Supplementary Material

Characterization of Silver Carbonate Nanoparticles Biosynthesized using Marine *Actinobacteria* and Exploring of their Antimicrobial and Antibiofilm Activity

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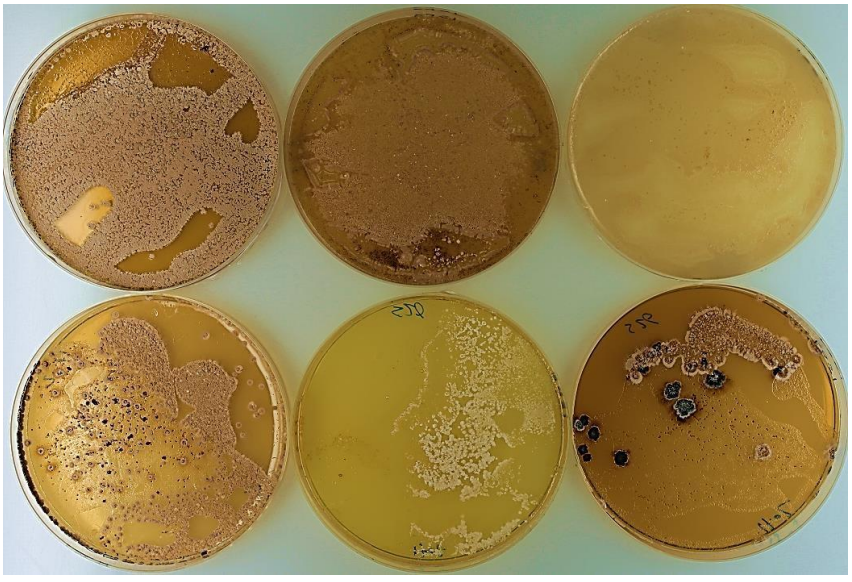
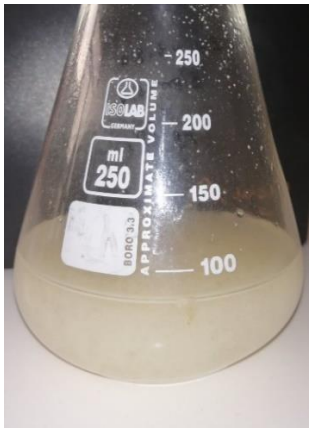


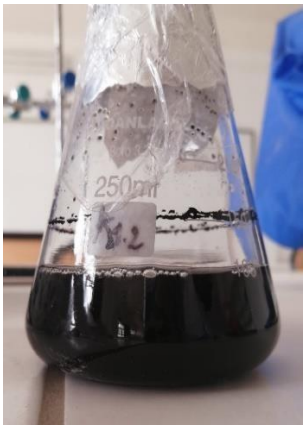
A

B **C**

Figure S1. A. Colony description and melanin pigment formation; from top left to bottom right: ISP2, ISP3, ISP4, ISP5, ISP6, and ISP7. **B.** Sodium chloride tolerance test; [NaCl] from top left to bottom right : 0%, 2.5%, 5%, 7.5%, 10%. **C.** Carbon utilization test; from top left to bottom right: glucose, arabinose, sucrose, xylose, inositol, mannose, fructose, rhamnose, raffinose, cellulose, water).

