

Supplementary figures

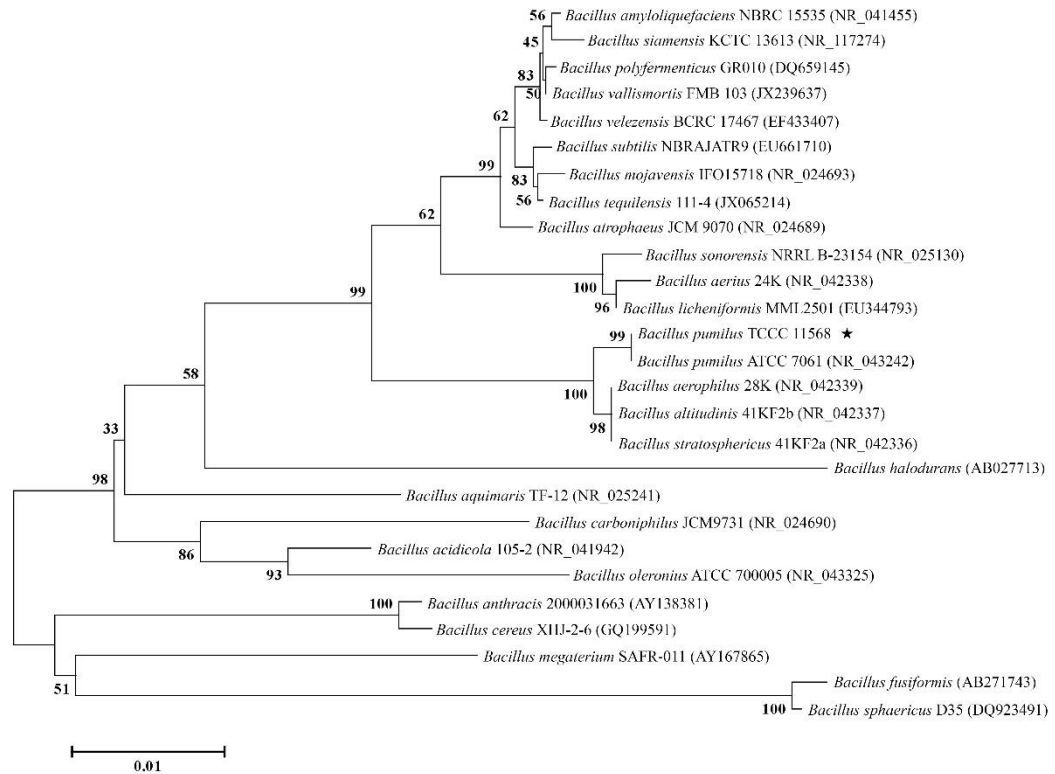


Figure S1. 16S rDNA-based phylogenetic comparison of *B. pumilus* TCCC 11568 and other *Bacillus* species. The phylogenetic tree was engineered using MEGA 6.0 software with the neighbor-joining method. The numbers at branch points suggested the percentage of bootstrap sampling from 1000 replications.

```

B. pumilus TCCC 11568 laccase      : MLEKFDELPIEYAPVKKNPRQITYEIAMGVELKVEIDLPPTQLTYNGSLPGPTI QANRNSNKKIKNINLPAGEFLPDHITHG—HDEEIVTVVHLHGGVTPASSDGYPEAW—SKD—EAGGPFHEVYVYVPMQOACTL
B. pumilus ATCC 7061 laccase     : MLEKFDELPIEYAPVKKNPRQITYEIAMGVELKVEIDLPPTQLTYNGSLPGPTI HANRNSNKKIKNINLPAGEFLPDHITHG—HDEEIVTVVHLHGGVTPASSDGYPEAW—SKD—EAGGPFHEVYVYVPMQOACTL
B. pumilus W3 laccase            : MLEKFDELPIEYAPVKKNPRQITYEIAMGVELKVEIDLPPTQLTYNGSLPGPTI KANRNSNKKIKNINLPAGEFLPDHITHG—HDEEIVTVVHLHGGVTPASSDGYPEAW—SKD—EAGGPFHEVYVYVPMQOACTL
B. vallismortis fmb 103 laccase : MLEKFDELPIEYAPVKKNPRQITYEIAMGVELKVEIDLPPTQLTYNGSLPGPTI EVKRNSNKKIKNINLPAGEFLPDHITHGSDSGHDEEIVTVVHLHGGVTPASSDGYPEAW—SKD—EAGGPFHEVYVYVPMQOACTL
B. amyoliquefaciens TCCC 111018 laccase : MALEKFDELPIEITLQVQKTSNGKITYEVIAMEGCHKLEIDLPPTQLTYNGSLPGPTI DVNQDENYIKNINLPAGEFLPDHITHGSDSGHDEEIVTVVHLHGGVTPASSDGYPEAW—TRO—KEAGGPFHEVYVYVPMQOACTL
B. subtilis X1 laccase          : MLEKFDELPIEITLQVQKTSNGKITYEVIAMEGCHKLEIDLPPTQLTYNGSLPGPTI DVNQDENYIKNINLPAGEFLPDHITHGSDSGHDEEIVTVVHLHGGVTPASSDGYPEAW—TRO—KEAGGPFHEVYVYVPMQOACTL
B. velezensis TCCC 111904 laccase : MALEKFDELPIEITLQVQKTSNGKITYEVIAMEGCHKLEIDLPPTQLTYNGSLPGPTI DVNQDENYIKNINLPAGEFLPDHITHGSDSGHDEEIVTVVHLHGGVTPASSDGYPEAW—TRO—KEAGGPFHEVYVYVPMQOACTL
B. licheniformis ATCC 14580 laccase : MALEKFDELPIEYVLPQVSKSKKITYEVIAMEGCHKLEIDLPPTQLTYNGSLPGPTI EVQKHENYIKNINLPAGEFLPDHITHGD—GHDEEIVTVVHLHGGVTPASSDGYPEAW—TRO—HAKGPFHEVYVYVPMQOACTL

B. pumilus TCCC 11568 laccase      : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. pumilus ATCC 7061 laccase     : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. pumilus W3 laccase            : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. vallismortis fmb 103 laccase : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. amyoliquefaciens TCCC 111018 laccase : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. subtilis X1 laccase          : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. velezensis TCCC 111904 laccase : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE
B. licheniformis ATCC 14580 laccase : WYDHAMATRLNRYAGLAGPYLISAFENISUBLPKQYTIPLDMQKRFQDGLSTYPSFENYTPEDSDTPPSIVPFCGFTILVNGKVPVMEEPRVYRFRINASNTRFYBLDIDATILQGS—DGGGLFVYVHQ—FSLAPAE

