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Agency Code: 21XD

Proposal Number: 72569EMREP **Agreement Number: W911NF-18-1-0444**
INVESTIGATOR(S):

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Report Date: 28-Dec-2022 Date Received: 08-Nov-2022
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Begin Performance Period: 29-Sep-2018 **End Performance Period:** 28-Sep-2022
Report Term: 0-Other
Submitted By: Qunying Yuan Email: qunying.yuan@aamu.edu
Phone: (256) 372-4241

STEM Degrees: 1 **STEM Participants:**

Major Goals: The overall research objective of this project at Alabama A&M University, one of the leading HBCUs, is to genetically engineer E. coli cells to simplify the processes of nanoparticle biosynthesis in bacteria and help scale up to large scale nanoparticle production for the biomedical and bioengineering applications in Army. Specifically, our goals include: 1) Clone or synthesize genes that have been implicated in nanoparticle synthesis in various bacteria and transform the genes into E. coli host cells; 2) Construct fosmid libraries that contain large fragment of genomic DNA from bacteria known to be able to synthesize nanoparticles and transform these libraries into E. coli cells; 3) Examine the ability of these engineered E. coli cells to resist heavy metal ions and to synthesize various nanoparticles; 4) Characterize the properties of nanoparticles using different methods; 5) Optimize the conditions that allow the engineered E. coli cells to produce monodisperse, morphologically uniform nanoparticles at large scale; 6) Analyze and measure the spectroscopic and optical properties of the biosynthesized nanoparticles and determine size/shape-property relationships; 7) Investigate the interaction of biosynthesized nanoparticles with other materials and explore the fabrication of nanoparticle-based nanostructures and devices. This project is potentially transformative, and could create a new nanoparticle synthesis paradigm using biological methods, and will benefit the research community by providing new approaches for the synthesis of nanoparticles. The project will greatly benefit Army research and contribute to the mission of ARL by providing biological synthesis of nanoparticles for tailored properties and applications. The engineered E. coli cells developed in this project will produce various nanoparticles ranging from metallic to semiconducting nanoparticles, and can be easily and conveniently used by the Army researchers for research, economical and rapid development of biotechnology. The biological synthesis of nanoparticles has advantages over chemical synthesis, and is a cost-effective, non-toxic, and environmentally benign green approach that do not use toxic chemicals to produce nanoparticles. The biosynthetic method using bacteria as a factory can produce shape and size controlled nanoparticles, and can be used for fast and large scale production.

Accomplishments: During the period from Sep. 2020 to Aug. 2021
We screened 1823 clones from 4 different bacteria: Shewanella oneidensis (340 clones) Pseudomonas putida (612 clones), Streptococcus pyogenes (429 clones), Desulfovibrio desulfuricans (440 clones), for their metal tolerance to metal ion, cadmium, nickel and zinc.
Among these, we tested 120 clones from Desulfovibrio desulfuricans, 25 out of 340 from Shewanella oneidensis,

RPPR Final Report
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205 clones out of 612 clones from Pseudomonas putida, 55 clones out of 429 clones from Streptococcus pyogenes for their capacity to synthesize selenium, platinum and palladium nanoparticles. The EPI300™-T1R E. coli strain that used as host cells of library construction was used as a control. Among the 1823 clones, all of them, including the control cells, successfully synthesized selenium nanoparticles. However, none of these clones synthesized either platinum or palladium nanoparticles.

Training Opportunities: During the time of Sep. 2021 to Aug. 2022, the funding of this project supported one master degree graduate student, and one undergraduate student. The undergraduate student was trained to synthesize nanoparticles, purify nanoparticles and other basic and important lab skills, such as bacterial culture, plasmid extraction, autoclave, PCR, DNA electrophoresis and protein electrophoresis.

Results Dissemination: The research during this period requires high through-put approaches. The work load has been heavy, but no novel and significant findings were revealed. The data were not presented at any conference or in any publications. However, a collaboration between my lab and Dr. Terrance Ravine at University of Southern Alabama is examining the antimicrobial activity of the biological silver nanoparticles. The paper was published on journal “Applied Nano”.
Ravine, T.*; Yuan, Q.; Howell, M. Biogenic Silver Nanoparticles Processed Twice Using 8M Urea Exhibit Superior Antibacterial and Antifungal Activity to Commercial Chemically Synthesized Counterparts. Appl. Nano 2022, 3, 187-201. https://doi.org/10.3390/applnano3040014 (Corresponding author)

