infection of e.g. pumpkin or watermelon fruits

First, precultures in three ml NB were prepared, either with cell material from the glycerol stocks or colonies directly from NB-agar.

For identification of bacterial growth during the TCVA later on, 12-well plates were filled with approximately one ml of NB-agar supplemented with the redox and pH indicator resazurin (7-hydroxy-10-oxidophenoxazin-10-ium-3-one sodium salt; orange at pH 3.8 and violet at pH 6.5). Resazurin is reduced to resorufin by viable cells [Borra et al. 2009], what causes a decrease of the pH and thus, a color change from a violet-bluish to a pink or even orange agar [Sarker et al. 2007]. The indicator was prepared as 100-fold concentrated stock (20 mM, i.e. 5.024 mg/ml ddH_2O) and filter sterilized, before it was added to the media in a final concentration of 0.2 mM.

Subsequently, the precultures were diluted to an OD_{600} of 0.1 using 0.89% (w/v) NaCl and 50 µl of the bacterial dilution streaked onto the NB-resazurin-agar of each well. After inoculation of the agar containing plates, five µl of an undiluted 1-alkene standard solution were pipetted onto a sterile filter paper (Ø 1.6 cm) per well of the opposing empty plate. The assay was conducted with the terminal olefins 1-C8, 1-C9, 1-C10, 1-C11, 1-C12, 1-C13, 1-C14, 1-C15, 1-C17 and 1-C18. Before incubation at 30 °C for 24 to 72 h, in dependence of the prior observed growth speed of the bacteria, each plate pair was fixated with two clamps.

As many plates were needed, the plant pathogenic bacterium species used for the negative and positive control, respectively was changed from plate to plate.

2.6.3 *In vitro* assay with a selection of plant pathogenic fungi

Four plant pathogenic fungi were chosen from the strain collection (Institute of Environmental Biotechnology, Graz University of Technology, Austria, [Tab 3]).

Tab 3. Phytopathogenic fungi chosen for *in vitro* TCVAs with 1-alkenes.

Species	Strain code	Target plant
Botrytis cinerea	*	Broad host range e.g. grapes
Fusarium culmorum	-	Mainly crop
Rhizoctonia solani	*	Broad host range e.g. potatoes
Verticillium longisporum	V25	Mainly oilseed rape, causing wilt

*strains used for the practical laboratory courses (Institute of Environmental Biotechnology, Graz University of Technology, Austria)

Previously, the fungi were grown for approximately one week on PDA. Mycelial plugs (Ø five mm) were cut from each fungus (*Botrytis cinerea*, *Fusarium culmorum*, and *Rhizoctonia solani*, [Tab 3]) from the corresponding donor plate. Then, always one plug was placed in the center of a well of a 6-well plate, each filled with approximately three ml PDA. For *Verticillium longisporum* V25, 35 µl of an undiluted ($1.4 \cdot 10^6$) and a 1:1 diluted ($0.7 \cdot 10^6$) spore suspension, respectively were applied. The concentration of the spores was determined beforehand using Equation (1).

After inoculation of the plates, five µl of an undiluted 1-alkene standard were pipetted onto a sterile filter paper (Ø 1.6 cm) per well of the opposing empty plate. The assay was conducted with the terminal unsaturated olefins 1-C11, 1-C12, 1-C13, as well as 1-C14. Before incubation at room temperature for one week, each plate pair was fixated with two clamps.

Again, on the side of the 1-alkene standards 25 µl DMSO, pipetted onto a sterile filter paper, were taken as negative control and a paper towel soaked with bacillol as positive control.

3 Results

For this thesis *Jeotgalicoccus* sp. ATCC 8456 was used as model organism for 1-alkene formation. Genomic and phenotypic aspects as well as physiological and metabolic properties were considered in order to not only gain an insight into a relatively uncharacterized bacterium and to search for possible reasons for the microbial production of terminal olefins, but also to evaluate their potential for applications in environmental biotechnology. Therefore, also a screening for other bacterial terminal unsaturated olefin producers was included.

3.1 Genome based identification and characterization of 1-alkene production in *Jeotgalicoccus* sp. ATCC 8456

3.1.1 Verification of *Jeotgalicoccus* sp. ATCC 8456

After revitalization of the *Jeotgalicoccus* sp. ATCC 8456 strain, received from the ATCC, approximately 123 ng/µl genomic DNA were isolated.
Gel electrophoresis of the amplified and purified 16S rRNA gene allowed identifying the size of the PCR-fragment. The size of approximately 1.5 kbp [line 2; Fig 7] corresponded to the expected length of the 16S rRNA gene sequence (1512 bp, accession number HQ709267, [Rude et al. 2011]).

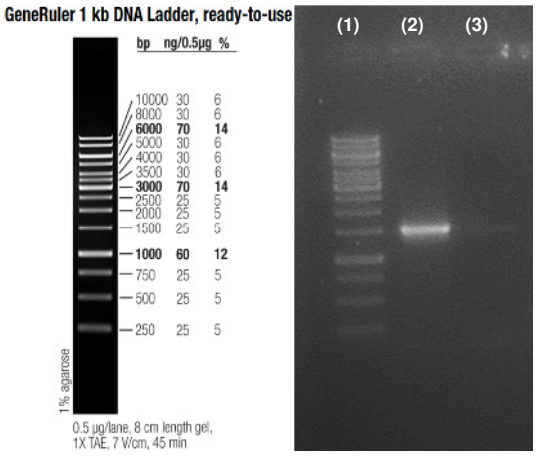

Fig 7. Amplified 16S rRNA gene from *Jeotgalicoccus* sp. ATCC 8456. The PCR-product showed a size of ca. 1.5 kb (lane 2). Lane 1: 1 kb ready-to-use DNA ladder; lane 3: negative control.

The slightly visible band for the negative control at 1.5 kbp [lane 3; Fig 7] appeared most likely due to overloading of the PCR-product.

The subsequent sequencing verified *Jeotgalicoccus* sp. ATCC 8456, whereby two gaps occurred at positions 52 and 724 [Appendix; Seq 1] after alignment with the available sequence deposited on GenBank (accession number HQ709267).
At position 52 an A and at position 724 a G were submitted by the authors in a previous sequencing approach [Rude et al. 2011]. The presence of the first nucleotide (A) was corroborated in this study by comparing the amplicon sequence with the 16S rRNA gene from the whole genome sequencing. For the latter nucleotide (G), the amplicon sequencing as well as the whole genome sequencing revealed the absence in the 16S rRNA gene.
Furthermore, the unknown nucleotides R and Y (representing A or G and C or T, respectively [NC-IUB 1985]) could be identified as G (position 377), T (position 1013), and A (position 1424) again with both sequencing techniques.
Comparison of both 16S rRNA sequences (amplicon vs. genome) showed differences in length at the 3'-end (49 bp) as well as at the 5'-end (51 bp), resulting in a shorter sequence of 1410 bp. This difference in size is a result of the Sanger sequencing method due to the binding of the forward and reverse primer and therefore missing sequencing at the ends, respectively.

3.1.2 Genome features based on gene annotation

Genome assembly of *Jeotgalicoccus* sp. ATCC 8456 depicted it as a circular chromosome built from 2090457 bp, containing in total a nucleotide composition of 387464 C, 384348 G, 663305 T and 655340 A.
The GC-content of the genome was determined to be 36.92%.
Furthermore, 2117 genes were annotated using BASys [Van Domselaar et al. 2005].

Regarding the genome information on protein level, 95.9% of all identified open reading frames showed a length between 50 and 650 amino acids. The abundance of each amino acid stayed ≤ 10% for all encoded proteinogenic amino acids. Among the eight most abundant amino acids, four are considered hydrophobic, namely leucine (9.4%), isoleucine (8.3%), valine (7.1%), as well as alanine (6.4%). Furthermore, both acidic amino acids, glutamic acid (7.8%) and aspartic acid (6.3%) were also present, followed by one basic and one polar amino acid, lysine (6.5%) and glycine (6.5%), respectively. All other amino acids occured less frequently.

Additionally, 3% of the proteins were addressed functionally to the lipid metabolism, whereby the functions of 5% of all ORFs that were retrieved from the genome are not identified yet and almost one third of the proteins (24%) remained completely unknown. For further 8%, the functionality was predicted, i.e. a total of 37% of the 2117 genes obtained no or only assumed assignments [Fig 8].

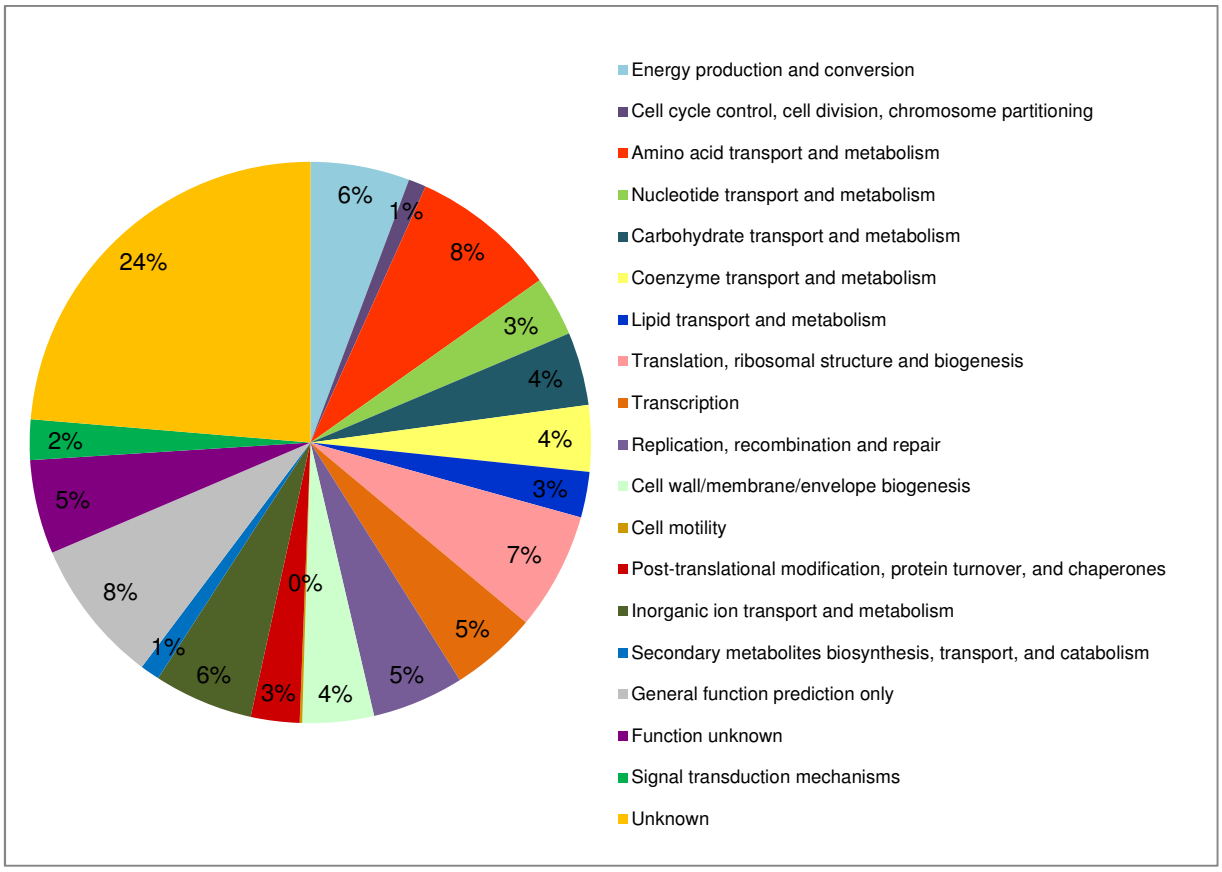

Fig 8. Assumed protein functions according to Clusters of Orthologous Groups (COGs). For the lipid metabolism 3% presumed proteins were assigned from the genome. Almost one third (24%) of the ORFs are completely unknown.

3.1.3 Phylogenetic clustering of *Jeotgalicoccus* sp. ATCC 8456

A first phylogenetic placement of *Jeotgalicoccus* sp. ATCC 8456 was accomplished by comparing the 16S rRNA gene to the sequences deposited in the 16S rRNA gene database of NCBI, whereby the highest 16S rRNA gene sequence similarities were seen almost equally to *Jeotgalicoccus marinus* (99.7%) and *Jeotgalicoccus huakuii* (99.6%). The sequence identity to all other species of this genus was ≤ 96.1%.

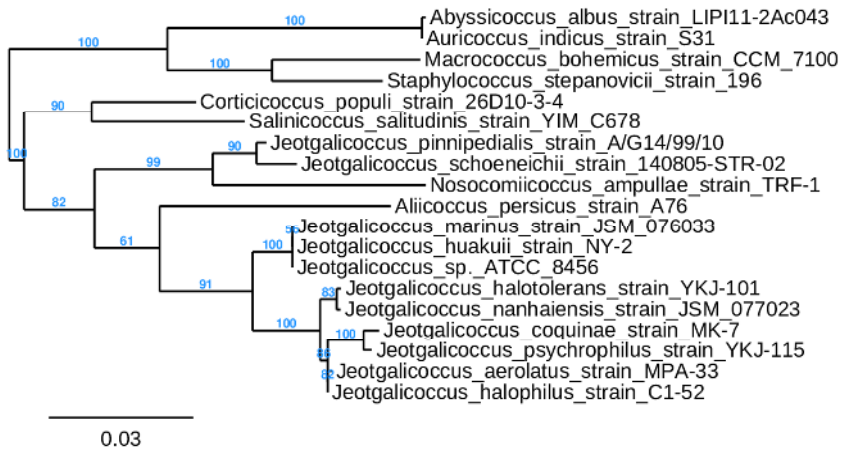

Fig 9. Maximum-likelihood tree based on the 16S rRNA gene sequences visualizing the phylogenetic positioning of *Jeotgalicoccus* sp. ATCC 8456 within the family of Staphylococcaceae. The model organism was compared to ten other *Jeotgalicocci* as well as to one strain of each of the genera that were the closest related to strain ATCC 8456 using NCBI BLASTn. The tree is based on 100 resamplings. The scale bar represents 3% sequence divergence.

Maximum likelihood phylogeny analysis with MAB revealed the same clustering result, although with 56% the bootstrap values were the lowest for these three *Jeotgalicocci* strains based on 100 replications. However, they were, together with the other strains of this genus, the least related to *Jeotgalicoccus schoeneichii* and *Jeotgalicoccus pinnipedialis*.

3.1.4 Analysis of the formation of terminal olefins

Formation of 1-alkenes was quantified using SPME GC-MS.

Jeotgalicoccus sp. ATCC 8456 produced the terminal olefins 1-C11, 1-C13, 1-C15, 1-C17, and 1-C19 [Fig 10]. Seventeen independent quantifications were conducted in total [Appendix; Tab 21 and Tab 22]. Not all 1-alkenes were formed during all measurements and thereof only a limited number was representative and used for calculation of the mean values. Hence, the amount of measurements performed differed from the number that was regarded as representative. The representative values were quantified [Tab 4].
Values of all standard deviations were ≥ 41% of the respective concentration [Tab 4], indicating a high variation in the quantification using the SPME-based system.

However, a strong tendency for a favored formation of long-chained 1-alkenes could be observed. 1-Alkenes ≥ 1-C15 occurred in more of the SPME GC-MS measurements, compared to 1-C11 and 1-C13. Also, especially 1-C17 and 1-C19 were produced in ca. 100-fold greater concentrations than the other terminal unsaturated olefins [Tab 4].

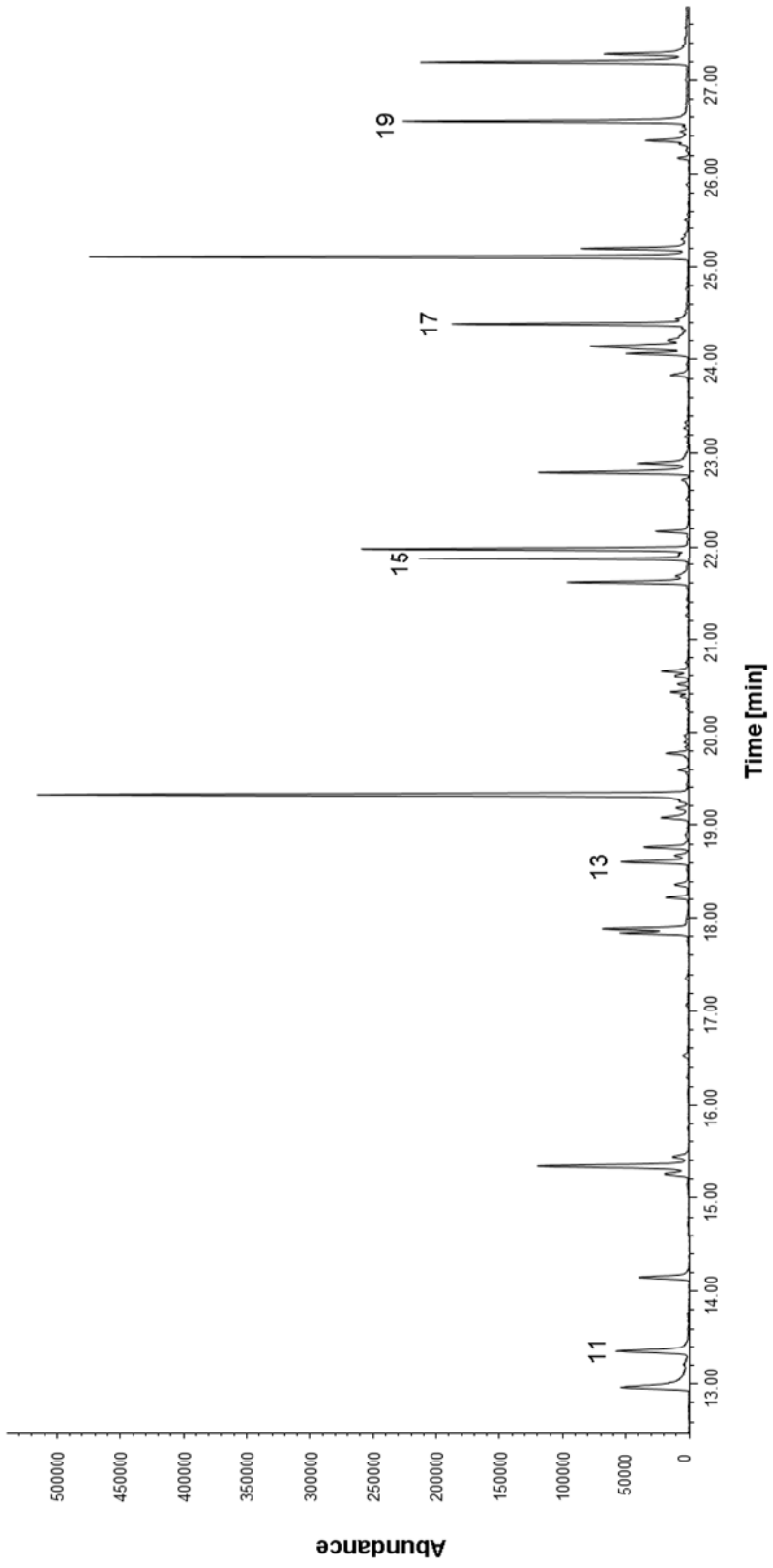

Fig 10. Chromatographic representation of the 1-alkenes produced by *Jeotgalicoccus* sp. ATCC 8456. Identified as 1-C11, 1-C13, 1-C15, 1-C17, and 1-C19, here named 11, 13, 15, 17, and 19. Product detection was performed by SPME GC-MS.

Tab 4. Terminal alkenes found to be produced by *Jeotgalicoccus* sp. ATCC 8456. Seventeen SPME GC-MS quantifications were done in total [Appendix; Tab 22]. Quantification of 1-alkenes was calculated as a mean value from the representative measurements.

1-Alkene	1-C11	1-C13	1-C15	1-C17	1-C19
Total measurements	17	17	17	17	17
Present in	8	10	16	14	15
Representative	5	8	14	8	10
Mean and SD [nM]	21.2 ± 8.7	3.9 ± 4.7	2.2 ± 2.9	137.0 ± 67.5	351.3 ± 181.5

Under standard conditions, *Jeotgalicoccus* sp. ATCC 8456 produced only α-olefins with an uneven number of carbon atoms.