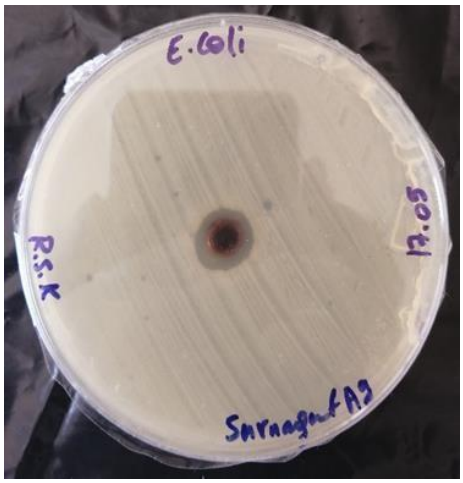


After the addition of $AgNO_3$ to the biomass of strain S26

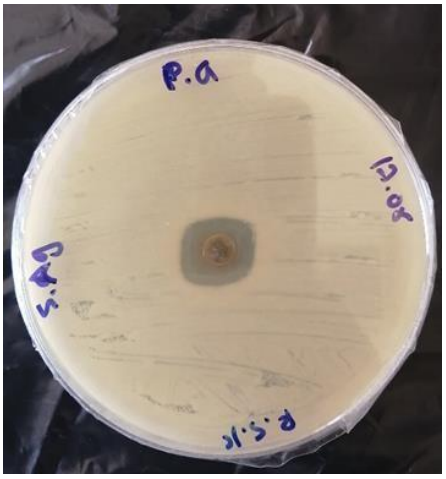


Before the addition of $AgNO_3$ to the biomass of strain S26

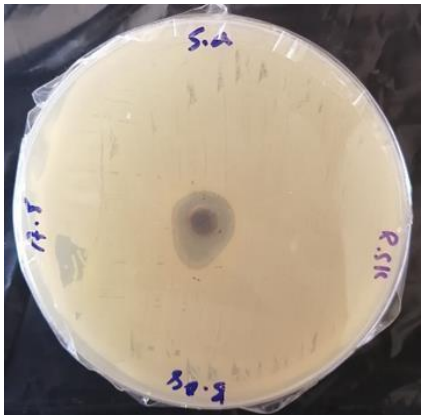
Figure S2. Biosynthesis of silver carbonate nanoparticles by the biomass of *Saccharopolyspora erythraea*
S26 strain.



Escherichia coli **(ATCC 25922)**

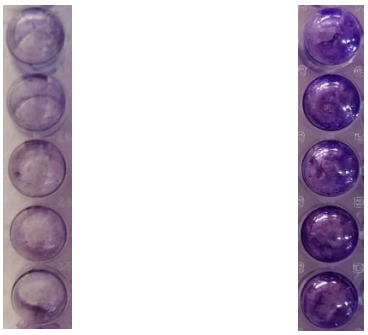


Pseudomonas aeruginosa **(ATCC 19606)**



Staphylococcus aureus **(ATCC 25923)**

Figure S3. Antimicrobial activity of silver carbonate nanoparticles prepared from the biomass of strain *Saccharopolyspora erythraea* S26.



BioAg$_2$CO$_3$NPs Negative control

Figure S4. Microtiter plate biofilm assay for the evaluation of the antibiofilm activity of Bio-Ag$_2$CO$_3$NPs against the biofilm formed by strain *Klebsiella pneumoniae* kp6 strain

Figure S5. Marine samples used for Actinobacteria isolation. A : Marine algae Codium bursa; B : Marin sponge *Chondrosia reniformis*.

Table S1. Antimicrobial activity (mm) of *Actinobacteria* strains against different pathogenic microorganisms.

Origine of isolates	Isolates	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*M. luteus*	*B. cereus*	*L. monocytogenes*	*C. albicans*
Marine sediment	M51	-	8	-	-	-	21	-	15
	M52	-	-	-	-	16	-	-	8.5
	BS4	-	-	-	-	8.5	-	-	-
	BC8	-	-	-	8	-	-	-	-
	M2S9	-	-	8	-	-	-	-	-
	M2S10	-	-	-	-	-	17	-	14
	M2S12	-	-	-	12	-	-	-	-
	M19	-	-	-	11	-	-	-	-
	MHB2	-	-	-	9	10	-	-	9
	RHS22	-	-	-	-	-	-	-	10
	S26	-	-	-	31	21.5	29	-	20.5
	M2S27	-	-	-	-	-	8	-	-
	M2S28	-	-	-	-	-	12	-	12
	M29	-	-	-	11	-	9	-	10
	M2S30	-	-	-	12.5	-	-	-	11
	M5S34	-	-	-	-	9.5	-	-	8.5
	CS3	-	-	-	-	-	13	-	-
	CC54	-	-	-	-	15.5	-	-	-
	RR56	-	-	-	16	-	12	-	12.5
	RR57	-	-	-	8	-	15	-	19
	M2S59	-	-	-	-	-	9	-	11
	MRH94	-	-	-	30	29.5	28.5	-	21.5
	M2S108	-	-	-	-	-	-	-	10.5
	CS114	-	-	-	-	19	-	-	-
	M2S115	-	-	-	-	-	13	-	14
	MS44	-	-	-	20.5	-	12	-	19
	M2C6	-	-	-	-	-	16	-	-
	CS7	-	-	-	8.5	-	-	-	-
	CS16	-	-	-	-	-	10	-	-
	CS58	22	-	-	-	27	-	-	20
Marine algae	SW1V5	-	-	-	-	-	-	-	13.5
	W7V9	-	-	9	-	-	-	-	11.5
	W3V10	-	-	10	9	-	-	-	11
	W4V12	13.5	16.5	11	18.75	15.75	13	13.5	20
	V4	-	-	-	-	-	-	-	11.25
	S17	-	-	-	11.5	-	13	-	-
	W6V18	-	-	-	7.5	-	-	-	7
Marine sponge	SA6M7	-	-	10	-	-	-	-	-
	A6M14	-	-	-	8.75	-	-	-	7
	S23	-	-	-	14	-	9	-	13