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Qunying Yuan
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Co PD/PI
Participant: Zhigang Xiao
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Undergraduate Student
Participant: Lauren Williams
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
National Academy Member: N

Participant Type: Undergraduate Student
Participant: Adrian Rhoden
Person Months Worked: 6.00 **Funding Support:**
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RPPR Final Report
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Authors: Qunying Yuan, Manjula Bomma, Zhigang Xiao
Keywords: biosynthesis; silver nanoparticles; engineered Escherichia coli; metallothionein
Abstract: Phytochelatins, the enzymatic products of phytochelatin synthase, play a principal role in pro-tecting the plants from heavy metal and metalloid toxicity due to their ability to scavenge metal ions. In the present study, we investigated the capacity of soluble intracellular extracts from E. coli cells expressing R. tropici phytochelatin synthase to synthesize gold nanoparticle. We discov-ered that the reaction mediated by soluble extracts from the recombinant E. coli cells had a high-er yield of gold nanoparticles, compared to that from the control cells. The compositional and morphological properties of the gold nanoparticles synthesized by the intracellular extracts from recombinant cells and control cells were similar. In addition, this extracellular nanoparticle synthesis method produced purer gold nanoparticles, avoiding the isolation of nanoparticles from cellular debris when whole cells are used to synthesize nanoparticles. Our results suggest-ed that phytochelatins can improve
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Article Title: . Expression of Rhizobium tropici phytochelatin synthase in Escherichia coli resulted in increased bacterial selenium nanoparticle synthesis
Authors: Qunying Yuan*, Manjula Bomma Haley Hill, Zhigang Xiao.
Keywords: Selenium Nanoparticles; Recombinant Escherichia coli; Phytochelatin Synthase; Phytochelatins
Abstract: Phytochelatins, the enzymatic products of phytochelatin synthase, play a principal role in pro-tecting the plants from heavy metal and metalloid toxicity due to their ability to scavenge metal ions. In the present study, we investigated the capacity of soluble intracellular extracts from E. coli cells expressing R. tropici phytochelatin synthase to synthesize gold nanoparticle. We discov-ered that the reaction mediated by soluble extracts from the recombinant E. coli cells had a high-er yield of gold nanoparticles, compared to that from the control cells. The compositional and morphological properties of the gold nanoparticles synthesized by the intracellular extracts from recombinant cells and control cells were similar. In addition, this extracellular nanoparticle synthesis method produced purer gold nanoparticles, avoiding the isolation of nanoparticles from cellular debris when whole cells are used to synthesize nanoparticles. Our results suggest-ed that phytochelatins can improve
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Article Title: Enhanced Extracellular Synthesis of Gold Nanoparticles by Soluble Extracts from Escherichia Coli Transformed with Rhizobium Tropici Phytochelatin Synthase Gene
Authors: Qunying Yuan 1, *, Manjula Bomma 1 and Zhigang Xiao
Keywords: gold nanoparticles; extracellular nanoparticle synthesis; recombinant Escherichia coli; phytochela-tin synthase; phytochelatins
Abstract: Phytochelatins, the enzymatic products of phytochelatin synthase, play a principal role in pro-tecting the plants from heavy metal and metalloid toxicity due to their ability to scavenge metal ions. In the present study, we investigated the capacity of soluble intracellular extracts from E. coli cells expressing R. tropici phytochelatin synthase to synthesize gold nanoparticle. We discov-ered that the reaction mediated by soluble extracts from the recombinant E. coli cells had a high-er yield of gold nanoparticles, compared to that from the control cells. The compositional and morphological properties of the gold nanoparticles synthesized by the intracellular extracts from recombinant cells and control cells were similar. In addition, this extracellular nanoparticle synthesis method produced purer gold nanoparticles, avoiding the isolation of nanoparticles from cellular debris when whole cells are used to synthesize nanoparticles. Our results suggest-ed that phytochelatins can improve
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Article Title: Biogenic Silver Nanoparticles Processed Twice Using 8M Urea Exhibit Superior Antibacterial and Antifungal Activity to Commercial Chemically Synthesized Counterparts
Authors: Ravine, T.*; Yuan, Q.; Howell, M.
Keywords: biogenic; silver nanoparticles; 8M urea; antimicrobial activity; minimum inhibitory concentration; functionalized
Abstract: Biogenic silver nanoparticles (b-AgNPs) were produced extracellularly using a cell lysate of genetically modified Escherichia coli and subdivided into three groups. Each group received a different treatment to determine which one best removed residual cell lysate material. The first group was treated twice using only water (water ×2), the second using 8M urea once (8M urea ×1), and the third using 8M urea twice (8M urea ×2). Subsequently, each group was assessed for its ability to inhibit the growth of six bacterial and two fungal pathogens. Testing was accomplished using the minimum inhibitory concentration (MIC) method. Commercially produced c-AgNPs were included for comparison. In all cases, the b-AgNPs (8M urea ×2) demonstrated the greatest inhibition of microbe growth. Conversely, the commercial AgNPs failed to show any growth inhibition at 10 µg/mL the highest concentration tested. The greater antibacterial activity of the b-AgNPs (8M urea ×2) over both b-AgNPs (8M urea ×1) and b
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Acknowledged Federal Support: **Y**