Additional evidence of the abundance of longer-chained and branched 1-alkenes was given as judged from the GC-MS spectra, although not verified with proper standards.

In total, BASys could annotate 2117 genes in the chromosome of the model microorganism, thereof KEGG could map 1220 of them to functional orthologues in its database.

The functional *in vivo* redox chain for the terminal unsaturated olefin production consists of $OleT_{JE}$ (CypC, BASYS00724, fatty acid peroxygenase, 442 amino acids) and most likely of a flavin-comprising NADPH-dependent ferredoxin reductase as well as an iron-sulfur cluster containing ferredoxin (BASYS00361, ferredoxin, 84 aa). For the ferredoxin reductase two candidates were identified (TrxB, BASYS01288, ferredoxin-NADP reductase, 329 aa and TrxB, BASYS02089, ferredoxin-NADP reductase 1, 343 aa).
Also, a paralogue of $OleT_{JE}$ (CypC, BASYS00261, fatty acid peroxygenase, 416 aa) was found within the genomic sequence of *Jeotgalicoccus* sp. ATCC 8456, which shared 38% sequence similarity with $OleT_{JE}$.

Three other ORFs annotated as monooxygenases were found in the genome of *Jeotgalicoccus* sp. ATCC 8456: putative monooxygenases MoxC (BASYS01119) and YxeK (BASYS01433), as well as a probable nitronate monooxygenase (BASYS02075). However, there is most likely no relationship to the formation of 1-alkenes.

It was reported that fatty acids would serve as precursors for the $OleT_{JE}$-catalyzed formation of terminal olefins [Du et al. 2017]. Therefore, the pathway for synthesis of free fatty acids in *Jeotgalicoccus* sp. ATCC was investigated.
Searching for fatty acid metabolism in the BASys annotation result revealed 17 ORFs that could be involved [Tab 5].

Tab 5. Probable ORFs involved in the production of free fatty acids in *Jeotgalicoccus* sp. ATCC 8456. Possible proteins assigned in BASys as well as in the KEGG pathway map are highlighted in green, those not present in the map [Fig 11] are highlighted in blue, and ORFs only found in BASys are not highlighted. The comparably poor BLASTp result for FabG (BASYS01407) is highlighted in grey.

Name	Annotation	Annotated as	AA	kDa	Closest homology
AccA	BASYS00175	Acetyl-CoA carboxylase carboxyltransferase, subunit α	314	34.89	Acetyl-CoA carboxylase carboxyltransferase subunit alpha, 99.7% identity, *Jeotgalicoccus marinus* (WP_026866858)
AccB	BASYS00401	Biotin carboxyl carrier protein of acetyl-CoA carboxylase	142	15.84	Acetyl-CoA carboxylase biotin carboxyl carrier protein, 99.3% identity, *Jeotgalicoccus marinus* (WP_026866378)
AccB	BASYS02010		142	15.40	Biotin/lipoyl-binding protein, 98.6% identity *Jeotgalicoccus marinus* (WP_026866787)
AccC1	BASYS00400	Biotin carboxylase 1	449	50.08	Acetyl-CoA carboxylase biotin carboxylase subunit, 99.6% identity, *Jeotgalicoccus marinus* (WP_026866377)
AccC1	BASYS02011		448	49.68	Acetyl-CoA carboxylase biotin carboxylase subunit, 99.1% identity *Jeotgalicoccus marinus* (WP_026866788)
AccD	BASYS00174	Acetyl-CoA carboxylase carboxyltransferase, subunit β	283	31.61	Acetyl-CoA carboxylase carboxyltransferase subunit beta, 100.0% identity *Jeotgalicoccus marinus* (WP_026866857)
FabD	BASYS00709	Malonyl CoA-acyl carrier protein transacylase	305	33.00	[Acyl-carrier-protein] S-malonyltransferase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026867574)
AcpP	BASYS00707	Acyl carrier protein	78	8.83	Acyl carrier protein, 100.0% identity, multi-species *Jeotgalicoccus** (WP_026867576)
FabH	BASYS00889	3-Oxoacyl-[acyl-carrier-protein] synthase 3	321	35.23	Ketoacyl-ACP synthase III, 100.0% identity, *Jeotgalicoccus marinus* (WP_026867409)
FabG	BASYS00708	3-Oxoacyl-[acyl-carrier-protein] reductase	222	23.98	3-Oxoacyl-[acyl-carrier-protein] reductase, 99.6% identity, *Jeotgalicoccus marinus* (WP_026867575)
FabG	BASYS01407		103	10.76	Glucose 1-dehydrogenase, 92.2% identity, *Jeotgalicoccus psychrophilus* (WP_026859161)
FabG	BASYS01432		147	15.72	3-Oxoacyl-ACP reductase, 99.3% identity, *Jeotgalicoccus marinus* (WP_026867267)
FabZ	BASYS01880	(3R)-Hydroxymyristoyl-[acyl-carrier-protein] dehydratase	148	16.09	3-Hydroxyacyl-ACP dehydratase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026865993)
FabI	BASYS00876	Enoyl-[acyl-carrier-protein] reductase	229	24.69	Enoyl-[acyl-carrier-protein] reductase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026867422)
FabF	BASYS00888	3-Oxoacyl-[acyl-carrier-protein] synthase 2	392	41.44	Beta-ketoacyl-[acyl-carrier-protein] synthase II, 100.0% identity, *Jeotgalicoccus marinus* (WP_026867410)
FabF	BASYS01401		411	43.56	Beta-ketoacyl-[acyl-carrier-protein] synthase II, 100.0% identity *Jeotgalicoccus marinus* (WP_026865964)
YneP	BASYS01797	Uncharacterized protein YneP	142	16.56	Acyl-CoA thioesterase, 100.0% identity, *Jeotgalicoccus marinus* (WP_035855668)

*species *Jeotgalicoccus saudimassiliensis*, *Jeotgalicoccus halophilus*, *Jeotgalicoccus halotolerans*

Based on the gene annotation, a possible pathway for production of fatty acids could be hypothesized as follows:
first, acetyl-CoA might be carboxylated to malonyl-CoA by AccABC1D. Then, malonyl-CoA could be transferred to the ACP (AcpP) by FabD. Under emission of CO_2, malonyl-ACP condenses with another acetyl-CoA, catalyzed possibly by FabH. Thereby, acetoacetyl-ACP (β-ketoacyl-ACP for further cycles) is generated. Acetoacetyl-ACP (β-ketoacyl-ACP) might be reduced to β-hydroxyacyl-ACP by FabG. Dehydration with presumed FabZ leads to *trans*-2-enoyl-ACP and subsequent reduction to acyl-ACP catalyzed by the ORF assigned as FabI. For elongation, malonyl-ACP could be added to acyl-ACP by FabF [Tab 5].
The predicted thioesterase YneP was the only thioesterase found within the genomic sequence. It could be responsible for the release of fatty acids from the acyl carrier protein resulting in free fatty acids [Torto-Alalibo et al. 2014], serving as precursor for the direct conversion to terminal unsaturated olefins by $OleT_{JE}$.

Comparing the BASys annotation results with the pathways of orthologue genes deposited in the KEGG database [Fig 11], the initiation of the fatty acid synthesis as well as the elongation cycles could be confirmed [highlighted in green; Tab 5].
Thereby, the second FabF gene (BASYS01401) was assigned as orthologue of FabB (KEGG, K00647), and either FabG (BASYS00708) or the uncharacterized oxidoreductase (YmfI, BASYS00661) as orthologues of FabG (KEGG, K00059). YmfI was referred to be responsible for the first reduction step in the fatty acid biosynthesis pathway with BASys, but shared only 30% sequenced identity with FabG (BASYS00708).
Additionally, the other two FabG (BASYS01407 and BASYS01432) [not highlighted; Tab 5] were not assigned to the corresponding KEGG orthology (KO) identifier, assuming that they might have been misclassified with the previous annotation, or due to a low sequence similarity were not found in the KEGG database. This is further supported by the fact, that one of these two FabG genes (BASYS01407) obtained a comparably poor BLAST result [highlighted in grey; Tab 5].
ORFs YneP as well as AcpP were found in the KEGG database and assigned to acyl-CoA thioester hydrolase and acyl carrier protein, respectively, supporting their postulated function in the fatty acid biosynthesis pathway.

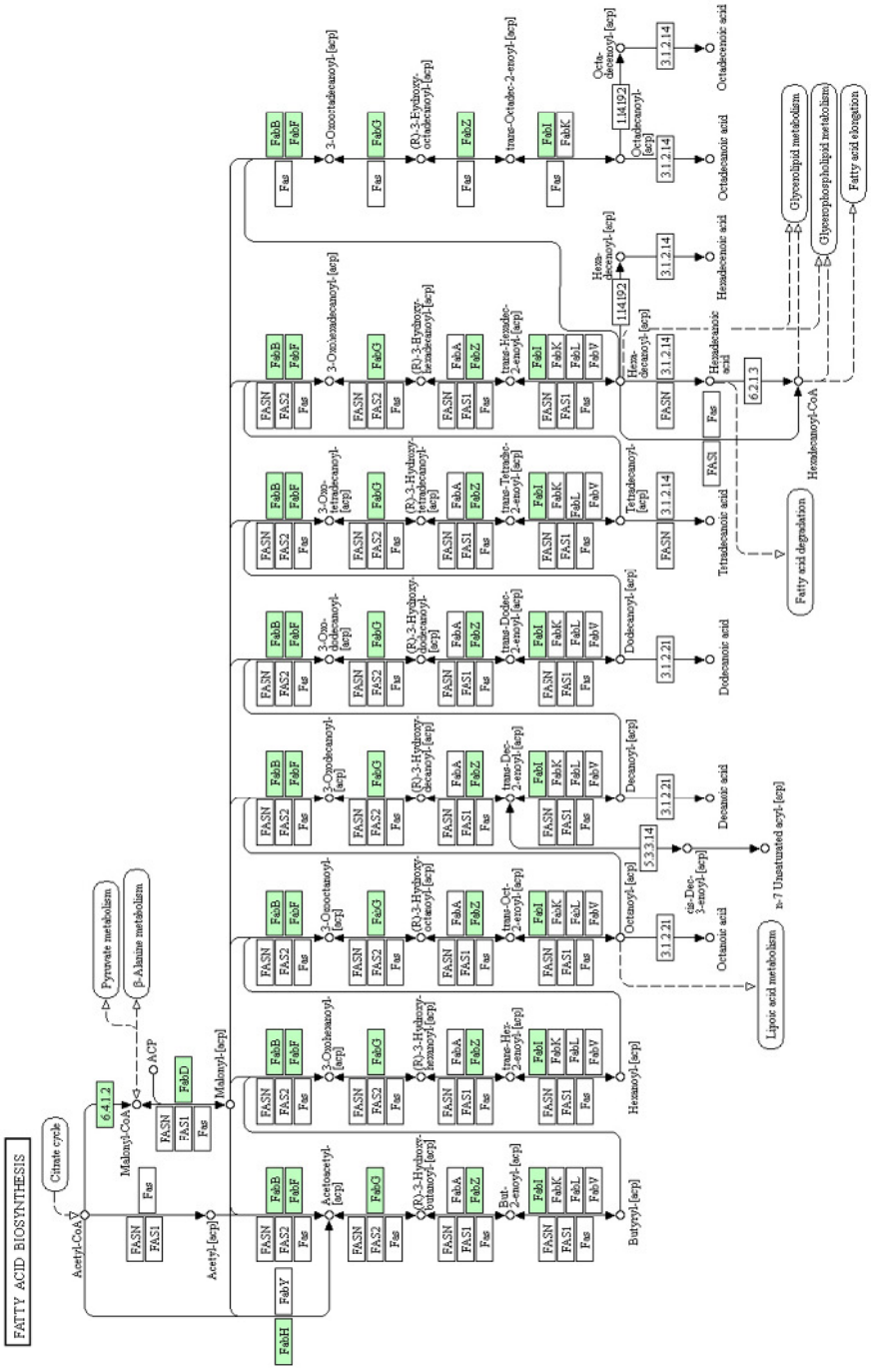

Fig 11. KEGG pathway map of the fatty acid biosynthesis in *Jeotgalicoccus* sp. ATCC 8456. Orthologous genes found in the genome of the model organism are highlighted in green.

Furthermore, a biosynthetic gene cluster analysis using AntiSMASH bacterial version implied the absence of genes in the model organism for production of antibiotics or other secondary metabolite compounds.

3.1.5 Genes involved in the oxidative stress resistance

As $OleT_{JE}$ was first classified as a peroxygenase, *Jeotgalicoccus* sp. ATCC 8456 was also analyzed with respect to its oxidative and metal stress response.

Thus, the annotation identified the very likely presence of the peroxide sensing transcriptional repressor PerR (BASYS00011, peroxide operon regulator, 147 aa) [Tab 6].

Tab 6. Oxidative and metal stress regulation by transcription factor PerR. Genes presumed to be controlled by the PerR repressor. Name, annotation information and number of amino acids (AA) were gathered from the results on the genome of *Jeotgalicoccus* sp. ATCC 8456 using BASys. The closest homology was defined using BLASTp.

Name	Annotation	Annotated as	AA	Closest homology
PerR	BASYS00011	Peroxide operon regulator	147	Peroxide-responsive transcriptional repressor PerR, 100.0% identity, *Jeotgalicoccus marinus* (WP_051220550)
HemA	BASYS00239	Glutamyl-tRNA reductase	449	Glutamyl-tRNA reductase, 99.8% identity, *Jeotgalicoccus marinus* (WP_026866229)
HemX	BASYS00240	Protein hemX	274	Hypothetical protein, 98.9% identity, *Jeotgalicoccus marinus* (WP_026866230)
HemC	BASYS00241	Porphobilinogen deaminase	309	Hydroxymethylbilane synthase, 100.0 % identity, *Jeotgalicoccus marinus* (WP_026866231)
HemD	BASYS00242	Uroporphyrinogen-III Synthase	229	Uroporphyrinogen-III synthase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026866232)
HemB	BASYS00243	Delta-aminolevulinic acid dehydratase	327	Porphobilinogen synthase, 99.1% identity, *Jeotgalicoccus marinus* (WP_026866233)
HemL2	BASYS00244	Glutamate-1-semialdehyde 2,1-aminomutase 2	428	Glutamate-1-semialdehyde-2,1-aminomutase, 99.8% identity, *Jeotgalicoccus marinus* (WP_026866234)
Fur	BASYS00374	Ferric uptake regulation protein	152	Transcriptional repressor, 100.0% identity, *Jeotgalicoccus marinus* (WP_026866352)
KatA	BASYS01907	Catalase A	500	Catalase, 99.6% identity, *Jeotgalicoccus marinus* (WP_026867236)
BsaA	BASYS00644	Glutathione peroxidase homolog BsaA	165	Glutathione peroxidase, 96.9% identity, *Jeotgalicoccus marinus* (WP_035857287)
AhpF	BASYS01405	Alkyl hydroperoxide reductase subunit F	221	NAD(P)/FAD-dependent oxidoreductase, 100.0% identity, *Jeotgalicoccus marinus* (WP_051220433)
TrxA	BASYS00204	Thioredoxin	103	Thioredoxin, 100.0% identity, *Jeotgalicoccus marinus* (WP_026866887)
TrxB	BASYS00992	Thioredoxin reductase	311	Thioredoxin-disulfide reductase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026867312)
ZurA	BASYS00433	Zinc uptake system ATP-binding protein ZurA	260	Metal ABC transporter ATP-binding protein, 99.2% identity, *Jeotgalicoccus marinus* (WP_026866411)

It controls the expression of a regulon (independent genes) responsible for elimination of H_2O_2 [Ricci et al. 2002] in dependence of the metal homeostasis in the cell. It probably includes the regulation of the expression of genes for heme biosynthesis HemAXCDBL2 (BASYS00239 to BASYS00244), iron uptake (Fur, BASYS00374), the catalase (KatA, BASYS01907), and assumedly also a glutathione peroxidase (BsaA, BASYS00644), an

alkyl hydroperoxide reductase (AhpF, BASYS01405; AhpC could not be identified), thioredoxin (TrxA, BASYS00204), and a zinc uptake system (ZurA, BASYS00433).

Other, presumably to the oxidative stress response correlated enzymes found are a peroxidase that recognizes efficiently organic hydroperoxide (OsmC, BASYS00968), further a DNA-binding protein (Dps, BASYS01170), as well as the superoxide dismutase (SodA, BASYS00428).

Tab 7. Additional enzymes probably involved in the elimination of reactive oxygen species. Name, annotation information and number of amino acids (AA) were gathered from the results on the genome of *Jeotgalicoccus* sp. ATCC 8456 using BASys. The closest homology was defined using BLASTp.

Name	Annotation	Annotated as	AA	Closest homology
Dps	BASYS01170	General stress protein 20U	150	DNA starvation/stationary phase protection protein, 100.0% identity, *Jeotgalicoccus marinus* (WP_026865742)
SodA	BASYS00428	Superoxide dismutase [Mn/Fe] 1	199	Superoxide dismutase, 100.0% identity, *Jeotgalicoccus marinus* (WP_026866406)
OsmC	BASYS00968	Organic hydroperoxide resistance protein-like 1	135	Ohr family peroxiredoxin, 98.5% identity, *Jeotgalicoccus marinus* (WP_026867334)

3.2 Growth and terminal unsaturated olefin formation of *Jeotgalicoccus* sp. ATCC 8456 under various cultivation conditions

3.2.1 Characterization of the phenotype and growth behavior determined by external abiotic factors

The phenotype of the model organism was first investigated regarding colony morphology, motility, as well as respiration. Additionally, the optimal growth temperature was determined.

Fig 12. Observed colony morphology of model organism *Jeotgalicoccus* sp. ATCC 8456. Growth on solid agar was circular with a smooth surface and an entire margin. The colonies were cream-colored, opaque, viscous and showed a convex elevation.

Separate colonies of *Jeotgalicoccus* sp. ATCC 8456 showed a circular, cream-colored and opaque morphology with a smooth surface and an entire margin during growth on solid agar. Further, the elevation was convex and the consistency of the colonies viscous [Fig 12] [Breakwell et al. 2007].

Jeotgalicoccus sp. ATCC 8456 lacked motility, resulting in cell growth only at the injection site and otherwise translucent semi-solid agar. Growth appeared along the total depth of the previous inoculation, revealing a facultative anaerobic metabolism [Fig 12]. Control strain *Escherichia coli* BL21(DE3) showed the same respiratory and growth behavior.
Compared to other *E. coli* strains, mutation of the *fli*-operon, coding for flagellar genes, lead to immobility of *Escherichia coli* BL21(DE3) [Studier et al. 2009]. The negative control was not contaminated.

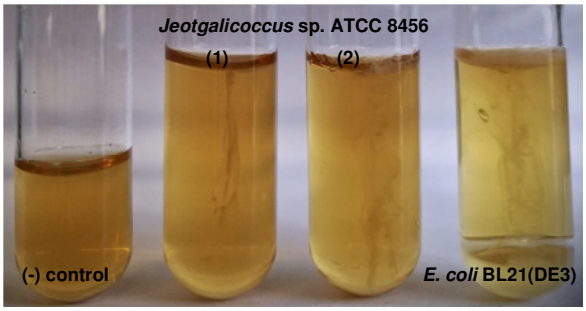

Fig 13. Identified immobility and facultative anaerobic metabolism for *Jeotgalicoccus* sp. ATCC 8456. Results shown compared to *Escherichia coli* BL21(DE3) and a negative control.

For determination of the temperature optimum, *Jeotgalicoccus* sp. ATCC 8456 was cultivated on solid agar at 26 °C, 30 °C and 37 °C, respectively. First colonies were visible after 72 h for the first two temperatures with 26 °C being superior over 30 °C with respect to the amount of grown cells. Remaining constant until the sixth day, the cultivations at 30 °C showed approximately only one third of colony enlargement compared to those grown at 26 °C [Fig 14]. No growth was observed at 37 °C during the entire cultivation.

