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Inexpensive Alternative to M&S Medium for Selection of *Arabidopsis* Plants in Culture

Mary A. Pollock & David G. Oppenheimer

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each oligonucleotide eluted with 2-ME could be almost quantitatively re-immobilized after removal of 2-ME (Table 2). Pretreatment of streptavidin agarose with 2 M 2-ME for 30 min at 23°C, heating to 95°C for 2 min and washing with buffers W1–W3 did not affect the ability of streptavidin to bind biotinylated oligonucleotides. Thus, it should be possible to recycle the streptavidin matrix after bound oligonucleotides have been removed. Control, nonbiotinylated versions of the same oligonucleotides were not detectably immobilized on streptavidin. The fact that the eluted oligonucleotides were not degraded was confirmed by polyacrylamide gel electrophoresis (PAGE) analysis (Figure 1).

Table 2 shows that between 1% and 6% of the various biotinylated RNA molecules immobilized on streptavidin agarose can be eluted after 3 min incubation at room temperature with an elution buffer containing 0.2 M 2-ME. Up to 60% of immobilized biotinylated RNA is eluted when the concentration of 2-ME is raised to 2.0 M. Without 2-ME, the RNA could not be eluted. The similar data obtained with the different RNA oligonucleotides indicate that the elution behavior does not depend on the length or sequence of the RNA.

With 5′-biotinylated short synthetic DNA oligonucleotides (Table 1), which have a spacer arm of 7 atoms between the biotin moiety and the 5′-terminal phosphate, the disruption of the biotin/streptavidin interaction with 0.2 M 2-ME was below 0.5%. However, about 3% elution was detected with 2.0 M 2-ME after incubation for 3 min at ambient temperature and up to 72.5% elution after incubation with a buffer containing 2.0 M 2-ME heated to 95°C for 2 min. The labeled biotin control was not detectably eluted under any conditions at room temperature, and only 3% was eluted with 2.0 M 2-ME after heating to 95°C for 2 min. This suggests that an oligonucleotide attached to biotin might favor the disruption of the streptavidin/biotin interaction and/or prevents rebinding in the presence of 2-ME. It might also be possible that the 2′-OH groups on the RNA contribute to effects that facilitate the elution by 2-ME or DTT when compared to the less efficient elution properties of DNA oligonucleotides. The differences in spacer arm length might also contribute to the elution efficiency to some extent.

In summary, Table 2 shows that 2′-biotinylated RNAs could be eluted, although inefficiently, from streptavidin agarose with 0.2 M 2-ME at room temperature. With 2.0 M 2-ME again at room temperature, elution of between 50% and 60% of the RNA oligonucleotides, regardless of sequence or length, was possible. However, 5′-biotinylated DNA primers were only inefficiently eluted with 2.0 M 2-ME at room temperature, but after heating to 95°C for 2 min, more than 50% of the labeled DNA was released from the matrix. Low levels of labeled biotin alone were only eluted with 2.0 M 2-ME after heating. The oligonucleotides eluted with 2-ME were still covalently bound to the biotin tag, as demonstrated by their efficient re-immobilization on streptavidin agarose, while checking the integrity of the eluted oligonucleotides confirmed that they were not degraded.

REFERENCES

1. **Chapman, K.B. and J.W. Szostak.** 1995. Isolation of a ribozyme with 5′-5′ ligase activity. Chem. Biol. *2*:325-333.
2. **Delius, H., H. van Heerikhuizen, J. Clarke and B. Koller.** 1985. Separation of complementary strands of plasmid DNA using the biotin-avidin system and its application to heteroduplex formation and RNA/DNA hybridizations in electron microscopy. Nucleic Acids Res. *13*:5457-5469.
3. **Folsom, V., M.J. Hunkeler, A. Haces and J.D. Harding.** 1989. Detection of DNA targets with biotinylated and fluoresceinated RNA probes. Anal. Biochem. *182*:309-314.
4. **Green, N.M. and E.J. Toms.** 1973. The properties of subunits of avidin coupled to sepharose. Biochem. J. *133*:687-698.
5. **Jenne, A. and M. Famulok.** 1998. A novel ribozyme with ester transferase activity. Chem. Biol. *5*:23-34.
6. **Wilson, C. and J.W. Szostak.** 1995. *In vitro* evolution of a self-alkylating ribozyme. Nature *374*:777-782.

