SYRACUSE UNIVERSITY

TECHNOLOGY TRANSFER AND INDUSTRIAL DEVELOPMENT

The Boddy Protocol

A new method for generating new chemical entities from bacteria

Pharmaceutical pipelines today are challenged by a shortage of promising new drugs. The cause of the problem is a lack of chemical diversity from either natural sources or combinatorial chemistry.

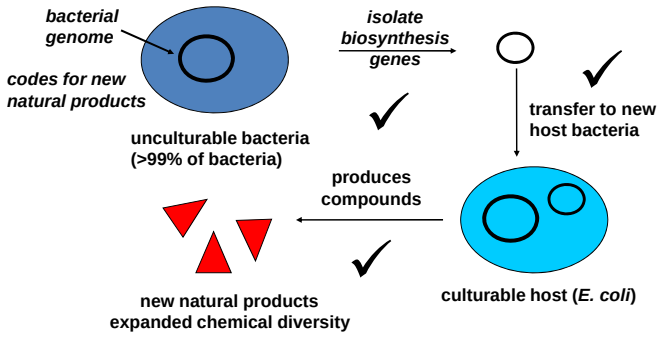
One promising source of great diversity is bacteria. However, 99.9% of bacteria cannot be cultured. Therefore a new mechanism is needed for accessing the huge potential of this resource.

Some have sought heterologous biosynthetic expression of desirable genes but this does not generate the desired metabolites unless all the synthetic genes are expressed. Achieving this by traditional methods is extremely time and labor intensive.

The Boddy protocol is based on an ancient evolutionary capability that uses genetic pathways to activate new behaviors and abilities. Predatory bacteria routinely make such adaptations to compete and obtain nutrients, and to develop resistance to drugs. Tests show that they can even use promoters of gene expression from unrelated bacteria.

Now the Boddy lab has identified one of these promoters as a potentially universal transcriptional activator for turning on production of polyketides and non-ribosomal peptides. This finding has been demonstrated by successfully producing oxytetracycline from E. coli.

This important new key to genetically engineering bacteria holds the potential for identifying and producing many useful new bioactive natural materials and ultimately refilling pharmaceutical pipelines.



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Publications:

Houghton, Stephen R.; Furst, Laura; Boddy, Christopher N.. Biomimetic Transannular Oxa-Conjugate Addition Approach to the 2,6-Disubstituted Dihydropyran of Laulimalide Yields an Unprecedented Transannular Oxetane. *Journal of Organic Chemistry* ACS ASAP.

Wang, Meng; Boddy, Christopher N.. Examining the Role of Hydrogen Bonding Interactions in the Substrate Specificity for the Loading Step of Polyketide Synthase Thioesterase Domains. *Biochemistry* (2008), 47(45), 11793-11803.

Boddy, Christopher N.; Lundgren, Benjamin R. Metabolically engineered microorganisms for enhanced production of sialic acid. U.S. Pat. Appl. Publ. (2008),17pp.

Burlina, Fabienne; Dixson, David D.; Doyle, Robert P.; Chassaing, Gerard; Boddy, Christopher N.; Dawson, Philip; Offer, John. Orthogonal ligation: a three piece assembly of a PNA-peptide-PNA conjugate. *Chemical Communications* (Cambridge, United Kingdom) (2008), (24), 2785-2787.

Lundgren, Benjamin R.; Boddy, Christopher N.. Sialic acid and N-acyl sialic acid analog production by fermentation of metabolically and genetically engineered Escherichia coli. *Organic & Biomolecular Chemistry* (2007), 5(12), 1903-1909.

Sharma, Krishna K.; Boddy, Christopher N.. The thioesterase domain from the pimaricin and erythromycin biosynthetic pathways can catalyze hydrolysis of simple thioester substrates. *Bioorganic & Medicinal Chemistry Letters* (2007), 17(11), 3034-3037.

Watanabe, Kenji; Hotta, Kinya; Praseuth, Alex P.; Koketsu, Kento; Migita, Akira; Boddy, Christopher N.; Wang, Clay C. C.; Oguri, Hiroki; Oikawa, Hideaki. Total biosynthesis of antitumor nonribosomal peptides in Escherichia coli. *Nature Chemical Biology* (2006), 2(8), 423-428.