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as of 09-Nov-2022

Partners

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I certify that the information in the report is complete and accurate:
Signature: Qunying Yuan
Signature Date: 11/8/22 12:32PM

Annual Report (September 2021 to August. 2022)

Biological Synthesis of Nanoparticles for the Bioengineering and Biotechnology Application in Army

The overall research objective of this project at Alabama A&M University, one of the leading HBCUs, is to genetically engineer *E. coli* cells to simplify the processes of nanoparticle biosynthesis in bacteria and help scale up to large scale nanoparticle production for the biomedical and bioengineering applications in Army. **Specifically, our goals include**: 1) Clone or synthesize genes that have been implicated in nanoparticle synthesis in various bacteria and transform the genes into E. *coli* host cells; 2) Construct fosmid libraries that contain large fragment of genomic DNA from bacteria known to be able to synthesize nanoparticles and transform these libraries into *E. coli* cells; 3) Examine the ability of these engineered *E. coli* cells to resist heavy metal ions and to synthesize various nanoparticles; 4) Characterize the properties of nanoparticles using different methods; 5) Optimize the conditions that allow the engineered E. coli cells to produce monodisperse, morphologically uniform nanoparticles at large scale; 6) Analyze and measure the spectroscopic and optical properties of the biosynthesized nanoparticles and determine size/shape-property relationships; 7) Investigate the interaction of biosynthesized nanoparticles with other materials and explore the fabrication of nanoparticle-based nanostructures and devices. This project is potentially transformative, and could create a new nanoparticle synthesis paradigm using biological methods, and will benefit the research community by providing new approaches for the synthesis of nanoparticles. The project will greatly benefit Army research and contribute to the mission of ARL by providing biological synthesis of nanoparticles for tailored properties and applications. The engineered E. coli cells developed in this project will produce various nanoparticles ranging from metallic to semiconducting nanoparticles, and can be easily and conveniently used by the Army researchers for research, economical and rapid development of biotechnology. The biological synthesis of nanoparticles has advantages over chemical synthesis, and is a cost-effective, non-toxic, and environmentally benign green approach that do not use toxic chemicals to produce nanoparticles. The biosynthetic method using bacteria as a factory can produce shape and size controlled nanoparticles, and can be used for fast and large scale production.

Accomplished under Goal:

During the period from Sep. 2020 to Aug. 2021

We screened 1823 clones from 4 different bacteria: *Shewanella oneidensis (340 clones) Pseudomonas putida (612 clones), Streptococcus pyogenes (429 clones), Desulfovibrio desulfuricans (440 clones*), for their metal tolerance to metal ion, cadmium, nickel and zinc. Among these, we tested 120 clones from *Desulfovibrio desulfuricans*, 25 out of 340 from *Shewanella oneidensis*, 205 clones out of 612 clones from *Pseudomonas putida*, 55 clones out of 429 clones from *Streptococcus pyogenes* for their capacity to synthesize selenium, platinum and palladium nanoparticles. The EPI300™-T1^{R} *E. coli* strain that used as host cells of library construction was used as a control. Among the 1823 clones, all of them, including the control cells, successfully synthesized selenium nanoparticles. However, none of these clones synthesized either platinum or palladium nanoparticles.

I. Construct of DNA libraries

Genomic DNA from 4 different bacteria: Shewanella oneidensis, Pseudomonas putida, Streptococcus pyogenes, Desulfovibrio desulfuricans was sheared and inserted into fosmid vector. Fosmid vector was transformed into EPI300™-T1^{R} *E. coli* cells. A total of 1823 clones were obtained (Figure 1). After the libraries were constructed, each clone was stored as 20% glycerol stock at -80^{o}c until further use.

