Screening the Farallon Island Bacterial Collection for Ionic Liquid Tolerance to Create Genomic Libraries

Alison Richins, Ann Nguyen, Thomas Rüegg, Steven Singer Michael Thelen

Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

ABSTRACT

Bacteria isolates from South East Farallon Island exposed to salty conditions were screened for resistance to ionic liquids, in an attempt to improve the microbial synthesis of biofuels. The bacteria were cultivated on different media that contained either 1-Ethyl-3-methylimidazolium chloride (EMiM-Cl) or 1-Ethyl-3-methylimidazolium acetate (EMiM-Ac) and examined for growth characteristics. The most resistant bacteria were used to create a genomic library to screen for genes conferring ionic liquid resistance.

INTRODUCTION

The biofuel engineering pathway begins with plants high in lignin and cellulose. The plant matter is dried and finely ground. The plant matter is then washed in an ionic liquid (IL) to dissolve the lignin and cellulose. After the lignin and cellulose are fractionated out it is rinsed to remove the high concentration of IL, however 2-6% remains. Enzymes are then added to convert cellulose to glucose, and finally bacteria or yeast use the sugar to produce liquid fuel. For this process to work, the bacteria and yeast must be resistant to IL, but currently there are no such biofuel hosts (1).

To find a gene that confers ionic liquid resistance in bacteria, environmental samples of bacteria were taken from a high salt environment in the Farallon Islands, see Figure 1. The bacteria were grown on different media that contained either EMiM-Cl or EMiM-Ac to determine which bacteria should be used to create a genomic library to screen for genes conferring ionic liquid resistance (1).

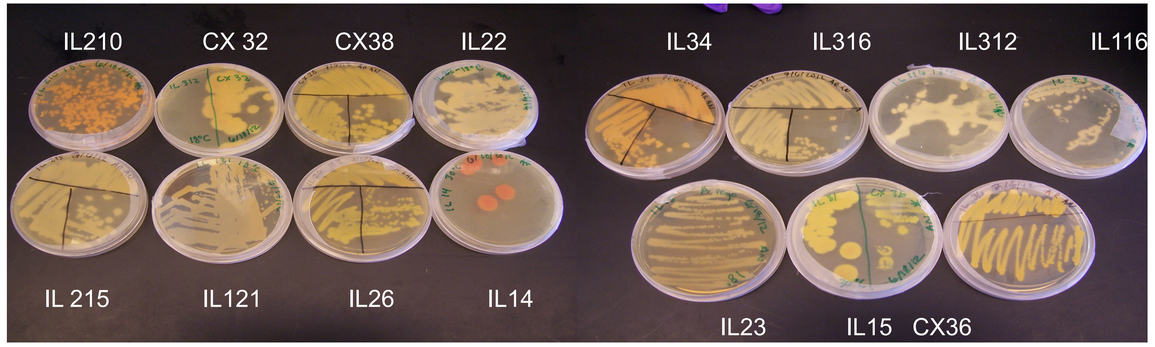
Figure 1. Biofilm where bacterial samples were collected.

MATERIALS AND METHODS

The Farallon Island collection of bacterial samples were originally isolated on Marine Agar containing 2% EMiM-Ac. Glycerol stocks of the isolates were transported to JBEI and stored at -80 °C. The isolates were plated on trypticase-soy agar (TSA) and cultured at both 18°C and 30°C (Figure 1). Bacteria cultures were then screened for growth on different IL backgrounds in both minimal and enriched media containing EMIM-AC and EMIM-CL (Table 1).

RESULTS

Figure 2.



Farallon Island bacterial isolates plated on TSA. The name of each isolate is above or below the plate. Note the many different colony morphologies and colors.

Table 1.

Isolates	1/5 MA + 2% EMiM-AC RT.	1/5 MA + 2% EMiM-CL RT.	TSB+2% EMiM-Ac @ 18C	TSB+4% EMiM-Cl @ 18C	TSB+8% EMiM-Cl @ 18C	TSB only @ 18 C	TSA ONLY @ RT
IL 210	+	+	+	+	+	+	+
CX 32	+	+	+	+	+	+	+
CX38	-	+	+	+	+	+	+
IL22	-	+	-	+	+	+	+
IL 215	-	+	+	+	+	+	+
IL121	-	+	-	+	+	+	+
IL 26	-	+	-	+	+	+	+
IL14	-	+	+	+	-	+	+
IL34	-	+	+	+	+	+	+
IL 316	-	+	-	+	+	+	+
IL321	-	+	+	+	+	+	+
IL116	+	+	+	+	+	+	+
IL23	+	+	-	+	+	+	+
IL15	+	+	+	+	+	+	+
CX36	-	+	-	+	+	+	+
IL31	+	+	+	+	+	+	+

Results for the growth of each isolate on different types of media. + indicates growth, - indicates no growth.

CONCLUSIONS

Bacterial isolates IL 210, IL 15, CX32 AND CX 38 grow on media containing EMiM-Cl as well as media containing EMiM-Ac. These isolates are likely to contain a gene or genes responsible for ionic liquid resistance, and therefore genomic libraries will be constructed and screened in E. coli for IL tolerance.

Figure 3.

Map of the Farallon Islands

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1. J. Khudyakov, et al (2012) Global transcriptome response to ionic liquid by a tropical rain forest soil bacterium, Enterobacter lignolyticus. PNAS, epub 14 May 2012

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