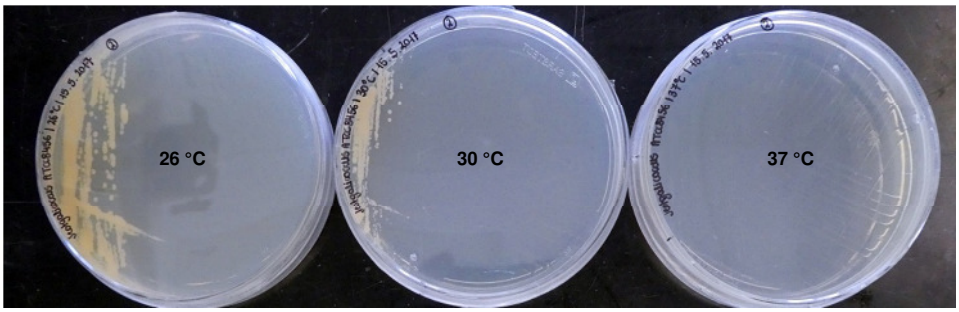

Fig 14. Temperature optimum of *Jeotgalicoccus* sp. ATCC 8456. Growth on solid agar decreased with increasing incubation temperature being the highest at 26 °C, lower at 30 °C and absent at 37 °C.

3.2.2 Insight into metabolic and enzymatic properties

The utilization of glucose as the only carbon source was studied via cultivation of the model microorganism in Hugh-Leifson medium.

Jeotgalicoccus sp. ATCC 8456 did not utilize glucose as sole carbohydrate source neither oxidatively (aerobically) nor fermentative (anaerobically). The agar remained green under anaerobic conditions [tube 6; Fig 15] and turned bluish-green for the respective aerobic cultivation after 24 h [tube 5; Fig 15].

Comparing to the controls, *Pseudomonas aeruginosa* was not able to convert glucose anaerobically [tube 2; Fig 15], but aerobically [tube 1; Fig 15], resulting in a yellow color for the first and an unchanged green agar color for the latter [Hunt and Phibbs 1983]. On the other hand, *Escherichia coli* BL21(DE3) oxidized [tube 3; Fig 15] and also fermented glucose [tube 4; Fig 15] [Fuhrer et al. 2005]. The color of the agar shifted in both cases from green to yellow.

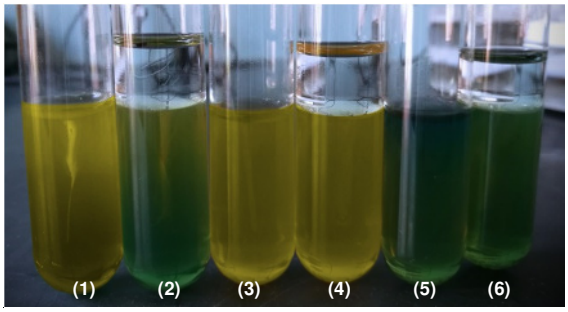

Fig 15. Glucose utilization identified to be nonexistent in *Jeotgalicoccus* sp. ATCC 8456 (tubes 5+6). Anaerobic condition provided with a layer of sterile mineral oil (even numbers); aerobic cultivation done without mineral oil (odd numbers). *Pseudomonas aeruginosa* (tubes 1+2; oxidative glucose metabolism only) and *Escherichia coli* BL21(DE3) (tubes 3+4; oxidative and fermentative glucose metabolism) were taken as control organisms.

Furthermore, the genome of *Jeotgalicoccus* sp. ATCC 8456 did not reveal any specific glucose metabolizing or transporting enzymes, but presumably parts of the general multicomponent phosphotransferase system for (mono)saccharide uptake [Kotrba et al. 2001] were found: the enzyme I (EI) as well as the phosphocarrierprotein HPr (PtsI, BASYS00818 and PtsH, BASYS00819) and further the transmembrane enzyme IIC (EIIC) and a fructose specific transmembrane enzyme IIC (BASYS00114 and BASYS01270).
All of these ORFs displayed a sequence similarity of ≥ 98.9% to the corresponding proteins of *Jeotgalicoccus marinus*.

As these results indicated, *Jeotgalicoccus* sp. ATCC 8456 might prefer other carbohydrates than glucose. It was found, that starting from fructose-6-phosphate, all genes coding for enzymes of the glycolysis were assumedly present in the genome of the model organism. The same holds true for the reverse reaction of the gluconeogenesis using oxaloacetate as precursor.

As foam is produced during the oxidation of H_2O_2 to O_2 and H_2O, it enables identification of catalase positive organisms.
Bacteria expressing cytochrome c oxidase are able to oxidize a detection reagent, resulting in purple-bluish colored colonies.

The existence of the enzymatic activities of catalase as well as cytochrome c oxidase was proven in *Jeotgalicoccus* sp. ATCC 8456 visually [A) and B); Fig 16] as well as with the genomic annotation on hand (catalase KatA [BASYS01907], a ba_3-type [CbaA, BASYS00504 and CbaB, BASYS00503] and a aa_3-type cytochrome c oxidase [CtaC, BASYS00777; CtaD, BASYS00776; CtaE, BASYS00775; CaaD, BASYS00774]).

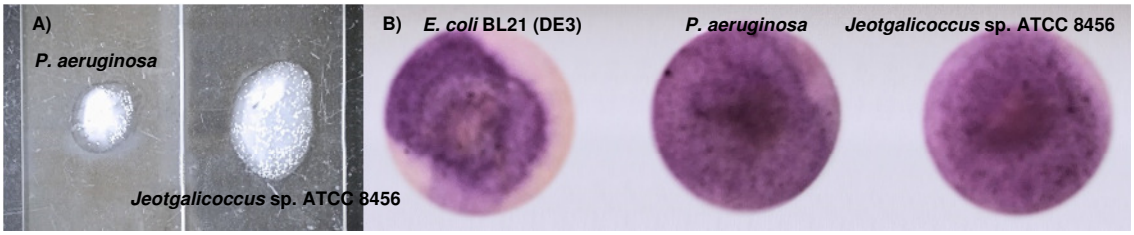

Fig 16. Expression of catalase (A) and cytochrome oxidase (B) verified in *Jeotgalicoccus* sp. ATCC 8456. *Pseudomonas aeruginosa* (catalase and oxidase positive) and *Escherichia coli* BL21(DE3) (oxidase negative) served as control organisms.

Pseudomonas aeruginosa was positive in both assays, as it is a catalase [A); Fig 16] and also oxidase positive microorganism [B); Fig 16] [Palleroni 2015; Gaby and Hadley 1957]. The biomass taken from *Escherichia coli* BL21(DE3) stayed colorless during testing [B); Fig 16], as a cytochrome oxidase is not present [Scheutz and Strockbine 2015].

3.2.3 Optimization of the media composition

The cell density in terms of OD_{600} of *Jeotgalicoccus* sp. ATCC 8456 was evaluated in four different liquid media, ATTC® Medium 3, MB, NB and TSBYE, to identify the optimum growth conditions with respect to nutrient supply. Therefore, the maximum reached cell density was determined for each medium [Appendix; Tab 15 to Tab 18], besides of an approximate estimate of the exponential growth phases and the doubling times.

Jeotgalicoccus sp. ATCC 8456 displayed the second longest exponential growth phases in TSBYE and NB, namely for each medium ca. 11 h. It was only surpassed by the log-phase reached with cultivation of this strain in ATTC® Medium 3 (ca. 23 h). The exponential growth phase in MB did not exceed ca. 6 h [Fig 17].

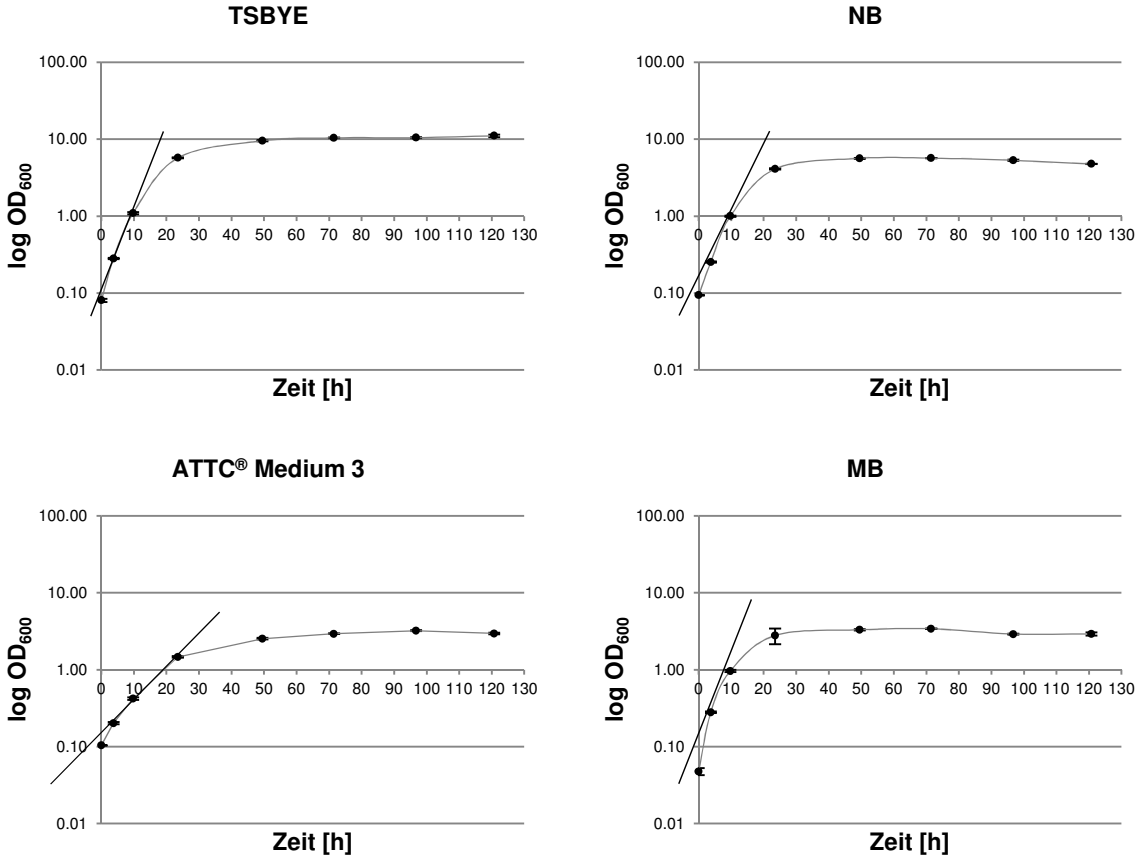

Fig 17. Evaluation of the log-phase of *Jeotgalicoccus* sp. ATCC 8456 from the growth curve obtained for cultivation in four different liquid media. The exponential growth phases decreased from ca. 23 h (ATTC® Medium 3) to ca. 11 h (TSBYE and NB) and ca. 6 h (MB). Values for the SD of the OD_{600} measurements were ≤ 0.15, except for TSBYE at 120.75 h (SD = 0.42) and also for MB at 23.5 h (SD = 0.65).

In TSBYE a maximal cell density of 11.06 was obtained, followed by 56.9% less growth of *Jeotgalicoccus* sp. ATCC 8456 in NB with a maximum OD_{600} of 4.77. Similar cell densities were seen during cultivation in ATTC® Medium 3 (OD_{600} of 2.96) and MB (OD_{600} of 2.91), both representing roughly only one third of the maximal cell density possible in TSBYE [Fig 18].

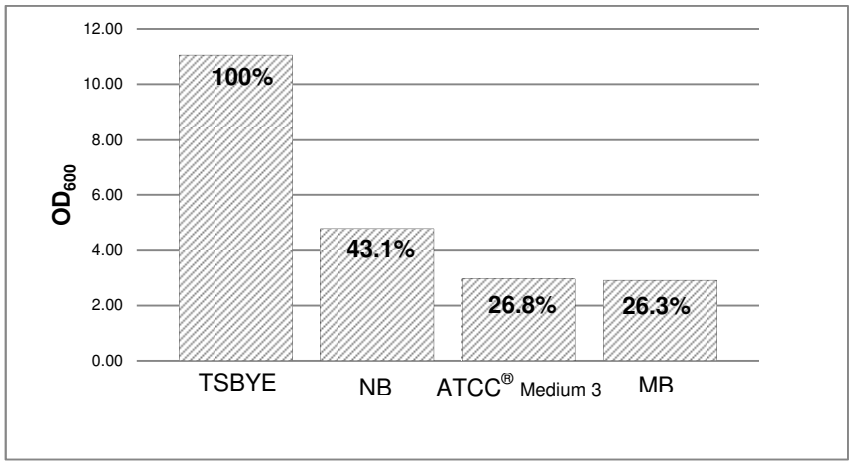

Fig 18. Maximum cell density reached for *Jeotgalicoccus* sp. ATCC 8456 in four different liquid media. Showing the highest cell density in TSBYE (OD_{600} of 11.06; 100%), all other absorptions were set relative to this value to calculate the percentages. Cultivations in NB, ATCC® Medium 3 and MB resulted in a maximum OD_{600} of 4.77 (43.1%), 2.96 (26.8%), and 2.91 (26.3%), respectively.

Furthermore, the doubling times, calculated from two replicates, were found to be 2 h 36 min (156 min) for growth of *Jeotgalicoccus* sp. ATCC 8456 in TSBYE, slightly surpassed by cultivation in MB with 2 h 18 min (138 min) under the same conditions. In NB, the model organism showed a generation time of 2 h 54 min (174 min). The strain required twice as much time to double when grown in ATTC® Medium 3, namely 6 h 24 min (384 min) [Fig 19].

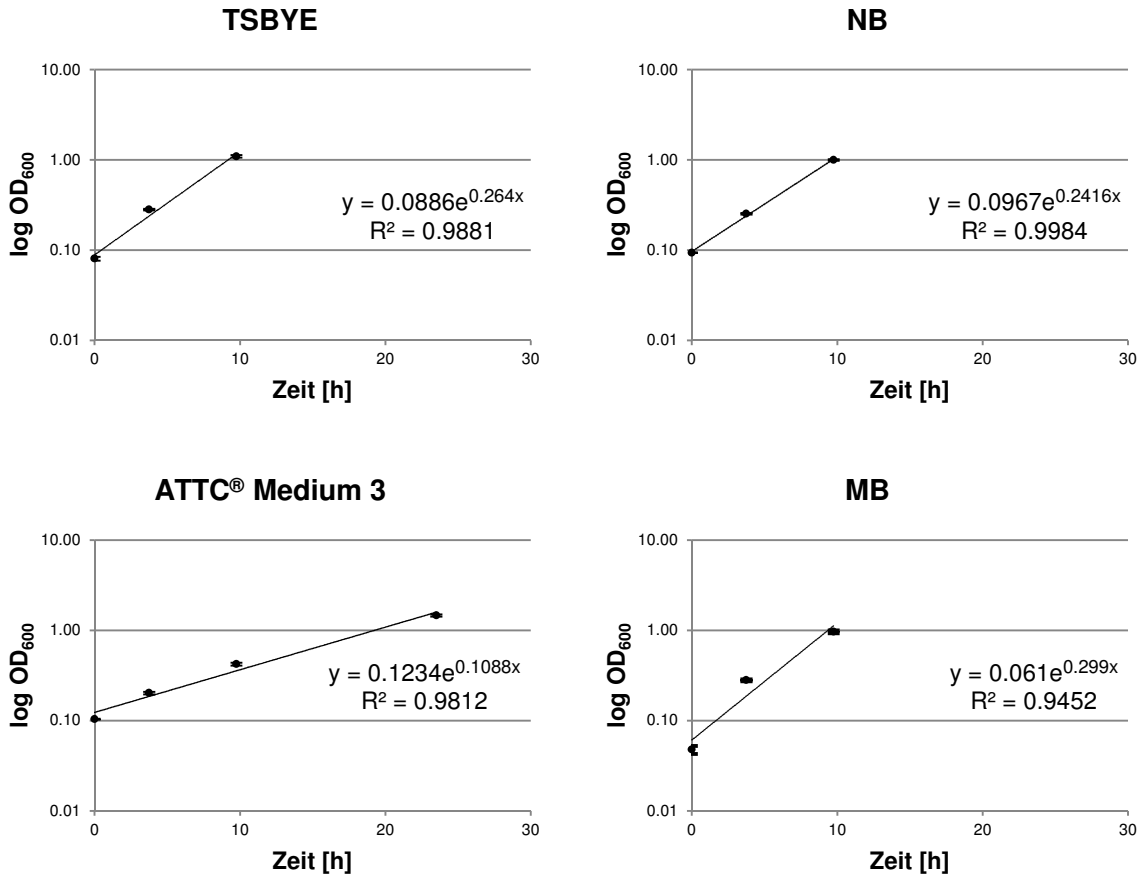

Fig 19. Exponential growth phase of *Jeotgalicoccus* sp. ATCC 8456, retrieved from the growth curve obtained for cultivation in four different liquid media. Used for determination of the doubling time, being 2 h 36 min (156 min) for cultivation in TSBYE, 2 h 54 min (174 min) in NB, 2 h 18 min (138 min) in MB and 6 h 24 min (384 min) for growth in ATTC® Medium 3.

3.2.4 Influence of increasing osmotic pressure on cell growth and 1-alkene formation

Two independent measurements revealed *Jeotgalicoccus* sp. ATCC 8456 to be slightly halotolerant [Larsen 1986]. A halophilic growth behavior was not demonstrated [Fig 20], as the strain did not require salt concentrations above isotonic solutions for maintenance [Gargaud et al. 2015; Margesin and Schinner 2001].

Thereby, the relationship between growth and salinity (NaCl) was invers correlated. The higher the salt concentration, the less growth occurred and the formation of cell clusters in the medium was augmented. At salt concentrations ≥ 10% (w/v) (maximum used for this approach) cells of *Jeotgalicoccus* sp. ATCC 8456 were still viable, but the effect of decreased proliferation and simultaneous presence of aggregated cells was especially observed at this level of salinity [Fig 20].

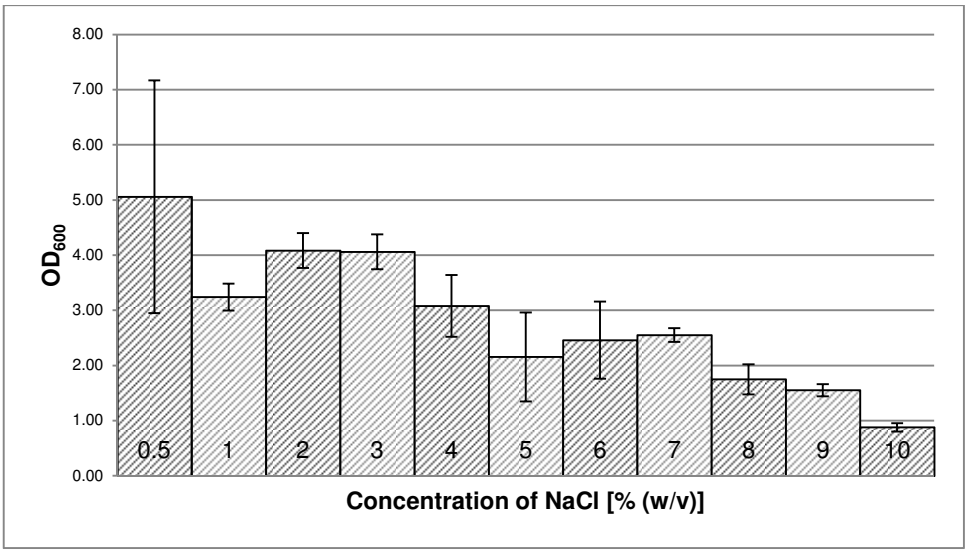

Fig 20. *Jeotgalicoccus* sp. ATCC 8456 found to be halotolerant. At 10% NaCl (w/v) cell aggregates were formed. No significant differences were observed up to 3% (w/v) NaCl, considering the great SD of 2.11 for growth under standard conditions with 0.5% (w/v) NaCl. Values for the SD were in all other cases ≤ 0.81.

The aggregation of cells at higher salt concentrations appeared similar to the clusters of cells known from *Staphylococcus* spp. [Fuchs et al. 2007]. Both, *Staphylococcus* species as well as *Jeotgalicoccus* sp. ATCC 8456 belong to the family of Staphylococcaceae [Schwaiger et al. 2010].

