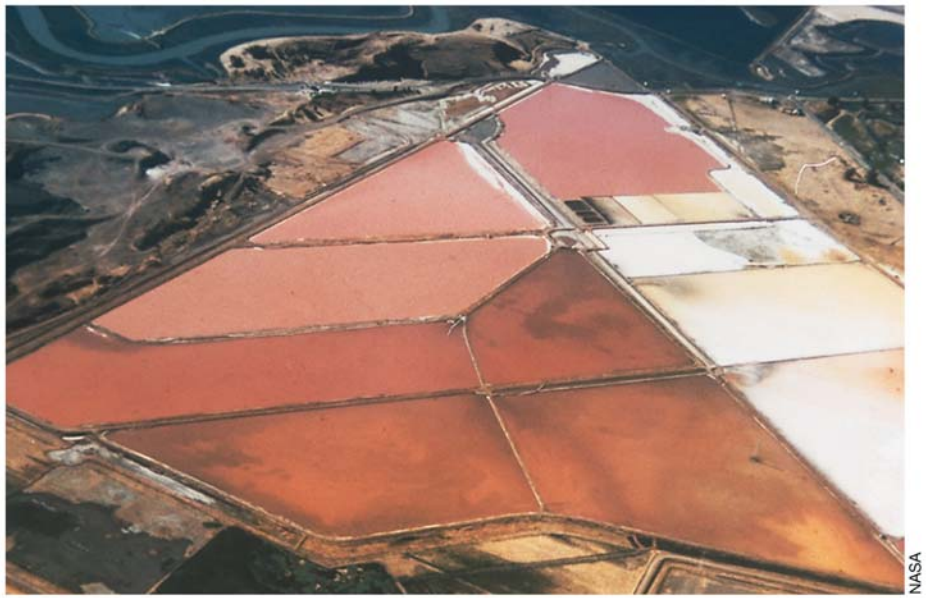
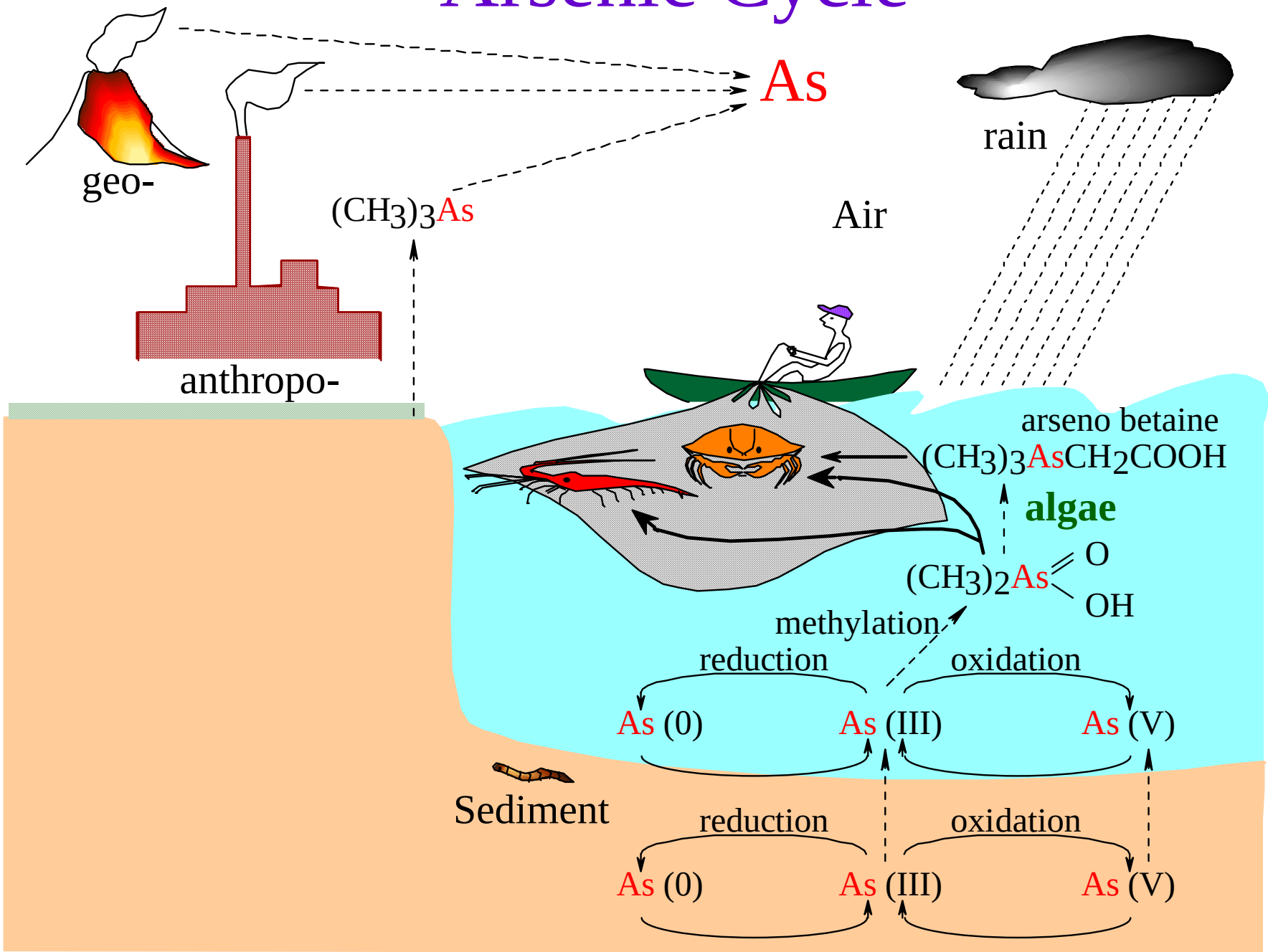
Arsenic Resistance in *Halobacterium* sp. NRC-1 Examined Using an Improved Gene Knockout System



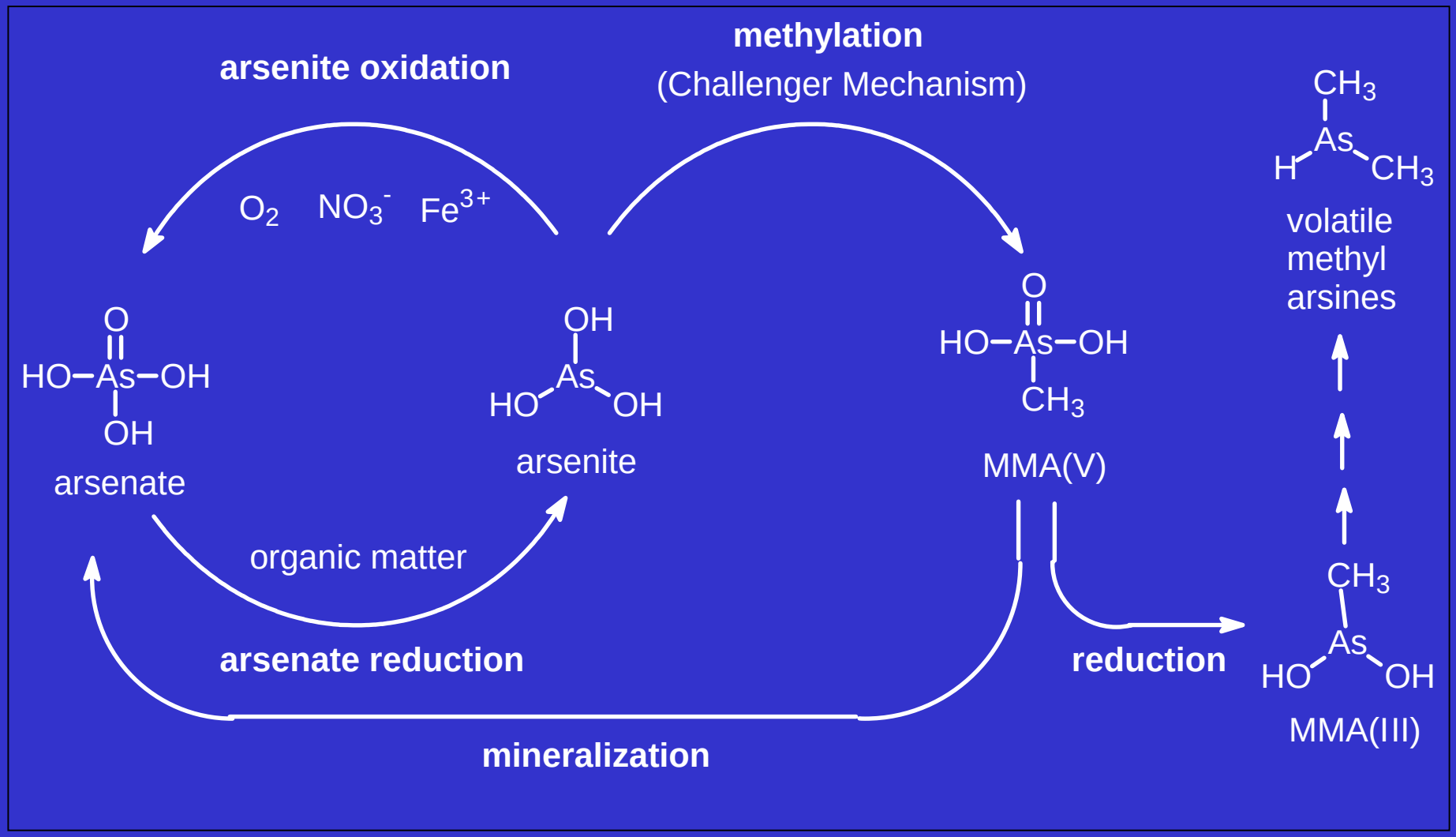
(b)

