Dolosigranulum pigrum Primer Design for Isolation From Nasal Samples

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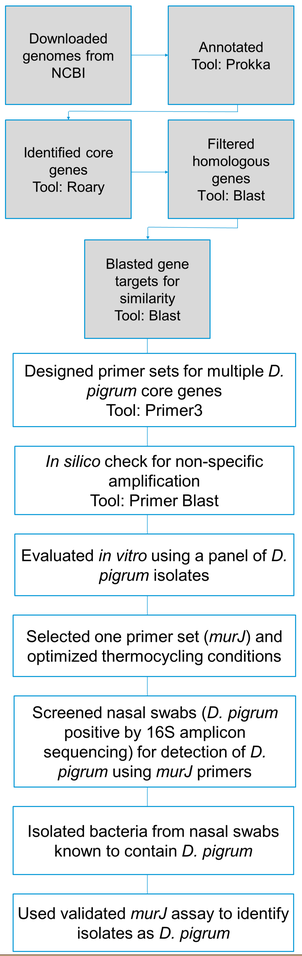
Background

Dolosigranulum pigrum is a gram-positive, non-spore forming bacterium from the family *Carnobacteriaceae* that can be found in the nasal cavity. We want to culture *D. pigrum* to understand how it can grow and survive, as well as to use in in vtro models. Our previous research shows that D. pigrum is associated with the presence or absence of S. aureus. To better investigate its role we need to be able to identify, isolate, culture and study D. pigrum. Our effort here is focused on the identification step of the process. *D. pigrum* is fastidious and difficult to identify using standard biochemical models so we aim to design species specific primers for identification of *D. pigrum*. These primers will be used for the detection and confirmation of *D. pigrum* isolated from nasal swabs.

Methods



Assay Development and Validation

Organism	murJ	group_1020
D. pigrum 1	+	+
D. pigrum 2	+	+
D. pigrum 3	+	+
D. pigrum 4	+	+
D. pigrum 5	+	+
D. pigrum 6	+	+
D. pigrum 7	+	+
D. pigrum 8	+	+
D. pigrum 9	+	+
D. pigrum 10	+	+
D. pigrum 11	+	+
D. pigrum 12	+	+
D. pigrum 13	+	+
D. pigrum 14	+	+
S. aureus	-	-
E. coli	+	+
E. coli	+	-
Water	-	-

Table 2: Results form the initial screening of two potential targets against a panel of *D. pigrum, E. coli,* and *S. aureus* isolate DNA.

Gene name	Gene size	Amplicon size	Function
murJ	1665	224	Lipid II flippase
group_1020	129	107	Hypothetical protein

Table 1: Possible gene targets descriptive information.

- On the initial screening of potential gene targets (Table 1), visible bands were observed for all of the *D. pigrum* isolates using the *murJ* primers summarized in table 2.
- With increased annealing temperatures, non-specific amplification of *E. coli* was reduced but sensitivity to *D, pigrum* was retained (data not shown).
- At an annealing temperature of 54C there was the least amount on non-specific amplification observed.
- Using optimized conditions, amplification at the expected base pair size was observed for entire *D. pigrum* isolate collection (Table 3).
- Non-specific amplification was easily differentiated from salient product by size. There was a different banding pattern seen for two of the isolates and we suspect that the unexpected band between 800-2000 base pairs long is also indicative of *D. pigrum.*

Organism	murJ
D. pigrum 1	+
D. pigrum 2	+
D. pigrum 3	+
D. pigrum 4	+
D. pigrum 5	+
D. pigrum 6	+
D. pigrum 7	+
D. pigrum 8	+
D. pigrum 9	+
D. pigrum 10	+
D. pigrum 11	+
D. pigrum 12	+
D. pigrum 13	+
D. pigrum 14	+
E. coli	-
S. aureus	-
Water	-

Table 3: Results from the optimized conditions (54C annealing temperature) on *D. pigrum* isolate DNA.

Sample	murJ	Expected
Swab 1	+	Y
Swab 2	-	N/A
Swab 3	-	N/A
Swab 4	-	N/A
Swab 5	-	Y
Swab 6	-	N/A
Swab 7	+	Y
Swab 8	+	N/A
Swab 9	-	N/A
Swab 10	-	N/A
Swab 11	+	Y
Swab 12	-	N/A
Swab 13	+	N/A
Swab 14	-	N/A
Swab 15	-	N/A
D. pigrum 5	+	N/A
D. pigrum 9	+	N/A
Water	-	N/A

Table 4: Results from screening the murJ primers on nasal swabs from ARAC volunteers for *D. pigrum.*

- Nasal samples collected from ARAC volunteers for *D. pigrum* carriage
- Table 4 shows the e-gel results when the *murJ* assay was applied to the samples.
- We expected to see *D. pigrum* in swabs 1,5,7 and 11 based on previous 16S amplicon sequencing (data not shown).
- Amplification of *D. pigrum* was observed in swabs 1,7 and 11, though not in swab 1 using our assay.
- Amplification of *D. pigrum* was also observed for 2 samples with no 16S data (swabs 8 and 13).

Assay Application

Nasal Swab plate growth

Time	Media	# Positive	# Missed
48 hr	Blood	2	2
48 hr	Schaedler	2	2
48 hr	Chocolate	2	2
72 hr	Blood	2	2
72 hr	Schaedler	2	2
72 hr	Chocolate	3	1

Table 5: Summary of results indicating number of samples identified as *D. pigrum* positive by media type and time of incubation

- Samples were streaked in a lawn pattern onto 3 media types and incubated for 48 and 72 hours.
- After screening samples of mixed bacterial growth from each condition, we found that using chocolate agar with a 72 hour incubation allowed for recovery of *D. pigrum* from 3 out of the 4 samples tested, compared to 2 out of 4 samples for the other growth conditions (Table 5).
- Putative *D. pigrum* was then isolated and confirmation testing was performed using the validated *murJ* assay.

Conclusions

- We developed and optimized an assay specific to *D. pigrum* that allowed us to consistently identify isolates already known to be *D. pigrum.*
- We further validated our assay using nasal swabs shown by 16S sequencing to contain *D. pigrum.*
- We were also able to identify isolates grown from these nasal swabs as *D. pigrum*.
- These results indicate that our novel PCR assay targeting the *murJ* gene can be used to identify *D. pigrum* isolates and detect *D. pigrum* in nasal samples.

References

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