Supplementary Table 1. Composition of media used in generating actinomycetes library.

<1> G.S.S. medium	**<2> Bennett's medium**	**<3> DYC medium**
Soluble starch 10g	Glucose 10g	Dextrine 25g
Glucose 20g	Yeast extract 1g	Dry yeast 12g
Soybean meal 25g	Bacto-peptone 2g	CSL 20g
Beef extract 1g	Beef extract 1g	NaBr 1g
Yeast extract 4g	D.I. Water 1L	$CoCl_2$ 1g
NaCl 2g	pH 7.2	D.I. Water 1L
K_2HPO_4 0.25g		pH 7.0
$CaCO_3$ 2g		
D.I. Water 1L		
pH 7.2		

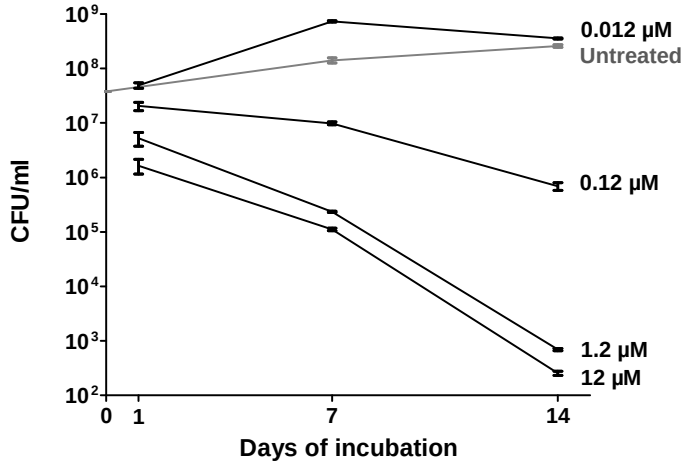
Supplementary Table 2. Strains ITR5, ITR9 and ITR13 were genetically resistant to ecumicin (ECM). PCR-sequencing was used identify *clpC1* mutation of each strain.

Strain ID	ITR5		ITR9		ITR13	
Passage	1st	2nd	1st	2nd	1st	2nd
clpC1 mutation	L92S	L92S	L92F	L92F	L96P	L96P
MIC vs. ECM (μM)	0.66-1.35	0.52	0.49-0.68	0.41	0.75-1.02	0.75

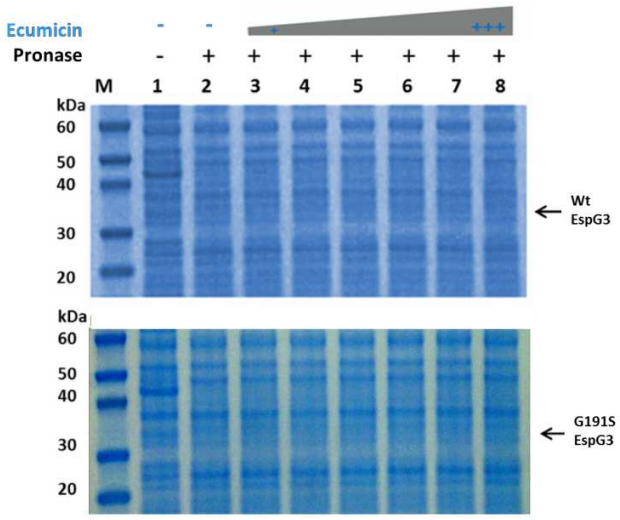
Supplementary Table 3. PpsC was not the target of ecumicin (ECM). *Mtb*, *M. tuberculosis*; RMP, rifampin; INH, isoniazid; SM, streptomycin; CAP, capreomycin. *M. tuberculosis* CDC1551 *ppsC* Tn Mutant (NR-18565) was acquired from ATCC.

	MIC (μM) v.s.					
	ECM	RMP	INH	SM	CAP	PA-824
Mtb CDC1551 *ppsC* Tn Mutant	0.16	0.08	0.29	1.2	1.9	<0.08
Mtb H37Rv	0.16	<0.012	0.29	1.2	1.9	<0.08

Supplementary Figure 1**.** Ecumicin has time and concentration-dependent bactericidal activity against *M. tuberculosis*. Inoculum size is 3.8×10^7 cfu/ml. Error bars = SD. Each data point indicates the mean of two measurements.