II. Screen the *E. coli* EPI300™-T1R clones for their resistance to metal ions.

To test the metal tolerance of these clones, 15% of glycerol stock of each clone was inoculated in 600µL of LB with 28µg/mL of chloramphenicol in 2mL 96-well block, and grew at 37^{o}C for 18-20hours with continuous shake at 250rpm. The next morning, the cells were centrifuged and supernatant was discarded. The pellets were resuspended in 1.2mL of fresh LB with 28µg/mL of chloramphenicol, further grew at 37oC for two hours. Then OD600nm was taken. 200µL Cells from each well was transferred to the corresponding well of three different new plates that has 1mL of fresh LB with 28µg/mL of chloramphenicol in the presence of 2mM metal ion: cadmium, nickel and zinc, respectively. The prepared cells in the presence of metal ions were incubated at 37oC for 18-20 hours with continuous shaking at 250rpm. The next morning, OD600nm was determined.

The green highlighted clones indicated that the growth of bacteria was slower. The OD600nm of these clones was 20% lower than the average OD600nm of the clones of the whole plate; the red highlighted clones indicated that their OD600nm was 10% higher than the average of the OD600nm of the clones in whole plate, after cultivation at the same condition.

Table I. 440 clones Constructed from *Desulfovibrio desulfuricans* DNA Library.

	1	2	3	4	5	6	7	8	9	10	11
A	1	2	3	4	5	6	7	8	9	10	11
B	12	13	14	15	16	17	18	19	20	21	22
C	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44
E	45	46	47	48	49	50	51	52	53	54	55
F	56	57	58	59	60	61	62	63	64	65	66
G	67	68	69	70	71	72	73	74	75	76	77
H	78	79	80	81	82	83	84	85	86	87	88

	1	2	3	4	5	6	7	8	9	10	11
A	89	90	91	92	93	94	95	96	97	98	99
B	100	101	102	103	104	105	106	107	108	109	110
C	111	112	113	114	115	116	117	118	119	120	121
D	122	123	124	125	126	127	128	129	130	131	132
E	133	134	135	136	137	138	139	140	141	142	143
F	144	145	146	147	148	149	150	151	152	153	154
G	155	156	157	158	159	160	161	162	163	164	165
H	166	167	168	169	170	171	172	173	174	175	176

	1	2	3	4	5	6	7	8	9	10	11
A	177	178	179	180	181	182	183	184	185	186	187
B	188	189	190	191	192	193	194	195	196	197	198
C	199	200	201	202	203	204	205	206	207	208	209
D	210	211	212	213	214	215	216	217	218	219	220
E	221	222	223	224	225	226	227	228	229	230	231
F	232	233	234	235	236	237	238	239	240	241	242
G	243	244	245	246	247	248	249	250	251	252	253
H	254	255	256	257	258	259	260	261	262	263	264

	1	2	3	4	5	6	7	8	9	10	11
A	265	266	267	268	269	270	271	272	273	274	275
B	276	277	278	279	280	281	282	283	284	285	286
C	287	288	289	290	291	292	293	294	295	296	297
D	298	299	300	301	302	303	304	305	306	307	308
E	309	310	311	312	313	314	315	316	317	318	319
F	320	321	322	323	324	325	326	327	328	329	330
G	331	332	333	334	335	336	337	338	339	340	341
H	342	343	344	345	346	347	348	349	350	351	352

	1	2	3	4	5	6	7	8	9	10	11
A	353	354	355	356	357	358	359	360	361	362	363
B	364	365	366	367	368	369	370	371	372	373	374
C	375	376	377	378	379	380	381	382	383	384	385
D	386	387	388	389	390	391	392	393	394	395	396
E	397	398	399	400	401	402	403	404	405	406	407
F	408	409	410	411	412	413	414	415	416	417	418
G	419	420	421	422	423	424	425	426	427	428	429
H	430	431	432	433	434	435	436	437	438	439	440

Table II. 332 clones Constructed from *Shewanella oneidensis* DNA Library.