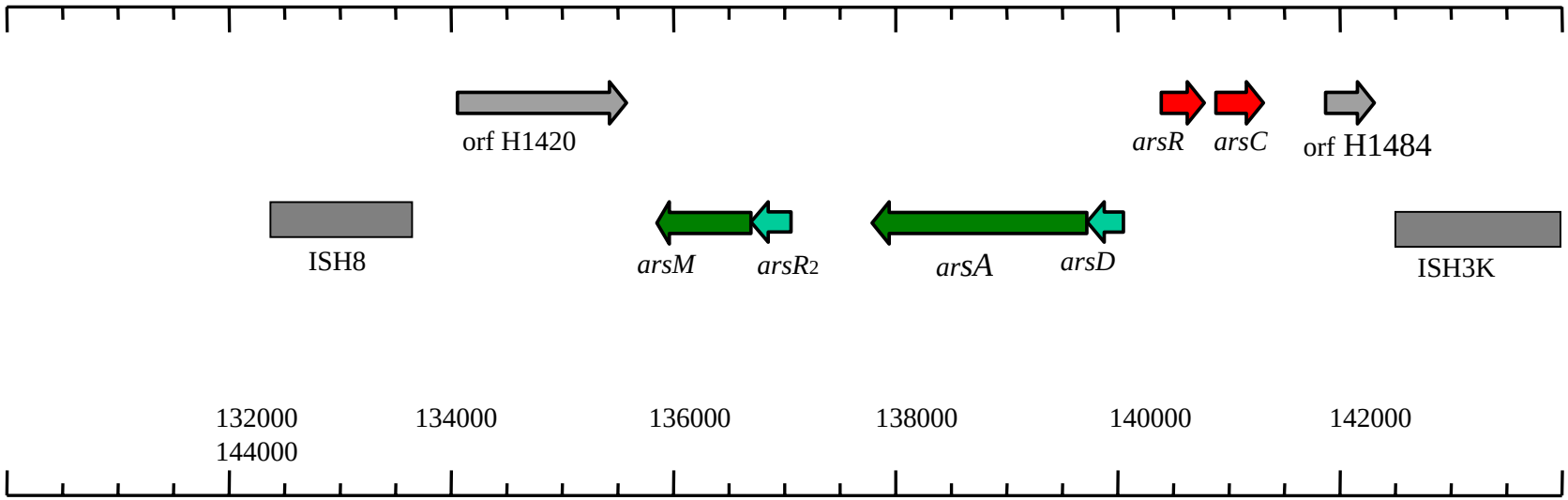
- Halophilic archaea
- Unusal arrangement of *ars* operon
- Contains putative As(III)-methyltransferase

Arsenic Cycle



Biological Arsenic Cycle



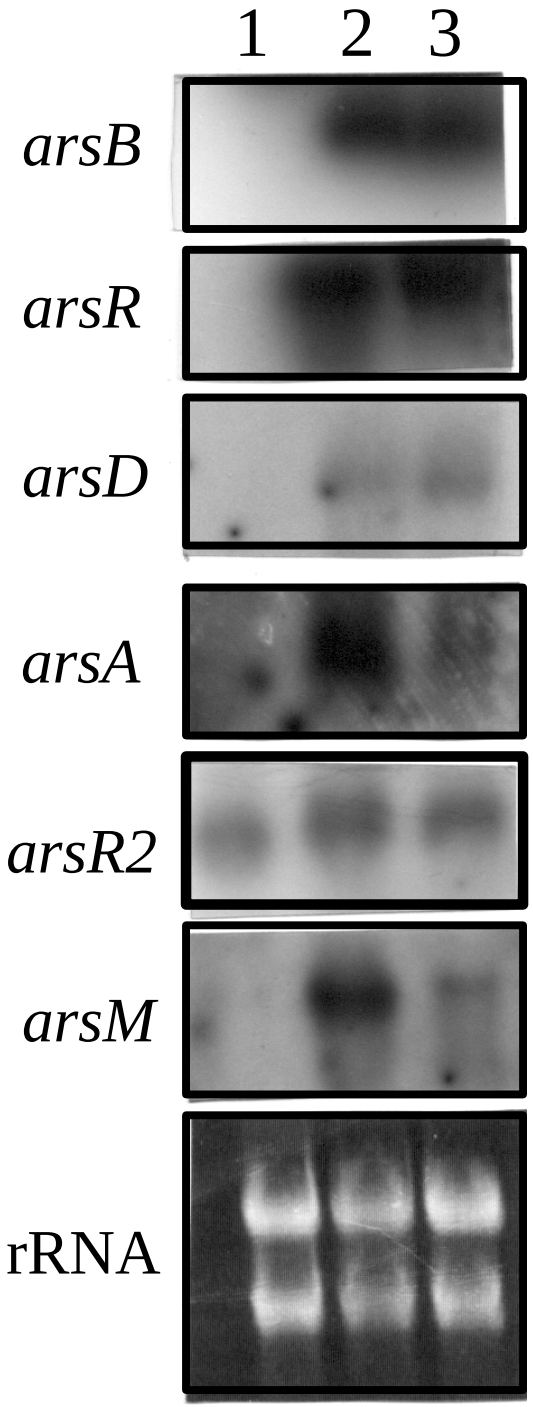

orf H1420
arsR
arsC
orf H1484
ISH8
arsM
arsR2
arsA
arsD
ISH3K
132000
134000
136000
138000
140000
142000
144000

(14000 bps)

