Process Biochemistry 61 (2017) 137–146

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Assessment of new environmental quorum quenching bacteria as a solution for membrane biofouling

Bahar Yavuztürk Gül, Ismail Koyuncu*

Department of Environmental Engineering, Istanbul Technical University, 34469, Maslak, Istanbul, Turkey

ARTICLE INFO

Keywords:
Membrane bioreactor
Quorum quenching
N-Acyl-homoserine lactone
AHL degradation
Quorum sensing

ABSTRACT

This study evaluated quorum quenching (QQ), which exists among bacteria that are isolated from saltern, pond and marine habitats, and applied selected QQ bacterium to inhibit biofilm formation in the membrane bioreactor (MBR). We identified nine genera belonging to 19 *N*-octanoyl-homoserine lactone (C8-HSL)-degrading bacteria, namely *Shewanella, Acinetobacter, Klebsiella, Bacillus, Deftia, Vibrio, Comamonas, Microbacterium*, and *Pseudomonas*. Both *Bacillus* sp. T5 and *Delftia lacustris* T6 were isolated from a saltern and degraded all the C8-HSL; thus, demonstrating the highest QQ capability. T5 was immobilized in a new immobilization medium named QQ-fiber, which was composed of floating 20-cm hollow fibers. To enlighten the antibiofouling potential of newly isolated *Bacillus* sp. T5, it was applied to an MBR and the QQ effect was monitored during 12 days of operation. According to transmembrane pressure (TMP) values, QQ bacterium led to less biofouling than the control reactor. In MBRs operated with T5, TMP was decreased with 25% efficiency. The decrease in biofouling resulted from QQ-fiber activity, and it was seen that T5 could be used successfully to inhibit biofilm formation in MBR.

1. Introduction

Quorum sensing (QS) is a phenomenon, where bacteria communicate through chemical signals and organize their group behavior by sensing their population density. QS is mediated by the production and emission of autoinducers. Autoinducers are small signaling molecules that amass in the extracellular environment. Bacteria release, identify and react to the accumulation of these molecules in order to control the gene expression of a community [1]. When bacteria sense that their population density has reached a threshold level, autoinducers are released. These trigger the expression of multiple genes in the population which, in turn, serve to regulate a range of significant biological functions such as aggregation, antibiotic biosynthesis, secondary metabolite production, biofilm maintenance and differentiation, luminescence, motility, plasmid conjugal transfer, siderophore production, swarming, symbiosis or virulence [1–4]. A range of different classes of signals exist within a QS system such as: (1) *N*-Acyl-homoserine Lactones (AHLs), (2) Furanosyl borate (Autoinducer-2), (3) Oligopeptides (5–10 amino acid cyclic thiolactone), (4) Hydroxyl-palmitic acid methylester, and (5) Methyl dodecanoic acid. Of these, the best characterized is AHLs. [5,6].

The term *quorum quenching* (QQ) describes the mechanisms that are responsible for deactivating the QS communication systems by interrupting the signaling process. The QQ mechanism uses competitive binding to inactivate the autoinducer synthases/receptors or switches off signal transmission through the degradation of the signaling molecules. Existing research to date has identified QQ mechanisms in bacteria [7–9], marine algae [10], root associated fungi [11], plants [12,13], and mammalian cells [14].

Many studies have focused in depth on the enzymatic degradation of AHL. Research in this area has developed three main categories of AHL degradation enzyme that correlate with their enzymatic mechanisms; (i) AHL lactonases: these hydrolyze the lactone ring of AHL, generating the corresponding *N*-acyl-homoserine [15,16]. The process of hydrolysis can also occur spontaneously in the presence of alkaline pH and can be reversed at lower pH levels [17], (ii) AHL acylases: these cleave the AHL amide bond and generate the corresponding free fatty acid and lactone ring [8,18], (iii) AHL oxidases and reductases (oxidoreduction) [19].

Existing studies have proven the presence of QQ activities in a range of different environments, including soil [20–22], and aquatic environments [23–25], and a number of researchers have successfully

Abbreviations: QS, quorum sensing; QQ, quorum quenching; AHL, acyl-homoserine lactone; AIP, autoinducing peptides; AI-2, autoinducer-2; MBR, membrane bioreactor; EPS, extracellular polymeric substances; TMP, trans-membrane pressure; C8-HSL, *N*-octanoyl-homoserine lactone; LB, Luria–Bertani medium

* Corresponding author.
E-mail address: koyuncu@itu.edu.tr (I. Koyuncu).

http://dx.doi.org/10.1016/j.procbio.2017.05.030
Received 20 December 2016; Received in revised form 12 May 2017; Accepted 17 May 2017
Available online 10 June 2017
1359-5113/