Besides of a comparably high standard deviation of 2.11 [Fig 20] for the cells under standard conditions (0.5% [w/v] NaCl), in contrast to ≤ 0.81 for all other salt concentrations, there was no significant difference in cell densities up to 3% (w/v) salt.

Screening of the genome of the model microorganism outcropped the existence of genes coding for possible enzymes and channels related to osmotic adaptation, but those for the exclusive production of osmolytes could not be disclosed.

Thus, mechanosensitive channels of the MscL (large conductance) and MscS (small conductance) family (MscL, BASYS00249 and YkuT, BASYS01479) for release of turgor pressure [Booth and Blount 2012], a KtrCD potassium uptake transporter system (KtrC, BASYS00814 and KtrD, BASYS00862) [Holtmann et al. 2003], and also a natrium-hydrogen antiporter comprised of the subunits A1 to G1 [Ito et al. 2017] encoded in one operon (MnhA1, MrpB, MnhC1, MnhD1, MnhE1, MnhF1, and MnhG1, BASYS00924 to BASYS00930) could be presumably identified.

Thereby, all proteins could be assigned with ≥ 99.1% to the corresponding enzymes of *Jeotgalicoccus marinus*.

Additionally, the machinery for accumulation of organic solutes e.g. glycine betaine [Oren 2002] within the cell to keep the osmotic balance (OpuCD, OpuCC, OpuCB, and

OpuCA, BASYS01800 to BASYS01803) was found. Here, the sequence similarities showed a greater deviation as for other aligned genes and were localized within the genome of *Jeotgalicoccus marinus* as ABC transporter ATP-binding protein (OpuCA, 99.5%), ABC transporter permease (OpuCD 91.6%), or osmoprotectant ABC transporter substrate-binding protein (OpuCC, 83.2%). OpuCB was detected to be with 87.6% similarity closest related to an ABC transporter permease of *Jeotgalicoccus saudimassiliensis*.

Furthermore, it was investigated how an increasing salinity (1 to 10% [w/v] NaCl) alters the amount of 1-alkenes produced by *Jeotgalicoccus* sp. ATCC 8456 or whether there are differences in the presence of individual α-olefins compared to the control (0.5% [w/v] NaCl).

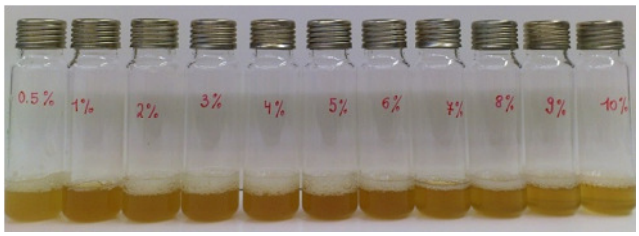

Fig 21. Cultivation of *Jeotgalicoccus* sp. ATCC 8456 with increasing salt concentrations for SPME GC-MS analysis of terminal olefin production. Cultures in media containing 1 to 10% (w/v) NaCl were measured. A concentration of 0.5% (w/v) NaCl served as control.

As all values of the first measurement [Appendix; Tab 23 and Tab 24] strongly deviated from measurements two and three, they were disregarded for calculation of mean values and standard deviations [Tab 8].

For comparison between the control under standard conditions (i.e. 0.5% [w/v] NaCl) [Tab 4] and cultivations with increasing salinity (1 to 10% [w/v] NaCl) [Tab 8] standard deviations were not considered.

Tab 8. 1-Alkene formation of *Jeotgalicoccus* sp. ATCC 8456 for cultivation with increasing salt concentrations (1 to 10% [w/v] NaCl). Three measurements were conducted in total, whereby one was not considered for the calculation. 1-Alkene concentrations in nM are given as mean values and with SD.

1-Alkene	1%	2%	3%	4%	5%
1-C11	19.2 ± 3.6	6.4 ± 2.4	7.4 ± 1.0	9.0 ± 1.8	19.1± 0.7
1-C13	32.8 ± 10.8	9.2 ± 3.2	5.3 ± 1.9	10.4 ± 2.0	14.1 ± 0.9
1-C15	16.8 ± 13.1	1.2 ± 0.5	2.7 ± 2.2	3.7 ± 2.1	3.5 ± 0.4
1-C17	297.9 ± 94.0	14.5 ± 3.7	35.1 ± 37.2	36.6 ± 21.5	60.2 ± 6.2
1-C19	253.8 ± 23.2	35.0 ± 9.5	43.6 ± 10.1	42.9 ± 7.1	27.3 ± 9.6
	6%	**7%**	**8%**	**9%**	**10%**
1-C11	16.2 ± 1.6	27.4 ± 0.4	8.1 ± 5.5	11.9 ± 1.3	9.8 ± 0.0
1-C13	11.3 ± 2.0	17.4 ± 4.1	10.9 ± 4.5	6.0 ± 2.3	4.1 ± 1.2
1-C15	4.5 ± 2.2	8.3 ± 1.1	4.7 ± 2.5	2.5 ± 0.2	1.7 ± 0.5
1-C17	146.1 ± 105.6	82.1 ± 33.4	32.3 ± 13.1	14.3 ± 4.7	7.2 ± 0.6
1-C19	17.3 ± 2.1	38.9 ± 8.7	3.7 ± 1.0	0	0

According to the quantified SPME GC-MS results, 1% (w/v) NaCl was the most favorable salt concentration for production of 1-C13, 1-C15, and 1-C17 in *Jeotgalicoccus* sp. ATCC 8456. 1-C17 was found to be present in 2-fold and 1-C13 as well as 1-C15 in 8-fold higher concentrations, respectively. On the contrary, terminal olefins 1-C11 as well as 1-C19 were produced in 1.2-fold and 1.4-fold lower amounts.
If 1-C11 should be formed in the highest possible concentration, growth of the model microorganism in 7% (w/v) NaCl gave the best results, with a 1.4-fold increase compared to the control medium. For the formation of 1-C19, cultivation of *Jeotgalicoccus* sp. ATCC 8456 under standard conditions outperformed all other salt concentrations.

Moreover, all 1-alkenes present in the control under standard conditions were also seen for the cultivations containing 1 to 10% (w/v) salt. Only 1-C19 was not formed in media supplemented with 9% and 10% (w/v) NaCl.
Although, less growth occurred with increasing concentration of NaCl compared to the standard conditions [Fig 20], a general dependency between salinity and the formation of terminal alkenes could not be identified [Tab 8].

3.2.5 Correlation between fatty acid metabolism and 1-alkene formation

It was analyzed, if the presence of fatty acids as substrates would cause a shift in the corresponding 1-alkene formation during growth of *Jeotgalicoccus* sp. ATCC 8456.
As fatty acid substrates, saturated hexanoic (6:0), octanoic (8:0), nonanoic (9:0), decanoic (10:0), undecanoic (11:0), dodecanoic (12:0), tetradecanoic (14:0), hexadecanoic (16:0), and octadecanoic acid (18:0) were added to the medium, either dissolved in DMSO or ethanol [raw data, Appendix; Tab 25 to Tab 28].
The expected terminal olefins, based on the proposed $OleT_{JE}$-catalytic reaction would therefore be the C_{n-1} products 1-C5, 1-C7, 1-C8, 1-C9, 1-C10, 1-C11, 1-C13, 1-C15, and 1-C19, respectively [highlighted in yellow; Tab 9].

For the approach where DMSO was used as co-solvent, feeding hexa- (6:0) and octanoic acid (8:0) did not induce the expected formation of 1-C5 and 1-C7, respectively, but other terminal olefins were detected. The terminal olefins 1-C14 (477.6 ± 114.3 nM) and 1-C18 (4150.2 ± 987.9 nM) were formed for hexanoic acid as well as 1-C10 (48.3 ± 7.0 nM) and 1-C15 (757.4 ± 73.9 nM) for octanoic acid [Tab 9]. Furthermore, the concentration of 1-C15 occurring during supplementation with octanoic acid was ca. 250-fold higher compared to the not induced conditions of the standard medium (2.2 nM vs. 757.4 nM), without taking the high standard deviation into account [Tab 4 and Tab 9].

Where, on the one hand feeding nonanoic acid (9:0) led to formation of 1-C12 (7.9 ± 3.1 nM) and 1-C8 in four out of five measurements, the addition of decanoic acid (10:0) on the other hand did not result in the detection of any 1-alkene [Tab 9].

In general, terminal olefins ≤ 1-C9 were not quantified, as calibration graphs could not be established due to the high volatility.

Tab 9. Terminal olefins produced by *Jeotgalicoccus* sp. ATCC 8456 upon feeding fatty acids. Three to seven measurements were conducted in total. The 1-alkene formation was either increased (highlighted in green), decreased (highlighted in red), remained the same (highlighted in blue) or not present (colorless) compared to/in the control (TSBYE without fatty acids). α-Olefins are given as mean values with SD. The expected C_{n-1} products from $OleT_{JE}$-catalysis are highlighted in yellow.

Fatty acid	6:0 [a]	8:0 [a]	9:0 [a]	10:0 [a]	11:0 [a]	12:0 [a]	14:0 [b]	16:0 [c]	20:0 [d]
1-Alkenes [nM]									
1-C5	0	0	0	0	0	0	0	0	0
1-C6	0	0	0	0	0	0	0	0	0
1-C7	0	0	0	0	0	0	0	0	0
1-C8	0	0	Not quantified	0	0	0	0	0	0
1-C9	0	0	0	0	0	0	0	0	0
1-C10	0	48.3 ± 7.0	0	0	2.0 ± 0.6	0	0	0	0
1-C11	0	0	0	0	0	37.0 ± 25.9	3.1 ± 2.0	164.2 ± 5.4	25.7 ± 9.9
1-C12	0	0	7.9 ± 3.1	0	0	0.5 ± 0.2	0.5 ± 0.2	0	0
1-C13	0	0	0	0	0	0	1896.2 ± 502.9	48.3 ± 4.8	0
1-C14	477.6 ± 114.3	0	0	0	0	0.6 ± 0.3	1.2 ± 1.2	1.5 ± 0.3	0
1-C15	0	757.4 ± 73.9	0	0	0	0	0	362.1 ± 137.8	0
1-C17	0	0	0	0	0	0	0	58.2 ± 24.3	0
1-C18	4150.2 ± 987.9	0	0	0	0	0	0	0	0
1-C19	0	0	0	0	0	0	19.0 ± 9.5	235.8 ± 147.4	1208.9 ± 213.5

Number of replicates: [a] 5 measurements, [b] 7 measurements, [c] 4 measurements, [d] 3 measurements

Inducing 1-alkene formation in the model microorganism with the fatty acids undecanoic (11:0), dodecanoic (12:0), tetradecanoic (14:0), hexadecanoic (16:0), and eicosanoic acid (20:0) yielded as expected the corresponding terminal olefins 1-C10 (2.0 ± 0.6 nM), 1-C11 (37.0 ± 25.9 nM), 1-C13 (1896.2 ± 502.9 nM), as well as 1-C15 (362.1 ± 137.8 nM), and 1-C19 (1208.9 ± 213.5 nM) (highlighted in yellow) [GC-MS spectrum for supplement with 14:0; Fig 22].

Compared to the not induced measurements, the values were thereby increased (highlighted in green) ca. 2-fold (1-C11), 475-fold (1-C13), 120-fold (1-C15), and 3.5-fold (1-C19), respectively [Tab 9]. The standard deviation was again not considered for comparing the data.

Further 1-alkenes were detected for the fatty acid substrates 12:0, 14:0, 16:0, and 20:0. *Jeotgalicoccus* sp. ATCC 8456 produced 1-C12 (37.0 ± 25.9 nM) and also 1-C14 (0.6 ± 0.3 nM) when dodecanoic acid was added, and 1-C11 (3.1 ± 2.0 nM, ca. 7-fold decrease compared not induced conditions, highlighted in red), 1-C12 (0.5 ± 0.2 nM), 1-C14 (1.2 ± 1.2 nM), as well as 1-C19 (19.0 ± 9.5 nM, ca. 18-fold decrease) when tetradecanoic acid was used as substrate.
Hexadecanoic acid led to the additional formation of 1-C11 (164.2 ± 5.4 nM, ca. 8-fold increase), 1-C13 (48.3 ± 4.8 nM, ca. 12-fold increase), 1-C14 (1.5 ± 0.3 nM), 1-C17 (58.2 ± 24.3 nM ca. 2.3-fold decrease), and 1-C19 (235.8 ± 147.4 nM, ca. 1.5-fold decrease). Whereas, for eicosanoic acid 1-C11 (25.7 ± 9.9 nM, same as in the control, highlighted in blue) was detected as additional terminal olefin.

The most frequently observed terminal olefins were 1-C11 and 1-C14, both present in four out of nine induced conditions, followed by 1-C12 and 1-C19 that were produced each in three cases where the medium was supplemented with a fatty acid [Tab 9].
Furthermore, a decreased cell density was seen for media containing 6:0 and 11:0, indicating a preference for fatty acids ≥ 12:0, where higher abundance of 1-alkenes was also identified.

For measurements where ethanol was used for dissolution of the fatty acids, for each of the acids only two SPME GC-MS runs were performed. Therefore, in a lot of cases no mean values and standard deviations could be calculated.

Jeotgalicoccus sp. ATCC 8456 produced 1-C6, 1-C8 as well as 1-C9 during growth in media where decanoic acid was added. All three 1-alkenes could not be quantified, as establishing of calibration graphs of these terminal olefins was not feasible, due to the high volatility of the compounds. Additionally, 1-C12 (1.9 nM) was formed [Appendix; Tab 28].
In media containing undecanoic acid, 1-C10 (0.8 ± 0.8 nM) and 1-C14 (2.1 ± 1.7 nM) were detected. When dodecanoic acid was added, the model organism formed 1-C10 (4.9 nM) as well as 1-C11 (3.9 ± 5.8 nM). Tetradecanoic acid led to 1-C11 (0.4 nM), 1-C12 (1.0 nM), 1-C13 (412.2 ± 322.9 nM), 1-C15 (2.7 nM), and also 1-C17 (4.5 nM).
Compared to the control sample cultivated in TSBYE [Tab 4], only the concentration of 1-C13 was induced and increased upon feeding with 14:0, all other 1-alkenes with an odd number of carbon atoms were produced in a lower amount.

Tab 10. Comparison of the formation of 1-alkenes by *Jeotgalicoccus* sp. ATCC 8456 induced with fatty acids, using DMSO or ethanol as co-solvent. The comparison is based on the terminal olefins detected using DMSO as co-solvent, thereby they were formed (highlighted in light grey), not formed (highlighted in dark grey) and either present in ca. equal concentration (highlighted in blue) or in less concentration (highlighted in red) using ethanol as co-solvent.

1-Alkene	Fatty acid							
	10:0		11:0		12:0		14:0	
	DMSO	ethanol	DMSO	ethanol	DMSO	ethanol	DMSO	ethanol
1-C5								
1-C6								
1-C7								
1-C8								
1-C9								
1-C10								
1-C11								
1-C12								
1-C13								
1-C14								
1-C15								
1-C17								
1-C18								
1-C19								

Moreover, lower (highlighted in red) or in two cases approximately equal amount (highlighted in blue) of terminal alkenes was detected using ethanol as co-solvent compared to DMSO. Providing 11:0 and 14:0 as substrates resulted in approximately equal concentrations of 1-C10 and 1-C12, respectively. For 12:0, lower amount of 1-C11, and for 14:0 lower amounts of 1-C11 as well as 1-C13 were induced using ethanol as co-solvent.

It means, the feeding of fatty acids dissolved in ethanol did not improve the 1-alkene production in *Jeotgalicoccus* sp. ATCC 8456 [Tab 10].

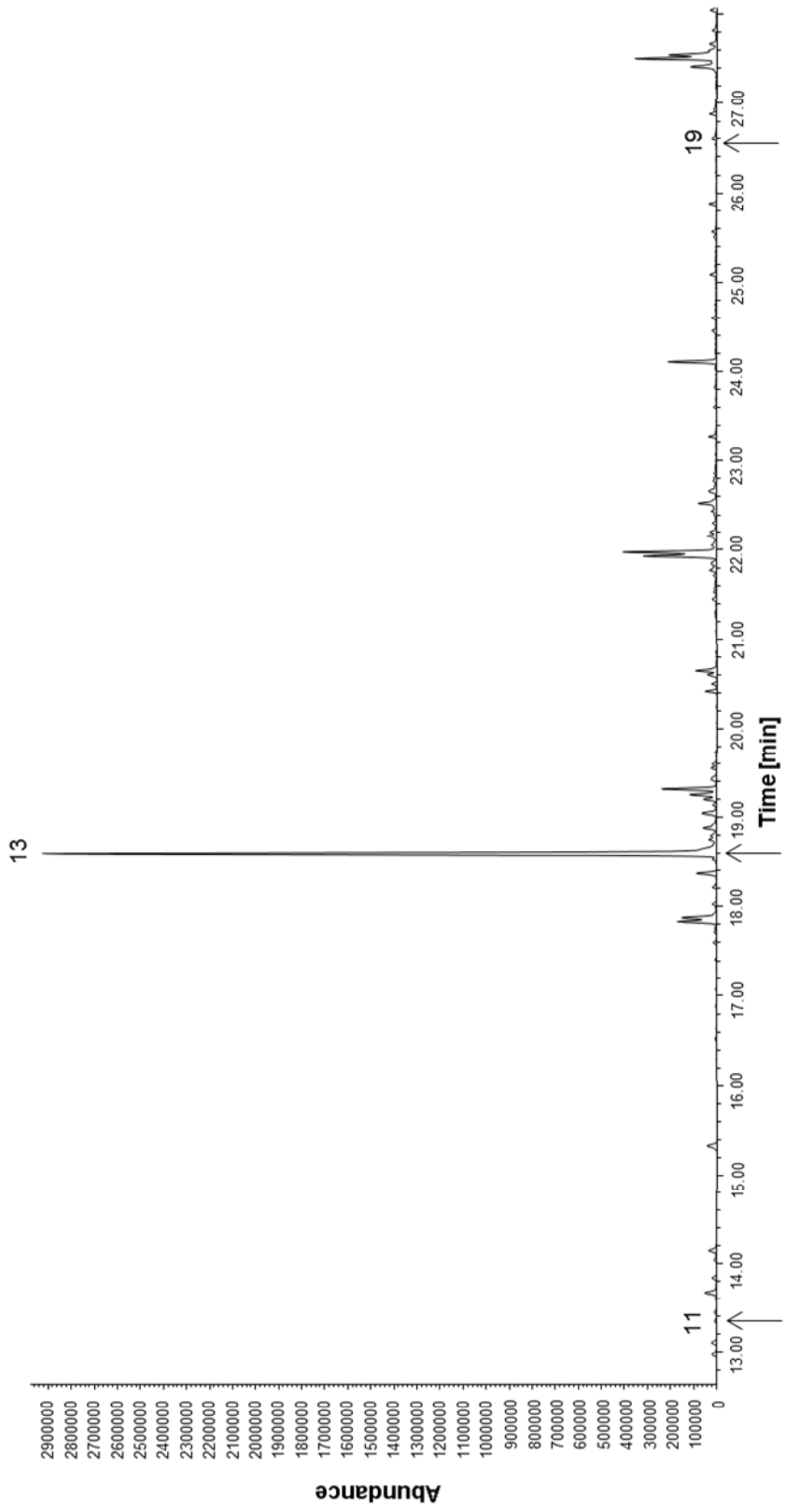

Fig 22. Feeding tetradecanoic acid as substrate caused a highly enhanced production of 1-C13 (here: 13) in *Jeotgalicoccus* sp. ATCC 8456. The fatty acid was provided in DMSO and product detection was performed by SPME GC-MS. Formation of the other terminal olefins found to be present for not induced standard conditions [Fig 10] was strongly reduced or not existent.

3.3 Identification of other promising bacterial 1-alkene producers

In order to expand the spectrum of terminal olefin producers, bacteria from the culture collection were screened for 1-alkene formation using SPME GC-MS.