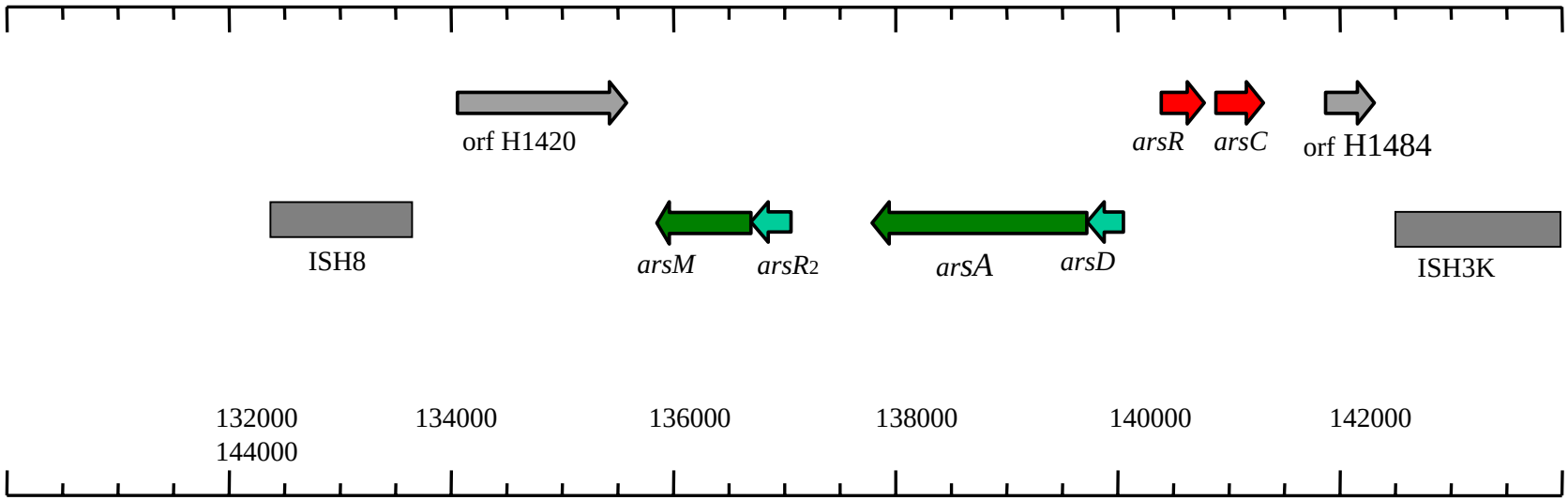
```
mousecyt19         maa srdadeihkd vqnyygnvlk tsad...... lqtn...acv
  ratcyt19         maa prda.eihkd vqnyygnvlk tsad...... lqtn...acv
humancyt19         maa lrda.eiqkd vqtyygqvlk rsad...... lqtn...gcv
  halo5177  msndnetmva drdpeetrem vreryagiat sgqdccgdvg ldvsgdggcc
**  *     *   *         *       *       *

            51                                                 100
mousecyt19  ...trakpvp s..yireslq nvhedvssry ygcg..ltvp erlencrild
  ratcyt19  ...tpakgvp e..yirkslq nvheevisry ygcg..lvvp ehlencrild
humancyt19  ...ttarpvp k..hirealq nvheevalry ygcg..lvip ehlencwild
  halo5177  sdeteasgse rlgydaddva svadgadlg. lgcgnpkafa amapgetvld
               *                   *          * *               **

            101                                                150
mousecyt19  lgsgsgrdcy vlsqlvgekg hvtgidmtev qvevaktyle hhmekfgfqa
  ratcyt19  lgsgsgrdcy vlsqlvgqkg hitgidmtkv qvevakayle yhtekfgfqt
humancyt19  lgsgsgrdcy vlsqlvgekg hvtgidmtkg qvevaekyld yhmekygfqa
  halo5177  lgsgagfdcf laaqevgpdg hvigvdmtpe miskarenva kn......da
            **** * **     *  *  * *  * ***

            151                                                200
mousecyt19  pnvtflhgri eklaeagiqs esygivisnc vinlvpdkqq vlqevyrvlk
  ratcyt19  pnvtflhgqi emlaeagiqk esydivisnc vinlvpdkqk vlrevyqvlk
humancyt19  snvtffhgni eklaeagikn eshdivvsnc vinlvpdkqq vlqeayrvlk
  halo5177  envefrlgei ghlp...vad esvnvvisnc vvnlapekqr vfddtyrvlr
             ** *  * *            **   * *** * ** * **  *    * **

            201                                                250
mousecyt19  hggelyfsdv yaslevpedi kshkvlwgec lggalywkdl aiiaqkigfc
  ratcyt19  yggelyfsdv yaslevsedi kshkvlwgec lggalywkdl aviakkigfc
humancyt19  hggelyfsdv ytslelpeei rthkvlwgec lggalywkel avlaqkigfc
  halo5177  pggrvaisdv vqtapfpddv qmdpdsltgc vagastvddl kamldeagfe
             **    *** *                       *      *

            251                                                300
mousecyt19  pprlvtadii t..venkele gvlgdcrfvs atfrvfklpk tepaercrvv
  ratcyt19  pprlvtanii t..vgnkele rvlgdcrfvs atfrlfklpk tepagrcqvv
humancyt19  pprlvtanli t..iqnkele rvigdcrfvs atfrlfkhsk tgptkrcqvi
  halo5177  aveiapkdes tefisdwdad rdlge.ylvs atiearkpar dd
                        *             *    ** **    *

            301                                                350
mousecyt19  ynggikghek elifdanftf kegeavavde etaavlknsr fapdflftpv
  ratcyt19  ynggimghek elifdanftf kegeavevde etaailrnsr fahdflftpv
humancyt19  ynggitghek elmfdanftf kegeivevde etaailknsr faqdflirpi

            351                                            395
mousecyt19  daslpapqgp f
  ratcyt19  easllapqtk viirdpfkla eesdkmkprc apegtggccg krksc
humancyt19  geklptsgav llws
```

Question:

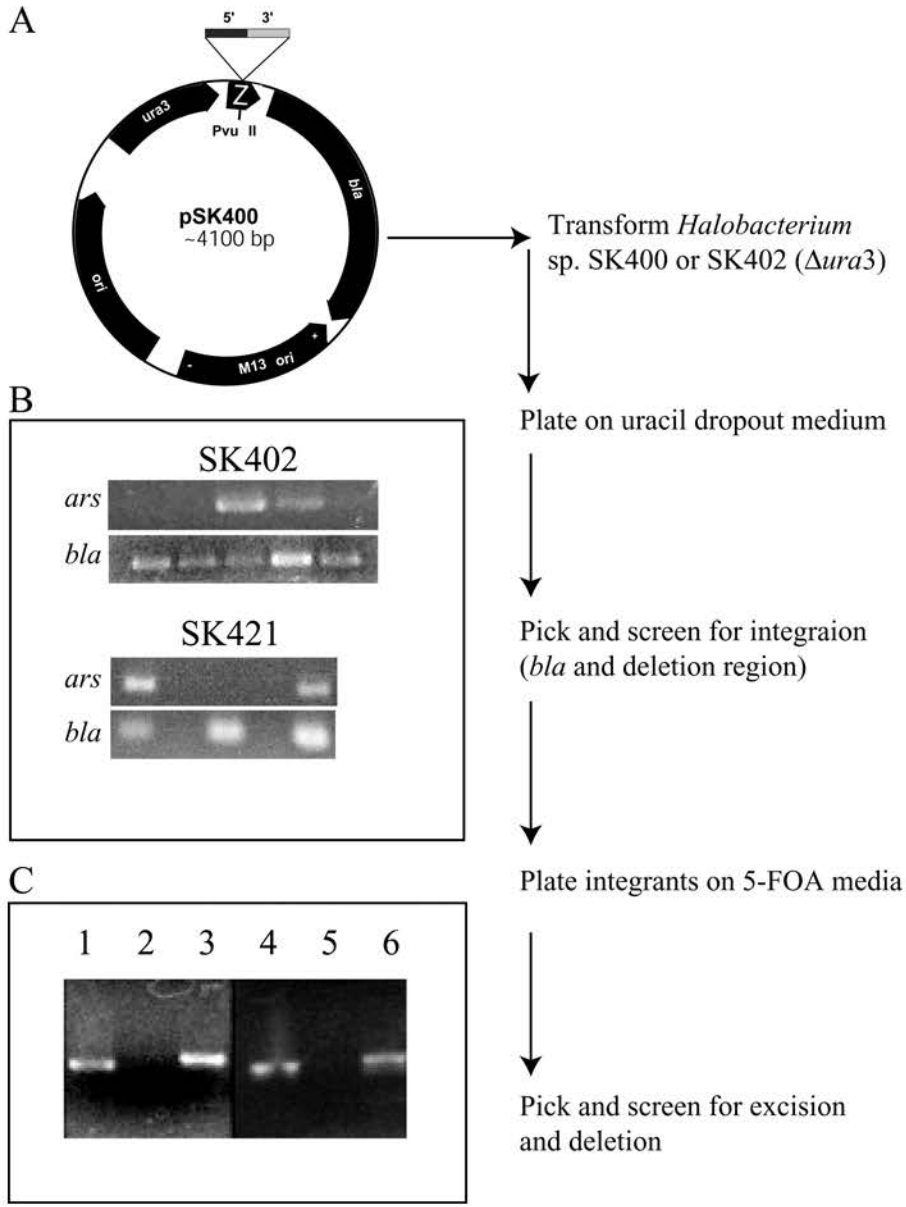
Is ArsM from *Halobacterium* NRC-1 involved in As(III) resistance