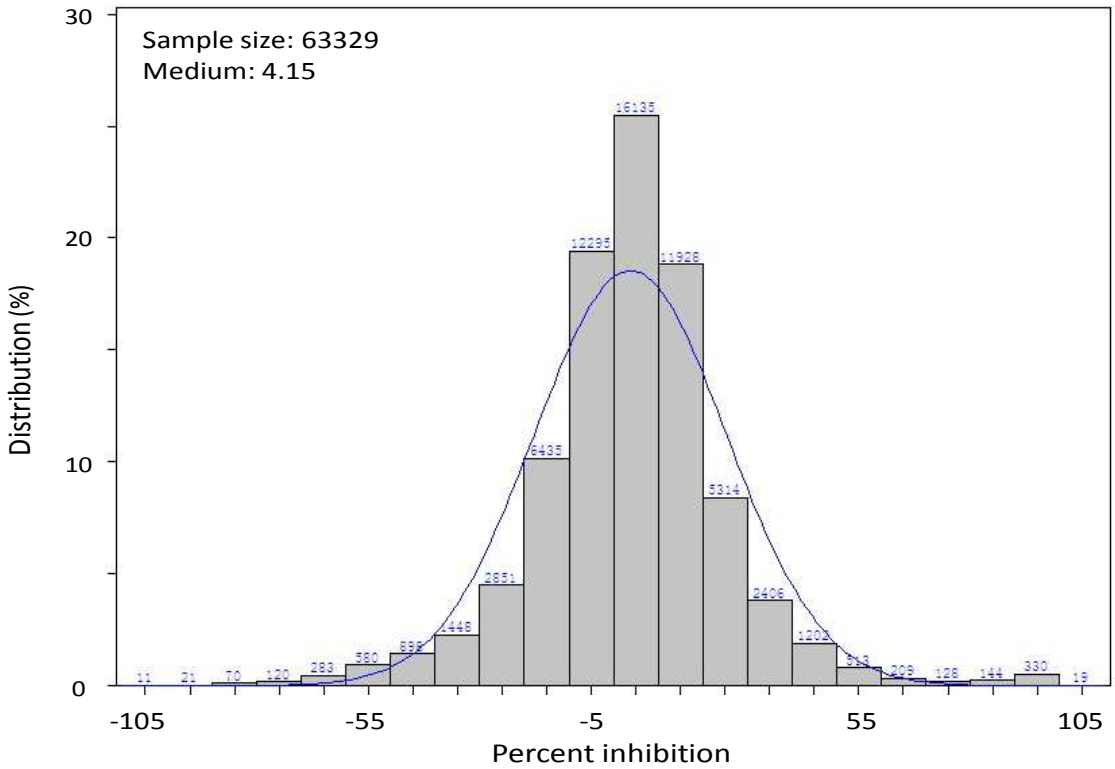


Supplementary Figure 2. Ecumicin does not protect either wild-type or mutated *M. tuberculosis* EspG3. Recombinant *E. coli* BL21 over-expressing *M. tuberculosis* wild type or mutant EspG3 were lysed, treated with ecumicin at different concentrations, subjected to pronase digestion, and analyzed by SDS-PAGE. M, size marker; 1, whole cell lysate without any treatment; 2, whole cell lysate digested by pronase; 3-8, whole cell lysates treated with ecumicin at increasing concentrations (0.004,0.04, 0.4, 4, 40, and 80 µg/ml), and subjected to pronase digestion.