In total, 59 strains were analyzed for α-olefin production [Appendix; Tab 12], which was found in eight of them. One strain belonged to the genus *Bacillus* (phylum of Firmicutes) and the remainder to *Pseudomonas* (phylum of Proteobacteria, class of Gammaproteobacteria).
1-C11 was not only produced by all eight 1-alkene positive strains, but also appeared in significantly higher concentrations, ranging from 145 to 1404 nM.
1-C13 was formed by five of these bacteria, 1-C12 as well as 1-C14 by one (*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, respectively), whereby the concentrations did not exceed 5 nM [Tab 11 and Appendix; Tab 29].
Further, the second round of measurement yielded always higher concentrations of 1-C11, resulting in a lowered or no production of the other terminal olefins, as observed in the first quantification.

Tab 11. Bacterial strains from the culture collection identified to be 1-alkene producers. Terminal olefin formation is given in nM concentrations, calculated from the respective GC-MS areas [Appendix; Tab 29]. Two independent SPME GC-MS measurements were performed.

Species	Strain code	Measurement	1-Alkene production in TSBYE [nM]			
			1-C11	1-C12	1-C13	1-C14
Bacillus thuringiensis	3R2-29	1	661.6	0	4.8	0
		2	695.8	0	0	0
Pseudomonas aeruginosa	QC14-3-8	1	331.1	0.7	4.5	0
		2	456.8	0.5	3.4	0
Pseudomonas brassicacearum[a]	L13-6-12	1	754.0	0	4.1	0
		2	1140.9	0	0.8	0
Pseudomonas brassicacearum[b]	L13-6-12	1	682.6	0	2.3	0
		2	1197.7	0	1.0	0
Pseudomonas brenneri	Baz30	1	434.1	0	0	0
		2	536.5	0	0	0
Pseudomonas fluorescens	Baz53	1	395.9	0	0	3.1
		2	587.3	0	0	0
Pseudomonas poae	RE*1-1-14	1	732.6	0	0.6	0
		2	1404.3	0	0.1	0
Pseudomonas putida	1T1	1	144.7	0	0	0
		2	885.2	0	0	0

[a, b] are different glycerol stocks from the culture collection

3.4 The role of terminal olefins as (bio-) control agents against plant pathogens

The inhibitory effect of selected 1-alkenes, applied in their highest possible concentration, was surveyed *in vitro* on the growth of important bacterial as well as fungal plant pathogens. In addition, the antagonism of *Jeotgalicoccus* sp. ATCC 8456 was tested *in vivo* against four fungal plant pathogens.

3.4.1 *In vitro* determination of growth inhibition of bacterial plant pathogens by 1-alkenes

As examples of relevant plant pathogenic bacteria *Bacillus pumilus*, *Burkholderia cepacia*, *Clavibacter michiganensis*, *Pectobacterium carotovorum*, *Pseudomonas viridiflava* [Fig 23], *Xanthomonas campestris* and *Xanthomonas cucurbitae* [Fig 24] were used to identify if pure 1-alkenes are able to inhibit their growth. For this purpose, *in vitro* two clamp VOC assays with the strains grown on NB-agar supplemented with the redox and pH indicator resazurin were set up. Resazurin is reduced to resorufin by viable cells [Borra et al. 2009], what causes a decrease of the pH and thus, a color change from a violet-bluish to a pink or orange agar [Sarker et al. 2007].

Except of *Clavibacter michiganensis* and also *Bacillus pumilus* growth could be seen clearly for all bacteria after 72 h of cultivation at 30 °C, resulting in differently colored agar [Fig 23].

Thereby, agar with grown *Burkholderia cepacia* appeared in most cases in an intense dark pink, except for cultivation against 1-C12, 1-C15, and 1-C18 were the color of the agar changed to orange [Fig 23].
Growth of *Pseudomonas viridiflava* as well as *Pectobacterium carotovorum* was uniform, represented as an intense dark pink agar in the first and a light pink agar in the second case.
Agar with grown *Bacillus pumilus* occurred in very light pink (1-C11 [one of two replicates], 1-C13, 1-C14 [one of two replicates], 1-C15, 1-C17), dark pink (1-C9, 1-C11 [one of two replicates]) and also blue (1-C8, 1-C10, 1-C14 [one of two replicates], 1-C18). For the latter, the cultures seemed to be parched. The bacterium appeared to be inhibited by 1-C12.
For all cases from 1-C8 to 1-C14, *Clavibacter michiganensis* did not grow in wells next to the positive control (+, bacillol), changing the agar otherwise to light pink [Fig 23].

As results for *Bacillus pumilus* and *Clavibacter michiganensis* were not clear, their assays were repeated, together with the assay of the remaining test organisms [Fig 24].

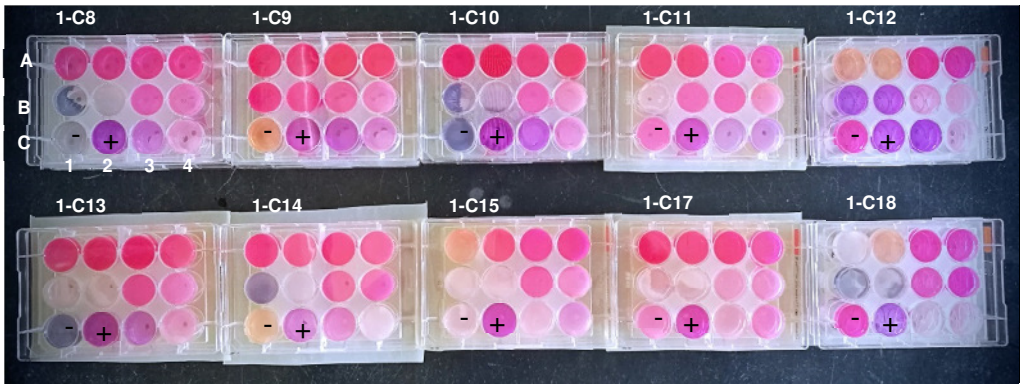

Fig 23. Growth inhibition assay of terminal alkenes (1-C8 to 1-C18) on bacterial phytopathogens. The application order is A1+2: *Burkholderia cepacia*, A3+4: *Pseudomonas viridiflava*, B1+2: *Bacillus pumilus*, B3+4: *Pectobacterium carotovorum*, C1+2: alternating bacteria and C3+4: *Clavibacter michiganensis*. Alternating bacteria for the controls were used as follows: *Bacillus pumilus* (1-C8, 1-C13), *Burkholderia cepacia* (1-C9, 1-C14), *Clavibacter michiganensis* (1-C10, 1-C15), *Pectobacterium carotovorum* (1-C11, 1-C17), *Pseudomonas viridiflava* (1-C12, 1-C18).

For the repeated assays, both bacteria were not inhibited by any terminal olefin tested. For this purpose, *Clavibacter michiganensis* was grown against all 1-alkenes (1-C8 to 1-C18) and *Bacillus pumilus* against 1-C12 [Fig 24].

Also, *Xanthomonas cucurbitae* as well as *Xanthomonas campestris* were not limited in their growth by any of the 1-alkenes (1-C8 to 1-C18). The agar changed always uniformly from purple to light pink for *Xanthomonas cucurbitae* and to an intense darker pink for *Xanthomonas campestris* [Fig 24].

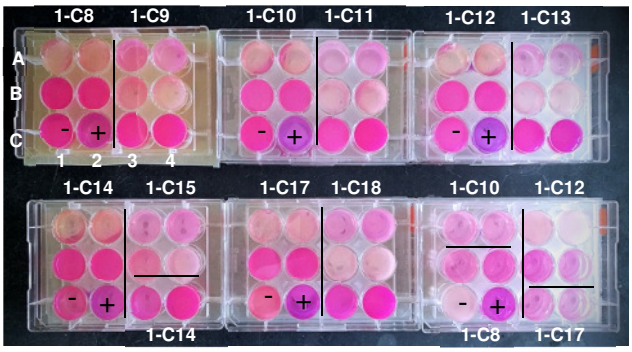

Fig 24. Growth inhibition assay of terminal alkenes (1-C8 to 1-C18) on bacterial phytopathogens. The application order for all assays except the one in the bottom right corner is A1+2 and B3+4: *Xanthomonas cucurbitae*, A3+4: *Clavibacter michiganensis*, B1+2 and C3+4: *Xanthomonas campestris*. Alternating bacteria for the controls were used as follows: *Xanthomonas campestris* (1-C8/1-C9, 1-C10/1-C11, 1-C12/1-C13), *Xanthomonas cucurbitae* (1-C14/1-C15, 1-C17/1-C18).
The application order for the last assay in the bottom right corner is A1+2, B1 to B4 as well as C3+4: *Clavibacter michiganensis*, A3+4: *Bacillus pumilus*. For the positive (+) as well as the negative (-) control *Bacillus pumilus* was used (C1+2).

Additionally, for the positive control (+, bacillol) in all cases no bacterial growth was visible as expected i.e. the agar remained purple. For the negative control (-, DMSO pipetted onto a sterile filter paper) the respective bacterium always grew [Fig 23 and Fig 24].

3.4.2 *In vitro* determination of growth inhibition of fungal plant pathogens by a selection of 1-alkenes

The persistent fungal agricultural crop pathogens *Botrytis cinerea*, *Fusarium culmorum*, *Rhizoctonia solani*, and *Verticillium longisporum* V25 were grown against 1-C11, 1-C12, 1-C13, as well as 1-C14 in two clamp VOC assays at room temperature.

After 24 h of co-incubation against the 1-alkenes [results for 1-C13 and 1-C14 shown; Fig 25], the mycelia increased 7 mm (*Botrytis cinerea* and *Fusarium culmorum*) and 8 mm (*Rhizoctonia solani*) in diameter, whereby *Botrytis cinerea* displayed a more uneven growth behavior compared to the other two fungi. Within the next 48 h, all mycelia reached the side walls of the wells (results not shown).

Fungi grown as negative control (-, co-incubation with DMSO pipetted onto a sterile filter paper) showed the same growth behavior and same increase in size over time compared to the fungi growing in the presence of 1-alkenes. As expected, the positive growth-inhibition control (+, bacillol) did not grow [Fig 25].

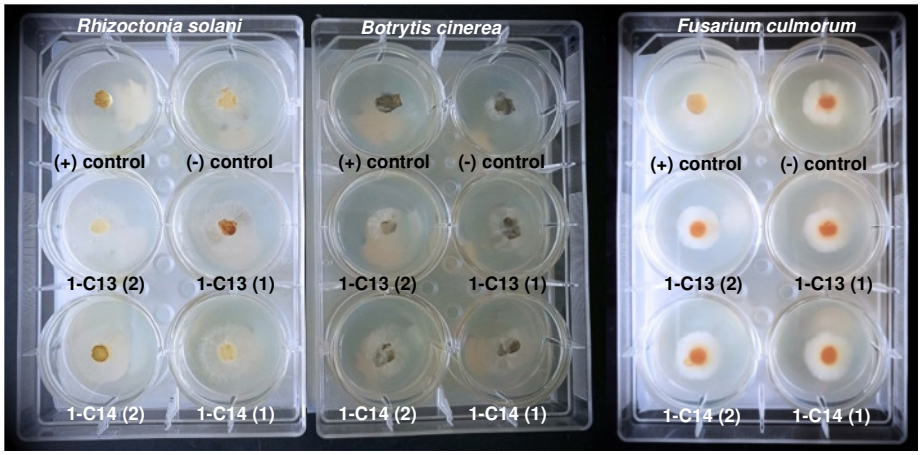

Fig 25. Growth of fungal plant pathogens was not inhibited by any 1-alkene (1-C11 to 1-C14) applied in the TCVAs. Here: 24 h of cultivation of *Botrytis cinerea*, *Fusarium culmorum* and *Rhizoctonia* solani with co-incubated with 1-C13 as well as 1-C14. Growth was visible for the negative control (-), but not seen for the positive control (+).

Also, for *Verticillium longisporum* V25, using an undiluted as well as a 1:1 diluted spore suspension, growth was observed after 24 h of cultivation against the four 1-alkenes.
During the following 24 h, the color of the fungi changed from opaque to cream-white [again 1-C13 and 1-C14 shown; Fig 26] and after five days the mycelia switched to black. The same growth characteristics arose for the negative control (-, co-incubation with DMSO pipetted onto a sterile filter paper), whereby *Verticillium longisporum* V25 did not grow in the positive control (+, bacillol).

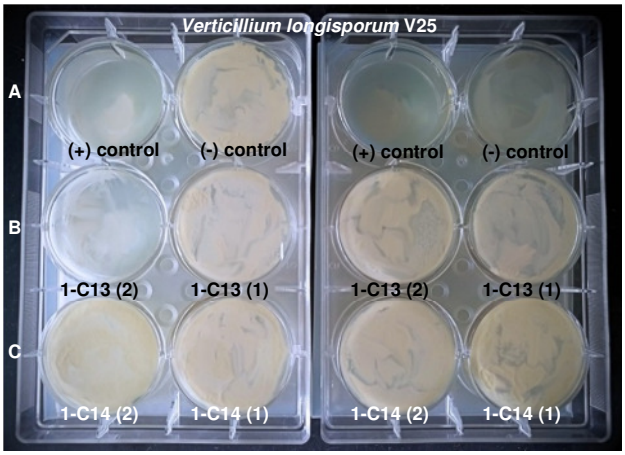

Fig 26. *Verticillium longisporum* V25 was not inhibited by terminal olefins 1-C11 to 1-C14. An undiluted (A and B) and a 1:1 diluted spore suspension (C) was used. Growth was visible for the negative control (-), but not visible for the positive control (+). Here: 48 h of cultivation.

Summed up, selected bacterial as well as fungal plant pathogens could not be inhibited by the tested terminal olefins under the used conditions.

3.4.3 Examination of the antagonism of *Jeotgalicoccus* sp. ATCC 8456 against fungal plant pathogens

Whether, volatiles emitted by *Jeotgalicoccus* sp. ATCC 8456 are able to inhibit growth of fungal agricultural crop pathogens, was evaluated using *in vivo* TCVAs. Thereby, the model organism was applied on NB-agar, solidified in the wells of one plate, and on the opposing plates *Botrytis cinerea*, *Fusarium culmorum*, *Rhizoctonia solani*, as well as *Verticillium longisporum* V25 were grown on PDA-agar [Fig 27].

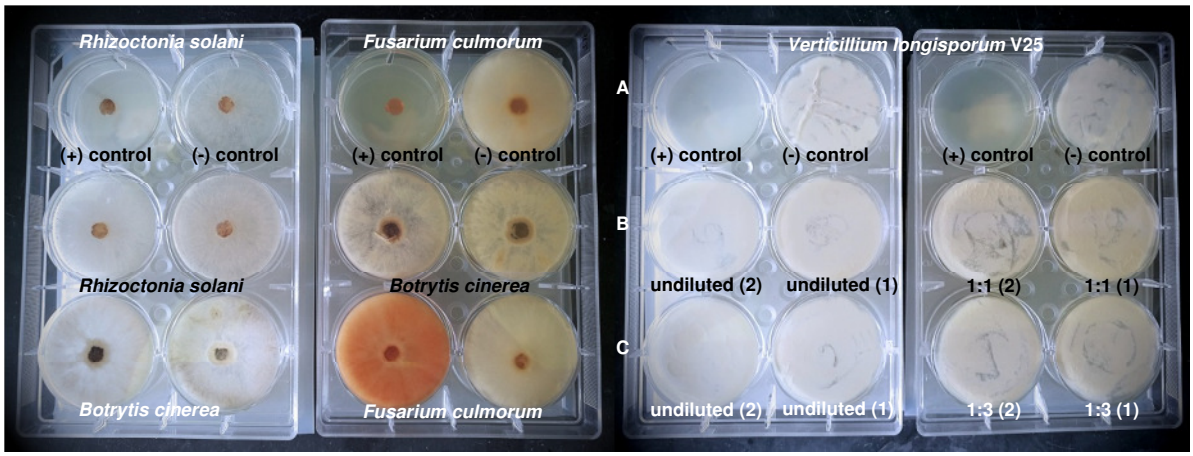

Fig 27. Antagonistic activity of *Jeotgalicoccus* sp. *ATCC 8456* against selected fungal plant pathogens. Growth was visible for the negative control (-), but not seen for the positive control (+). An undiluted (A to C), a 1:1 (B) and a 1:3 (C) diluted spore suspension of *Verticillium longisporum* V25 was applied.

For all selected fungi the same mycelium formation with respect to time dependent growth and color was detected [Fig 27] compared to the *in vitro* assays. The same holds true for the positive and the negative controls.

The plant pathogen *Verticillium longisporum* V25 was additionally applied as a 1:3 diluted spore solution, showing also any growth-inhibition as for the undiluted as well as the 1:1 diluted spore suspension, compared to the negative control.

Thus under the used conditions, *Jeotgalicoccus* sp. ATCC 8456 did not show an antagonistic activity against any of the selected fungal phytopathogens.

4 Discussion

4.1 Characterization of *Jeotgalicoccus* sp. ATCC 8456

The genus of *Jeotgalicoccus* is relatively unknown until today. Assumptions which might explain this phenomenon is that *Jeotgalicoccus* seems to disappear during cultivation of environmental samples, due to acceptance of only few media or the sequencing of only partial genomes as well as high genome similarities to *Salinicoccus* and *Staphylococcus* [Schwaiger et al. 2010]. Additionally, sequencing techniques and culture independent methods are in widespread use only for a comparable short time.

Due to the high similarities of almost all analyzed genes of *Jeotgalicoccus* sp. ATCC 8456, like that for fatty acid biosynthesis, compared to the genes of *Jeotgalicoccus marinus* (≥ 83.2%, but in most cases ca. 98 to 100%) the question arose, if it could be a subspecies of *Jeotgalicoccus marinus*. Arguments against were, that *Jeotgalicoccus* sp. ATCC 8456 cannot utilize glucose and tolerates only lower salinity (probably ca. 10% [w/v] NaCl) compared to *Jeotgalicoccus marinus* (25% [w/v] salt) [Chen et al. 2009]. Additionally, growth at 37 °C was not possible, but *Jeotgalicoccus marinus* is able to survive even up to 45 °C [Chen et al. 2009]. Further explanation could be, that *Jeotgalicoccus huakuii* was not fully sequenced, thereby *Jeotgalicoccus marinus* appears to be the closest related species of this genus.
Generally, the model microorganism has one of the lowest GC-contents (36.92%), compared to the other sequenced *Jeotgalicocci* [Liu et al. 2011a], e.g. *Jeotgalicoccus marinus* and *Jeotgalicoccus huakuii* were found to have 40.3% and 36.8% GC-content, respectively.

Furthermore, *Jeotgalicoccus* sp. ATCC 8456 is a non-motile and facultative anaerobe bacterium what holds also true for other isolated *Jeotgalicoccus* species [Yoon 2003], expect of *Jeotgalicoccus nanhaiensis* that grows strictly under aerobic conditions [Liu et al. 2011b].

One problem that occurred for the genome-based assignment of metabolic functionalities, like the presence of certain enzymes or the 1-alkene formation pathway, is that almost one third of all identified ORFs remained completely unknown and further 13% were assigned to unknown or only predicted protein functions. This resulted in a considerable large black-box of possible missed enzymes. For further verification and clarification of probable proteins involved in interesting synthesis routes other tools, e.g. using metabolomics,

knock-out mutant libraries or chemical assays have to be considered. That would have exceeded the scope of this master thesis.

Also, *Jeotgalicoccus* sp. ATCC 8456 can be considered not to be a human pathogen, as it did not grow at 37 °C [Price and Frey 2003].

Carbohydrate utilization of *Jeotgalicoccus* sp. ATCC 8456 was evaluated with the Hugh-Leifson-medium, where glucose was neither oxidized nor fermented. Together with the genomic analysis, it was assumed that this strain does not contain specific glucose transporting or converting enzymes and another saccharide would be the favored sugar source. The aerobic cultivation turned bluish-green, what could result from the increase in pH due to breakdown of peptone to amines instead of metabolizing glucose. This is an already known phenomenon from other bacteria e.g. *Alcaligenes faecalis* [Harada et al. 1965].
Previous studies also revealed an unreactive OF reaction for *Jeotgalicoccus halotolerans*, *Jeotgalicoccus pinnipedialis*, *Jeotgalicoccus psychrophilus*, and also *Jeotgalicoccus marinus*, [Schwaiger et al. 2010] implying that the inability to use glucose as sole carbon source is quite common among species of this genus.
Some controversies occurred, as *Jeotgalicoccus marinus* was depicted as glucose-utilizing bacterium in a previous analysis by other authors [Chen et al. 2009].