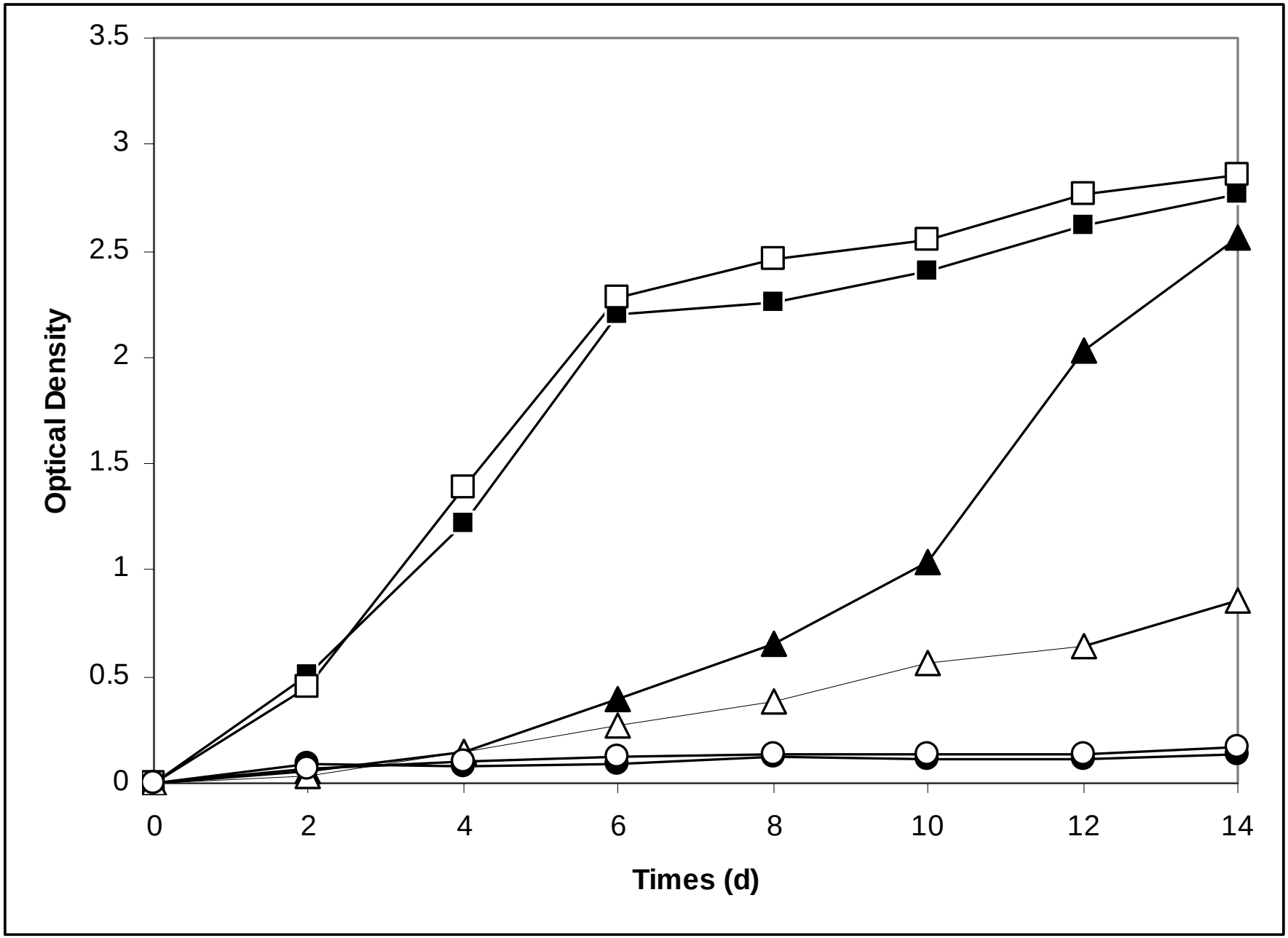


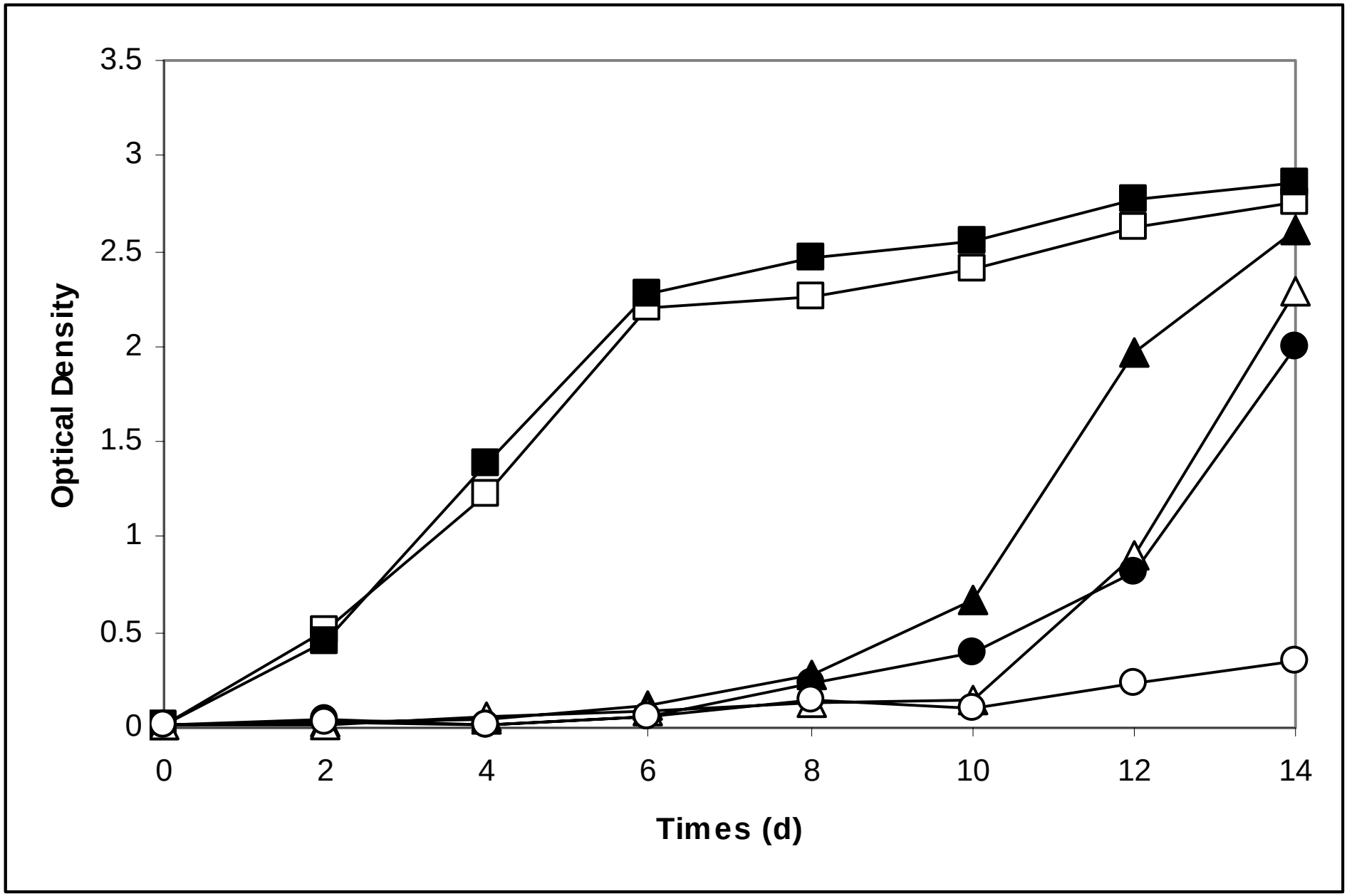
Induction of *ars* genes by As(III) And Sb(III)