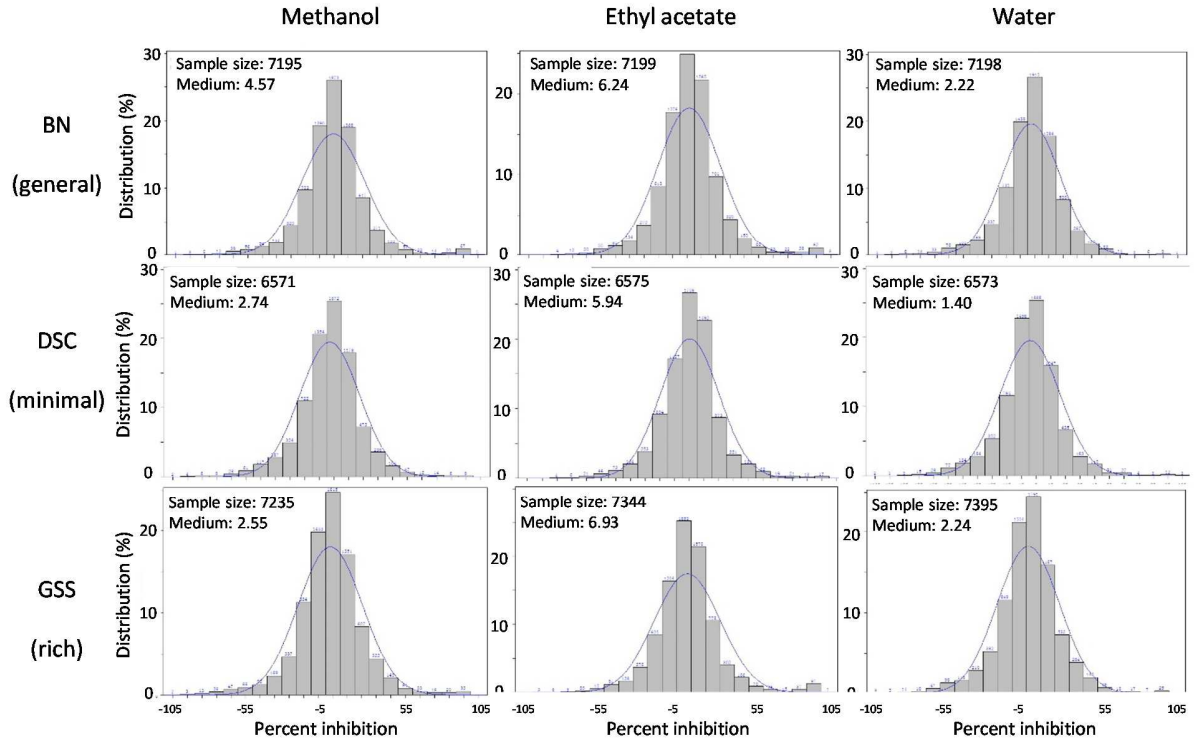


Supplementary information on the high-throughput screening.

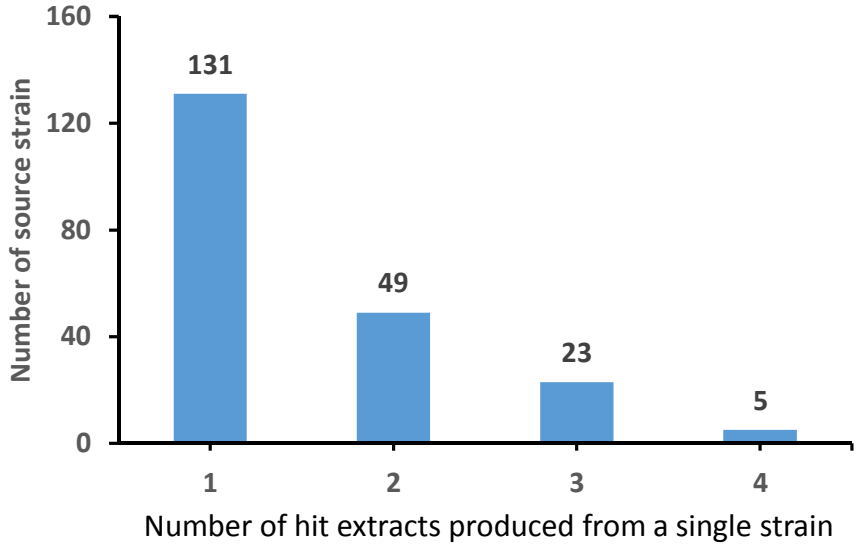
The primary screening encompassed 63,329 actinomycete extracts from ECUM. A total of 318 extracts (0.55%) exhibited ≥ 90% inhibition of fluorescence relative to untreated control cultures when screened against replicating *M. tuberculosis*. (Supplementary Figure 3). The activity was mostly found in the organic solvent extracts of normal or rich media (Supplementary Figure 3). The majority of active samples were found in only one of the 9 extracts that were produced from each culture. However some were found in as many of four of the extracts (usually both of the organic extracts and of the richer media). As a result, the secondary library of only active anti-*M. tuberculosis* actinomycete extracts contained a total of 208 source strains (3.0% of whole library).



