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CHALLENGES AND PROSPECTS OF ALGAL BIOTECHNOLOGY FOR THE ISOLATION AND CHARACTERIZATION OF ACTIVE PHARMACEUTICAL INGREDIENT (API) ON THE EXAMPLE OF NEW GLUTAMINYL CYCLASE (QC) INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Algae are phototrophic organisms (Phycophyceen) with a great heterogeneity in morphologic organisation and physiology. Algae have a ubiquitous presence and so they grow in diverse habitats such as lakes, the sea, the soil, rocks, air, or in hot springs but also they can be found in the snow. Only about 43000 of the 500000 estimated species are classified and only 200 macroalgae and 15 microalge are used commercial. However, their use is currently limited to high value added products such as carotenoids like ß–carotene and astaxanthin, cosmetic active ingredients and PUFFA's such as DHA or EPA. Mainly *Chlorella vulgaris*, *Haematococcus pluvialis*, *Porphyridium* sp. *Crypthecodinium* sp., *Schizochytrium* sp. and *Nannochloropsis* sp. are commercially cultivated for the production of biomass, astaxanthin, phycoerythrin and DHA. So algae are use in food and cosmetic as well as in aquaculture and in environmental technology. However, today there is an intensive research for the use of algae for biofuels such as biodiesel, ethanol or hydrogen. Further it is known that these organisms produce a range of bioactive compounds, which are not commonly available from other plants or animals. This enormous potential of algae is proven previously by a multitude of identified secondary metabolites with, for example, cytostatic, antibacterial, antiviral, anti–inflammatory, or antifungal properties, many acting via the specific inhibition of enzymes (Lindequist and Schweder, 2001; Kelecom, 2002; Sakai et al., 2014; Blunt et al., 2009, 2011, 2013, 2014). The first antibacterial compound was isolated from the microalgae *Chlorella* sp. in 1944 by Pratt. Since then microalgae are examined for the presence of new anti–bacterial compounds because of the steady increase of multi–resistant pathogens. Approximately 60 % of cancer drugs originally derived from plant or microbial substances. In various anti–cancer screening approaches aquatic natural compounds showed high inhibition effects against different human cancer cell lines (Ulber

et al., 2002). Therefore the search for compounds from algae is considered particularly promising (Mayer et al, 2003; Amador et al., 2003).

In recent years, more and more enzymes could be associated with pathophysiological processes and represent targets for many diseases, so that enzyme–inhibiting properties of natural substances are becoming increasingly important. Algae are known for the ability to synthesize complex and highly diverse compounds with specific enzyme inhibitions and neuroactive properties (Blunt et al, 2009; 2011; 2013; 2014; Sakai et al., 2014). Therefore algae represent a potential almost untouched resource and research source in search of new leads for the treatment of heretofore incurable diseases including Alzheimer's disease.

Alzheimer's disease is characterized by the aggregation of Aβ protein fragments to senile plaques. These Aβ peptides have glutamine at the N–terminus, which can be modified to a pyroglutamyl residue (pE). This modification results in a resistance to a proteolytic N–terminal degradation and to the loss of N–terminal charge. The resulting hydrophobic residual charge (decrease in solubility) results in accelerated aggregation of the pE–peptides. Various studies have shown that these neurotoxic pE–Aβ peptide fragments represent a major component of senile plaques. Further current study was able to confirm in vivo that QC catalyzes the N–terminal pE–modification. Thus, the expression of QC is upregulated in the cortex of Alzheimer's patients and correlates with the presence of pE–modified Aβ peptides. The oral application of QC inhibitors showed in two different transgenic mouse models of Alzheimer's disease as well as a Drosophila model a reduced strain of $A\beta_{3(pE)–42}$. The reduction of pE–Aβ peptide fragments by the inhibition of the QC provides an approach for the treatment of Alzheimer's disease (Schilling et al., 2008; Demuth et. al., 2010; Jawhar et al., 2011).

Due to the fact that algae have the ability to produce a large variety of bioactive compounds with specific inhibition of enzymes, algae can be expected to be a potential source of new QC inhibiting compounds. Therefore, we analyzed algal strains and crude extracts of different algae species belonging to Chlorophyceae and Eustigmatophyceae.

Different algae species were cultivated in a 100 L tubular photobioreactor and the ingredients were extracted from the biomasses. This was followed by the screening of the algae extracts in a QC enzyme assay. Here 27 of the 72 tested extracts inhibited the QC. The QC inhibiting metabolites were identified by Activity–correlation analysis. This are carried out by a direct correlation of the mass spectrometry metabolite profiles with the inhibition value of the QC of each extract. By further mass spectrometric investigations the structural characterization of the correlated compounds could be realized directly from the crude extracts without purification. Five QC inhibiting compounds were identified. Molecular modeling (docking studies) confirmed QC–inhibiting potential of all five compounds. The compounds showed a similar QC inhibition like a standard substance with 81 % and 76 % in the two tested concentrations of 0.25 mg/mL and 0.025 mg/mL.

In summary QC inhibitors could be identified, characterized and isolated from algae for the first time.

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