	1	2	3	4	5	6	7	8	9
A	1	9	17	25	33	41	49	57	65
B	2	10	18	26	34	42	50	58	66
C	3	11	19	27	35	43	51	59	67
D	4	12	20	28	36	44	52	60	68
E	5	13	21	29	37	45	53	61	69
F	6	14	22	30	38	46	54	62	70
G	7	15	23	31	39	47	55	63	71
H	8	16	24	32	40	48	56	64	

	1	2	3	4	5	6	7	8	9	10	11	12
A	102	103	104	105	106	107	108	109	110	111	112	113
B	114	115	116	117	118	119	120	121	122	123	124	125
C	126	127	128	129	130	131	132	133	134	135	136	137
D	138	139	140	141	142	143	144	145	146	147	148	149

	1	2	3	4	5	6	7	8	9	10	11	12
A	150	151	152	153	154	155	156	157	158	159	160	161
B	162	163	164	165	166	167	168	169	170	171	172	173
C	174	175	176	177	178	179	180	181	182	183	184	185
D	186	187	188	189	190	191	192	193	194	195	196	197
E	198	199	200	201	202	203	204	205	206	207	208	209
F	210	211	212	213	214	215	216	217	218	219	220	221
G	222	223	224	225	226	227	228	229	230	231	232	233
H	234	235	236	237	238	239	240	241	242	243	244	

	1	2	3	4	5	6	7	8	9	10	11
A	245	246	247	248	249	250	251	252	253	254	255
B	256	257	258	259	260	261	262	263	264	265	266
C	267	268	269	270	271	272	273	274	275	276	277
D	278	279	280	281	282	283	284	285	286	287	288
E	289	290	291	292	293	294	295	296	297	298	299
F	300	301	302	303	304	305	306	307	308	309	310
G	311	312	313	314	315	316	317	318	319	320	321
H	322	323	324	325	326	327	328	329	330	331	332

Table III. 612 clones Constructed from *Pseudomonas pudita* DNA Library

	1	2	3	4	5	6	7	8	9	10	11
A	1	2	3	4	5	6	7	8	9	10	11
B	12	13	14	15	16	17	18	19	20	21	22
C	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44
E	45	46	47	48	49	50	51	52	53	54	55
F	56	57	58	59	60	61	62	63	64	65	66
G	67	68	69	70	71	72	73	74	75	76	77
H	78	79	80	81	82	83	84	85	86	87	88

	1	2	3	4	5	6	7	8	9	10	11
A	89	90	91	92	93	94	95	96	97	98	99
B	100	101	102	103	104	105	106	107	108	109	110
C	111	112	113	114	115	116	117	118	119	120	121
D	122	123	124	125	126	127	128	129	130	131	132
E	133	134	135	136	137	138	139	140	141	142	143
F	144	145	146	147	148	149	150	151	152	153	154
G	155	156	157	158	159	160	161	162	163	164	165
H	166	167	168	169	170	171	172	173	174	175	176

	1	2	3	4	5	6	7	8	9	10	11
A	177	178	179	180	181	182	183	184	185	186	187
B	188	189	190	191	192	193	194	195	196	197	198
C	199	200	201	202	203	204	205	206	207	208	209
D	210	211	212	213	214	215	216	217	218	219	220
E	221	222	223	224	225	226	227	228	229	230	231
F	232	233	234	235	236	237	238	239	240	241	242
G	243	244	245	246	247	248	249	250	251	252	253
H	254	255	256	257	258	259	260	261	262	263	264

	1	2	3	4	5	6	7	8	9	10	11
A	265	266	267	268	269	270	271	272	273	274	275
B	276	277	278	279	280	281	282	283	284	285	286
C	287	288	289	290	291	292	293	294	295	296	297
D	298	299	300	301	302	303	304	305	306	307	308
E	309	310	311	312	313	314	315	316	317	318	319
F	320	321	322	323	324	325	326	327	328	329	330
G	331	332	333	334	335	336	337	338	339	340	341
H	342	343	344	345	346	347	348	349	350	351	352

	1	2	3	4	5	6	7	8	9	10	11
A	353	354	355	356	357	358	359	360	361	362	363
B	364	365	366	367	368	369	370	371	372	373	374
C	375	376	377	378	379	380	381	382	383	384	385
D	386	387	388	389	390	391	392	393	394	395	396
E	397	398	399	400	401	402	403	404	405	406	407
F	408	409	410	411	412	413	414	415	416	417	418
G	419	420	421	422	423	424	425	426	427	428	429
H	430	431	432	433	434	435	436	437	438	439	440

	1	2	3	4	5	6	7	8	9	10	11
A	441	442	443	444	445	446	447	448	449	450	451
B	452	453	454	455	456	457	458	459	460	461	462
C	463	464	465	466	467	468	469	470	471	472	473
D	474	475	476	477	478	479	480	481	482	483	484
E	485	486	487	488	489	490	491	492	493	494	495
F	496	497	498	499	500	501	502	503	504	505	506
G	507	508	509	510	511	512	513	514	515	516	517
H	518	519	520	521	522	523	524	525	526	527	528