Supplementary Figure 3. Activity of all extracts follows the normal distribution with medium mean at 4.52% inhibition. Number on top of each bar represents number of samples found with corresponding percentage inhibition range.



Supplementary Figure 4. Distribution of anti-*M. tuberculosis* activity in all extracts analyzed by media and extraction types.



Supplementary Figure 5. Media and/or extraction type dependence for production of active metabolites. Most hit strains (63%) would only produce anti-*M. tuberculosis* activity in one extract when cultured in a certain media.

Retesting against $H_{37}Rv$ for MIC determination confirmed 278 hits from the initial hit list (87.4%). When tested against mammalian VERO cells, 154 of the confirmed hits showed no obvious toxicity (IC_{50} > 10 µl/ml or SI > 10). When tested against the isogenic strains that are resistant to KM, RMP or SM, 124 of the confirmed non-toxic hits showed no cross-resistance with any of these drugs.

Most (67) of the 124 prioritized hits were found in only one of the 9 extracts that were produced from a single culture; 38 were found in two of the extracts; and 18 were found in three extracts. Removal of duplicate strains resulted in a list of 93 hits, representing 1.3% of strains in the original library. Only 10 of the 93 hits were water fractions.

The screening profiles for all nine extracts produced by the 93 strains were examined, to pick the most active fraction for further prioritization. Although the methanolic extract of strain MJM7049 cultured in Bennett's medium had a selectivity index of only 6.5 and the ethylacetate extract of the same strain cultured in GSS medium had IC_{50} > 10, the former was included in the hit list in place of the later due to its over 10-fold higher activity against all tested *M. tuberculosis* strains.

The activity of the final 93 hits against non-replicating *M. tuberculosis* as well as a spectrum of other microbes (*S. aureus*, *E. coli*, *C. albicans*, *M. smegmatis*) were identified.

Together with their anti-TB activity profile, they were divided into four priority groups. The first group contained three hits – two were selected for their highly selective potency against replicating *M. tuberculosis* with no cross-resistant with RMP, KM, SM or CS; another one was included for its high potency against non-replicating *M. tuberculosis*. Compared to hits in group 1, hits in the second group had one of the following undesirable features, (a) less active against replicating *M.*

tuberculosis, (b) active against at least one of the nontuberculous microbes, (c) some toxicity against mammalian cells. Compared to group 2, hits in group 3 had some of the following undesirable features, (a) less active against replicating *M. tuberculosis*, (b) highly active against at least one nontuberculous microbe, (c) toxicity against mammalian cells, (d) cross-resistance with RMP, SM or KM. Group 4 consisted of hits with the least favorable biological profile; they were the hits with lest activity against replicating *M. tuberculosis*, bearing cross-resistance to RMP, SM or KM.

After the groups were determined, an *M. tuberculosis* strain resistant to CS became available, and the top three groups were tested against it. The biological profile of the top three groups (22 hits) is summarized in Supplementary Table 4.

An analysis of their 16S rRNA suggests that the majority of the hits in the top three groups (17) were produced by strains belonging to the *Streptomyces* genus, and only one by a *Nonomuraea* species. The source strains of the remaining four were to be identified.