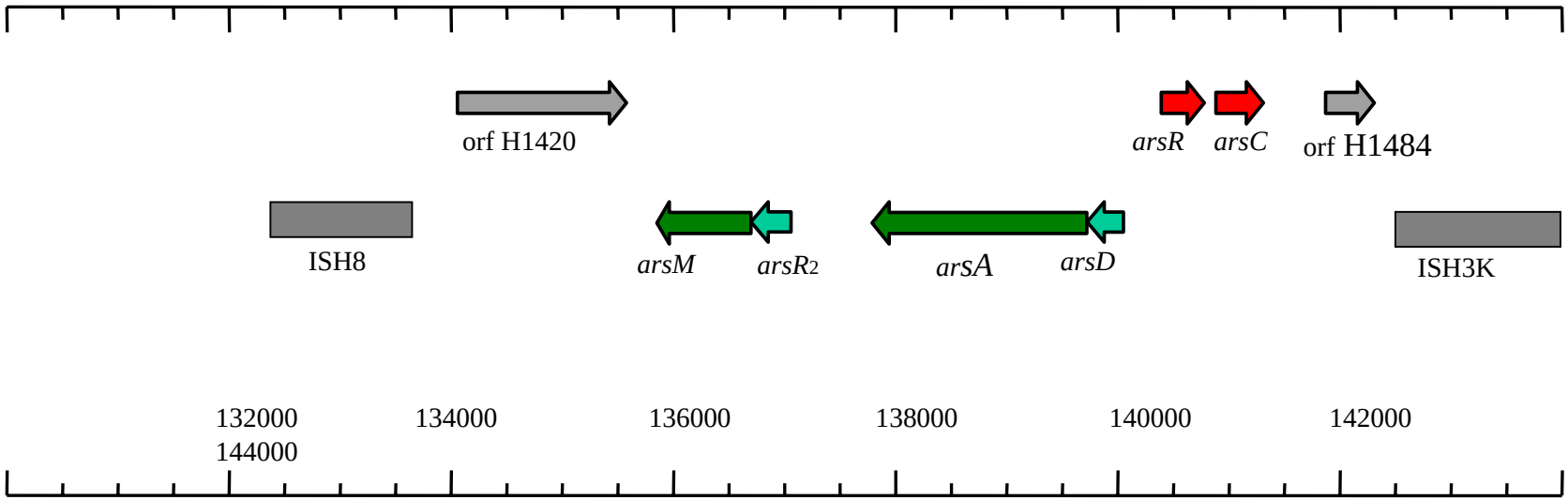

orf H1420
arsR
arsC
orf H1484
ISH8
arsM
arsR2
arsA
arsD
ISH3K
132000
134000
136000
138000
140000
142000
144000

(14000 bps)

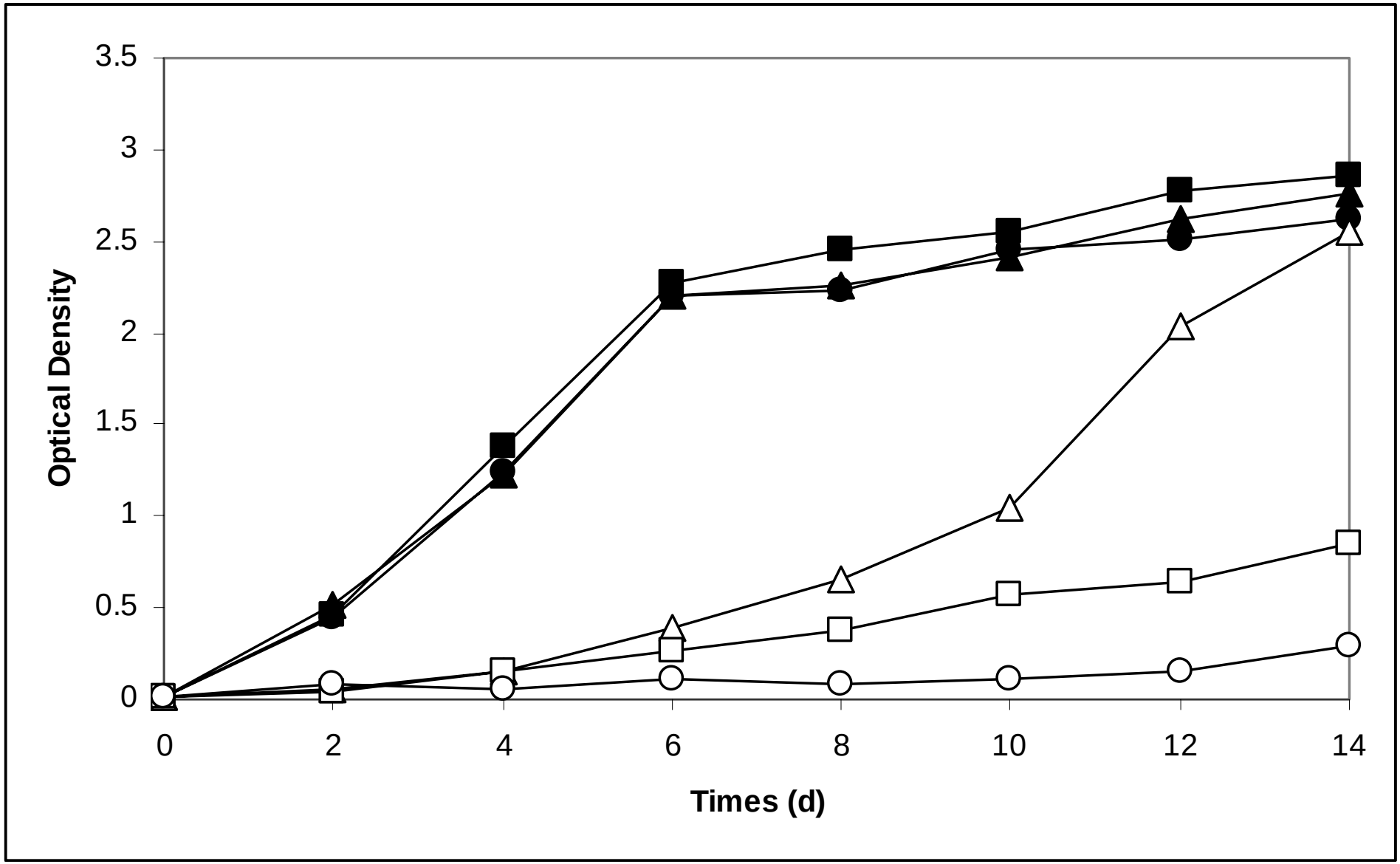

A
5'
3'
Z
Pvu II
ura3
bla
pSK400
~4100 bp
ori
M13 ori
-
+
Transform *Halobacterium* sp. SK400 or SK402 (Δ*ura*3)
Plate on uracil dropout medium
B
SK402
ars
bla
SK421
ars
bla
Pick and screen for integraion (*bla* and deletion region)
Plate integrants on 5-FOA media
C
1 2 3 4 5 6
Pick and screen for excision and deletion