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Andreas Jenne and Michael Famulok
*Institut für Biochemie der LMU München-Genzentrum
München, Germany*

Inexpensive Alternative to M&S Medium for Selection of *Arabidopsis* Plants in Culture

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The vacuum infiltration method is widely used to introduce foreign genes into *Arabidopsis thaliana* plants (1). To identify transformants, large numbers of seeds from the infiltrated plants must be screened for the presence of the dominant selectable marker. Many commonly used transformation vectors use the neomycin phosphotransferase II gene as the selectable marker. This confers kanamycin resistance to transformed plants. Kanamycin selection of transformants is commonly carried out on agar-solidified Murashige and Skoog (M&S) medium (2) supplemented with kanamycin. Although the vacuum infiltration method is easy, the transformation frequency is low; usually only a few percent of the seed from the infiltrated plants are transformed. Because of this low frequency, the screening and generation of large populations of transformed plants can be expensive.

We have found an alternative to M&S salts for the growth of *Arabidopsis* on agar-solidified medium. This alternative fulfills four time- and money-saving criteria: *(i)* it supports the growth of *Arabidopsis* plants on agar-solidified medium comparable to M&S medium, *(ii)* it is inexpensive, *(iii)* it is easy to prepare and *(iv)* it can be used as a complete nutrient solution for plants grown in soil or a soilless potting medium.

To find an alternative, we plated

seeds of the Rschew (RLD), Columbia (Col), Wassilewskija (WS) and Landsberg erecta (Ler) ecotypes on agar-solidified medium prepared from several commercially available plant fertilizers. Because agar-solidified medium is often used for antibiotic selection of *Arabidopsis* transformants, we also tested agar-solidified medium supplemented with kanamycin sulfate (Sigma Chemical, St. Louis, MO, USA) at a concentration of 50 μg/mL. All fertilizers used for making agar-solidified plates were prepared with double-distilled (dd)H_20 and contained 7 g/L of Bacto™-Agar (Life Technologies, Gaithersburg, MD, USA). Fertilizers were adjusted to pH 5.5 with either 1 M KOH or 1 M HCl before autoclaving.

We identified a commercial fertilizer that supported the growth of *Arabidopsis* plants: Peters Professional® General Purpose (PGP) 20-10-20 (Scotts Company, Marysville, OH, USA).

PGP medium supports *Arabidopsis* plant growth comparable to M&S medium when used in agar-solidified medium (Figure 1). Table 1 shows a comparison of PGP and M&S medium formulations. PGP can be used in the three concentrations with near identical results: *(i)* 0.762 g/L, *(ii)* 1.27 g/L and *(iii)* 2.54 g/L. When grown on each of these concentrations, the plants were dark green, had well-developed root systems and germinated and grew at rates comparable to plants grown on M&S medium. Also, when known transformants and wild-type (WT) seed were mixed and sown on agar-solidified PGP medium that was supplemented with 50 μg/mL kanamycin (PGP-Kan), the transformants were as healthy as those grown on M&S plates, and the non-transformants died as expected (Figure 1). In addition, when seed from a transformant known to be segregating for the kanamycin-resistance phenotype was plated on both PGP-Kan and M&S-Kan plates, no significant difference was seen in the ratio of kanamycin-resistant:kanamycin-sensitive seedlings on the two media (data not shown). We found that plants grown on PGP agar-solidified medium for longer than four weeks formed bolts but then turned slightly yellow. In contrast, plants on M&S salts medium remained a dark green. Although all the plants germinated on either PGP or M&S medium developed and set seed normally when transferred to soil before bolting, we do not suggest the substitution of PGP for M&S medium in *Arabidopsis* flowering or physiological experiments.