A new batch of the 22 hits were prepared from a medium scaled fermentation (1L), with their anti-TB activity confirmed by MABA. Subsequently, six fractions were generated for each extract by solid phase extraction on a RP-18 cartridge. Supplementary Table 5 shows the screening result of one top priority hit, strain MJM5123. The fractionation process concentrated the selective anti-TB activity of the methanolic extract of MJM5123 (MJM5123GM) into the two most lipophilic fractions. Furthermore, undetected in MJM5123GM, activity against non-replicating culture was seen in these two fractions.

Supplementary Table 4. Biological profile of priority group 1 – 3. Ext, extract; M, methanol; E, ethylacetate, W, water; rKM, kanamycin-resistant strain; rRMP, rifampin-resistant; rSM, streptomycin-resistant; rCS, cycloserine-resistant. MIC, the lowest concentration of a test agent that will inhibit 90% of the visible growth of a microorganism

				MIC vs *M. tuberculosis* in µl/ml (%, at 10 µl/ml)					IC50 in µl/ml		MIC vs *M. tuberculosis* in ul/ml (%, at 10 µl/ml)				LORA	
Priority group	ID	Media	Ext	Rv	rKM	rRMP	rSM	rCS	1	2	*E. coli*	*S. aureus*	*C. albicans*	*M. smegmatis*	% inhibition at 10µl/ml	MIC in µl/ml
1	5123	GSS	M	0.51	0.31	0.89	0.84	2.49	> 10	> 40	0%	0%	64%	0%	17%	>10
	7483	BN	E	1.55	2.25	2.27	2.26	4.68	> 10	> 40	17%	4.34	8.03	0%	101%	0.48
	8412	GSS	E	2.86	0.30	2.03	0.58	0.25	> 10	> 40	0%	12%	0%	2.3	22%	>10
2	1526	GSS	E	1.66	2.35	2.16	2.36	89%	> 10	> 40	23%	3.71	86%	0%	100%	1.68
	1543	BN	E	1.20	2.38	2.19	2.32	1.22	> 10	28.2	0%	2.94	7.22	0%	101%	0.11
	2526	BN	E	1.44	2.06	1.38	1.08	1.15	> 10	29.9	12%	1.78	4.27	0%	99%	0.08
	4824	BN	M	2.34	1.69	2.33	1.45	1.48	> 10	29.2	22%	2.01	4.26	56%	100%	4.23
	4977	BN	M	2.42	2.45	2.32	2.01	2.33	> 10	> 40	22%	2.14	4.84	44%	100%	0.04
	5560	GSS	E	2.28	2.29	3.66	1.90	3.74	> 10	> 40	0%	0.46	7.02	59%	99%	0.23
3	7049	BN	M	0.85	0.60	0.60	0.62	0.57	5.5	9.4	14%	0.90	1.88	77%	100%	0.58
	1401	GSS	E	4.66	4.88	4.46	4.68	4.30	> 10	> 40	13%	4.48	65%	16%	101%	0.30
	4302	GSS	M	4.76	4.84	4.90	4.44	4.77	> 10	> 40	26%	8.02	4.97	11%	98%	0.45
	4413	BN	M	2.49	2.52	4.36	2.24	2.50	> 10	> 40	19%	3.02	85%	19%	101%	>10
	4721	BN	M	4.94	4.73	6.81	4.59	4.66	> 10	> 40	15%	4.22	22%	16%	101%	8.85
	4887	BN	E	4.83	5.94	4.82	4.66	4.74	> 10	> 40	10%	4.82	6%	6%	101%	>10
	5132	BN	E	4.53	4.82	4.19	4.45	21%	> 10	> 40	1%	39%	0%	11%	58%	7.58
	5960	DYC	E	2.20	0.63	2.40	3.59	0.96	> 10	3.2	13%	0.22	64%	12%	14%	0.25
	6477	DYC	W	2.57	2.36	3.34	4.81	4.80	> 10	> 40	2%	35%	73%	87%	101%	0.24
	6833	GSS	W	1.51	3.60	1.16	0%	0%	> 10	> 40	0%	26%	88%	24%	101%	0.15
	8106	DYC	W	8.14	2.42	1.00	2.26	2.92	> 10	> 40	4.75	4.42	4.91	2.38	83%	0.26
	8427	BN	M	2.92	2.60	2.40	4.02	2.35	> 10	> 40	4%	3.17	88%	17%	100%	0.46
	8915	BN	M	2.37	3.74	3.48	3.87	3.73	> 10	> 40	2%	4.20	87%	8%	100%	>10