A New Mechanism for Benzopyrone Formation in Aromatic Polyketide Biosynthesis Zhang, Wenju; Wilke, Burkhardt I.; Zhan, Jixun; Watanabe, Kenji; Boddy,* Christopher N.; Tang,* Yi *J. Am. Chem. Soc.* 2007, ASAP. (* Corresponding authors)

Sialic acid and N-acyl sialic acid analog production by fermentation of metabolically and genetically engineered Escherichia coli Lundgren, Benjamin L.; Boddy, Christopher N. *Org. Biomol. Chem.* 2007, 5, 1903-1909. (Evaluated by Faculty of 1000.)

The thioesterase domain from the pimaricin and erythromycin biosynthetic pathways can catalyze hydrolysis of simple thioester substrates. Sharma, Krishna K.; Boddy, Christopher N. *Bioorg. Med. Chem. Lett.* 2007, 17, 3034-3037.

Total biosynthesis of antitumor nonribosomal peptides in Escherichia coli. Watanabe, Kenji; Hotta, Kinya; Praseuth, Alex P.; Kotetsu, Kento; Migita, Akira; Boddy, Christopher N.; Wang, Clay C. C.; Oguri, Hiroki; Oikawa, Hideaki *Nature Chemical Biology* 2006, 2, 423-428.

Sweetening Cyclic Peptide Libraries. Boddy, Christopher N. *Chem. Biol.* 2004, 11, 1599-1600.

Precursor-Directed Biosynthesis of Epothilone in Escherichia coli. Boddy, Christopher N.; Hotta, Kinya; Tse, Martha Lovato; Watts, R. Edward; Khosla, Chaitan. *J. Am. Chem. Soc.* 2004, 7436-7437.

Understanding Substrate Specificity of Polyketide Synthase Modules by Generating Hybrid Multimodular Synthases. Watanabe, Kenji; Wang, Clay C. C.; Boddy, Christopher N.; Cane, David E.; Khosla, Chaitan *J. Biol. Chem.* 2003, 278, 42020-42026.

Epothilone C Macrocyclization and Hydrolysis Are Catalyzed by the Isolated Thioesterase Domain of Epothilone Polyketide Synthase. Boddy, Christopher N.; Schneider, Tanya; Hotta, Kinya; Walsh, Christopher T.; Khosla, Chaitan *J. Am. Chem. Soc.* 2003, 125, 3428-3429.

Atropselective Macrocyclization of Diaryl Ether Systems: Application to the Synthesis of Vancomycin. Nicolaou, K. C.; Boddy, Christopher N. C. *J. Am. Chem. Soc.* 2002, 124, 10451-10455.

Extending Synthetic Access to Proteins with a removable acyl transfer auxiliary. Offer, John; Boddy, Christopher N. C.; Dawson, Philip E. *J. Am. Chem. Soc.* 2002, 124, 4642-4646.

Behind Enemy Lines. Nicolaou, K. C.; Boddy, Christopher N. C. *Scientific American* 2001, May, 54-61.

Does CIP nomenclature adequately handle molecules with multiple stereoelements? A case study of vancomycin and cognates. Nicolaou, K. C.; Boddy, Christopher N. C.; Siegel,Jay S. *Angew. Chem.* 2001, 113; 723-726; *Angew. Chem. Int. Ed.* 2001, 40, 701-704.

Total Synthesis of Vancomycin: Part 2. Retrosynthetic Analysis, Synthesis of Amino Acid Building Blocks and Strategy Evaluations. Nicolaou, K. C.; Boddy, Christopher N. C.; Li, Hui; Koumbis, A. E.; Hughes, Robert; Natarajan, Swaminathan; Jain, Nareshkumar F.; Ramanjulu, Joshi M.; Bräse, Stefan; Solomon, Michael E. *Chem. Eur. J.* 1999, 5, 2602-2621.

Total Synthesis of Vancomycin: Part 1. Design and Development of Methodology. Nicolaou, K. C.; Li, Hui; Boddy, Christopher N. C.; Ramanjulu, Joshi M.; Yue, Tai-Yuen; Natarajan, Swaminathan; Chu, Xin-Jie; Bräse, Stefan; Rübsam, Frank *Chem. Eur. J.* 1999, 5, 2584-2601.