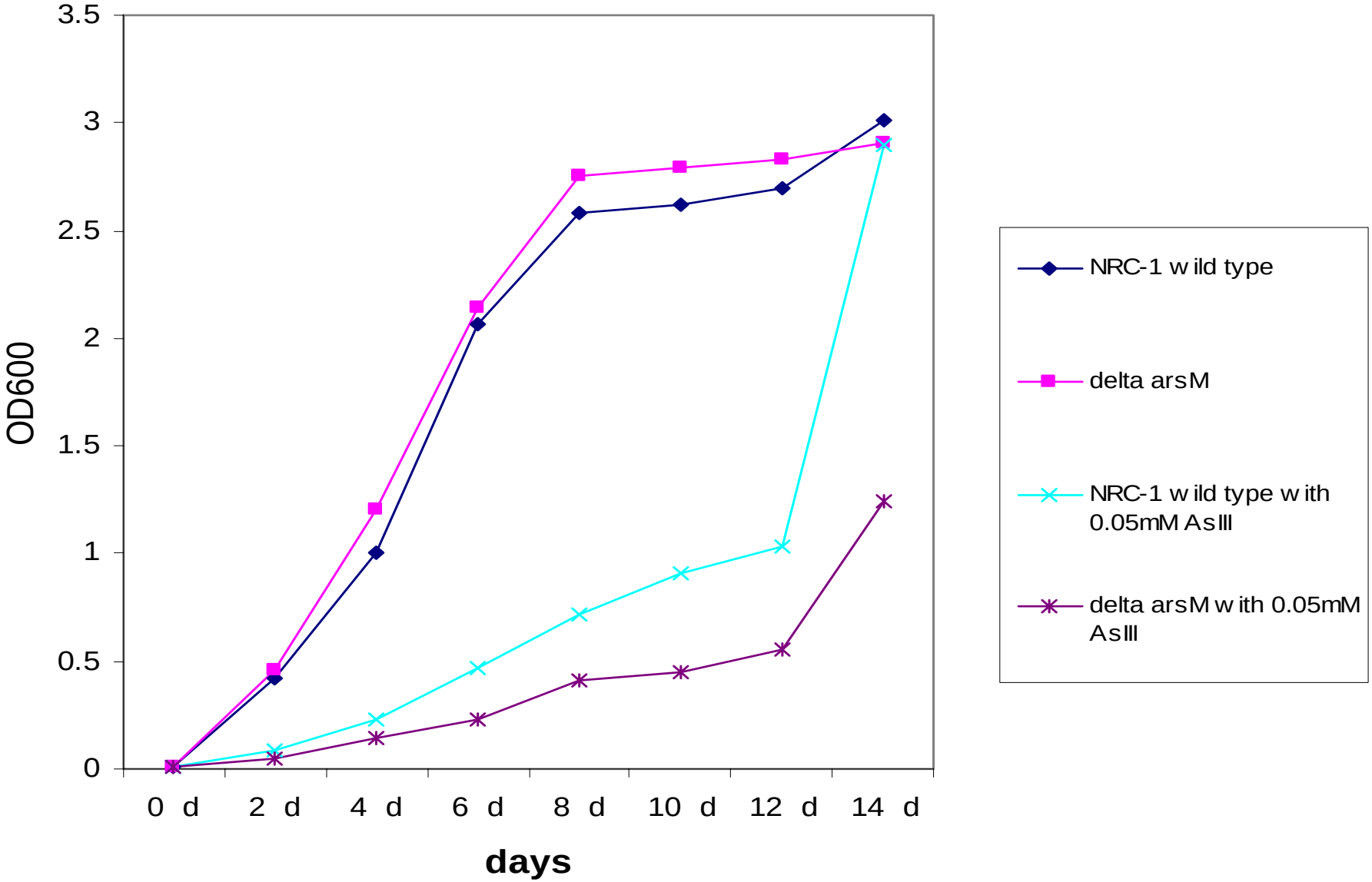

3.5
3
2.5
2
1.5
1
0.5
0
Optical Density
0
2
4
6
8
10
12
14
Times (d)

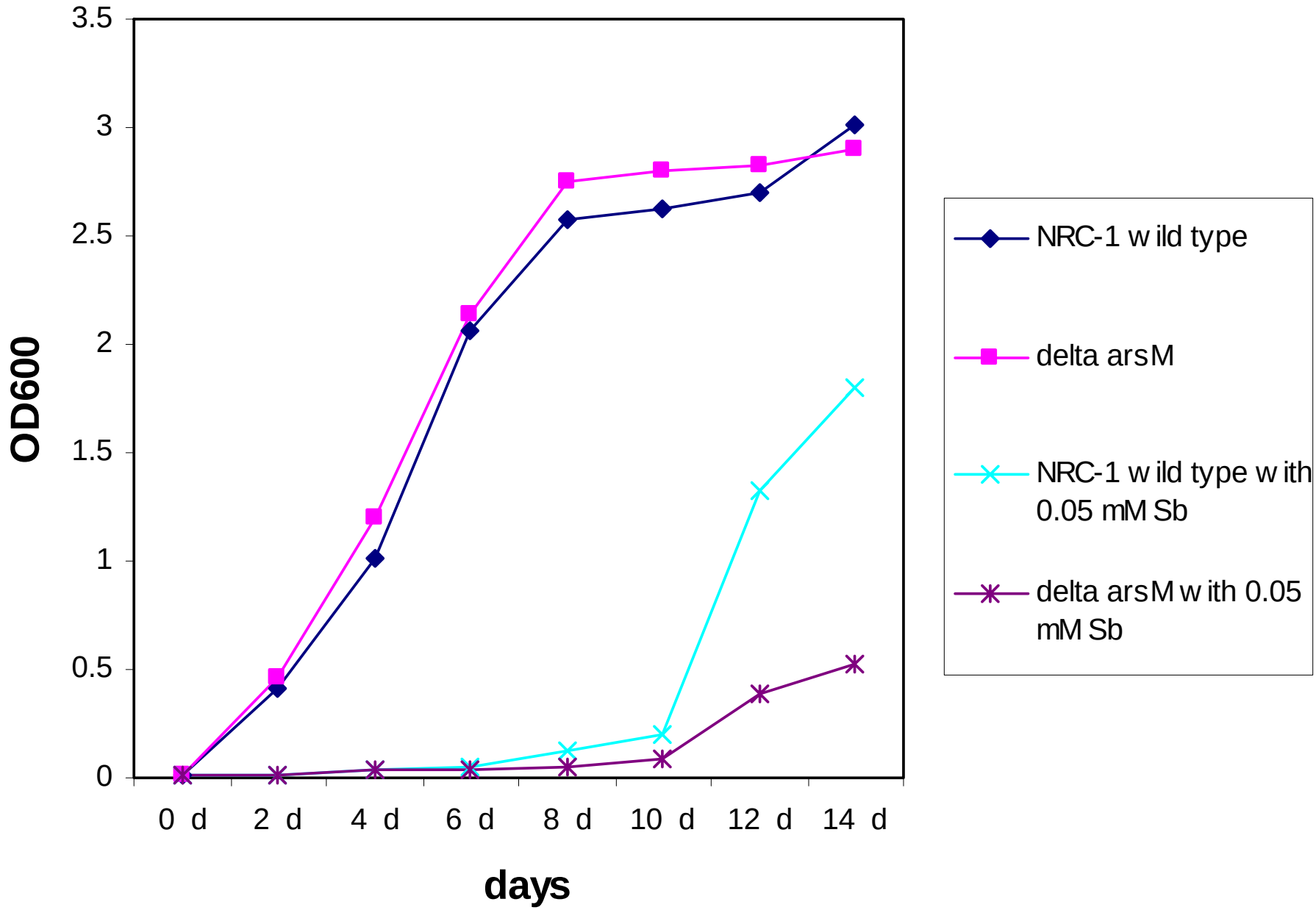

3.5
3
2.5
2
1.5
1
0.5
0
Optical Density
0
2
4
6
8
10
12
14
Times (d)


orf H1420
arsR
arsC
orf H1484
ISH8
arsM
arsR2
arsA
arsD
ISH3K
132000
134000
136000
138000
140000
142000
144000