Supplementary Table 5. Biological profiling of methanolic extract of prioritized culture MJM5123, and its fractions generated by solid phase extraction on an RP-18 cartridge and methanol/water gradient elution. G, GSS; M, methanolic extract. rKM, kanamycin-resistant strain; rRMP, rifampin-resistant; rSM, streptomycin-resistant; rCS, cycloserine-resistant.

Sample	MIC vs *M. tuberculosis* (µg/ml)					IC50 vs VERO (µg/ml)	LORA (µg/ml)	Spectrum Activity (% inhibition at 100 µg/ml)			
	Rv	rRMP	rSM	rKM	rCS			*E.coli*	*S. aureus*	*C. albican*	*M. smegmatis*
MJM5123GM	5.01	9.10	2.42	9.83	4.91	> 100	> 100	0%	12%	0%	33%
20% Methanol	25%	7%	6%	1%	7%	> 100	> 100	0%	5%	29%	12%
40% Methanol	13%	16%	0%	7%	12%	> 100	> 100	0%	3%	0%	18%
60% Methanol	17%	17%	0%	3%	0%	> 100	> 100	0%	5%	36%	10%
80% Methanol	8.48	9.34	2.46	9.42	4.80	> 100	> 100	0%	0%	0%	22%
100% Methanol	0.14	0.25	0.04	0.60	0.15	> 100	1.57	0%	1%	38%	9.72
Chloroform	0.19	0.51	0.08	0.40	0.15	> 100	6.70	0%	7%	71%	9.99

Supplementary information on the isolation, identification and MIC determination of clinical M. tuberculosis isolates from National Masan Hospital

Four *M. tuberculosis* strains were applied in this study; 1 reference (H37Rv) and 3 clinical strains isolated from pulmonary TB patients hospitalized in National Masan Hospital. All clinical strains were isolated and stored in the TB Specimen Biobank, National Masan Hospital. The clinical strains were identified as *M. tuberculosis* by both IS6110 specific PCR kit (Seegene, Seoul, Korea) and Antigen Test kit (SD, Seoul, Korea). Additionally, all isolates were incubated on Blood agar plates for 3 days in order to test for potential contamination. When no contamination was observed, the *M. tuberculosis* isolates were used for drug susceptibility tests by the agar proportion method against INH and RIF, and by the absolute concentration method against other injectable and FQ drugs. The DNA of *M. tuberculosis* isolates were extracted from the colonies grown on Ogawa medium. The extracted DNA samples were used for molecular drug susceptible tests (MTBDRplus and MTBDRsl, HAIN Lifesciences, Germany) against INH, RIF, Injectable, and FQ drugs. In addition, the DNA samples were used for checking potential non-tuberculous mycobacteria (NTM) contamination by Genotype Mycobacterium CM/AS kit (HAIN). The isolates used in this study contain no NTM contamination. For MIC measurement, the *M. tuberculosis* strains freshly grown on 7H11 agar medium were used.

The MIC values of TB drugs and the test compound ecumicin were measured by the previously published Microplate assay (1) with modification. In the test Alamar Blue was replaced by Resazurin (0.6 mM). Each sample was tested in triplicates. MIC value of each sample was determined as the lowest concentration that prevented a color change from blue to pink.

Reference:

1. **Collins L, Franzblau S.** 1997. Microplate Alamar Blue Assay versus BACTEC 460 System for High-Throuput Screening of Compounds against Mycobacterium tuberculosis and Mycobacterium avium. Antimicrob. Agents Chemother. **41:**1004-1009.