Chemistry, Biology, and Medicine of the Glycopeptide Antibiotics. Nicolaou, K. C.; Boddy, Christopher N. C.; Bräse, Stefan; Winssinger, Nicolas *Angew. Chem.* 1999, 111, 2230-2287; Angew. Chem. Int. Ed. 1999, 38, 2097-2152.

Total Synthesis of Vancomycin Aglycon - Part 1: Synthesis of Amino Acids 4-7 and Construction of the AB-COD Ring Skeleton. Nicolaou, K. C.; Natarajan, Swaminathan; Li, ; Jain, Nareshkumar F.; Hughes, Robert; Solomon, Michael E.; Ramanjulu, Joshi M.; Boddy, Christopher N. C.; Takayanagi, Masaru *Angew. Chem.* 1998, 110, 2872-2878; Angew. Chem. Int. Ed. 1998, 37, 2708-2714.

Probing the Ring Size of Epothilones: Total Synthesis of [14]-, [15]-, [17]-, [18]-Epothilones A. Nicolaou, K. C.; Sarabia, Francisco; Ninkovic, Sacha; Finlay, M. Ray V.; Boddy, Christopher N. C. Angew. Chem. 1998, 110, 85-89; *Angew. Chem.* Int. Ed. 1998, 37, 81-84.

A Suzuki Coupling-macrolactamization Approach to the AB-COD Bi-cyclic System of Vancomycin. Nicolaou, K. C.; Ramanjulu, Joshi M.; Natarajan, Swaminathan; Bräse, Stefan; Li, Hui; Boddy, Christopher N. C.; Rübsam, Frank Chem. *Commun.* 1997, 1899-1900.

New Technology for the Synthesis of Vancomycin-type Biaryl Ring Systems. Nicolaou, K. C.; Chu, Xin-Jie; Ramanjulu, Joshi M.; Natarajan, Swaminathan; Bräse, Stefan; Rübsam, Frank; Boddy, Christopher N. C. *Angew. Chem.* 1997, 109, 1551-1552; *Angew. Chem. Int. Ed.* 1997, 36, 1539-1540.