We also found that PGP could be used as a complete nutrient solution for watering plants grown in soil or a soilless potting medium when used at a concentration of 1.27 or 2.54 g/L. In addition, we found that a 50.8 g/L stock of PGP could be kept at room temperature for at least two months without supporting the growth of bacteria or algae. For these studies, 20 L of the 50.8 g/L stock were prepared and stored in a polypropylene carboy until needed. To water *Arabidopsis* plants grown in a soilless potting medium, the 50.8 g/L stock of PGP was diluted to 1.27 or 2.54 g/L with distilled H_2O, and the pH of the diluted stock was adjusted to 5.5 using either 1 M HCl or 1 M KOH.

During our investigations of several commercial fertilizers, we recorded three additional observations: *(i)* We found that fertilizers containing urea (a common source of nitrogen in fertilizers) did not adequately support the growth of *Arabidopsis* plants on agar-solidified medium. *(ii)* Plants grown on our acidic (ca. pH 5.5) mixture of vermiculite and Metro-Mix® 360 (Scotts

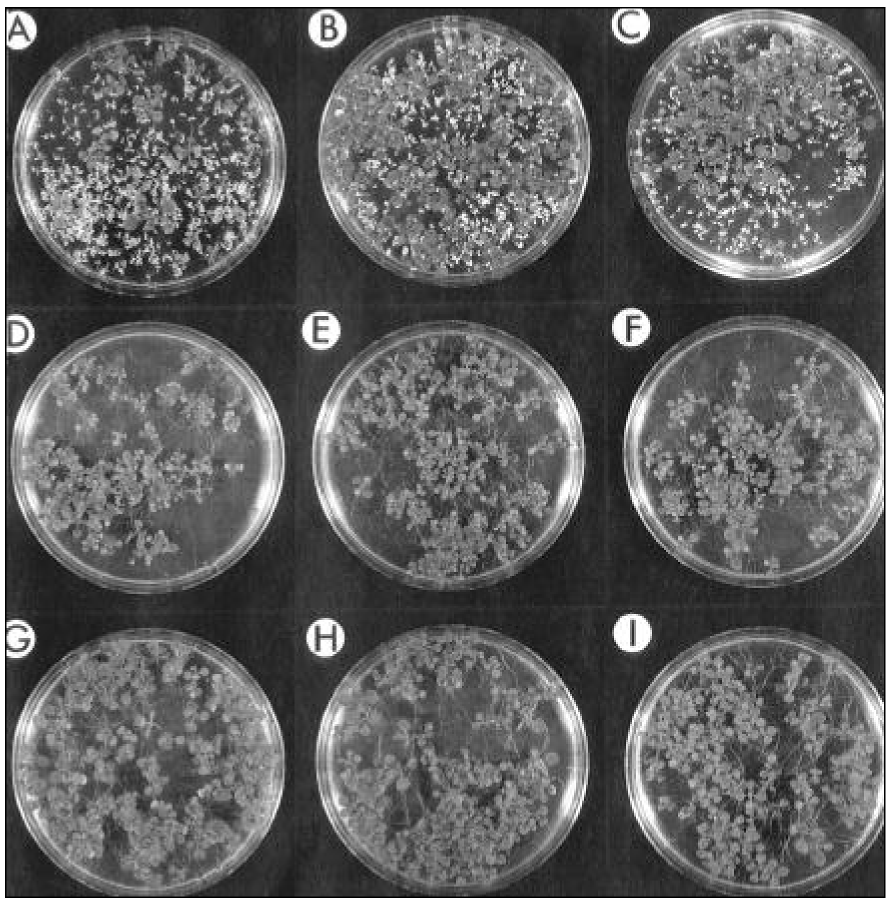

Figure 1. PGP 20-10-20 fertilizer is an effective substitute for M&S salts in agar-solidified medium. Seeds were surface-sterilized for 5 min in a solution containing 50% commercial bleach, 50% ddH_2O and 1–3 drops of lemon-scent Joy® dishwashing detergent per 100 mL of solution (as a wetting agent). Seeds were quickly rinsed once in 70% ethanol, immediately followed by four rinses in sterile ddH_2O. All plates were wrapped with a single layer of Micropore™ surgical tape (3M Health Care, St. Paul, MN, USA) and incubated at 22°C in a Percival Plant Tissue Culture Growth Chamber (Percival Scientific, Boone, IA, USA) under fluorescent illumination (16-h day:8-h night cycle). Plates A, B and C were supplemented with 50 μg/mL kanamycin sulfate. Seeds sown on plates A–C were a mixture of known transformants and non-transformants. All other seeds were non-transformants. Plates A, D and G contained M&S salts; plates B, E and H contained 2.54 g/L PGP 20-10-20 fertilizer; plates C, F and I contained 1.27 g/L PGP. Seeds sown on plates A–F were of the Col ecotype, whereas seeds sown on plates G–I were of the RLD ecotype.

Table 1. A Comparison of PGP and M&S Medium Formulations

PGP 20-10-20		M&S Medium	
Total Nitrogen (N)	20%	Total Nitrogen (N)	19.43%
Ammoniacal Nitrogen	7.76%	Ammoniacal Nitrogen	6.67%
Nitrate Nitrogen	12.24%	Nitrate Nitrogen	12.76%