B. pumilus TCCC 11568 laccase      : RDVYLDFSNYNKTIILAKKAGCG—D—VPEIDAMNQFRIKRELGRVPKTLRLPKPLPLRPSQADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. pumilus ATCC 7061 laccase     : RDVYLDFSNYNKTIILAKKAGCG—E—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. pumilus W3 laccase            : RDVYLDFSNYNKTIILAKKAGCG—E—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. vallismortis fmb 103 laccase : RDVYLDFSNYNKTIILAKKAGCG—D—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. amyoliquefaciens TCCC 111018 laccase : RDVYLDFSNYNKTIILAKKAGCG—D—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. subtilis X1 laccase          : RDVYLDFSNYNKTIILAKKAGCG—G—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. velezensis TCCC 111904 laccase : RDVYLDFSNYNKTIILAKKAGCG—DA—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI
B. licheniformis ATCC 14580 laccase : RDVYLDFSNYNKTIILAKKAGCG—DA—VPEIDAMNQFRIKRELGRVAKTLRPLPKPLPLRPSRADREKILLDGG—ADYGRFLLDNGWVPEV—TNGR—GSGV—VNLVNTGR—HPILHLVYFRILRPPEDTEVYSTGEI

B. pumilus TCCC 11568 laccase      : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. pumilus ATCC 7061 laccase     : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. pumilus W3 laccase            : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. vallismortis fmb 103 laccase : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. amyoliquefaciens TCCC 111018 laccase : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. subtilis X1 laccase          : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. velezensis TCCC 111904 laccase : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—
B. licheniformis ATCC 14580 laccase : VITGAVNEAPLLEKQKDTIQAFA—GVIRI—L—NTPYSGR—VYVHCILHE—EDYDAMRQ—IIQ—

```

Figure S2. Multiple alignment of rLAC protein sequence from *B. pumilus* TCCC 11568 and other laccases derived from *Bacillus* genus (*B. pumilus* ATCC 7061[23], *B. pumilus* W3, *B. vallismortis* fmb103, *B. amyoliquefaciens* TCCC 111018, *B. subtilis* X1, *B. velezensis* TCCC 111904, *B. licheniformis* ATCC 14580). The identical and similar amino acids are marked in black and solid grey, respectively. These protein sequences were obtained from NCBI database, and the alignment was carried out with DNAMAN software.

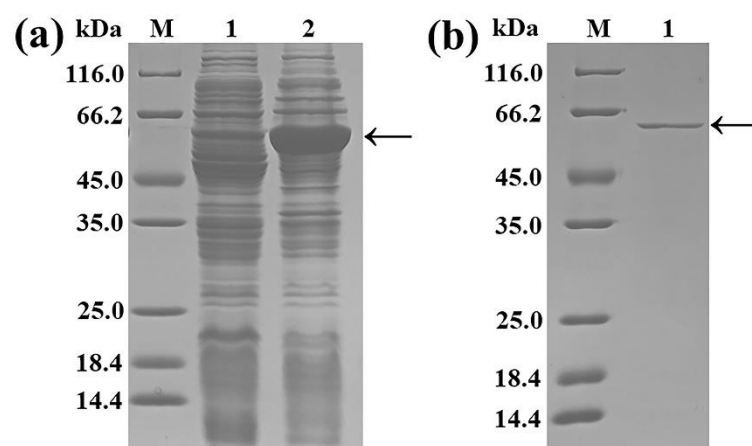


Figure S3. Detection of rLAC expressed in *E. coli*. (a) Lane M: protein standard ladder; Lane 1: cell extract of *E. coli* BL21/pET-22b; Lane 2: cell extract of *E. coli* BL21/pET-lac. (b) Lane M: protein standard ladder; Lane 1: the purified rLAC. Black arrowhead indicates the rLAC band.