(14000 bps)

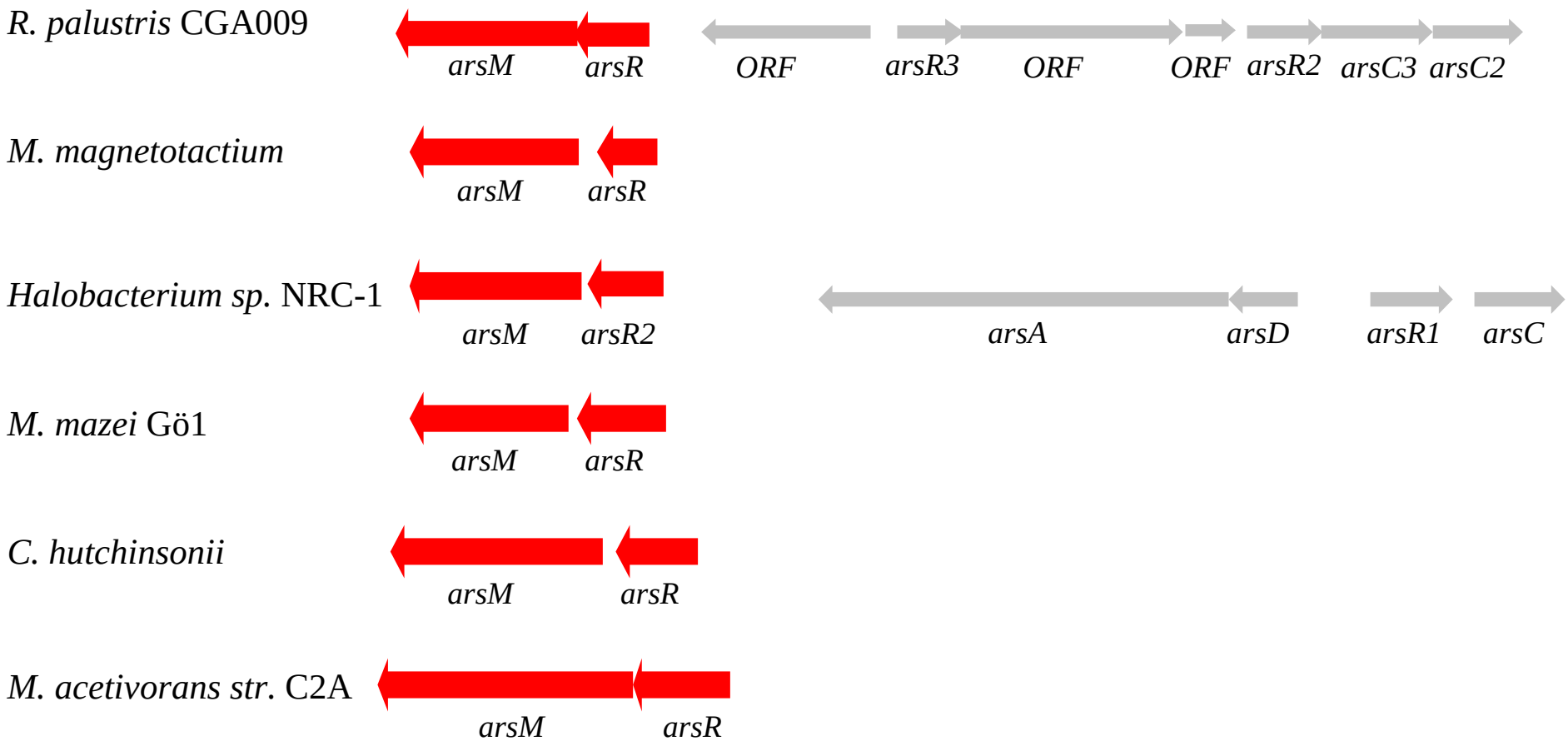

3.5
3
2.5
2
1.5
1
0.5
0
Optical Density
0
2
4
6
8
10
12
14
Times (d)


3.5
3
2.5
2
1.5
1
0.5
0
OD600
0 d
2 d
4 d
6 d
8 d
10 d
12 d
14 d
days
NRC-1 wild type
delta arsM
NRC-1 wild type with 0.05mM AsIII
delta arsM with 0.05mM AsIII


3.5
3
2.5
2
1.5
1
0.5
0
OD600
0 d
2 d
4 d
6 d
8 d
10 d
12 d
14 d
days
NRC-1 wild type
delta arsM
NRC-1 wild type with 0.05 mM Sb
delta arsM with 0.05 mM Sb

Problem:

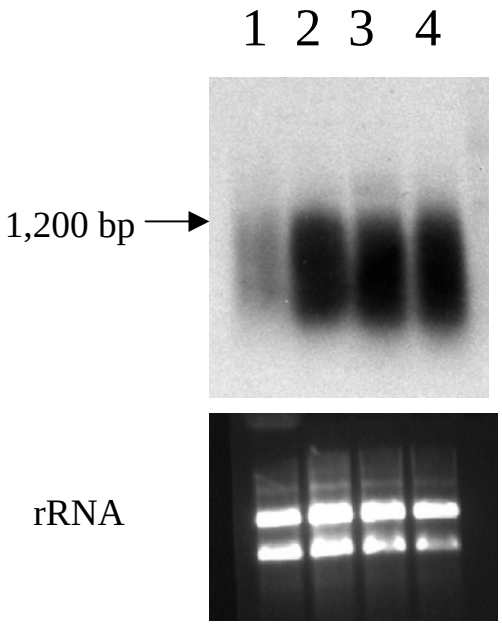
ArsM from *Halobacterium* NRC-1 is a halophilic enzyme


R. palustris CGA009
arsM
arsR
ORF
arsR3
ORF
ORF
arsR2
arsC3
arsC2
M. magnetotactium
arsM
arsR
Halobacterium sp. NRC-1
arsM
arsR2
arsA
arsD
arsR1
arsC
M. mazei Gö1
arsM
arsR
C. hutchinsonii
arsM
arsR
M. acetivorans str. C2A
arsM
arsR