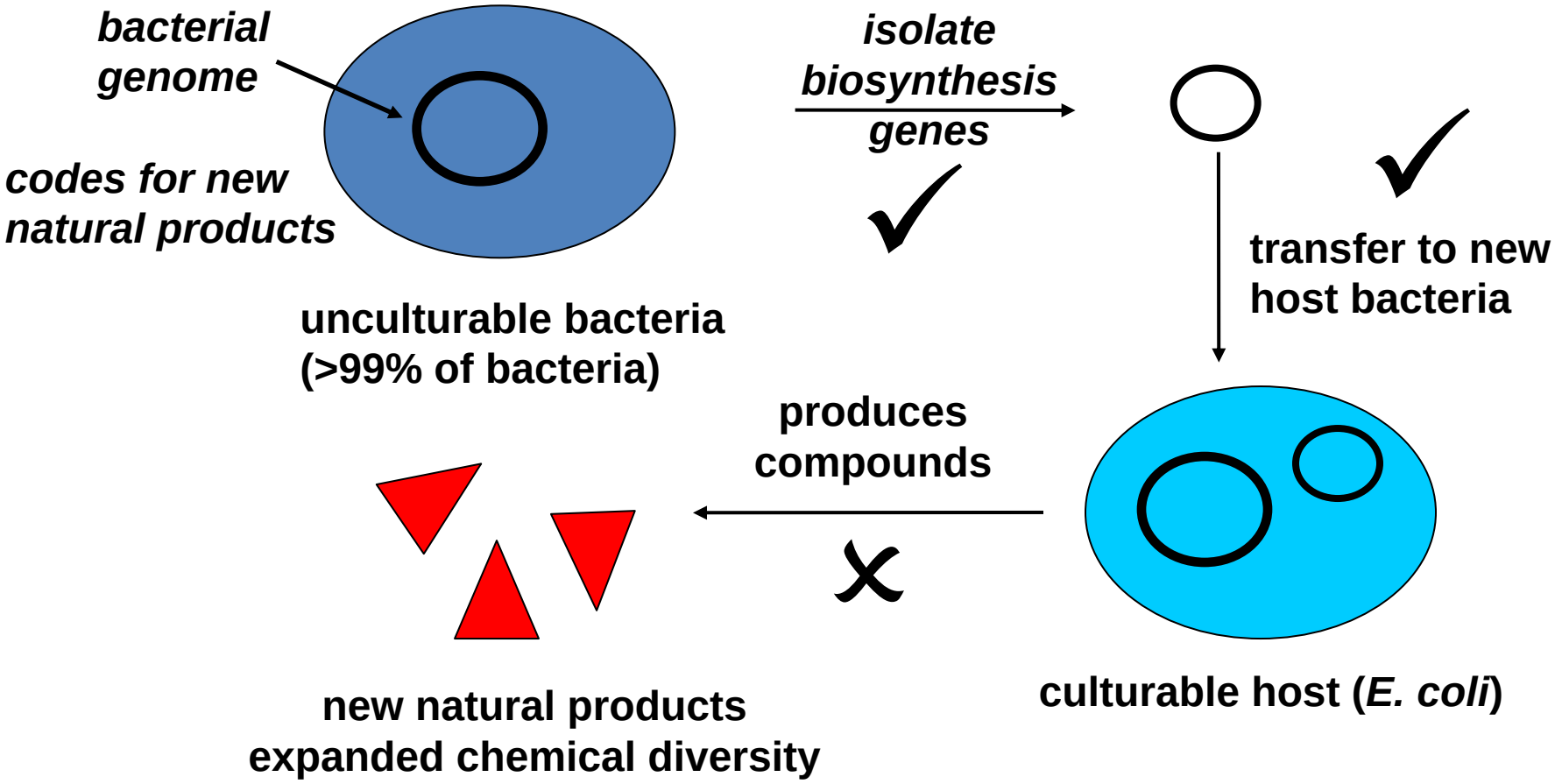
New Synthetic Technology for the Synthesis of Aryl Ethers: Construction of C-O-D and DO- D Ring Model Systems of Vancomycin. Nicolaou, K. C.; Boddy, Christopher N. C.; Natarajan, Swaminathan; Yue, Tai-Yuen; Li, Hui; Bräse, Stefan; Ramanjulu, Joshi M. *J. Am. Chem. Soc.* 1997, 119, 3421-3422.

Total Synthesis of Crystalline (±)-Fredericamycin A. Clive, Derrick L. J.; Tao, Yong; Khodabocus, Ahmad; Wu, Yong Jin; Angoh, A. Gaetan; Bennett, Sharon M.; Boddy, Christopher N.; Bordeleau, Luc; Cantin, Michel; Kleiner, Galit; Middleton, Donald S.; Nichols, Christopher J.; Richardson, Scott R.; Vernon, Peter G. *Stud. Nat. Prod. Chem.* 1995, 16, 27-74.

Total Synthesis of Crystalline (±)-Fredericamycin A. Use of Radical Spirocyclization. Clive, Derrick L. J.; Tao, Yong; Khodabocus, Ahmad; Wu, Yong Jin; Angoh, A. Gaetan; Bennett, Sharon M.; Boddy, Christopher N.; Bordeleau, Luc; Kellner, Dorit; Kleiner, Galit; Middleton, Donald S.; Nichols, Christopher J.; Richardson, Scott R.; Vernon, Peter G. *J. Am. Chem. Soc.* 1994, 116, 11275-11286.

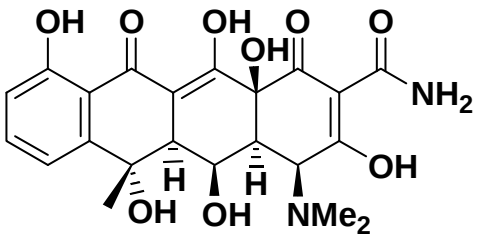
Total Synthesis of (±)-Fredericamycin A. Use of Radical Spirocyclization. Clive, Derrick L. J.; Tao, Yong; Khodabocus, Ahmad; Wu, Yong Jin; Angoh, A. Gaetan; Bennett, Sharon M.; Boddy, Christopher N.; Bordeleau, Luc; Kellner, Dorit; Kleiner, Galit; Middleton, Donald S.; Nichols, Christopher J.; Richardson, Scott R.; Vernon, Peter G. *J. Chem. Soc., Chem. Commun.* 1992, 1489-1490.