For characterization of bacteria, it is also relevant to identify the growth behavior in different media to optimize the cultivation, for instance avoiding limitation of product formation due to transition into the stationary phase.
From determining the growth phases of the model microorganism in four different media, it could be concluded, that *Jeotgalicoccus* sp. ATCC 8456 might not be an ideal workhorse for industrial production due to its very slow growth and low generation time. The exponential growth phases ranged from six to 23 h, where more than two h were required for doubling. Thereby, TSBYE was the most promising medium with respect to the finally obtained cell densities (OD_{600} values), probably outbalancing the fact that only the lowest doubling times could be reached, compared to all other tested media. For instance, *Escherichia coli* has a doubling time of approximately 20 min [Clark and Maaløe 1967].
The results obtained should be treated with caution, as *Jeotgalicoccus* sp. ATCC 8456 is a sensitive bacterium in handling. Especially, for calculation of the generation times, further repetitions of the experiment would be necessary.

For osmotic stress responses, *Jeotgalicoccus* sp. ATCC 8456 most likely contains both known mechanisms. On the one hand there is the salt-in strategy, where organisms accumulate potassium ions within the cell for osmotic balance. The genomic analysis of the model bacterium showed that channels of the MscL and MscS family, a potassium uptake system as well as a natrium-hydrogen antiporter were encoded. On the other hand the alternative strategy is to accumulate organic solutes like glycine betaine or glycerol in the cytoplasm [Oren 2002]. The glycine betaine transport system (Opu) was identified in the genome of *Jeotgalicoccus* sp. ATCC 8456, however, genes for the formation of osmolytes were not found.
As no direct correlation between increasing salinity and the formation of 1-alkenes was observed, these molecules serve probably not as osmoprotectants, but might have other yet unknown biological functions for the organism.

This thesis showed that *Jeotgalicoccus* sp. ATCC 8456 could not be applied as antagonist against the selected fungal plant pathogens, but a very recent study successfully employed *Jeotgalicoccus huakuii* NBRI 13E for plant growth promotion due to its natural abiotic stress resistance e.g. to salt [Misra et al. 2019]. This indicates the possible implementation of *Jeotgalicocci* spp. in environmental biotechnology approaches, meaning that the true potential of *Jeotgalicoccus* sp. ATCC 8456 for this field might not be deciphered yet.

4.2 Bacterial 1-alkene formation

Jeotgalicoccus sp. ATCC 8456 was found to be a 1-alkene producing microorganism in previous research [Rude et al. 2011]. In this thesis, it was possible to expand the scope of reported unbranched 1-alkenes (only 1-C19) to further terminal olefins. The natural formation under standard cultivation conditions of unbranched 1-C13, 1-C15, 1-C17, and also 1-C19 could be confirmed here.

It was the first time that α-olefin formation was quantified in *Jeotgalicoccus* sp. ATCC 8456 *in vivo*. For *Jeotgalicoccus halotolerans*, *Jeotgalicoccus huakuii*, as well as *Jeotgalicoccus psychrophilus*, 1-alkene concentrations between six to 13 mg/l for 1-C15, 1-C17 and 1-C19 were determined elsewhere [Nusantara Putra et al. 2019]. These values are maximally four magnitudes larger than the ones measured in this thesis, taking into account differences in the used methodology. The three other species were thereby grown in MB-medium and the terminal unsaturated olefins extracted from the corresponding pellets prior to GC-MS measurement using liquid injection [Nusantara Putra et al. 2019].

Cultivation with increasing salinity did not clearly affect the 1-alkene formation. This means that no beneficial role was detected for inducing 1-alkene formation trough increasing the salt concentration in the medium.

Providing fatty acid substrates induced considerably the production of the corresponding C_{n-1} 1-alkenes for nonanoic (9:0), undecanoic (11:0), dodecanoic acid (12:0), tetradecanoic (14:0), hexadecanoic (16:0) and eicosanoic acid (20:0). Due to high volatility, 1-C8 could not be quantified, but compared to the not induced measurements, the values for 1-C11, 1-C13, 1-C15, and 1-C19 were increased approximately 2-fold, 475-fold, 120-fold, and 3.5-fold, respectively. The formation of 1-C10 was also detected upon feeding with undecanoic acid, which was otherwise not measured before for cultivation under standard conditions.
Furthermore, a decreased cell density was seen for media containing hexanoic and undecanoic acid, indicating a preference for fatty acids ≥ 12:0 (dodecanoic acid), where higher abundance of 1-alkenes was also detected.
When fatty acids were fed, also α-olefins with an even number of carbon atoms could be quantified (especially ≤ 1-C14), indicating also other reaction mechanisms like a preceding decarboxylation of the fatty acids [Rude et al. 2011]. Alternatively, the unknown catalytic activity of the paralogue of $OleT_{JE}$ (CypC, BASYS00261, fatty acid peroxygenase, 416 aa), which was found within the genomic sequence of *Jeotgalicoccus* sp. ATCC 8456 could play a major role here. Both ORFs shared a low sequence similarity of 38%. It would be interesting to elucidate the role and importance of this second monooxygenase in the production of 1-alkenes.

Regarding the used quantification method itself, maybe it ought to be reconsidered if SPME GC-MS is properly reproducible, as very high standard deviations occurred, even when only the most representative values were used for calculation. In literature, standard deviation values between ten and at maximum 20% (for trace element analysis) are common, making SPME GC-MS indeed a reliable method for quantification approaches [Bojko et al. 2012; Verzera et al. 2001].
Perhaps, the origin of this phenomenon is to be sought in the relationship of growth to product formation. 1-Alkene production could be directly, indirectly or not associated with the bacterial growth. The underlying regulation mechanisms are still unknown, also due to the missing annotation of a high share or the genome, as a consequence of the low homologies of the putative protein coding sequences to characterized ones, makes it difficult to study.
For product formation not associated to growth, longer incubation times (i.e. more biomass) would lead to higher concentrations of the respective terminal olefins [Doran 2013].

Differences of only a few hours in the cultivation could be a reason for the variability of the measured concentrations of the terminal olefins. The time differences might have occurred during the long waiting times in between samples during the GC-MS measurment.

As it is possible to induce the formation of C_{n-1} 1-alkenes through feeding with fatty acids, this provides innovative biotechnological advantages to specifically produce certain terminal olefins, and reduce the concentration of other 1-alkene side products. This was proven *in vivo* [Rude et al. 2011], as well as *in vitro* [Dennig et al. 2015] in previous research. In this thesis, titers of the respective C_{n-1} α-olefins could be increased compared to the not induced standard conditions, e.g. for 1-C13 more than 1.8 mM (328 mg/l) and for 1-C19 more than 1.2 mM (320 mg/l) could be quantified. These concentrations are significantly higher than those achieved by other authors without feeding fatty acid substrates, e.g. ca. 10 mg/l for 1-C19 [Nusantara Putra et al. 2019]. Furthermore, fatty acids are a relatively cheap resource and might also be applied from waste oil [Wang and Zhu 2018].

The fatty acid biosynthesis pathway in connection to the formation of 1-alkenes in *Jeotgalicoccus* sp. ATCC 8456 could be reconstructed based on the annotation results derived from whole genome sequencing. This is important for further optimization or engineering of the pathway to achieve higher yields of terminal olefins *in vivo*.

Furthermore, as 1-alkenes are hydrophobic, they needed to be dissolved in a co-solvent prior to the addition to the cultivation medium. For this purpose, ethanol and DMSO were used. When ethanol was taken as co-solvent, in all cases for the 1-alkenes that were also produced when DMSO was applied as co-solvent, the concentration was the same or even lower.
The logP value of DMSO is -1.3 and that of ethanol -0.24, whereas the latter has the lower miscibility with water resulting in a lower bioavailability and transport of the fatty acids to the cells for effective 1-alkene production [Faber 2011]. This could also explain why the standard deviations for feeding with fatty acids dissolved in DMSO were more stable compared to ethanol.

$OleT_{JE}$ was reported as a peroxygenase [Rude et al. 2011], requiring hydrogen peroxide for the catalytic reaction to take place. Up to now, there is not much knowledge on how the availability of peroxide for the catalytic reaction in the cell works.
As reactive oxygen species can be lethal for cells, all aerobic living organisms must employ resistance mechanism [Fu et al. 2015]. Several of these systems for the detoxification of peroxide were found on the genomic level of the model microorganism, like the

transcription repressor PerR. The PerR system could be involved therefore in regulation of the availability of hydrogen peroxide for $OleT_{JE}$.
A direct interconnection between oxidative stress response and metal homeostasis for the PerR regulon has been described [Hillion and Antelmann 2015]. PerR also regulates the heme biosynthesis, and again this most likely could have an impact on $OleT_{JE}$ as it is a heme-containing monooxygenase.

Volatile organic compounds, like those emitted from microorganisms or plants, are used as infochemicals for defense mechanisms and also for communication across distances [Schmidt et al. 2015]. The operating range of secreted molecules is much shorter. Therefore, the importance of VOCs also in the field of environmental biotechnology increases, e.g. as biocontrol agents [Cernava et al. 2015].
It was seen that the terminal olefins produced by bacterial strains (seven *Pseudomonas* spp. and one *Bacillus* sp.) were 1-C11, 1-C12, 1-C13, as well as 1-C14. Therefore, they were applied in TCVAs to analyze their effect on the growth of fungal phytopathogens. This selection was further based on literature, where it was shown, that 1-C11 was able to impede oomycete plant pathogens like *Phytophthora infestans*, not only in their growth but also in their sporulation [Hunziker et al. 2015]. It is especially emitted by different *Pseudomonas* species isolated from the rhizosphere [Dandurishvili et al. 2011]. Also, 1-C12 was identified to impact the sporangia formation and germination of this oomycete [De Vrieze et al. 2015].
In this thesis, it was verified that under the tested conditions the unbranched 1-alkenes were not capable of inhibiting the selected bacterial and fungal plant pathogens.
One of their main function is probably to enhance the bioavailability of e.g. defensive secretes in various organisms as previously described [Gehlsen et al. 2009].

5 Conclusion and Outlook

Not only a broad variety of microorganisms, including *Jeotgalicoccus* sp. ATCC 8456, but also eukaryotes like insects or plants are able to produce 1-alkenes. They are of great industrial interest and naturally aid in survival and defense machineries of organisms due to their hydrophobic properties.

The research objective of this thesis was to provide a first insight into the metabolism, the survival, and the 1-alkene formation strategies of *Jeotgalicoccus* sp. ATCC 8456. Additionally, a screening for other potential α-olefin producers among bacterial species was performed.

With whole-genome sequencing and subsequent annotation, important genes for the production of terminal unsaturated olefins and fatty acid precursors could be elucidated. Besides, mechanisms involved in osmotolerance and detoxification of reactive oxygen species were identified in the model organism.

Furthermore, the induction of the formation of 1-alkenes through feeding with fatty acids was successfully proven.

For commercial applications, the α-olefin forming enzymes have to be analyzed in more detail in order to gain sophisticated knowledge about the catalysis of these industrially and biologically important bioactive substances.

Further investigations are required to completely decipher the production pathways of terminal olefins *in vivo* and to implement the responsible genes successfully into suitable host organisms for industrial large-scale processes. It also should be looked into the potential of these pathways to convert, e.g. waste fats and oils, providing an ecofriendly alternative to existing methods.

Appendix Material and Methods

Tab 12. Selection of bacteria from the strain collection (Institute of Environmental Biotechnology, Graz University of Technology, Austria) screened for 1-alkene formation using SPME GC-MS.
Proteobacteria and Bacteroidetes are gram negative, whereas Firmicutes and Actinobacteria are gram positive. The strains were chosen according to their relationship to known* 1-alkene forming bacteria: *Micrococcus* (belongs to the family of Micrococcaceae within the phylum of Actinobacteria), *Shewanella* (belongs to the family of Shewanellaceae within the phylum of Proteobacteria i.e. class of Gammaproteobacteria), *Synechococcus* (belongs to the family Synechococcaceae within the phylum of Cyanobacteria), *Xanthomonas* (belongs to the family of Xanthomonadaceae within the phylum of Proteobacteria i.e. class of Gammaproteobacteria), *Jeotgalicoccus* (belongs to the phylum of Firmicutes and the family of Staphylococcaceae) [Wackett 2014]. If the assignment of the bacteria from the strain collection was not possible based on family, phylum or class were taken. Seven bacteria did not grow** either on agar or in liquid medium (NB or TSBYE) and were therefore not further considered. In total, 59 strains were screened for α-olefin production.

Phylum or class	Family	Species	Strain code
Alphaproteobacteria	Rhizobiaceae	*Agrobacterium radiobacter*	1Pe2-5
Gammaproteobacteria	Vibrionaceae	*Aliivibrio fischeri***	DSM-7151
Firmicutes	Bacillaceae	*Bacillus amyloliquefaciens*	6R3-3
Firmicutes	Bacillaceae	*Bacillus cereus*	BE2-3-27
Firmicutes	Bacillaceae	*Bacillus licheniformis*	8B11-101
Firmicutes	Bacillaceae	*Bacillus megaterium*	24B1
Firmicutes	Bacillaceae	*Bacillus mycoides*	2P4-4
Firmicutes	Bacillaceae	*Bacillus subtilis*	1Pe4-134
Firmicutes	Bacillaceae	*Bacillus thuringiensis*	3R2-29
Betaproteobacteria	Burkholderiaceae	*Burkholderia bryophila*	A5
Betaproteobacteria	Burkholderiaceae	*Burkholderia cepacia*	L16-3-11
Betaproteobacteria	Burkholderiaceae	*Burkholderia gladioli*	850aiiA1S8
Betaproteobacteria	Burkholderiaceae	*Burkholderia phenazinium?*	MS1
Betaproteobacteria	Burkholderiaceae	*Burkholderia phytofirmans*	PSYN 17436
Actinobacteria	Microbacteriaceae	*Clavibacter michiganensis sub*sp. *michiganensis DSMZ 20134*	DSMZ20134
Actinobacteria	Microbacteriaceae	*Curtobacterium flaccumfaciens*	Buk6
Gammaproteobacteria	Enterobacteriaceae	*Enterobacter amnigenus*	Hgx4
Gammaproteobacteria	Enterobacteriaceae	*Enterobacter radicincitans D523*	D523
Gammaproteobacteria	Enterobacteriaceae	*Erwinia carotovora*	Eca DSM549
Gammaproteobacteria	Enterobacteriaceae	*Erwinia carotovora*	Eca DSM30168
Gammaproteobacteria	Enterobacteriaceae	*Klebsiella pneumoniae*	-
Gammaproteobacteria	Enterobacteriaceae	*Klebsiella terrigena*	Baz31
Gammaproteobacteria	Xanthomonadaceae	*Lysobacter antibioticus***	DSM 2044
Gammaproteobacteria	Xanthomonadaceae	*Lysobacter antibioticus*	DSM 2045
Alphaproteobacteria	Methylobacteriaceae	*Methylobacterium extorquens***	Rab-1 = DSM21961
Alphaproteobacteria	Methylocystaceae	*Methylosinus sporium DSM 17706***	-
Actinobacteria	Micrococcaceae	*Micrococcus luteus*	-
Firmicutes	Paenibacillaceae	*Paenibacillus illinoisensis*	T4B1c.7-B
Firmicutes	Paenibacillaceae	*Paenibacillus polymyxa*	PB71 = GnDBI71/1
Firmicutes	Paenibacillaceae	*Paenibacillus* sp.	Baze28
Gammaproteobacteria	Enterobacteriaceae	*Pantoea agglomerans*	Hgx5
Bacteroidetes	Sphingobacteriaceae	*Pedobacter cryoconitis***	Buk112
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas aeruginosa*	QC14-3-8
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas brassicacearum*[a]	L13-6-12
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas brassicacearum*[b]	L13-6-12
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas brenneri*	Baz30
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas fluorescens*	Baz53
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas graminis*	Baz90
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas poae*	RE*1-1-14
Gammaproteobacteria	Pseudomonadaceae	*Pseudomonas putida*	1T1

Gammaproteobacteria	Enterobacteriaceae	*Salmonella typhimurium LT2*	-
Gammaproteobacteria	Enterobacteriaceae	*Serratia marcescens*	-
Gammaproteobacteria	Enterobacteriaceae	*Serratia plymuthica*	DSM 4540
Gammaproteobacteria	Enterobacteriaceae	*Serratia plymuthica 3Re4-18*	3Re4-18
Gammaproteobacteria	Enterobacteriaceae	*Serratia rubidaea*	C27
Firmicutes	Staphylococcaceae	*Staphylococcus lugdunensis*	-
Firmicutes	Staphylococcaceae	*Staphylococcus aureus*	practical course
Firmicutes	Staphylococcaceae	*Staphylococcus aureus*	
Firmicutes	Staphylococcaceae	*Staphylococcus auricularis*	25
Firmicutes	Staphylococcaceae	*Staphylococcus capitis*	CM2734
Firmicutes	Staphylococcaceae	*Staphylococcus caprae*	10s
Firmicutes	Staphylococcaceae	*Staphylococcus epidermidis*	41608/80
Firmicutes	Staphylococcaceae	*Staphylococcus epidermidis*	ATCC14990
Firmicutes	Staphylococcaceae	*Staphylococcus hominis*	CCM2732
Firmicutes	Staphylococcaceae	*Staphylococcus saprophyticus*	11635
Firmicutes	Staphylococcaceae	*Staphylococcus saprophyticus*	S1877
Firmicutes	Staphylococcaceae	*Staphylococcus schleiferi*	-
Firmicutes	Staphylococcaceae	*Staphylococcus simulans*	-
Firmicutes	Staphylococcaceae	*Staphylococcus warneri*	CCM2730
Firmicutes	Staphylococcaceae	*Staphylococcus xylosus*	CCM2725
Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas maltophilia*	e-p3 = R3089
Gammaproteobacteria	Xanthomonadaceae	*Stenotrophomonas rhizophila*	P69
Actinobacteria	Streptomycetaceae	*Streptomyces ornatus***	Baz96
Actinobacteria	Streptomycetaceae	*Streptomyces* sp.	7W1
Gammaproteobacteria	Xanthomonadaceae	*Xanthomonas campestris***	X.C.B2-1
Gammaproteobacteria	Xanthomonadaceae	*Xanthomonas cucurbitae 6g5*	6g5

*known 1-alkene producers at the beginning of this thesis (October 2017).

Tab 13. Dilution series of 1-alkene standards for generation of the corresponding linear calibration graphs. Dilutions were done with DMSO. Step sequence is read from left to right, 30 µl of the end concentration were added to three ml TSBYE (i.e.100-fold dilution).