	1	2	3	4	5	6	7	8	9	10	11
A	529	530	531	532	533	534	535	536	537	538	539
B	540	541	542	543	544	545	546	547	548	549	550
C	551	552	553	554	555	556	557	558	559	560	561
D	562	563	564	565	566	567	568	569	570	571	572
E	573	574	575	576	577	578	579	580	581	582	583
F	584	585	586	587	588	589	590	591	592	593	594
G	595	596	597	598	599	600	601	602	603	604	605
H	606	607	608	609	610	611	612				

Table 4. 429 clones Constructed from *Streptolococcus pyrogens* DNA Library

	1	2	3	4	5	6	7	8	9	10	11
A	1	2	3	4	5	6	7	8	9	10	11
B	12	13	14	15	16	17	18	19	20	21	22
C	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44
E	45	46	47	48	49	50	51	52	53	54	55
F	56	57	58	59	60	61	62	63	64	65	66
G	67	68	69	70	71	72	73	74	75	76	77
H	78	79	80	81	82	83	84	85	86	87	88

	1	2	3	4	5	6	7	8	9	10	11
A	89	90	91	92	93	94	95	96	97	98	99
B	100	101	102	103	104	105	106	107	108	109	110
C	111	112	113	114	115	116	117	118	119	120	121
D	122	123	124	125	126	127	128	129	130	131	132
E	133	134	135	136	137	138	139	140	141	142	143
F	144	145	146	147	148	149	150	151	152	153	154
G	155	156	157	158	159	160	161	162	163	164	165
H	166	167	168	169	170	171	172	173	174	175	176

	1	2	3	4	5	6	7	8	9	10	11
A	177	178	179	180	181	182	183	184	185	186	187
B	188	189	190	191	192	193	194	195	196	197	198
C	199	200	201	202	203	204	205	206	207	208	209
D	210	211	212	213	214	215	216	217	218	219	220
E	221	222	223	224	225	226	227	228	229	230	231
F	232	233	234	235	236	237	238	239	240	241	242
G	243	244	245	246	247	248	249	250	251	252	253
H	254	255	256	257	258	259	260	261	262	263	264

	1	2	3	4	5	6	7	8	9	10	11
A	265	266	267	268	269	270	271	272	273	274	275
B	276	277	278	279	280	281	282	283	284	285	286
C	287	288	289	290	291	292	293	294	295	296	297
D	298	299	300	301	302	303	304	305	306	307	308
E	309	310	311	312	313	314	315	316	317	318	319
F	320	321	322	323	324	325	326	327	328	329	330
G	331	332	333	334	335	336	337	338	339	340	341
H	342	343	344	345	346	347	348	349	350	351	352

	1	2	3	4	5	6	7	8	9	10	11
A	353	354	355	356	357	358	359	360	361	362	363
B	364	365	366	367	368	369	370	371	372	373	374
C	375	376	377	378	379	380	381	382	383	384	385
D	386	387	388	389	390	391	392	393	394	395	396
E	397	398	399	400	401	402	403	404	405	406	407
F	408	409	410	411	412	413	414	415	416	417	418
G	419	420	421	422	423	424	425	426	427	428	429
H											

The red highlighted clones may have higher tolerance to metal ions. These clones were selected to test their capacity for nanoparticle synthesis. The green highlighted clones had poor growth under metal ion challenges.

II. Screen the *E. coli* EPI300™-T1R clones for their capacity to synthesize nanoparticles.

The clones that exhibited an OD600nm that was 10% higher than average were selected to further test their capacity for three types of nanoparticle synthesis: selenium, palladium and platinum nanoparticle. The rationale for choosing these metallic or metalloid ions as test ions is because we did not successfully produce any palladium or platinum nanoparticles using bacteria, while all bacteria in our previous studies successfully synthesized selenium nanoparticles. It would be of great interest if any of the fosmid clones synthesizes palladium or platinum nanoparticles.

A total of 330 clones were tested and *E. coli* EPI300™-T1R did not carry fosmid was used as control.

Preliminary data suggested that all clones were able to synthesize selenium nanoparticles (Figure A, B, C). However, only two of the 1830 clones exhibited a color change during nanoparticle synthesis reactions (Figure 2A, B). The reactions of palladium, platinum and selenium nanoparticle synthesis of two clones, clone 495 and 535 from *Pseudomonas pundit* library, all displayed a color change from yellowish to dark brown (Figure 2). Unfortunately, SEM did not identify nanoparticles in the reaction.

