Identification and Characterization of Ethanol-Resistant Bacteria from the Human Gut

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Introduction

In recent years, there has been a lot of research on the human gut microbiome, which consists of all the microorganisms and their genes living in our gut flora. This has increased our understanding of the interactions between those gut microbiome bacteria and the effect of them on human health problems, such as their effects on obesity, immunity and psychological effects via the 'gut-brain' axis.

In this project, two ethanol resistant bacteria, DJO101 and DJO201, those were isolated from the gut flora of people who regularly consume alcohol were characterized by comparing their alcohol-protecting abilities with another gut flora bacterium, *E. coli*, and identified with Polymerase Chain Reaction (PCR).

Methods

Basic morphological analysis including observing cell morphology under microscopy after gram staining was done. To test their ethanol-resistance performance, broth assays and Bioscreen were prepared by growing DJO 101, DJO 201, and E. coli into Brain Heart Infusion (BHI) Medium with different level of ethanol concentration to measure their Minimal Inhibitory Concentration and growth rate.

To sequence DNA, PCR, a technique used in molecular biology to amplify DNA, was conducted to isolate the 16S rRNA gene from these bacteria. In my research, The universal rRNA primers (16s-R 5'- ACG GGC GGT GTG TRC -3' and 16s F 5'- ACG GGC GGT GTG TRC -3') were used to target the unique regions conserved in selected bacteria and amplify the 16s rRNA gene sequence. Their genes were sequenced by the University of Minnesota Genomics Center (UMGC) to sequence the genes. Then bacteria were identified by comparing the DNA sequences with a database of 16S rRNA gene sequences at the NCBI. NCBI is a U.S. government-funded national resource for molecular biology information.

Results

Gram Stain Results

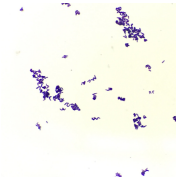


Figure 1: DJO101
Gram Positive Rods

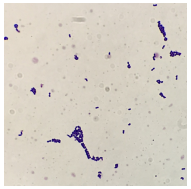
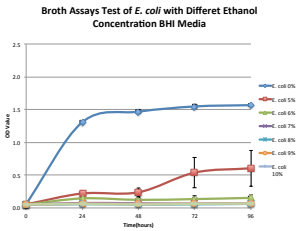
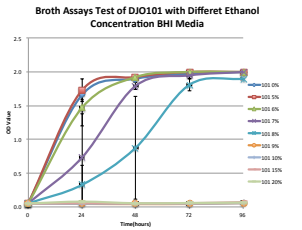
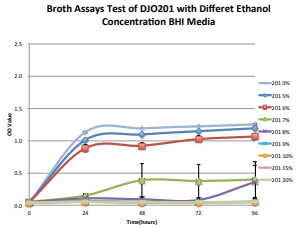


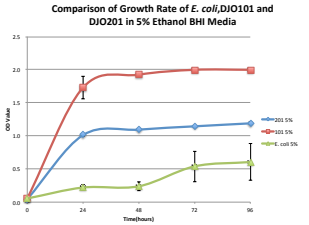
Figure 2: DJO201
Gram Positive Cocci

Figure 3: *E. coli*
Gram Negative Rods

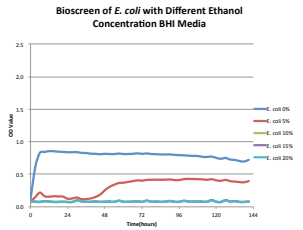


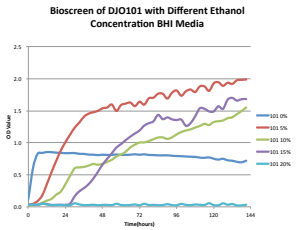


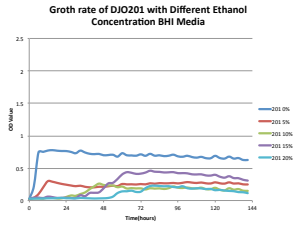




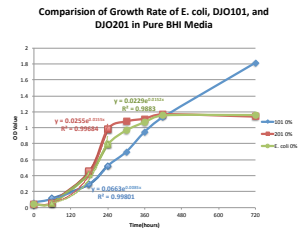
Duplicates for each bacteria were done. According to the broth assays, all of those three bacteria, E. coli, DJO101, and DJO 201 could fully grow in BHI media. E. coli could hardly grow in 5% ethanol BHI media. DJO101 could grow in up to 8% ethanol BHI media, while DJO201 could grow up around 6% and 7% ethanol BHI media. In 5% ethanol BHI media, DJO101 had the maximum growth rate and OD value, DJO201 was the second, while E. coli had lowest growth rate and OD value.







Bioscreen tests showed DJO101 and DJO201 could readily grow in 5% ethanol BHI medium, while E. coli could only start to grow after two days, in which evaporation of ethanol should be taken into consideration.



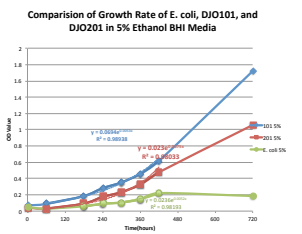


Table 1: Comparison of Growth Rate of *E. coli*, DJO101, and DJO201 in Pure BHI Medium and 5% Ethanol BHI Medium

Bacteria	*E.coli*		DJO101		DJO201	
Media	Pure BHI	5% Ethanol BHI	Pure BHI	5% Ethanol BHI	Pure BHI	5% Ethanol BHI
Growth Rate Constant (min^{-1})	0.0152	0.0053	0.0085	0.0053	0.0155	0.0075
Doubling Time (min)	46	131	82	131	45	92

In pure BHI medium, E. coli and DJO201 had similar growth rate constant, while DJO101 had the smallest growth rate constant. In 5% ethanol BHI media, the growth rate of E. coli was affected by a higher degree than DJO101 and DJO201.

The 16s rRNA bands of all of those bacteria had around 800bp. After sequencing, it was determined that the DJO101 is *Corynebacterium* sp. , and the DJO201 is *Enterococcus faecalis.*

Conclusion

DJO101 is gram-positive, small rods, *Corynebacterium* sp , and DJO201 is gram-positive small cocci, *Enterococcus faecalis*. They have higher ethanol tolerance level than E.coli that they could readily grow in 5% ethanol condition and even higher.

Acknowledgements

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