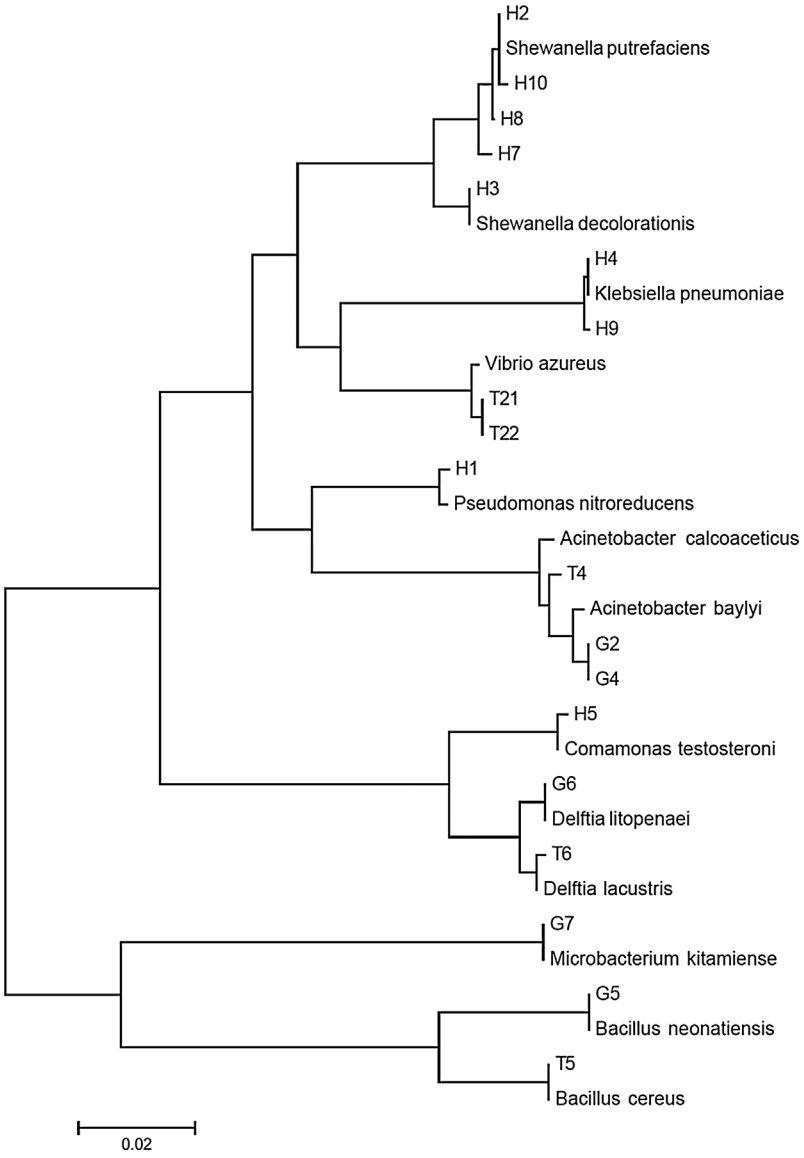

Fig. 1. Neighbour-joining tree based on the 16S rRNA gene, showing phylogenetic relationships among quorum quenching bacteria isolated from pond, estuary and saltern. Bootstrap coefficients below 50% were not shown. Scale bar, 0,05 substitutions per nucleotide position.

isolated several strains and genes that are capable of degrading AHLs. However, knowledge as to how QQ mechanisms suppress QS in saline environments remains relatively limited, although some existing studies do indicate that the QQ mechanisms in this environment inhibit QS through the compounds produced by *Marinobacter, Halomonas* and archaean strains [26].

Given the fact that QS is known to have the ability to control a variety of different functions, as outlined above, there is the potential that the compounds that interfere with bacterial QS can be employed for alternative purposes; for example, to produce of commercially desirable bacterial pigments [27], to eliminate pathogenic activity in aquacultural and agricultural environments [21,28,29], or to operate as antifouling agents [30–32]. Recent research explored the potential that quorum quenching has as an innovative method of controlling biofouling in a membrane bioreactor (MBR) for the advanced treatment of wastewater [33–35].

The goals of this study were: (i) To isolate and identify those bacteria found in estuary, pond and saltern environments that are capable of interfering with AHL-mediated QS, (ii) To determine the extent to which these forms of bacteria have quenching capabilities with using bacterial biosensor *Agrobacterium tumefaciens*, and (iii) To evaluate the inhibition of membrane biofilm that resulted from QQ activity during the MBR operation.

2. Materials and methods

2.1. Bacterial strains, growth media, culture condition

The AHL degrading bacteria were screened on a minimal medium containing sterile distilled water and 2,5 mM *N*-octanoyl-L-homoserine lactone (C8-HSL) (Sigma–Aldrich, USA) as a sole carbon source, which is the most abundant autoinducers in the membrane bioreactors [9,34,36]. By sodium requirements of halophilic marine and saltern QQ bacteria a modified minimal medium containing C8-HSL (2,5 mM) and NaCl (1,9%–5% w/vol) was used [37]. Ringer solution was used to dilute the soil samples. All of the isolated bacteria were cultivated in a Luria–Bertani (LB) medium at 30 °C. *Agrobacterium tumefaciens* A136(Ti-)- (pCF218)(pCF372) [38] the reporter strains for the bioassay, were cultured in LB at 30 °C and supplemented with spectinomycin and tetracycline when necessary [39].

2.2. Isolation of QQ consortia from environmental samples

Samples were taken from three different environmental origins for bacterial isolation and screening for QQ activity against C8-HSL. Sample locations were chosen for its different microbial diversity. One of the samples was collected in coastal environment in Haliç, Marmara