Phosphate (P_2O_5)	10%	Phosphate (PO_4)	2.74%
Soluble Potash (K_2O)	20%	Soluble Potash (K_2O)	—
Magnesium ($MgSO_4$)	0.05%	Magnesium ($MgSO_4$)	0.84%
Boron (H_3BO_4)	0.0068%	Boron (H_3BO_4)	0.025%
Copper (Cu EDTA)	0.0036%	Copper (Cu EDTA)	0.00015%
Iron (Fe EDTA)	0.05%	Iron ($FeSO_4$•$7H_2O$)	0.13%
Manganese (Mn EDTA)	0.025%	Manganese ($MnSO_4$•H_2O)	0.127%
Molybdenum (Na_2MoO_4•$2H_2O$)	0.0009%	Molybdenum (Na_2MoO_4•$2H_2O$)	0.0023%
Zinc (Zn EDTA)	0.0025%	Zinc ($ZnSO_4$•H_2O)	0.045%

Company) were healthy when watered with PGP 20-10-20. However, we found that plants grown on a more alkaline soil or soilless potting medium [such as Promix® BX (Primere Horticulture LTEE), ca. pH 6.2] fare better when watered with Peters Pete-lite Special 20-10-20 (Scotts Company). When used as a complete nutrient solution, Peters Pete-lite Special 20-10-20 should be prepared, stored and used in the same manner as PGP. *(iii)* Although we have not rigorously tested PGP medium as an infiltration medium with the vacuum-infiltration method, we have been successful with similar formulations of plant fertilizers.

PGP is an extremely economical substitute for M&S medium. Our cost for 20 agar-solidified plates of commercially available M&S medium (1 L) is approximately $1.00. PGP average retail price is $18.50 for a 25-lb bag of water-soluble crystals (plus an additional $30 for shipping and handling). Each bag contains enough fertilizer to make approximately 1080 L of a 50.8 g/L stock. Therefore, the average cost for 20 agar-solidified plates (1 L) of PGP ranges from 0.06–0.22 cents, depending on the final concentration used.

In conclusion, PGP 20-10-20 is a cost-effective and convenient alternative to M&S medium for growing *Arabidopsis* plants on agar-solidified plates. PGP fertilizer supports the growth of *Arabidopsis* comparable to M&S medium; it is inexpensive, easy to prepare and can be used as a complete nutrient solution for *Arabidopsis* plants in soil or soilless potting medium.

REFERENCES

1. **Bechtold, N., J. Ellis and G. Pelletier.** 1993. In planta *Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. Comptes Rendus de l'Academie des Sciences Serie lll Sciences de la Vie *316*:1194-1199.
2. **Murashige, T. and F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum *15*:473-497.

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Mary A. Pollock and David G. Oppenheimer
University of Alabama
Tuscaloosa, AL, USA