Table S2. Molecular identification of the 18 selected active strains, based on 16S rRNA gene sequencing.

Isolates	Clusters	Closest reference species	Similarity (%)
S17	**Cluster I**	*Streptomyces spinoverrucosus*	99.72
MRH94		*Streptomyces luridiscabiei Strep-*	99.86
MS44		*tomyces hydrogenans Strepto-*	99.46
M29		*myces artemisiae Streptomyces*	99.65
M2S28		*peucetius Streptomyces al-*	99.72
W3V10		*bogriseolus Streptomyces lav-*	100
W4V12		*endofoliae Streptomyces pactum*	99.88
W7V9		*Streptomyces hydrogenans*	100
M51		*Streptomyces qinglanensis Strep-*	99.49
MHB2		*tomyces qinglanensis*	99.67
W6V18			99.25
CS58	**Cluster II**	*Nocardiopsis terrae*	99.72
RR56		*Nocardiopsis terrae*	99.65
M52		*Nocardiopsis halotolerans*	100
RR57		*Nocardiopsis sinuspersici*	99.46
S23		*Nocardiopsis dassonvillei subsp. dassonvillei*	99.74
M2S10		*Nocardiopsis synnemataformans*	99.89
S26	**Cluster III**	*Saccharopolyspora erythraea*	99.87

Table S3. Characteristics of the S26 strain and the closest species within the genus *Saccharopolyspora*. 1. S26 ; 2. *Saccharopolyspora erythrea* DSM 40517^T (AM420293). 3. *Saccharopolyspora spinosporotrichia* DSM 44350^T (Y09571).

Characters	**1**	**2**	**3**
Spores arrangement	Open spirals	Open spirals	Spirals
Spores ornamentation	Spiny	Spiny	Spiny
Substrate mycelium	Branched	Branched	Fragmented
Color			
Substrate mycelium	Brown beige	Bright red	Beige
Aerial mycelium	Beige	White rose	White
Soluble pigment	Orange-brown		Pastel yellow
Growth on sole carbon sources			
Glucose	+	+	+
Arabinose	+	+	+
Saccharose	+	+	+
Xylose	+	+	+
Inositol	+	+	+
Mannose	+	+	+
Fructose	+	+	+
Rhamnose	+	+	+
Raffinose	-	+	+
Cellulose	-	+	+
NaCl tolerance	(0 - 5)%, Opt 0%	(0 - 5)%, Opt 0%	(0 - 7)%, Opt 0%
Nitrate reductase	-	+	-
Ureas	+	+	+

Table S4. Peak list of silver carbonate nanoparticles biosynthesis using the biomass strain S26.

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
17.6239	1446.11	0.1956	5.02833	18.26
19.5154	5645.86	0.2075	4.54503	71.31
30.3374	1502.61	0.8822	2.94387	18.98
30.6912	2351.64	0.1717	2.91073	29.70
33.8752	7917.86	0.7632	2.64408	100.00
35.5840	549.53	0.1120	2.52092	6.94
39.5070	1844.61	0.2524	2.27917	23.30
43.5341	577.84	0.8319	2.07721	7.30
48.5342	1515.54	0.7830	1.87425	19.14
53.0702	1022.72	0.2831	1.72424	12.92
54.3468	459.05	0.3933	1.68672	5.80
60.6894	1619.10	0.7809	1.52474	20.45
67.2534	237.20	0.5522	1.39099	3.00
71.5640	261.10	0.5183	1.31742	3.30