How do we get at this diversity? A culture independent mechanism for accessing bacterial natural products is needed



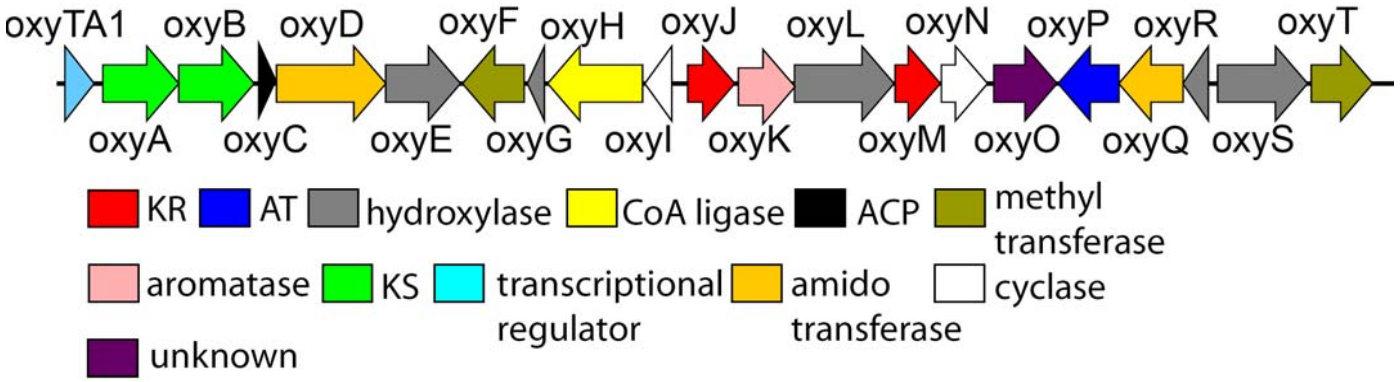
heterologous biosynthetic pathway expression is a known methodology with a major limitation

Heterologous pathway expression does not generate desired metabolites due to insufficient gene expression



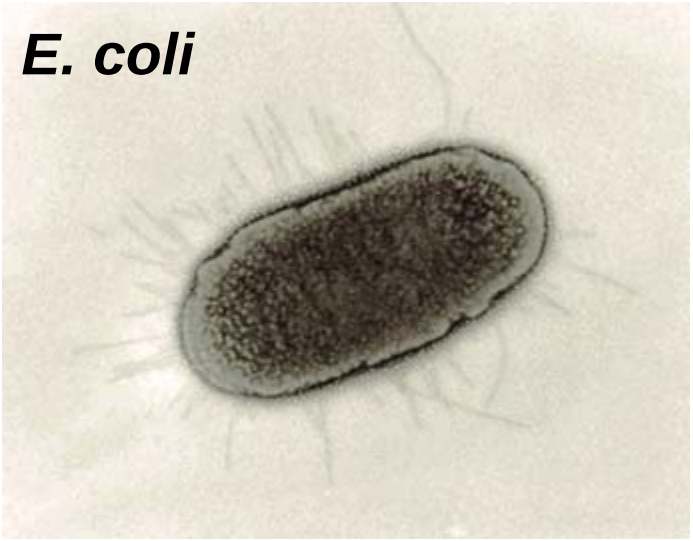
oxytetracycline

- polyketide natural product
- produced by *Streptomyces rimosus*
- over 20 genes involved in biosynthesis



If any of the synthetic genes are not expressed no oxytetracycline will be produced by the hetereologous host

Our “universal transcriptional activator” is sufficient to activate transcription of heterologous pathways in *E. coli*.