	Dilution	Volume stock	Volume DMSO	Total volume	End concentration	Concentration medium (3 ml)
		[µl]	[µl]	[µl]	[nM; nmol/l]	[nM; nmol/l]
Stock of analytical 1-decene standard diluted in DMSO						
A					$5.28 \cdot 10^9$	
B	from A	1	39	40	$1.32 \cdot 10^8$	$1.32 \cdot 10^6$
C	from B	10	990	1000	$1.32 \cdot 10^6$	$1.32 \cdot 10^4$
D	from C	20	100	120	$2.20 \cdot 10^5$	$2.20 \cdot 10^3$
E	from C	10	90	100	$1.32 \cdot 10^5$	$1.32 \cdot 10^3$
F	from C	10	990	1000	$1.32 \cdot 10^4$	$1.32 \cdot 10^2$
G	from E	10	990	1000	$1.32 \cdot 10^3$	13.21
H	from G	250	250	500	$6.60 \cdot 10^2$	6.60
I	from G	50	450	500	$1.32 \cdot 10^2$	1.32
Stock of analytical 1-undecene standard diluted in DMSO						
A					$4.86 \cdot 10^9$	
B	from A	1	39	40	$1.22 \cdot 10^8$	$1.22 \cdot 10^6$
C	from B	10	990	1000	$1.22 \cdot 10^6$	$1.22 \cdot 10^4$
D	from C	20	100	120	$2.03 \cdot 10^5$	$2.03 \cdot 10^3$
E	from C	10	90	100	$1.22 \cdot 10^5$	$1.22 \cdot 10^3$
F	from C	10	990	1000	$1.22 \cdot 10^4$	$1.22 \cdot 10^2$
G	from E	10	990	1000	$1.22 \cdot 10^3$	12.15
H	from G	250	250	500	$6.08 \cdot 10^2$	6.08
I	from G	50	450	500	$1.22 \cdot 10^2$	1.22
Stock of analytical 1-dodecene standard diluted in DMSO						
A					$4.50 \cdot 10^9$	
B	from A	1	39	40	$1.13 \cdot 10^8$	$1.13 \cdot 10^6$
C	from B	10	990	1000	$1.13 \cdot 10^6$	$1.13 \cdot 10^4$
D	from C	20	100	120	$1.88 \cdot 10^5$	$1.88 \cdot 10^3$
E	from C	10	90	100	$1.13 \cdot 10^5$	$1.13 \cdot 10^3$
F	from C	10	990	1000	$1.13 \cdot 10^4$	$1.13 \cdot 10^2$
G	from E	10	990	1000	$1.13 \cdot 10^3$	11.26
H	from G	250	250	500	$5.63 \cdot 10^2$	5.63
I	from G	50	450	500	$1.13 \cdot 10^2$	1.13
Stock of analytical 1-tridecene standard diluted in DMSO						
A					$4.20 \cdot 10^9$	
B	from A	1	39	40	$1.05 \cdot 10^8$	$1.05 \cdot 10^6$
C	from B	10	990	1000	$1.05 \cdot 10^6$	$1.05 \cdot 10^4$
D	from C	20	100	120	$1.75 \cdot 10^5$	$1.75 \cdot 10^3$
E	from C	10	90	100	$1.05 \cdot 10^5$	$1.05 \cdot 10^3$
F	from C	10	990	1000	$1.05 \cdot 10^4$	$1.05 \cdot 10^2$
G	from E	10	990	1000	$1.05 \cdot 10^3$	10.50
H	from G	250	250	500	$5.25 \cdot 10^2$	5.25
I	from G	50	450	500	$1.05 \cdot 10^2$	1.05
Stock of analytical 1-tetradecene standard diluted in DMSO						
A					$3.95 \cdot 10^9$	
B	from A	1	39	40	$9.87 \cdot 10^7$	$9.87 \cdot 10^5$
C	from B	10	990	1000	$9.87 \cdot 10^5$	$9.87 \cdot 10^3$
D	from C	20	100	120	$1.64 \cdot 10^5$	$1.64 \cdot 10^3$
E	from C	10	90	100	$9.87 \cdot 10^4$	$9.87 \cdot 10^2$
F	from C	10	990	1000	$9.87 \cdot 10^3$	98.67
G	from E	10	990	1000	$9.87 \cdot 10^2$	9.87
H	from G	250	250	500	$4.93 \cdot 10^2$	4.93
I	from G	50	450	500	98.67	0.99

Stock of analytical 1-pentadecene standard diluted in DMSO						
A					$3.68 \cdot 10^{9}$	
B	from A	1	39	40	$9.21 \cdot 10^{7}$	$9.21 \cdot 10^{5}$
C	from B	10	990	1000	$9.21 \cdot 10^{5}$	$9.21 \cdot 10^{3}$
D	from C	20	100	120	$1.53 \cdot 10^{5}$	$1.53 \cdot 10^{3}$
E	from C	10	90	100	$9.21 \cdot 10^{4}$	$9.21 \cdot 10^{2}$
F	from C	10	990	1000	$9.21 \cdot 10^{3}$	92.09
G	from E	10	990	1000	$9.21 \cdot 10^{2}$	9.21
H	from G	250	250	500	$4.60 \cdot 10^{2}$	4.60
I	from G	50	450	500	92.09	0.92
Stock of analytical 1-heptadecene standard diluted in DMSO						
A					$3.29 \cdot 10^{9}$	
B	from A	1	39	40	$8.23 \cdot 10^{7}$	$8.23 \cdot 10^{5}$
C	from B	10	990	1000	$8.23 \cdot 10^{5}$	$8.23 \cdot 10^{3}$
D	from C	20	100	120	$1.37 \cdot 10^{5}$	$1.37 \cdot 10^{3}$
E	from C	10	90	100	$8.23 \cdot 10^{4}$	$8.23 \cdot 10^{2}$
F	from C	10	990	1000	$8.23 \cdot 10^{3}$	82.30
G	from E	10	990	1000	$8.23 \cdot 10^{2}$	8.23
H	from G	250	250	500	$4.12 \cdot 10^{2}$	4.12
I	from G	50	450	500	82.30	0.82
Stock of analytical 1-octadecene standard diluted in DMSO						
A					$3.13 \cdot 10^{9}$	
B	from A	1	39	40	$7.81 \cdot 10^{7}$	$7.81 \cdot 10^{5}$
C	from B	10	990	1000	$7.81 \cdot 10^{5}$	$7.81 \cdot 10^{3}$
D	from C	20	100	120	$1.30 \cdot 10^{5}$	$1.30 \cdot 10^{3}$
E	from C	10	90	100	$7.81 \cdot 10^{4}$	$7.81 \cdot 10^{2}$
F	from C	10	990	1000	$7.81 \cdot 10^{3}$	78.13
G	from E	10	990	1000	$7.81 \cdot 10^{2}$	7.81
H	from G	250	250	500	$3.91 \cdot 10^{2}$	3.91
I	from G	50	450	500	78.13	0.78
Stock of analytical 1-nonadecene standard in DMSO						
A					$2.96 \cdot 10^{9}$	
B	from A	1	39	40	$7.41 \cdot 10^{7}$	$7.41 \cdot 10^{5}$
C	from B	10	990	1000	$7.41 \cdot 10^{5}$	$7.41 \cdot 10^{3}$
D	from C	20	100	120	$1.24 \cdot 10^{5}$	$1.24 \cdot 10^{3}$
E	from C	10	90	100	$7.41 \cdot 10^{4}$	$7.41 \cdot 10^{2}$
F	from C	10	990	1000	$7.41 \cdot 10^{3}$	74.11
G	from E	10	990	1000	$7.41 \cdot 10^{2}$	7.41
H	from G	250	250	500	$3.71 \cdot 10^{2}$	3.71
I	from G	50	450	500	74.11	0.74

Tab 14. SMPE GC-MS relevant information on the 1-alkene standards [Haynes 2012].

Standard	MW [g/mol]	Kp at 1 atm [°C]	Density [g/l]	Molarity [M; mol/l]	Retention time* [min]	RI*	RI (NIST library)
1-C5	70.1	30.0	641	9.14	1.656	502.42	482
1-C6	84.2	63.5	669	7.95	2.044	602.11	584
1-C7	98.2	93.6	697	7.10	2.948	692.78	685
1-C8	112.2	121.3	715	6.37	4.725	781.38	789
1-C9	126.2	146.9	730	5.78	7.352	885.71	889
1-C10	140.3	172.0	741	5.28	10.348	987.52	989
1-C11	154.3	193.0	750	4.86	13.402	1088.19	1091
1-C12	168.3	213.8	758	4.50	16.345	1189.01	1190
1-C13	182.4	233.0	766	4.20	18.645	1289.56	1292
1-C14	196.4	251	775	3.95	20.414	1389.00	1392
1-C15	210.4	269.0	775	3.68	21.904	1488.74	1492
1-C17	238.5	300	785	3.68	24.419	1687.92	1692
1-C18	252.5	314.4	789	3.13	25.535	1787.41	1793
1-C19	266.5	331	790	2.96	26.562	1884.80	1892

*Retention times and retention indices (RI) as measured using a HP-5MS column and SPME GC-MS [Chapters 2.5.1 to 2.5.2].

Appendix Results and Discussion

Seq 1. Verification of the 16S rRNA gene of *Jeotgalicoccus* sp. ATCC 8456 using BLASTn against the published sequence (accession number HQ709267, [Rude et al. 2011]). The lower line represents the query sequence annotated in GenBank. Relevant differences are marked in grey.

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EMBOSS_001         1 ------------------------------------------------G      1
                                                                   |
EMBOSS_001         1 AGAGGTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATG     50

EMBOSS_001         2 C-AGTCGAGCGCGAGCGTAAGGAGCTTGCTCCTTACAATCGAGCGGCGGA     50
                     | ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001        51 CAAGTCGAGCGCGAGCGTAAGGAGCTTGCTCCTTACAATCGAGCGGCGGA    100

EMBOSS_001        51 CGGGTGAGTAACACGTGGGCAACCTACCCTTTAGACTGGGATAACTACCG    100
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       101 CGGGTGAGTAACACGTGGGCAACCTACCCTTTAGACTGGGATAACTACCG    150

EMBOSS_001       101 GAAACGGTAGCTAATACCGGATAAGTTGGATTACACAAGTAATCTTAATG    150
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       151 GAAACGGTAGCTAATACCGGATAAGTTGGATTACACAAGTAATCTTAATG    200

EMBOSS_001       151 AAAGGCGGATTTATCTGTCACTAAAGGATGGGCCTGCGGTGCATTAGCTA    200
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       201 AAAGGCGGATTTATCTGTCACTAAAGGATGGGCCTGCGGTGCATTAGCTA    250

EMBOSS_001       201 GTTGGTGAGGTAGTGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGA    250
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       251 GTTGGTGAGGTAGTGGCTCACCAAGGCAACGATGCATAGCCGACCTGAGA    300

EMBOSS_001       251 GGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG    300
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       301 GGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG    350

EMBOSS_001       301 GCAGCAGTAGGGAATCTTCCGCAATGGACGCAAGTCTGACGGAGCAACGC    350
                     ||||||||||||||||||||||||||:||||||||||||||||||||||||||
EMBOSS_001       351 GCAGCAGTAGGGAATCTTCCGCAATGRACGCAAGTCTGACGGAGCAACGC    400

EMBOSS_001       351 CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAGGAAG    400
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       401 CGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAGGAAG    450

EMBOSS_001       401 AACACTTTAGGTAGTAACTGGCCTAGAGATGACGGTACTTAACCAGAAAG    450
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       451 AACACTTTAGGTAGTAACTGGCCTAGAGATGACGGTACTTAACCAGAAAG    500

EMBOSS_001       451 CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG    500
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       501 CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCG    550

EMBOSS_001       501 TTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTAGAATAAGTCT    550
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       551 TTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTAGAATAAGTCT    600

EMBOSS_001       551 GATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGTTTTA    600
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       601 GATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGTTTTA    650

EMBOSS_001       601 CTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCG    650
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       651 CTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCG    700

EMBOSS_001       651 CAGAGATATGGAGGAACACCAGT-GGCGAAGGCGGCTCTCTGGTCTGTAA    699
                     ||||||||||||||||||||||| ||||||||||||||||||||||||||||
EMBOSS_001       701 CAGAGATATGGAGGAACACCAGTGGGCGAAGGCGGCTCTCTGGTCTGTAA    750

EMBOSS_001       700 CTGACGCTGAGGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTG    749
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
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EMBOSS_001       751 CTGACGCTGAGGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTG    800

EMBOSS_001       750 GTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGGGGGTTTCCGCCC    799
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       801 GTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGGGGGTTTCCGCCC    850

EMBOSS_001       800 CTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCG    849
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       851 CTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCG    900

EMBOSS_001       850 CAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGC    899
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       901 CAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGC    950

EMBOSS_001       900 ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATC    949
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       951 ATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATC   1000

EMBOSS_001       950 CTCTGATCGCCATAGAGATATGGTTTCCCTTTTGGGCAGAGAGACAGGTG    999
                     ||||||||||||:||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1001 CTCTGATCGCCAYAGAGATATGGTTTCCCTTTTGGGCAGAGAGACAGGTG   1050

EMBOSS_001      1000 GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC   1049
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1051 GTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGC   1100

EMBOSS_001      1050 AACGAGCGCAACCCTTAAATTTAGTTGCCATCATTCAGTTGGGCACTCTA   1099
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1101 AACGAGCGCAACCCTTAAATTTAGTTGCCATCATTCAGTTGGGCACTCTA   1150

EMBOSS_001      1100 GATTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA   1149
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1151 GATTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA   1200

EMBOSS_001      1150 TCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGGCAGGTTAC   1199
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1201 TCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGGCAGGTTAC   1250

EMBOSS_001      1200 AAAGGGCAGCGAAGCCGCGAGGCCAAGCGAATCCCATAAAACTGTTCTCA   1249
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1251 AAAGGGCAGCGAAGCCGCGAGGCCAAGCGAATCCCATAAAACTGTTCTCA   1300

EMBOSS_001      1250 GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGAATCGCTAGTA   1299
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1301 GTTCGGATTGGAGTCTGCAACTCGACTCCATGAAGCTGGAATCGCTAGTA   1350

EMBOSS_001      1300 ATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACAC   1349
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1351 ATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACAC   1400

EMBOSS_001      1350 CGCCCGTCACACCACGAAAGTCGATAACACCTGAAGCCGGTGGACTAACC   1399
                     ||||||||||||||||||||||||:||||||||||||||||||||||||||||
EMBOSS_001      1401 CGCCCGTCACACCACGAAAGTCGRTAACACCTGAAGCCGGTGGACTAACC   1450

EMBOSS_001      1400 TTAATGGAGGT-----------------------------------------   1410
                     ||||||||||||
EMBOSS_001      1451 TTAATGGAGGTAGCCGTCAAAGGTGGGATTGATAATTGGGGTGAAGTCGT   1500