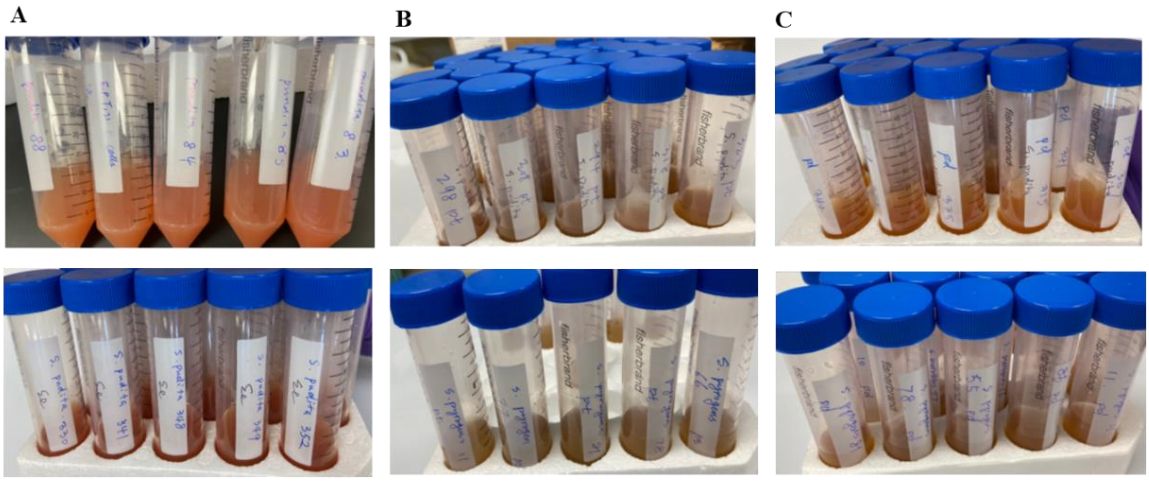

Figure 1. Representative reactions of nanoparticle synthesis. **A**. Reactions of some of the clones of *Pseudomonas pudita* and *Streptococcus pyogenes* that synthesized selenium nanoparticles. **B**. Reactions of some of the clones of *Pseudomonas pudita* and *Streptococcus pyogenes* that attempted to produce platinum nanoparticles. **C**. Reactions of some of the clones of *Pseudomonas pudita* and *Streptococcus pyogenes* that attempted to produce palladium nanoparticles.