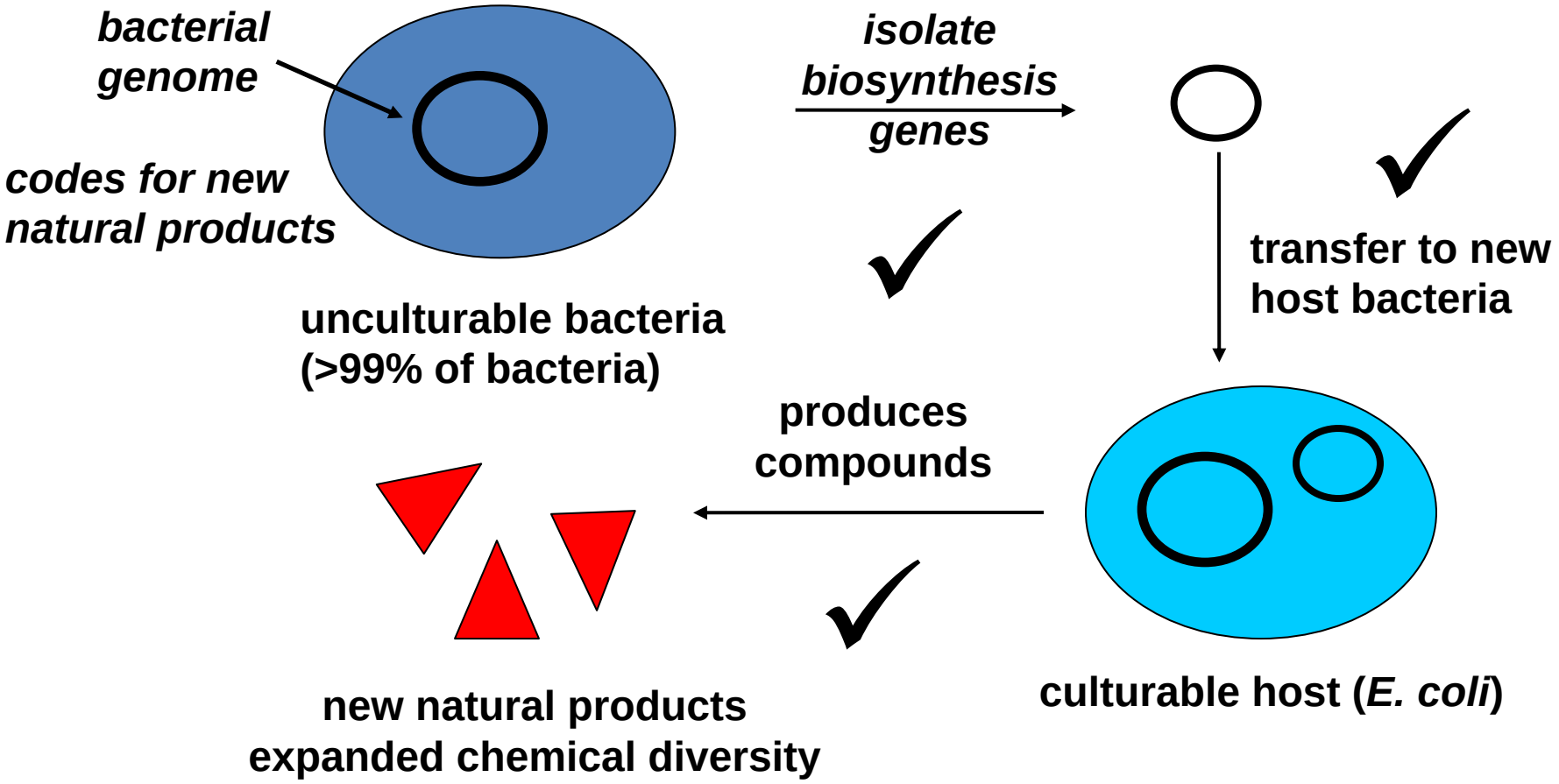


- *optimal host*
- *well understood metabolism*
- *easy to grow*
- *cannot normally express biosynthetic pathways*

addition of the “Universal transcriptional activator” enables *E. coli* to heterologously express biosynthetic pathways

Used this approach to make oxytetracycline in *E. coli*.

Provides a solution enabling culture independent production of new natural products


bacterial genome
codes for new natural products
unculturable bacteria (>99% of bacteria)
isolate biosynthesis genes
transfer to new host bacteria
culturable host (E. coli)
produces compounds
new natural products
expanded chemical diversity