925. ALKALIPHILIC SULPHUR RESPIRING STRAINS MEDIUM

Mineral base:

Na_2CO_3	20.0	g
$NaHCO_3$	10.0	g
NaCl	5.0	g
K_2HPO_4	1.0	g
Distilled water	1000.0	ml

Sterilize at 110°C 20 min in a closed vessel (i.e. a serum tube or bottle). pH after sterilization will about 10.

Trace element solution:

EDTA	5.0	mg
$FeSO_4$ x 7 H_2O	2.0	mg
$ZnSO_4$ x 7 H_2O	100.0	mg
$MnCl_2$ x 4 H_2O	30.0	mg
$CoCl_2$ x 6 H_2O	200.0	mg
$NiCl_2$ x 6 H_2O	20.0	mg
Na_2MoO_4 x 2 H_2O	30.0	mg
$CuCl_2$ x 2 H_2O	10.0	mg
H_3BO_3	300.0	mg
Distilled water	1000.0	ml

Final pH should be 3, add HCl if needed. Sterilization - 120°C 20 min.

After sterilization add:

Trace elements solution	2 ml/l
$MgCl_2$ x 6 H_2O (200.0 g/l)	1 ml/l

(a white colloid will form which will rapidly dissolve after mixing)

Prepare sterile stock solutions of the following

Sodium thiosulfate pentahydrate (2 M - 496 g/l)
KSCN solution (2 M - 194 g/l)
NH_4Cl - (1 M - 53.5 g/l)
KNO_3 - (1 M - 101 g/l)

Growth:
DSM 13531 = ARh1 (KSCN grown):
Growth with thiocyanate : add KSCN up to 15 mM, incubation - 30°C in conical flasks 1/10 liquid to air ratio, statically;
growth is slow, about 1 week

DSM 13541 = ARh2 (thiosulphate grown):
Growth with thiosulfate: add 40 mM thiosulfate and 5 mM NH_4Cl, incubate in closed flasks, 1/10 liquid to air on shaker 200 rpm; heavy sulfur formation is usual, it is necessary to wait until all the sulfur is consumed (about 1 week).

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DSM 13532 = ARh2 (KSCN grown):
Growth with 15 mM KSCN: same conditions as for DSM 13531 = ARh1.

DSM 13542 = ARh1 (thiosulphate grown)
Growth with thiosulfate (40 mM) and nitrate (10 mM); in conical flasks 1/5 liquid/air ratio on shaker 200 rpm. Growth takes 3-4 days, some sulfur can be formed during initial growth phase.

DSM 13533 = ALRh
strains ALRh: grows with 40 mM thiosulfate and 5 mM KSCN in
conical flasks 1/5 liquid to air ratio at 200 rpm.

Thioalkalivibrio versutus AL 2 = DSM 13738, *Tv.nitratus* ALJ 12 = DSM 13741, and *Tv.denitrificans* ALJD = DSM 13742
Use 40 mM thiosulphate, 5 mM KNO_3, reduce the amount of $MgCl_2$ to 0.5 mM

Thioalkalimicrobium aerophilum AL 3 = DSM 13739 and *Tm.sibericum* AL 7 = DSM 13740
Use 80 mM thiosulphate, 5 mM KNO_3, reduce the amount of $MgCl_2$ to 0.5 mM

Thioalkalispira microaerophila, ALEN 1 = DSM 14786. The strain is grown under nitrogen with 1% oxygen in the gas phase. The strain grows slowly with a potential lag phase of several days.
Use 30 mM thiosulphate and up to a maximum of 5 mM NH_4Cl

Thioalkalivibrio nitratireducens ALEN 2 = DSM 14787
Use 30 - 40 mM thiosulphate and 5-10 mM KNO_3