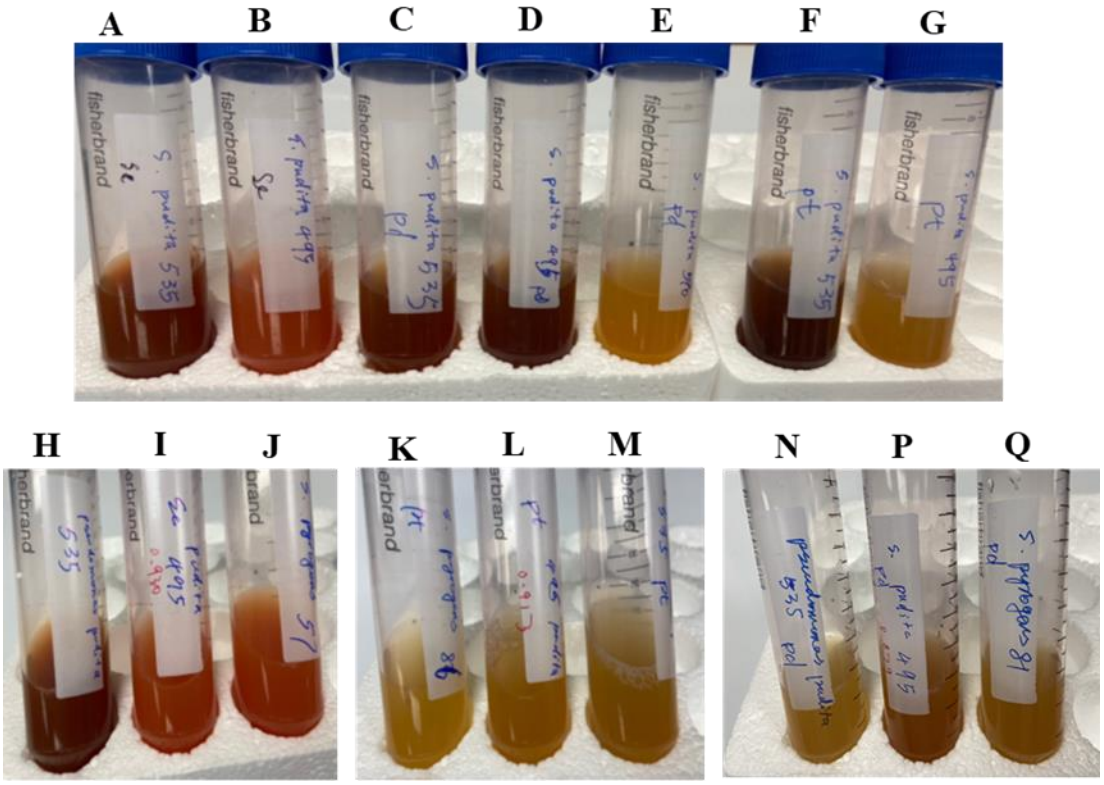

Figure 2.

III. Characterization of selenium nanoparticles using TEM

We examined the nanoparticles synthesized by one of the clones of *Pseudomonas pudtita* using TEM. The nanoparticles displayed a spherical shape and a size of 78.1±23.2 nm.

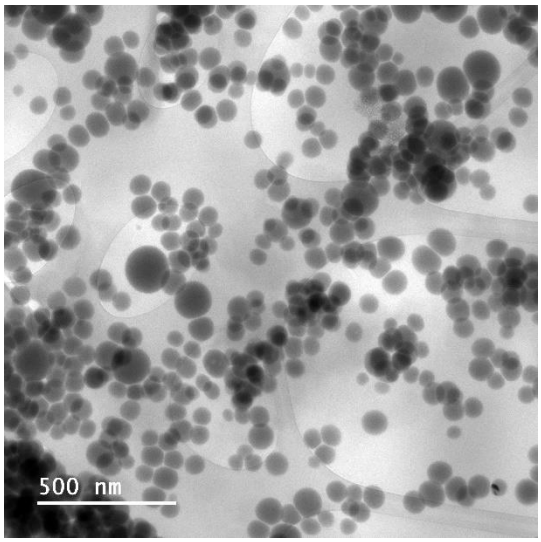

Figure 3. Analysis of selenium nanoparticles by TEM.

V. Opportunities for training during the reporting period:

During the time of Sep. 2021 to Aug. 2022, the funding of this project supported one master degree graduate student, and one undergraduate student. The undergraduate student was trained to synthesize nanoparticles, purify nanoparticles and other basic and important lab skills, such as bacterial culture, plasmid extraction, autoclave, PCR, DNA electrophoresis and protein electrophoresis.

VI. Dissemination during the reporting period:

The research during this period requires high through-put approaches. The work load has been heavy, but no novel and significant findings were revealed. The data were not presented at any conference or in any publications. However, a collaboration between my lab and Dr. Terrance Ravine at University of Southern Alabama is examining the antimicrobial activity of the biological silver nanoparticles. The paper was published on journal "Applied Nano".

VII. Plans for next reporting period

Up to now, no clones showed the capacity to synthesize other nanoparticles than those produced by the host bacterium. The project was ending in September. However, the DNA libraries constructed through the support by this project will be useful. The investigators plan to screen these clones for their antimicrobial activity, since some of the clones displayed a reduced growth even in the absence heavy metal ions. It may provide opportunities to seek new funding.

VIII. Honors and Awards Received During the Reporting Period: No.

IX. Products:

1. Presentation: No

2. Publication:

Ravine, T.*; Yuan, Q.; Howell, M. Biogenic Silver Nanoparticles Processed Twice Using 8M Urea Exhibit Superior Antibacterial and Antifungal Activity to Commercial Chemically Synthesized Counterparts. Appl. Nano 2022, 3, 187-201. https://doi.org/10.3390/applnano3040014 (Corresponding author)

3. Students' activities:

Lauren Williams had been trained and mentored to perform research in the project during the period. The students have been trained by the Co-PI, Dr. Xiao to use the AAMU-EE clean room fabrication facilities and operate the instruments in the clean room for the experiments and research. Lauren performed experiments, fabricated devices, measured the fabricated devices, and analyzed the experimental data for their research.

Adrian Rhoden worked on bacteria culture, extraction of nanoparticles from bacteria for this project. At the same time, he was also involved in other research work in the lab. He learned some important lab skills: bacteria transformation, plasmid isolation, DNA agarose gel electrophoresis and protein SDS-PAGE.

A

B

A. Adrian Rhoden was extracting selenium nanoparticles. **B**. Adrian Rhoden was working on protein SDS-PAGE.