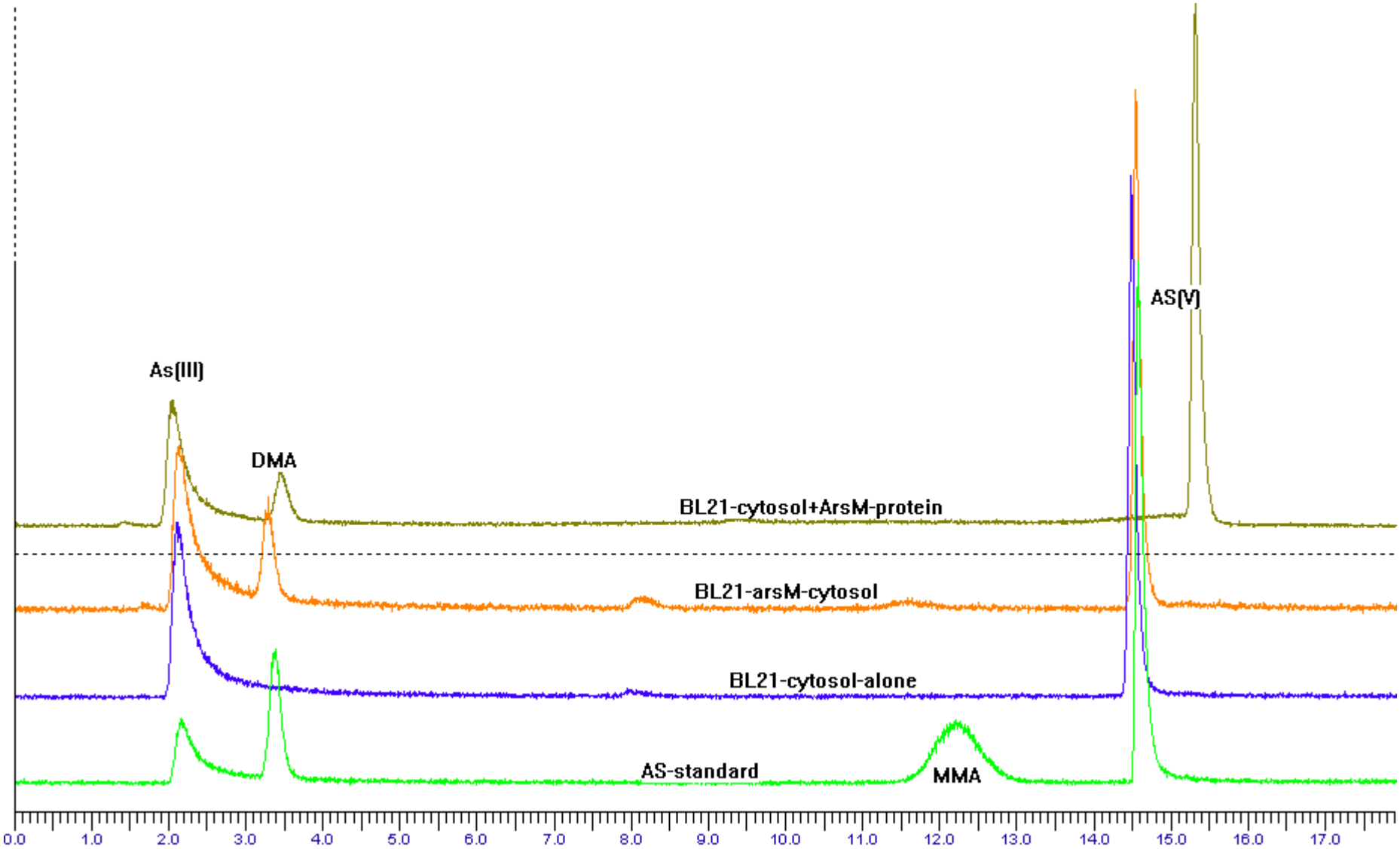
```
MM0661          ----MDANEKKEVIKKRFQEIAMSGG--------TCCSGGGCCGDSSSSDISRAIG---YSEADIEAVPD  55
MA3783          ----MDAAEKKEVIKKKYQEIATLGG--------SCCSGGGCCGDLSAADLSRSLG---YSEADVQAVPD  55
VNG7121         MVADRDPEETREMVRERYAGIATSGQDCCGDVGLDVSGDGGCCSDETEASGSERLG---YDADDVASVAD  67
Magn029446      ------------------------------------------------------MG---YDESDLAAAGD  13
RPA3562         --MPTDMQDVKDIVREKYASAA-----------LKVATGGASCCGSSALPGASPITSNLYDAAQEQGLPA  57
Chut02001955    --MKNNNQDVKEMVQQKYSEIANQ----------SKVQNETSCCGAGSCGTYTIMS---EDYSGLDGYNP  55
                                                                        :     .     .

MM0661          -ANMG-LGCGNPASIAELKPGDIVLDLGSGAGFDCFLAAQKVGNSGKVIGVDMTPEMVEKVQANARKYGY 123
MA3783          -ANLG-LGCGNPTAFAELKPGDIVLDLGSGAGFDSFLAAQRVGSLGKVIGVDMTQEMVKKAQDNARKYGY 123
VNG7121         GADLG-LGCGNPKAFAAMAPGETVLDLGSGAGFDCFLAAQEVGPDGHVIGVDMTPEMISKARENVAKNDA 136
Magn029446      GANLG-LGCGNPQAIAAMRPGEVVVDLGSGAGFDAFLAVRQVGESGRVIGVDMTHEMLAKARANAAKLGL  82
RPA3562         EAMLASLGCGNPTALAQLSPGETVLDLGSGGGIDVLLSARRVGPTGKAYGLDMTDEMLALARDNQRKAGL 127
Chut02001955    DADLG-LGCGLPTEYAKIKKGDTVVDLGSGAGNDAFVARTLTGEEGEVIGIDMTEAMIQKAKGNTEKLGF 124
                 * :. **** *   * :  *: *:*****.* * :::   .*  *.. *:***  *:  .: *  * .

MM0661          SNVEFRQGDIEALPVEDSSVDVIISNCVINLAPDKEKVFREAFRVLKLGGRLYISDLVLLDELPEDLKND 193
MA3783          SNVEFRQGDIEALPLDDRSVDVIISNCVINLAPDKEKVFREAFRVLKPGGRMYVSDMVLLEDLPEDLKND 193
VNG7121         ENVEFRLGEIGHLPVADESVNVVISNCVVNLAPEKQRVFDDTYRVLRPGGRVAISDVVQTAPFPDDVQMD 206
Magn029446      ANVEFRLGEIEHLPIADNTADVVISNCVINLSPDKPAVLNDAFRVLKPGGRVAISDVVMLRPLPPELAAM 152
RPA3562         DNVEFLKGEIEAIPLPDHSVDVIISNCVINLSGDKDRVLREAFRVLKPGGRFAVSDVVTRGEIPEALRRD 197
Chut02001955    TNVAFRLGDIEDIPLSSKRADVVVSNCVMNLVPDKAKAFSEVFRILKPLGHFSISDIVLKGDLPDAIKKE 194
                 ** *  *:*  :*: .  .:*::****:**  :*  .: :.:*:*:  *:. :**:*    :*  :

MM0661          RDLLAGCVAGAVLKEEYLKLLKRAGFSVEILAED----------PDISKSQYKGLP-----VESLKLKAW 248
MA3783          CDLLAGCVAGALLKEEYLGLLKKAGFSFKILAED----------SDVSKRQYEGLP-----VESLKLKAW 248
VNG7121         PDSLTGCVAGASTVDDLKAMLDEAGFEAVEIAPK-------DESTEFISDWDADRD-----LGEYLVSAT 264
Magn029446      KELLTGCAAGAATVAELSNWLEQAGFTDIRIEPK-------PESRELIANWAPGLG-----IEDYVASAT 210
RPA3562         VLLWVGCLAGALDEADYVAKLAAAGFAQISIEPTR--VYDIEDAREFLTGKGIDVDALAPQMQDKFFSGF 265
Chut02001955    GEMYAGCVSGAIKKSEYLGILAEQGFVNITVQKEKEIIIPVEVLSQYLSQEEINSYK-EKGMGIYSITVY 263
                    .** :**    :    *   **    :              :       .       :     .

MM0661          V----------------- 249
MA3783          V----------------- 249
VNG7121         IEARKPARDD-------- 274
Magn029446      IEARRPLV---------- 218
RPA3562         VRATKPGADGEVPARCCG 283
Chut02001955    AERPDASCGCDPRATCC- 280
```

Question:

Is ArsM from *Rhodopseudomonas palustis* an As(III)-methyltransferase



Northern Blot analysis of *arsM* using RNA of *Rhodopspeudomonas palustris* after different growth conditions. 1 control RNA; 2. Growth with 1mM As(V); 3. Growth with 0.1 mM As(III); 4. Growth with 0.05 mM Sb(III). Cells were cultured in LB medium at 27°C for 3 days.


As(III)
DMA
AS(V)
BL21-cytosol+ArsM-protein
BL21-arsM-cytosol
BL21-cytosol-alone
AS-standard
MMA
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0

Summary

- *ars* operon in *Halobacterium* NRC-1 is involved in conferring arsenic and antimonite resistance
- Several *ars* genes are induced by arsenite and antimonite
- ArsM is involved in arsenite resistance
- Improved method for creating deletions in *Halobacterium*

Future plans

- Transcriptional startpoint determination of *arsM* by primer extension
- Purification and enzymatic characterization of ArsM from different organisms
- Establishing an enzymatic assay
- As(III) methylation in different bacteria by HCLP-ICP-MS

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Jie Qin