EMBOSS_001      1411 ------------   1410

EMBOSS_001      1501 AACAAGGTAACC   1512
```

Tab 15. Optical density of *Jeotgalicoccus* sp. ATCC 8456 during growth in ATCC® Medium 3 at different time points. Used for determination of the exponential growth phase and the corresponding doubling time.

Time point	Time	OD_{600} [-]			
	[h]	1	2	Mean	SD [-]
t0	0.00	0.10	0.10	0.10	0.0013
t1	3.75	0.21	0.20	0.20	0.0068
t2	9.75	0.43	0.41	0.42	0.0161
t3	23.50	1.44	1.49	1.46	0.0368
t4	49.50	2.50	2.57	2.53	0.0509
t5	71.50	2.95	2.92	2.93	0.0247
t6	96.75	3.26	3.18	3.22	0.0559
t7	120.75	3.01	2.91	2.96	0.0714

Tab 16. Optical density of *Jeotgalicoccus* sp. ATCC 8456 during growth in MB at different time points. Used for determination of the exponential growth phase and the corresponding doubling time.

Time point	Time	OD_{600} [-]			
	[h]	1	2	Mean	SD [-]
t0	0.00	0.04	0.05	0.05	0.0049
t1	3.75	0.28	0.29	0.28	0.0068
t2	9.75	0.99	0.94	0.96	0.0331
t3	23.50	2.33	3.25	2.79	0.6452
t4	49.50	3.25	3.35	3.30	0.0707
t5	71.50	3.42	3.40	3.41	0.0163
t6	96.75	2.93	2.87	2.90	0.0431
t7	120.75	2.82	3.01	2.91	0.1344

Tab 17. Optical density of *Jeotgalicoccus* sp. ATCC 8456 during growth in NB at different time points. Used for determination of the exponential growth phase and the corresponding doubling time.

Time point	Time	OD_{600} [-]			
	[h]	1	2	Mean	SD [-]
t0	0.00	0.09	0.09	0.09	0.0008
t1	3.75	0.25	0.26	0.25	0.0052
t2	9.75	0.98	1.01	1.00	0.0208
t3	23.50	4.05	4.16	4.11	0.0795
t4	49.50	5.58	5.68	5.63	0.0764
t5	71.50	5.64	5.68	5.66	0.0346
t6	96.75	5.23	5.38	5.31	0.1061
t7	120.75	4.77	4.76	4.77	0.0099

Tab 18. Optical density of *Jeotgalicoccus* sp. ATCC 8456 during growth in TSBYE at different time points. Used for determination of the exponential growth phase and the corresponding doubling time.

Time point	Time	OD_{600} [-]			
	[h]	1	2	Mean	SD [-]
t0	0.00	0.08	0.08	0.08	0.0037
t1	3.75	0.28	0.28	0-28	0.0053
t2	9.75	1.12	1.07	1.09	0.0359
t3	23.50	5.78	5.67	5.73	0.0788
t4	49.50	9.40	9.67	9.53	0.1959
t5	71.50	10.32	10.49	10.40	0.1167
t6	96.75	10.35	10.56	10.46	0.1478
t7	120.75	10.76	11.35	11.06	0.4186

Tab 19. Optical density of *Jeotgalicoccus* sp. ATCC 8456 during growth in TSBYE with increasing salinity (1 to 10% [w/v] NaCl) after 24 h of cultivation. The control is represented by TSBYE containing 0.5% (w/v) salt. Percentages of the OD_{600} were calculated in comparison to the control medium (defined as 100% growth).

NaCl	OD_{600} [-]						
[%]	1.1	1.2	2.1	2.2	Mean	SD [-]	[%]
0.5	6.03	7.57	3.24	3.39	5.06	1.83	100.00
1	2.99	3.12	3.30	3.55	3.24	0.21	47.66
2	4.09	3.81	4.15	4.29	4.08	0.18	60.04
3	3.80	3.80	4.44	4.20	4.06	0.27	59.70
4	3.51	3.61	2.61	2.59	3.08	0.48	45.28
5	3.03	2.65	1.48	1.46	2.15	0.70	31.66
6	3.14	2.98	1.91	1.81	2.46	0.60	36.16
7	2.71	2.59	2.48	2.42	2.55	0.11	37.50
8	2.08	1.86	1.51	1.54	1.75	0.23	25.69
9	1.63	1.64	1.41	1.54	1.55	0.09	22.84
10	0.87	0.77	0.94	0.93	0.88	0.07	12.90

Tab 20. Linear calibration graphs (y = kx + d) for quantification of 1-alkenes 1-C10 to 1-C19 measured with SPME GC-MS. Due to enhanced volatility, calibration graphs could not be determined for standards 1-C5 to 1-C9.

Standard	Molarity	Linear calibration graph
	[M; mol/l]	y = [ ], x = [nmol/l; nM], k = [l/nmol; nM^{-1}]
1-Pentene	9.14	Quantification not possible
1-Hexene	7.95	Quantification not possible
1-Heptene	7.10	Quantification not possible
1-Octene	6.37	Quantification not possible
1-Nonene	5.78	Quantification not possible
1-Decene	5.28	y = 31373.80997x
1-Undecene	4.86	y = 39732.84457x
1-Dodecene	4.50	y = 65145.16859x
1-Tridecene	4.20	y = 20065.06713x
1-Tetradecene	3.95	y = 48660.03499x
1-Pentadecene	3.68	y = 151526.0647x
1-Heptadecene	3.68	y = 5968.774495x
1-Octadecene	3.13	y = 1615.253581x
1-Nonadecene	2.96	y = 5190.998307x

Tab 21. 1-Alkene formation of *Jeotgalicoccus* sp. ATCC 8456 in TSBYE. Seventeen SPME GC-MS measurements were conducted in total. Quantification of 1-alkenes, here given as area, derived from the corresponding chromatograms. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

1-Alkene	Measurement of 1-alkene production [area]								
	1	2	3	4	5	6	7	8	9
1-C5	0	0	0	0	0	0	0	0	0
1-C6	0	0	0	0	0	0	0	0	0
1-C7	0	0	0	0	0	0	0	0	0
1-C8	0	0	0	0	0	0	0	0	0
1-C9	0	0	0	0	0	0	0	0	0
1-C10	0	0	0	0	0	0	0	0	0
1-C11	0	0	0	0	0	0	0	0	0
1-C12	0	0	0	0	0	0	0	0	0
1-C13	0	0	0	25971	0	4998	0	0	0
1-C14	0	0	0	0	0	0	0	0	0
1-C15	21619	25115	24794	550889	67743	94451	0	22074	86761
1-C17	35062	0	0	74224	178284	346623	0	44901	227233
1-C18	0	0	0		0	0	0	0	0
1-C19	233143	0	0	86951	181236	413313	285203	109690	461161

Continuation Tab 21									
1-Alkene	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
1-C5	0	0	0	0	0	0	0	0	
1-C6	0	0	0	0	0	0	0	0	
1-C7	0	0	0	0	0	0	0	0	
1-C8	0	0	0	0	0	0	0	0	
1-C9	0	0	0	0	0	0	0	0	
1-C10	0	0	0	0	0	0	0	0	
1-C11	1384567	916177	633484	483931	233932	94238	787120	209372	
1-C12	0	0	0	0	0	0	0	0	
1-C13	1066797	555278	264216	180559	15355	23331	76153	40737	
1-C14	0	0	0	0	0	0	0	0	
1-C15	3060344	2134813	1224660	853133	430184	338378	566177	401016	
1-C17	2639392	815144	784014	632489	1585643	1224776	637724	516636	
1-C18	0	0	0	0	0	0	0	0	
1-C19	3224945	2553892	2214347	1747709	2447427	2565786	1367870	1237593	

Tab 22. 1-Alkene formation of *Jeotgalicoccus* sp. ATCC 8456 in TSBYE. Seventeen SPME GC-MS measurements were conducted in total. Quantification of 1-alkenes, here given as concentration (in nM), derived from the corresponding areas [Tab 21]. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

1-Alkene	**Measurement of 1-alkene production [nM]**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1-C5	0	0	0	0	0	0	0	0	0
1-C6	0	0	0	0	0	0	0	0	0
1-C7	0	0	0	0	0	0	0	0	0
1-C8	0	0	0	0	0	0	0	0	0
1-C9	0	0	0	0	0	0	0	0	0
1-C10	0	0	0	0	0	0	0	0	0
1-C11	0	0	0	0	0	0	0	0	0
1-C12	0	0	0	0	0	0	0	0	0
1-C13	0	0	0	1.29	0	0.25	0	0	0
1-C14	0	0	0	0	0	0	0	0	0
1-C15	0.14	0.17	0.16	3.64	0.45	0.62	0	0.15	0.57
1-C17	5.87	0	0	12.44	29.87	58.07	0	7.52	38.07
1-C18	0	0	0		0	0	0	0	0
1-C19	44.91	0	0	16.75	34.91	79.62	54.94	21.13	88.84
1-Alkene	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	
1-C5	0	0	0	0	0	0	0	0	
1-C6	0	0	0	0	0	0	0	0	
1-C7	0	0	0	0	0	0	0	0	
1-C8	0	0	0	0	0	0	0	0	
1-C9	0	0	0	0	0	0	0	0	
1-C10	0	0	0	0	0	0	0	0	
1-C11	34.85	23.06	15.94	12.18	5.89	2.37	19.81	5.27	
1-C12	0	0	0	0	0	0	0	0	
1-C13	53.17	27.67	13.17	9.00	0.77	1.16	3.80	2.03	
1-C14	0	0	0	0	0	0	0	0	
1-C15	20.20	14.09	8.08	5.63	2.84	2.23	3.74	2.65	
1-C17	442.20	136.57	131.35	105.97	265.66	205.20	106.84	86.56	
1-C18	0	0	0	0	0	0	0	0	
1-C19	621.26	491.98	426.57	336.68	471.48	494.28	263.51	238.41	

Tab 23. 1-Alkene formation of *Jeotgalicoccus* sp. ATCC 8456 in TSBYE with increasing salinity (1 to 10% [w/v] NaCl). Three SPME GC-MS measurements were conducted for each salt concentration in total. Quantification of 1-alkenes, here given as area, derived from the corresponding chromatograms. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

1-Alkene	Measurement	Measurement of 1-alkene production [area]				
		1% NaCl	2% NaCl	3% NaCl	4% NaCl	5% NaCl
1-C5	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C6	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C7	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C8	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C9	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C10	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C11	1	0	0	0	0	0
	2	863501	321052	320110	406393	779075
	3	659393	185342	266910	306425	741803
1-C12	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C13	1	0	0	0	0	0
	2	812471	230204	80403	236631	296104
	3	504995	138587	133393	179329	270330
1-C14	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C15	1	0	0	10044	0	0
	2	3942270	232234	179549	334166	575440
	3	1146216	136729	641532	783485	489281
1-C17	1	0	0	0	0	0
	2	2174808	102117	52577	127535	385157
	3	1381772	71123	366547	308677	333042
1-C18	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C19	1	0	0	0	0	0
	2	1232198	216410	189525	196500	176920
	3	1402275	147079	263380	248603	106347

Continuation Tab 23						
1-Alkene	**Measurement**	**6% NaCl**	**7% NaCl**	**8% NaCl**	**9% NaCl**	**10% NaCl**
1-C5	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C6	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C7	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C8	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C9	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C10	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C11	1	0	0	0	0	0
	2	685419	1101793	169481	436250	389721
	3	597975	1078309	476040	509548	390849
1-C12	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C13	1	0	0	0	0	0
	2	254497	291087	281905	88552	65967
	3	198436	407870	153777	152830	99404
1-C14	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C15	1	0	40864	49360	15649	17446
	2	913699	1371130	975322	357468	211867
	3	438164	1144717	451794	389234	308360
1-C17	1	0	0	0	0	0
	2	1317870	630956	248082	65523	40413
	3	426356	349340	137705	105616	45324
1-C18	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C19	1	0	0	0	0	0
	2	97391	233668	22795	0	0
	3	82330	169958	15536	0	0

Tab 24. 1-Alkene formation of *Jeotgalicoccus* sp. ATCC 8456 in TSBYE with increasing salinity (1 to 10% [w/v] NaCl). Three SPME GC-MS measurements were conducted for each salt concentration in total. Quantification of 1-alkenes, here given as concentration (in nM), derived from the corresponding areas [Tab 23]. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

1-Alkene	Measurement	Measurement of 1-alkene production [nM]				
		1% NaCl	2% NaCl	3% NaCl	4% NaCl	5% NaCl
1-C5	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C6	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C7	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C8	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C9	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C10	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C11	1	0	0	0	0	0
	2	21.73	8.08	8.06	10.23	19.61
	3	16.60	4.66	6.72	7.71	18.67
1-C12	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C13	1	0	0	0	0	0
	2	40.49	11.47	4.01	11.79	14.76
	3	25.17	6.91	6.65	8.94	13.47
1-C14	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C15	1	0	0	0.07	0	0
	2	26.02	1.53	1.18	2.21	3.80
	3	7.56	0.90	4.23	5.17	3.23
1-C17	1	0	0	0	0	0
	2	364.36	17.11	8.81	21.37	64.53
	3	231.50	11.92	61.41	51.72	55.80
1-C18	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C19	1	0	0	0	0	0
	2	237.37	41.69	36.51	37.85	34.08
	3	270.14	28.33	50.74	47.89	20.49

Continuation Tab 24						
1-Alkene	**Measurement**	**6% NaCl**	**7% NaCl**	**8% NaCl**	**9% NaCl**	**10% NaCl**
1-C5	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C6	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C7	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C8	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C9	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C10	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C11	1	0	0	0	0	0
	2	17.25	27.73	4.27	10.98	9.81
	3	15.05	27.14	11.98	12.82	9.84
1-C12	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C13	1	0	0	0	0	0
	2	12.68	14.51	14.05	4.41	3.29
	3	9.89	20.33	7.66	7.62	4.95
1-C14	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C15	1	0	0.27	0.33	0.10	0.12
	2	6.03	9.05	6.44	2.36	1.40
	3	2.89	7.55	2.98	2.57	2.04
1-C17	1	0	0	0	0	0
	2	220.79	105.71	41.56	10.98	6.77
	3	71.43	58.53	23.07	17.69	7.59
1-C18	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
1-C19	1	0	0	0	0	0
	2	18.76	45.01	4.39	0	0
	3	15.86	32.74	2.99	0	0

Tab 25. Induced formation of 1-alkenes by *Jeotgalicoccus* sp. ATCC 8456 during feeding with 0.5% fatty acid. The fatty acids 6:0, 8:0, 9:0, 10:0, 11:0, 12:0, 14:0, 16:0, and 20:0 were added to TSBYE medium dissolved in DMSO. Three to seven SPME GC-MS measurements were conducted for each fatty acid in total. Quantification of 1-alkenes, here given as area, derived from the corresponding chromatograms. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

Fatty acid	Measurement	Measurement of 1-alkene production [area]				
		1-C5	1-C6	1-C7	1-C8	1-C9
6:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
8:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
9:0	1	0	0	0	0	0
	2	0	0	0	125457	0
	3	0	0	0	291729	0
	4	0	0	0	243818	0
	5	0	0	0	155554	0
10:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
11:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
12:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
14:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	0	0
16:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
20:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0

Continuation Tab 25						
Fatty acid	**Measurement**	**1-C10**	**1-C11**	**1-C12**	**1-C13**	**1-C14**
6:0	1	0	0	0	0	7727674
	2	0	0	0	0	19626512
	3	0	0	0	0	17368776
	4	0	0	0	0	27534273
	5	0	0	0	0	28420980
8:0	1	451090	0	0	0	0
	2	1630595	0	0	0	0
	3	1287826	0	0	0	0
	4	1761543	0	0	0	0
	5	1379645	0	0	0	0
9:0	1	0	0	310897	0	0
	2	0	0	346867	0	0
	3	0	0	638065	0	0
	4	0	0	795706	0	0
	5	0	0	486501	0	0
10:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
11:0	1	42504	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	82224	0	0	0	0
	5	67336	0	0	0	0
12:0	1	0	663353	10245	0	52280
	2	0	351099	33468	0	150952
	3	0	493229	49021	0	22293
	4	0	2354084	23052	0	16320
	5	0	2362796	38802	0	21192
14:0	1	0	43620	21431	26728226	16446
	2	0	0	0	4995762	0
	3	0	0	0	5135752	0
	4	0	73369	48639	35221812	125606
	5	0	90678	31357	31140049	27322
	6	0	229841	0	49550969	0
	7	0	185899	37069	47597543	0
16:0	1	0	6380124	0	932427	59956
	2	0	6389597	0	850454	58353
	3	0	6837249	0	1055637	93951
	4	0	6495382	0	1034681	71439
20:0	1	0	163002	0	0	0
	2	186621	743402	0	0	0
	3	0	1297321	0	0	0

Continuation Tab 25						
Fatty acid	**Measurement**	**1-C15**	**1-C17**	**1-C18**	**1-C19**	
6:0	1	0	0	2902247	0	
	2	0	0	5035771	0	
	3	0	0	5656826	0	
	4	0	0	7867084	0	
	5	0	0	8255030	0	
8:0	1	23247251	0	0	0	
	2	113455017	0	0	0	
	3	130907069	0	0	0	
	4	105995798	0	0	0	
	5	108679143	0	0	0	
9:0	1	0	0	0	0	
	2	0	0	0	0	
	3	0	0	0	0	
	4	0	0	0	0	
	5	0	0	0	0	
10:0	1	0	0	0	0	
	2	0	0	0	0	
	3	0	0	0	0	
	4	0	0	0	0	
	5	0	0	0	0	
11:0	1	0	0	0	0	
	2	0	0	0	0	
	3	0	0	0	0	
	4	0	0	0	0	
	5	0	0	0	0	
12:0	1	0	0	0	0	
	2	0	0	0	0	
	3	0	0	0	0	
	4	0	0	0	0	
	5	0	0	0	0	
14:0	1	0	126092	0	69866	
	2	0	0	0	0	
	3	0	0	0	0	
	4	0	0	0	50217	
	5	0	0	0	112758	
	6	0	0	0	161449	
	7	0	0	0	0	
16:0	1	39090916	180543	0	539497	
	2	34634876	107330	0	583881	
	3	72845656	443159	0	1853819	
	4	72915270	418430	0	1918132	
20:0	1	0	0	0	0	
	2	0	0	0	7059200	
	3	0	0	0	5492038	

Tab 26. Induced formation of 1-alkenes by *Jeotgalicoccus* sp. ATCC 8456 during feeding with 0.5% fatty acid. The fatty acids 6:0, 8:0, 9:0, 10:0, 11:0, 12:0, 14:0, 16:0, and 20:0 were added to TSBYE medium dissolved in DMSO. Three to seven SPME GC-MS measurements were conducted for each fatty acid in total. Quantification of 1-alkenes, here given as concentration (in nM), derived from the corresponding areas [Tab 25]. Only representative values were used for further calculation of mean values and standard deviations (highlighted in blue).

Fatty acid	Measurement	Measurement of 1-alkene production [nM]				
		1-C5	1-C6	1-C7	1-C8	1-C9
6:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
8:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
9:0	1	0	0	0	0	0
	2	0	0	0	Not quantified	0
	3	0	0	0	Not quantified	0
	4	0	0	0	Not quantified	0
	5	0	0	0	Not quantified	0
10:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
11:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
12:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
14:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	7	0	0	0	0	0
16:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
20:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0

Continuation Tab 26						
Fatty acid	**Measurement**	**1-C10**	**1-C11**	**1-C12**	**1-C13**	**1-C14**
6:0	1	0	0	0	0	158.81
	2	0	0	0	0	403.34
	3	0	0	0	0	356.94
	4	0	0	0	0	565.85
	5	0	0	0	0	584.07
8:0	1	14.38	0	0	0	0
	2	51.97	0	0	0	0
	3	41.05	0	0	0	0
	4	56.15	0	0	0	0
	5	43.97	0	0	0	0
9:0	1	0	0	4.77	0	0
	2	0	0	5.32	0	0
	3	0	0	9.79	0	0
	4	0	0	12.21	0	0
	5	0	0	7.47	0	0
10:0	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
11:0	1	1.35	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	2.62	0	0	0	0
	5	2.15	0	0	0	0
12:0	1	0	16.70	0.16	0	1.07
	2	0	8.84	0.51	0	3.10
	3	0	12.41	0.75	0	0.46
	4	0	59.25	0.35	0	0.34
	5	0	59.47	0.60	0	0.44
14:0	1	0	1.10	0.33	1332.08	0.34
	2	0	0	0	248.98	0
	3	0	0	0	255.95	0
	4	0	1.85	0.75	1755.38	2.58
	5	0	2.28	0.48	1551.95	0.56
	6	0	5.78	0	2469.51	0
	7	0	4.68	0.57	2372.16	0
16:0	1	0	160.58	0	46.47	1.23
	2	0	160.81	0	42.38	1.20
	3	0	172.08	0	52.61	1.93
	4	0	163.48	0	51.57	1.47
20:0	1	0	4.10	0	0	0
	2	5.95	18.71	0	0	0
	3	0	32.65	0	0	0

Continuation Tab 26						
Fatty acid	**Measurement**	**1-C15**	**1-C17**	**1-C18**	**1-C19**	
6:0	1	0	0	1796.77	0	
	2.1	0	0	3117.63	0	
	2.2	0	0	3502.13	0	
	3.1	0	0	4870.49	0	
	3.2	0	0	5110.67	0	
8:0	1	153.42	0	0	0	
	2.1	748.75	0	0	0	
	2.2	863.92	0	0	0	
	3.1	699.52	0	0	0	
	3.2	717.23	0	0	0	
9:0	1	0	0	0	0	
	2.1	0	0	0	0	
	2.2	0	0	0	0	
	3.1	0	0	0	0	
	3.2	0	0	0	0	
10:0	1	0	0	0	0	
	2.1	0	0	0	0	
	2.2	0	0	0	0	
	3.1	0	0	0	0	
	3.2	0	0	0	0	
11:0	1	0	0	0	0	
	2.1	0	0	0	0	
	2.2	0	0	0	0	
	3.1	0	0	0	0	
	3.2	0	0	0	0	
12:0	1	0	0	0	0	
	2.1	0	0	0	0	
	2.2	0	0	0	0	
	3.1	0	0	0	0	
	3.2	0	0	0	0	
14:0	1	0	21.13	0	13.46	
	2.1	0	0	0	0	
	2.2	0	0	0	0	
	3.1	0	0	0	9.67	
	3.2	0	0	0	21.72	
	4.1	0	0	0	31.10	
	4.2	0	0	0	0	
16:0	1.1	257.98	30.25	0	103.93	
	1.2	228.57	17.98	0	112.48	
	2.1	480.75	74.25	0	357.12	
	2.2	481.21	70.10	0	369.51	
20:0	1	0	0	0	0	
	2.1	0	0	0	1359.89	
	2.2	0	0	0	1057.99	

Tab 27. Induced formation of 1-alkenes by *Jeotgalicoccus* sp. ATCC 8456 during feeding with 0.5% fatty acid. The fatty acids 10:0, 11:0, 12:0, and 14:0 were added to TSBYE medium dissolved in ethanol. Two SPME GC-MS measurements were conducted for each fatty acid in total. Quantification of 1-alkenes, here given as area, derived from the corresponding chromatograms.

1-Alkene	Measurement	Measurement of 1-alkene production [area]			
		10:0	11:0	12:0	14:0
1-C5	1	0	0	0	0
	2	0	0	0	0
1-C6	1	196508	0	0	0
	2	0	0	0	0
1-C7	1	0	0	0	0
	2	0	0	0	0
1-C8	1	10054748	0	0	0
	2	0	0	0	0
1-C9	1	2297379	0	0	0
	2	0	0	0	0
1-C10	1	0	51632	154220	0
	2	0	26493	0	0
1-C11	1	0	0	374037	16683
	2	0	0	85318	0
1-C12	1	124405	0	0	65338
	2	0	0	0	0
1-C13	1	0	0	0	14377595
	2	0	0	0	10434531
1-C14	1	0	131584	0	0
	2	0	180633	0	0
1-C15	1	0	0	0	409275
	2	0	0	0	0
1-C17	1	0	0	0	26674
	2	0	0	0	0
1-C18	1	0	0	0	0
	2	0	0	0	0
1-C19	1	0	0	0	0
	2	0	0	0	0

Tab 28. Induced formation of 1-alkenes by *Jeotgalicoccus* sp. ATCC 8456 during feeding with 0.5% fatty acid. The fatty acids 10:0, 11:0, 12:0, and 14:0 were added to TSBYE medium dissolved in ethanol. Two SPME GC-MS measurements were conducted for each fatty acid in total. Quantification of 1-alkenes, here given as concentration (in nM), derived from the corresponding chromatograms.

1-Alkene	Measurement	Measurement of 1-alkene production [nM]			
		10:0	11:0	12:0	14:0
1-C5	1	0	0	0	0
	2	0	0	0	0
1-C6	1	Not quantified	0	0	0
	2	0	0	0	0
1-C7	1	0	0	0	0
	2	0	0	0	0
1-C8	1	Not quantified	0	0	0
	2	0	0	0	0
1-C9	1	Not quantified	0	0	0
	2	0	0	0	0
1-C10	1	0	1.65	4.92	0
	2	0	0.84	0	0
1-C11	1	0	0	9.41	0.42
	2	0	0	2.15	0
1-C12	1	1.91	0	0	1.00
	2	0	0	0	0
1-C13	1	0	0	0	716.55
	2	0	0	0	520.03
1-C14	1	0	2.70	0	0
	2	0	3.71	0	0
1-C15	1	0	0	0	2.70
	2	0	0	0	0
1-C17	1	0	0	0	4.47
	2	0	0	0	0
1-C18	1	0	0	0	0
	2	0	0	0	0
1-C19	1	0	0	0	0
	2	0	0	0	0

Tab 29. Bacterial strains from the culture collection identified to be 1-alkene producers. Terminal olefin formation is shown as area of the peak from the chromatogram.

Species	Strain code	Measurement	Measurement of 1-alkene production [area]			
			1-C11	1-C12	1-C13	1-C14
Bacillus thuringiensis	3R2-29	1	26288309	0	95670	0
		2	27645957	0	0	0
Pseudomonas aeruginosa	QC14-3-8	1	13156934	47348	90858	0
		2	18148567	29840	67240	0
Pseudomonas brassicacearum[a]	L13-6-12	1	29957689	0	82424	0
		2	45329956	0	16192	0
Pseudomonas brassicacearum[b]	L13-6-12	1	27122292	0	46031	0
		2	47589507	0	19969	0
Pseudomonas brenneri	Baz30	1	17248707	0	0	0
		2	21317601	0	0	0
Pseudomonas fluorescens	Baz53	1	15730183	0	0	151988
		2	23335355	0	0	0
Pseudomonas poae	RE*1-1-14	1	29106610	0	11727	0
		2	55797915	0	1246	0
Pseudomonas putida	1T1	1	5751768	0	0	0
		2	35169799	0	0	0

[a, b] are different glycerol stocks from the culture collection

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